

**REDUCING HYDROGEN CYANIDE IN CASSAVA BASED DIETS TO IMPROVE
GROWER PIG PERFORMANCE IN KENYA**

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**A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for
the Master of Science Degree in Animal Nutrition of Egerton University**


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DECLARATION AND RECOMMENDATION

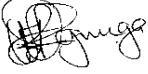
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DEDICATION

This work is dedicated to Alpha and Omega, God for His protection and guidance throughout my studies. Lastly, my parents (Faustin MUNYAMPIRWA and late Josephine MUKAKARANE), my husband (Jerome NDIZIHIWE), and children (Cynthia, Clement, and Ange Princesse), and siblings, thank you for constantly encouraging and supporting me in prayers. May the Almighty God bless you abundantly.

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ABSTRACT

In Kenya, the benefits of pig farming help many households; however, its growth is constrained by the high cost of commercial pig feeds, mainly caused by the constant dependence on maize. Cassava has been identified as a substitute for maize in feeds, however, its elevated levels of crude fibre and the hydrogen cyanide (HCN) contribute to poor nutrient digestibility in animals. This study evaluated the effect of Fermentation and Enzyme Treatment on the nutritional composition, *in-vitro* digestibility, cyanide reduction of Cassava (*Manihot Esculenta*) based diets (In a ratio of 1: 0.3; Root: 1; Leaf: 0.3, CRLM), and its impact on grower pig performance. In the first experiment, CRLM was subjected to five treatments: untreated (T1), fermented with *Aspergillus niger* (T2), spontaneously fermented (T3), enzyme-treated with Natuzyme® (T4), and fermented with *Lactobacillus brevis* (T5). Nutritional composition, digestibility, were analyzed. What the data reveals is that the optimal treatment was reconfirmed in the following feeding trials. In experiments two and three, 18 Landrace x Large White crosses (20 +/- 3 kg) were allotted three diets; 0% fermented CRLM (T1), 20% FCRLM (T2) and 40% FCRLM (T3). These diets were evaluated for their impact on growth performance, carcass traits and sensory characteristics of the pork. The *in-vitro* digestibility data, feeding trials and carcass data were all two-way analysed using the ANOVA procedures available in the Statistical Analysis System (SAS, 2023). Differences between means were subjected to Tukey's test for significance at $p < 0.05$. In the clinical trial results obtained from the pretreatment phase, it was inconclusive that during the course of spontaneous fermentation (T3) the CRLM quality was improved by the decrease of HCN concentration from 45.00 ppm to 8.00 ppm and CF from 5.16% to 3.87%, and increase in crude protein from 7.47% to 11.09% and dry matter digestibility from 93.67% to 98.62%. The trial results indicated that the feeding of 20% of fermented CRLM resulted in the optimal average daily gain of 0.68 kg/day and the best feed conversion ratio of 4.16, with no detrimental effects on carcass traits and meat quality. These results indicated that spontaneous fermentation substantially increases the nutritive value of CRLM and as such, its use as a feed ingredient for pigs is safe and nutritionally adequate. Incorporating up to 20% FCRLM in grower pig diets can reduce feed costs while maintaining growth performance and pork quality.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADF	Acid Detergent Fiber
ADG	Average Daily Gain
<i>Ad libitum</i>	Free choice feeding
ANFs	Anti-Nutitive Factors
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ASAL	Arid and Semi-Arid Lands
ATTD	Apparent Total Tract Digestibility
CO₂	Carbon dioxide
CH₄	Methane
FCR	Feed Conversion Ratio
FCRLM	Fermented Cassava Root-Leaf Meal
GDP	Gross Domestic Product
GLM	General Linear Model
GMOs	Genetically Modified Organisms
HCN	Hydrogen Cyanide
HSD	Honest Significance Difference
IMF	Intramuscular Fat
IVDMD	<i>in-vitro</i> dry matter digestibility
ISAPP	International Scientific Association on Probiotics and Prebiotics
KALRO	Kenya Agricultural and Livestock Research Organization
NaOH	Sodium Hydroxide
NDF	Neutral Detergent Fiber
NRC	National Research Council
NSP	Non-Starch Polysaccharides
SAS	Statistical Analysis System
SDGs	Sustainable Development Goals.
SW	Slaughter Weight
TAP	Tatton Agriculture Park
VFA	Volatile Fatty Acids

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Across low and middle income nations, the population is steadily increasing, and the demand for food, particularly meat, increases proportionally (Fróna *et al.*, 2021). In Kenya, the human population is expected to reach 96 million from 47 million. The population in the Urban region is predicted to be three times the current of 12 million up to 40 million in 2050 (FAO, 2017). As the population increases, the amount of land per household decreases.

However, 83% of Kenya's land is classified as arid or semi-arid, suitable for livestock production such as cattle raising and grazing (Nalinya *et al.*, 2020). This has necessitated that the available land in high-rainfall regions intensifies from agricultural practices to produce more in sustaining food security and poverty reduction. The economic reality of developing countries is food production instead of its import. Due to the available land and resources, this must be achieved by producing animal species that have faster growth with cheaper feeds (Weishaupt *et al.*, 2020), especially Pigs. Livestock sector remains the main driver of the Kenyan economy, accounting for about 15% of the GDP and almost 50% of the resources of the agricultural sector (Munywoki, 2021).

Smallholder pig production contributes to food and nutrition stability and also to the creation of employment in many rural households in Kenya (Micheni *et al.*, 2020). In 2023, Kenya produced 40,055 tonnes of pork from about 840,160 pigs, generating revenue of KSh 19.5 billion (KNS, 2023). Consumption of pork in Kenya has pitched by 60% in the years 2016 and 2017 (Muthui *et al.*, 2019). Such consumption, therefore, increases demand and encourages more people to engage in pig farming or the associated value chain, which promotes employment.

Pigs exhibit high reproductive potential, characterized by short breeding cycles, efficient feed utilization, and faster economic returns relative to most other livestock species (Schild *et al.*, 2020). This, combined with improved pig farming techniques, is leading to more farmers adopting pig farming (Muthui *et al.*, 2019). Pig farming in Kenya is mostly done by small farmers who have 10-100 pigs at a time (FAO, 2012). Small-scale pig farming is characterized by different production systems, including free-range, semi-intensive, and intensive production. Local and crossbreds (exotic x local) dominate, while exotics are kept by a few farmers. However, poor disease and parasite control and inadequate nutrition reduce productivity. However, the high cost and seasonal shortages of conventional feeds like maize and soybean continue to constrain the

sector's rapid development (Mugumaarhahama *et al.*, 2020). This necessitates investigating the potential of locally available alternative feed resources like Cassava (*Manihot esculenta* Crantz).

Cassava (*Manihot esculenta* Crantz) ranks as the second most widely cultivated root crop in Kenya, following the Irish potato. Its notable tolerance to drought and adaptability make it a vital subsistence crop among rural communities, contributing significantly to household food security and poverty reduction (Gatto *et al.*, 2021). Cassava production in Kenya is mainly concentrated in a few agro-ecospheres. These include Western Kenya, the coastal and eastern regions of the country. In these regions, cassava accounts for a large proportion 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. The average yields of cassava in Kenya are 12.3 t/ha (Senkoro *et al.*, 2018). It can potentially completely replace maize as an energy source in pigs. Cassava also contains some anti-nutrients that affect animal health when ingested in high amounts (Bayata, 2019). Its energy yield per unit land area is between 25 and 60 tonnes /ha (Sanusi *et al.*, 2023). Cassava and its foliage are promising options for livestock feed, given the high production levels in Kenya (Githunguri *et al.*, 2021).

Cassava leaves have been recognized as a rich source of antioxidants, dietary fiber, vitamins, and essential amino acids. The protein content of the leaves has been documented to range between 17.7 and 38.1% on a dry matter basis (Da Silva Santos *et al.*, 2020). Soybean, a widely recognized plant-based protein source, contains 39.39-44.5% crude protein on a dry matter basis; however, cassava leaves represent a viable alternative protein source. In addition, they are rich in vitamins, including B1, B2, C, Carotenoids, and β -carotene (pro-vitamin A), ranging from 40.6 to 80.4 mg per 100 g dry matter, corresponding to 6.77-13.40 mg retinol equivalents (RE)/100 g dry basis. This indicates that just 10 g of dried cassava leaves could supply the recommended daily intake of vitamin A, which is essential for maintaining ocular health (Rodriguez, 2016). Cassava leaves also are concentrated in minerals, like calcium, magnesium, and potassium (Alamu *et al.*, 2021).

Cassava leaves contain cyanogenic glycosides, such as linamarin and lotaustralin, which can release toxic hydrogen cyanide and therefore restrict the safe level of consumption. These compounds are harmful to animals and can be fatal if they hydrolyze to hydrogen cyanide after ingestion. Long-term use of cyanide causes various health problems such as goiter, cretinism, Konzo disease, and neurodegeneration (Mburu, 2012; Rivadeneyra & Rodríguez, 2020).

Previous studies have shown that anti-nutritional factors such as tannins, crude fiber, phytates, and trypsin inhibitors can impair livestock growth and reproductive efficiency by reducing nutrient bioavailability (Koné *et al.*, 2022). However, other studies argue that fermentation improves the digestibility of proteins, reduces anti-digestive constituents, and improves microbial safety (Alrosan *et al.*, 2023). There is, however, a paucity of scientific information concerning the nutritive value of cassava root and leaf meals, in particular fermented ones, in growing pig diets in Kenya. This study, therefore, sought to fill this gap by determining the effects of fermented cassava root and leaf meals in grower pig diets on their performance.

1.2 Statement of the Problem

In Kenya, the swine farming sector serves as a primary economic activity for many families. The thriving expansion of the sector is, however, hampered by the economic burden of the cost of animal feeds, which accounts for an average of 60 - 70% of the total costs of production. One of the biggest pyromid energy feeds is a major constituent of pig feeds, however, its availability is limited and its cost is continuously rising. Consequently, smallholder farmers may resort to low-cost, substandard feed options, which frequently cause nutrient deficiencies and reduce pig growth performance by 30-40% relative to animals fed nutritionally balanced diets. Cassava is a widely available crop that could substitute maize due to its high carbohydrate content. However, its utilization in pig diets is restricted by anti-nutritive factors like hydrogen cyanide (HCN) that accounts about 40 to 50 ppm while untreated. These levels exceed the safe threshold of 10 ppm, and crude fiber levels of 5 to 7%, both of which limit nutrient absorption, feed efficiency, and growth. These challenges reduce its value as a reliable feed ingredient for monogastric animals. Fermentation using a probiotic could reduce the anti-nutritive factors like high cyanogenic glycosides (HCN), and crude fiber content, but there is insufficient knowledge on the performance of pigs fed on probiotic fermented cassava root-leaf-based diets; thus, the reason to conduct this study.

1.3 Objectives of the Study

1.3.1 General Objective

To contribute to food and nutrition security through sustainable pig production using cassava-based diets.

1.3.2 Specific Objectives

- i. To determine the content of cyanogenic glycosides (HCN), fiber, and *in-vitro* digestibility of fermented, probiotic, enzyme-treated, and untreated cassava-based diets.
- ii. To determine the feed intake, feed conversion ratio, and growth rate of grower pigs offered treated cassava-based diets.
- iii. To determine the effect of treated cassava-based diets on carcass quality characteristics (Dressing percentage, Carcass pH, Sensory attributes) of grower pigs.

1.4 Hypotheses

- i. There is no significant difference in the content of cyanogenic glycosides (HCN), fiber, and *in-vitro* digestibility of fermented, probiotic, enzyme-treated, and untreated cassava-based diets.
- ii. Inclusion of treated cassava-based diets in grower pig diets has no significant effect on feed intake, feed conversion ratio, and growth rate.
- iii. Treated cassava-based diets have no significant effect on carcass quality characteristics of grower pigs.

1.5 Justification of the Study

Pig farming in Kenya is critical in closing the food and nutrition gap. The sector has experienced rapid growth in pig production and demand for pork, but faces constraints such as seasonal scarcity of feed and high prices of locally available feeds, which limit the productivity and income of smallholder farmers. Affordable alternative feed resources sustain growth and reduce losses during periods of supply fluctuations. These feed resources should also be affordable and meet the nutrient requirements of pigs with less or no competition with humans. Cassava is a drought-tolerant crop that provides a basic diet for most rural households to address food insecurity and mitigate poverty.

In Kenya, Cassava is cultivated on about 90,394 Ha of land, with a total annual production of close to 1,112,000 metric tonnes (FAO, 2018). It is therefore readily available. Cassava, particularly its roots and leaves, contains an amino acid-derived anti-nutrient called Cyanogenic glucoside (HCN). Cassava leaves are nutrient-dense, particularly in protein, with content ranging from 17.7 to 38.1% on a dry matter basis. Nevertheless, they contain a relatively high crude fiber content of 15-35%, whereas growing pigs can efficiently utilize feeds containing up to 6% crude fiber; exceeding this threshold impairs nutrient absorption. Consequently, it is necessary to process

cassava root-leaf meal based diets through fermentation or supplementation with exogenous enzymes to improve their digestibility.

Various processing techniques and methods have been used to reduce these anti-nutritional components in plant materials, such as sun-drying, oven-drying, steaming, shredding, steeping, steaming and sun drying. Several studies have suggested the use of microbial fermentation of solid feeds to enhance nutrient bioavailability and boost the nutrient content of feeds. In these studies, microorganisms like lactic acid bacteria, filamentous fungi (*Rhizopus oligosporus*), fungi, and yeast were used to ferment peas and cassava. The results showed an increase in nutritional value and a reduction in anti-nutritional factors.

This study examined the possibility that fermentation processed value -added cassava root and leaf products could be used as affordable, nutritious substitutes for traditional pig feed ingredients. The focus was on evaluating their nutritional composition, safety (reduced cyanogenic glycosides content), and impact on pig performance and carcass quality. Additionally, the study aimed to promote local cassava utilization , thereby supporting circular Agriculture and enhancing the sustainability of pig production systems in environments with limited resources. As a result, these efforts support the attainment of Sustainable Development Goals (SDGs) 1 and 2, which focus on poverty alleviation and the elimination of hunger. Furthermore, this study offers valuable insights into the application of fermented cassava root-leaf meal in the formulation of pig diets.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of the Livestock Industry in Kenya

The livestock sector is a key component of Kenya's economy, accounting for roughly 15% of the national gross domestic product (GDP) and representing nearly 50% of the total value generated by the agricultural sector (Munywoki, 2021). Livestock products were valued at Ksh 1.891 billion in 2016 (DLP Report, 2016). Approximately 70% of the livestock are in arid and semi-arid lands (ASAL) which make up about 80% of the country. It is estimated that the 13 million Kenyans living in the ASALS region derive their livelihood mainly from animal husbandry (Wambua, 2019). Livestock production is the main source of livelihood for people in arid and semi-arid lands (ASAL), accounting for 13.90% of employment in these areas and more than 95% of family income.

The livestock industry continues to be a significant employment and income generator through dairy farming, poultry and pig production (Kimaru *et al.*, 2023). It makes up about 30% of all marketed agricultural products within the country while also satisfying a significant share of the domestic demand for meat, milk, and other livestock products. The exports of live animals and meat, dairy products, and even processed pork also bring in valuable foreign exchange (Herrero *et al.*, 2013). Additionally, the sector provides the necessary primary products for agro-based industries. Having noted its dominance in rural areas, livestock farming plays a critical role in terms of food and nutritional security, income and employment opportunities, and even poverty alleviation (Wezi *et al.*, 2023).

For women and other disadvantaged members of society, the livestock industry provides one of the few productive assets that are available to them. The total livestock population in Kenya includes 18 million cattle of which approximately 5 million are crossbred and exotic dairy breeds, 17 million sheep, 27.7 million goats, 334,689 pigs, 32 million poultry, 0.8 million rabbits, and 3 million camels (FAO, 2017). The livestock industry in the country has the potential to enhance the livelihoods of the people and strengthen the food self-sufficiency of the country (Mutsami & Karl, 2020).

2.2 Pig Production in Kenya

Piggeries make a meaningful contribution to the socioeconomic development of both the urban and rural areas of Kenya. Nationally, about 70% of pig production comes from smallholder farmers, and the rest is from commercial producers (Mbuthia *et al.*, 2015). Small-scale farmers are

attracted to pig rearing as it is economically viable and correlates with low opportunity costs of labour, high farrowing rates, short periods of reproduction, and high feed efficiency (Murungi *et al.*, 2021). In Kenya, pig production is predominantly subsistence and small scale for self-sufficient and income-earning households, a trend related to land congestion (Motsa'a *et al.*, 2019). Because of the limited spatial requirements, pigs can be reared in a way that saves space and are therefore valuable livestock for farmers with fewer resources. This system is particularly evident in urban informal settlements, where smallholder pig farming is practiced on a minor scale. These favorable characteristics allow farmers to venture into pig production with minimal startup capital and achieve quick financial returns. The national pig population is estimated at 334,689, with most found in Central (27.48%) and Western (26.24%) regions of Kenya (Murungi *et al.*, 2021).

2.3 Pig Production System in Kenya

In Kenya, pig farming is classified into three main systems: large-scale intensive operations, small-scale commercial farms, and free-range or backyard production systems (Mutua *et al.*, 2022). Large-scale intensive farms are typically well-structured commercial enterprises, such as Farmers Choice, maintaining herds of 5,000 to 30,000 pigs. These operations participate in the complete value chain, encompassing pig rearing, slaughter, and subsequent meat processing. Small-scale production of commercial pig farming is mainly carried out by farmers in the city and the suburbs surrounding the city; Characterized by the use of exotic breeds in captivity and providing better nutrition, therefore better performance (Mengesha *et al.*, 2022).

In western Kenya, farmers implement free-range or backyard systems where pigs are tethered outside. Pigs under this system are left to graze and forage, so they are characterized by low production (Mbuthia *et al.*, 2015; Mengesha *et al.*, 2022). The average daily gain (ADG) of pigs in this system is relatively lower at 0.182-0.480 kg/day compared to pigs kept in the intensive system with an average daily gain (ADG) of 0.570-0.831 kg/day (Carter *et al.*, 2016).

2.4 Sources of Ingredients for Pigs in Kenya

In pig enterprise, feed costs constitute nearly 80% of total production expenses, making it the most crucial factor in determining profitability. The feed ingredients, typically by-products of grain milling and oil cakes, are often imported from neighboring countries (Muthui *et al.*, 2019). The concentration of feed producers in urban areas in Kenya increases costs for smallholders due to transportation expenses. Seasonal fishing restrictions also cause fluctuations in fishmeal prices (Chia *et al.*, 2019). Consequently, many pig farmers resort to using surplus agricultural products,

market and kitchen waste, and food scraps, which leads to nutritional imbalances and reduced pig productivity.

2.5 Pig Nutrition

Pigs are monogastric animals whose digestive physiology closely resembles that of humans and poultry, although minor anatomical and functional variations exist among their digestive organs (Ramalanjaona *et al.*, 2016). Ingested feed undergoes enzymatic digestion within the stomach and small intestine, allowing soluble nutrients to be absorbed across the intestinal walls of the small intestine (Liu, 2015). According to (Liu, 2015), indigestible components such as non-starch polysaccharides (NSPs), resistant starch, and undigested proteins move into the colon and cecum, where microbial fermentation occurs. During this process, fiber is degraded by gut microbes, leading to the production of methane (CH₄), Carbon dioxide (CO₂), and volatile fatty acids including acetic, butyric, and propionic acids (Lukitawesa *et al.*, 2020).

Due to the nature of their digestive system, pigs are inefficient at digesting high-fiber feeds such as grass, silage, and hay, which makes them largely dependent on grain-based diets. For feed to be nutritionally beneficial, the process of digestion and nutrient absorption must take place effectively (Jacyno *et al.*, 2016). Nutrient digestibility depends on several factors and can be classified into feed and environmental factors. For example, young, especially recently weaned pigs have limited enzyme secretion and less absorption capacity (Pitarch *et al.*, 2017). This is in contrast to adult pigs, which have a more developed and wider digestive tract and a relatively longer residence time in the digestive tract (Jacyno *et al.*, 2016).

2.6 Nutrient Requirements of Pigs

Nutrients are chemical compounds contained in feed that are necessary for the maintenance of animal body functions, growth, lactation, and pregnancy (Shurson *et al.*, 2021). These include fats, proteins, carbohydrates, vitamins, minerals, and water. The nutritional requirements of pigs vary according to body weight and physiological condition, performance potential, and the environmental conditions to which the animal is exposed (NRC, 2012; Pomar *et al.*, 2021).

Table 1: Nutrient requirement of growing-finishing pigs fed ad-libitum amount /kg diet (90% dry matter) based on body weight

Nutrient	Live weight in Kilograms			
	11-25	25-50	50-75	75-100
DE Kcal/Kg	3402	3402	3402	3402
ME kcal/kg	3350	3300	3300	3300
CP(g/kg)	209	180	155	132
Amino acid (g/kg)				
Lysine	14.0	11.2	9.7	8.4
Methionine	4	3.2	2.8	2.5
Threonine	8.7	7.2	6.4	5.6
Methionine cysteine	7.9	6.8	5.9	5.1
Arginine	6.2	5.0	4.4	3.8
Leucine	14.1	11.3	9.8	8.5
Isoleucine	7.3	5.9	5.2	4.5
Phenylalanine	8.3	6.8	5.9	5.3
Minerals (g/kg)				
Phosphorus	6.0	5.6	5.2	5.2
Calcium	7.0	6.6	5.9	5.2
Magnesium	3	2	2	2
Chlorine	3.2	0.8	0.8	0.8

Source: NRC (2012).

2.7 Dietary Fiber

Dietary fiber consists of a complex mixture of structural and non-structural polysaccharides, together with lignin from plant cell walls, which are resistant to degradation by mammalian endogenous enzymes (Torhemen *et al.*, 2017). This limitation arises from the inability of endogenous enzymes to hydrolyze the glycosidic linkages present in dietary fiber. Consequently, the undigested fiber fractions are subjected to partial or complete microbial fermentation in the colon and cecum, leading to the production of volatile fatty acids (VFAs) and gases such as methane (CH₄), Carbon dioxide (CO₂), and hydrogen (H₂) (NRC, 2012).

Among the non-starch polysaccharides (NSPs), cellulose, hemicellulose, and pectin. Cellulose, hemicellulose, and pectin constitute the major structural components of plant cell walls, whereas glucomannans, fructans, and gums occur in smaller quantities (Kotatha *et al.*, 2023). The digestibility of fiber varies widely and depends on physicochemical characteristics, as sugar beet fibers are completely soluble, while bran is insoluble in growing pigs (Do *et al.*, 2023). Many studies have shown that age can affect the digestibility of fibre in pigs where mature pigs, sows, and boars have higher levels of cellulolytic bacteria than young pigs (Jarrett & Ashworth, 2018).

2.8 Overview of Non-Conventional Feedstuffs

In tropical regions, pig production is generally encouraged only when it relies on feed resources that do not compete directly with human food supplies (Kambashi *et al.*, 2014). However, with the increasing population, competition for food resources becomes unavoidable, leading to the exploration and utilization of alternative feed sources. Non-conventional feed ingredients comprise agricultural residues, by-products from slaughterhouses, agro-industrial wastes, household leftovers, and cassava components such as roots and leaves. Although these materials are inexpensive and readily available, they are generally characterized by high fiber content, low energy, and carbohydrate levels, and the presence of various anti-nutritional compounds (Kambashi *et al.*, 2014). These alternative feed resources have demonstrated considerable potential for use in livestock feeding, particularly cassava (*Manihot esculenta* Crantz), which serves as an excellent carbohydrate source and may effectively substitute maize as the primary energy ingredient in diets for growing pigs (Williams *et al.*, 2023).

2.8.1 Cassava (*Manihot esculenta* Crantz) in Kenya

Cassava is a perennial woody plant cultivated for its edible roots, believed to have originated in tropical America, and was introduced to the African continent through the Congo Basin by Portuguese explorers around 1558 (Githunguri & Njiru, 2021). Currently, cassava contributes significantly to the livelihoods of more than 300 million people across Africa, owing to its high levels of carbohydrates, vitamins B and C, and essential minerals (Bayata, 2019; Zekarias *et al.*, 2019).

Cassava's drought tolerance makes it a crucial crop for enhancing food security and reducing poverty, particularly within rural communities. In Kenya, cassava ranks second among root crops after the Irish potato and is mainly cultivated in the western, coastal, and eastern parts of the country. In 2017, national production reached approximately 1.11 million tonnes from 90,400 hectares of land, translating to an average yield of 12.3 MT/ha; this is significantly lower

than the estimated potential yield of 50 MT/ha (Kidasi *et al.*, 2021) mainly due to the continued cultivation of traditional landraces that possess inherently low yield potential and are frequently vulnerable to locally prevalent viral diseases (Githunguri & Njiru, 2021).

2.8.2 Nutritional value of cassava feed products

The nutrient profile of cassava varies according to the plant part considered (root or leaf), the cultivar grown, geographical location, plant maturity, and prevailing environmental conditions (Bayata, 2019). In regions with poor soil fertility or limitations such as high phosphorus fixation, erosion, low levels of exchangeable bases, and elevated aluminium concentrations, cassava roots can remain in the field, thereby allowing more fertile land to be allocated to high-value crops (Ndare, 2019). Cassava leaves are a protein-rich feed source, with total essential amino acid levels surpassing those found in soybean (Chaiareekitwat *et al.*, 2022).

2.8.3 Cassava whole roots

Cassava roots serve as an excellent source of energy and act as a physiological energy reserve, containing carbohydrates at levels of 32-35% on a fresh weight basis (FW) and 80-90% on a dry matter basis (DM) (Zekarias *et al.*, 2019). Starch constitutes the majority of carbohydrates in cassava roots, representing for approximately 80% of the total carbohydrate content, with amylopectin representing 83% and amylose 17%. In addition, cassava roots contain minor amounts of simple sugars, including sucrose, glucose, fructose, and maltose (Zekarias *et al.*, 2019).

The fiber concentration in cassava roots is influenced by both the cultivar and the maturity of the root, generally remaining below 1.5% in fresh roots and 4% in powdered form. The lipid content ranges from 0.1% to 0.3% on a fresh weight basis, with linoleic, oleic, and palmitic acids being the predominant fatty acids present (Onyango, 2019). Cassava roots have a relatively low protein content, ranging from 1% to 3% on a dry matter basis, and from 0.4 to 1.5 g per 100 g on a fresh weight basis (Zekarias *et al.*, 2019).

Cassava roots contain many minerals like calcium, iron, potassium, magnesium, copper, zinc, and manganese, and Zekarias *et al.* (2019) have shown that calcium content in cassava roots is ranged between 15 and 35 mg/ 100 g of consumable part. Cassava roots also contain vitamin C which ranges between 15 to 45 mg/100 g of edible portions. The mineral and vitamin concentrations present in cassava roots are deficient; therefore, it is recommended to combine them with cassava leaves (Montagnac *et al.*, 2009).

2.8.4 Nutritional value of cassava leaves

The concentration of nutrients found in cassava leaves, in terms of both quality and quantity, is subject to varietal differences, growth stage, and proportion of leaves and stems in each plant (Zekarias *et al.*, 2019). Fujimoto *et al.* (2017) indicated that cassava leaves are good sources of protein, essential minerals, and vitamins B1, B, C, and carotene. On the basis of the dry matter of the leaves, several authors (Chaiareekitwat *et al.*, 2022; Zekarias *et al.*, 2019) have reported that cassava leaves have an average crude protein content of between 14 to 40%.

Cassava leaves have a crude protein of 10.9g/100g and the amino acid profile of cassava leaf protein is considered well balanced (Zekarias *et al.*, 2019). Other forms of carbohydrates found in cassava leaves include starch and amylose which range between 19% to 24% (Zekarias *et al.*, 2019). Other studies indicated that cassava leaves contain minerals such as iron, zinc, manganese, magnesium, and calcium which are found in high concentrations (Chaiareekitwat *et al.*, 2022; Zekarias *et al.*, 2019).

2.8.5 Anti-nutritive compounds in cassava root-leaves

Although cassava is a great source of nutrients, it is still considered to have anti-nutrients like cyanides, oxalates, tannins, and phytates that inhibit the absorption of essential nutrients and damage digestion (Benson *et al.*, 2023). Cyanides, for instance, vary between 75 to 1,000 mg/kg, with the age, variety, and environment contributing to the cyanide level (Benson *et al.*, 2023). The leaves do possess linamarin and lotaustralin, which are cyanogenic glycosides, in high concentration although these tend to decrease with the age of the leaves due to other leaves maturing faster (Olayemi *et al.*, 2020). Linamarin hydroxynitrile lyase and linamarase enzymes convert the glycosides to toxic hydrogen cyanide (HCN). This poses a great health risk to living beings (Olayemi *et al.*, 2020). In neo, the leaves are finely shredded and soaked in water to dampen them, then pounded, boiled, and fried which greatly reduces the cyanide concentration to about 63-73%, and other combined methods reduce it by 97% (Ojiambo *et al.*, 2017). Fermentation with probiotics and the leaves tends to improve the leaves' nutritional value while greatly lowering the anti-nutrients (Hawashi *et al.*, 2019).

2.9 Approaches to Improve Utilization of Fibrous Diets by Pigs

The efficient use of fibrous diets in growing pigs is contingent on the successful digestion of complex structural barriers like cellulose, hemicellulose, and lignin. Varied processes like chemical, mechanical, and biological treatments have been shown to improve the degradability of dietary fibre, thus improving feed efficacy (Kanengoni *et al.*, 2015).

2.9.1 Application of exogenous enzymes

According to Vries *et al.*, (2012) removing cell wall-associated nutrients entails using external enzymes (particularly those dismantling cell walls) to remove side chains and fragment intricate polymers. Individual or combined carbohydrases, proteases, and phytases are among the most utilised enzymes. Factors like substrate compatibility, feed particle size, enzyme dosage, enzyme activity, and the level of non-starch polysaccharides (NSPs) present govern their impact on improving nutrient utilisation (Vries *et al.*, 2012). Besides enhancing nutrient availability and digestibility, enzyme supplements also support the developing digestive systems of young pigs (Moeser & Kempen, 2002).

2.9.2 Fermentation treatment

Fermentation is a transformative process where starches and sugars are converted into lactic acid, volatile fatty acids (VFA), carbon dioxide (CO₂), alcohols, and methane (Humer & Schedle, 2016). It can occur naturally or be aided by an inoculum containing specific *Lactobacillus* starter cultures, which helps achieve a pH below 4.5, a lactic acid bacteria count of about 9 log₁₀ colony-forming units, and a lactic acid concentration of around 150 mmol/l (Missotten *et al.*, 2010). The organic acids produced during anaerobic fermentation have antibacterial properties because they can permeate bacterial cell membranes and lower the internal pH, disrupting enzyme activity inside the bacteria (Plumed & Von, 2009).

Fermentation is also effective at reducing anti-nutritional factors (ANFs). Yousif and El Tinay (2003), in his work with natural fermentation of pearl millet showed a decrease in total polyphenols and phytic acid accompanied by enhanced *in-vitro* protein digestibility. Similarly, it can lower glucosinolate levels in rapeseed meal and enhance energy and calcium absorption in broilers. Additionally, fermentation by bacteria producing NSP-degrading enzymes reduces fiber content and increases amino acid digestibility in fermented corn-soybean mixes (Shi *et al.*, 2017). The pH reduction that occurs during fermentation further promotes mineral absorption, as ferrous iron, which is poorly utilized, is converted into the more absorbable ferric form (Nkhata *et al.*, 2018). Jørgensen *et al.* (2010) also noted that fermenting barley and wheat grains for two days enhanced apparent total tract nutrient digestibility by 2–3% and 1–2%, respectively.

2.9.3 Fermentation using a probiotic

The International Scientific Association on Probiotics and Prebiotics (ISAPP) has recently reaffirmed the FAO/WHO definition of probiotics, with minor revisions, describing them as “live microorganisms that, when administered in adequate amounts, provide health benefits to the host”

(Hill *et al.*, 2014). Common probiotic species include bacterial genera such as *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Bacillus*, and *Streptococcus*, along with yeast strains like *Saccharomyces* (Maftai *et al.*, 2024). Widely documented probiotic use in food and animal feed formulations started many years ago (Yirga, 2015). Probiotics must endure an intravenous odyssey, including hostile environmental factors, such as passage through acid(s) in the stomach and bile, variable levels of enzymes, and dilation of the intestinal mucosa, and must manage to stick to and populate the intestinal mucosa to provide assistance (He *et al.*, 2019). Probiotic microorganisms include, but are not limited to, *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Escherichia coli*, *Lactococcus lactis*, and some *Enterococcus* species, not forgetting the non-pathogenic yeasts like *Saccharomyces*, which are highly valued in the field of food for their beneficial health effects (Fijan, 2014) To maintain low production costs and greater probiotic consistency, probiotic starter cultures must have certain desired characteristics traits (Hill *et al.*, 2014). Fermentation of fresh raw materials such as vegetables, acid milk, meat, and cereals in food and feed is greatly supported by lactic acid bacteria (LAB) which have not undergone such vigorous fermentation (Kembabazi *et al.*, 2021).

2.9.4 Overview of *Aspergillus niger* as a probiotic used in feed fermentation

With respect to the taxonomy of fungi, the filamentous fungus known as *Aspergillus niger* is in the genus *Aspergillus*. It can be found as a contaminant in food products as well as in soil, the decaying remains of organisms, and certain decaying organic materials. Additionally, it is prevalent in the soil, the decaying remnants of organisms, in food products, and in organic matter, and is predominantly found in industrial barracks, as well as in other places indoors (Concepción *et al.*, 2023). Morphologically, *Aspergillus niger* has distinctly ‘V’ shaped bifurcating branches and a hyaline and septate culture medium. Such coloration can be black, brown, or yellow, and the colonies can assume these colours depending on the specific strain intermixed with the optimal growth conditions (Barros *et al.*, 2020). This organism is a significant microbiological resource, with its foremost use being the production of fermented products, as well as in industrial enzyme production. Out of all the species in the *Aspergillus* genus, the most contrasting and dominant species is *Aspergillus brasiliensis*, whose distinguishing physiologic and metabolic traits are the basis of its bioindustrial domination.

2.9.5 Use of *Aspergillus niger* in fermentation of cassava root-leaf based diets

Adesokan *et al.* (2023) improved quantification of the fermentation of *Aspergillus niger* on cassava roots for gari production while lowering the cyanogenic glucosides and positively

altering the sensory traits of the fermentation products. Fawole *et al.* (2022); and Susan (2021) noted *Aspergillus niger*'s ability to produce and secrete amylases and cellulases during fermentation, which hydrolyse and assimilate complex polysaccharides. Ramos *et al.* (2022) demonstrated *Aspergillus niger*'s detoxification of cassava leaves for fufu, improved the nutritive value by lowering cyanide and phytic acid, and increasing the proteolytic and digestible activity for the product. *Aspergillus niger* is capable of accomplishing cyanide concentration of cassava products for fermentation by the fungi to 95 percent, according to recent studies, by employing linamarase to hydrolyse cyanogenic glycosides into HCN, which the cassava products will be metabolised by the fungi ((Banwo *et al.*, 2023; Elie *et al.*, 2022). *Aspergillus niger* and *Lactobacillus brevis* significantly contribute to the fermentation of cassava-based diets; however, they can both pose a number of potential risks for the production of feeds.

The synthesis of toxins, notably ochratoxin A during the production of citric acid by *Aspergillus niger*, may lead to the deterioration of feed quality, potentially harmful to animal health (Ghafouri & Alhamiri, 2024) In addition, the effectiveness of fermentation is extremely variable, while the impractical detoxification of residual cyanide could be left within a perilous range. The worth of soluble vitamins and proteins may also be lost with excessive fermentation. On the other hand, the employed probiotic *Lactobacillus brevis* does not provide a guarantee. Its activity and survival are challenged by fermentative and intestinal microflora (Oloya *et al.*, 2024). Lastly, and probably most crucial, the attainment of adequate fermentation conditions: the temperature and pressure ranges, moisture, and especially the economic addition of a proteinaceous growth factor, all set profound limits to smallholders.

2.9.6 Spontaneous fermentation

Fermentation is also referred to as spontaneous, natural, or uncontrolled fermentation; it is an ancient fermentation practice that occurs independently of any commercial starter culture. It is based on the microorganisms that exist on the cassava roots and leaves, on utensils, in water, and even in the air (Halake & Chinthapalli, 2020; Spitaels *et al.*, 2017; Wang *et al.*, 2023).

This process, which is traditionally practised in rural settings and in bottom of the pyramid (BoP) markets, is used because of its simplicity, low cost, and effective detoxification of raw cassava. The substrate and the local bacteria, as well as the biochemical (which are often complex and varied) processes that occur during fermentation, determine the bacterial community. Biochemically important substrates in ammonia, that is, high protein substrates, proteolytic enzymes attack the substrates to produce peptides and amino acids (Read, 2024). The high alkaline

pH of the cassava serves as a buffer to other bacteria, such as members of the genus *Bacillus*, which are benevolent, and harmful bacteria (Güven & BenliKaya, 2005; Yokaryo & Tokiwa, 2014). The safety and suitability of cassava for animal feeding is improved by the spontaneous fermentation process which reduces the anti-nutritional value of cassava and other anti-nutritional factors surrounding cassava.

The process of fermentation improves the detoxification of cassava and the digestibility and nutritive value for animals (Assohoun *et al.*, 2023; Mehloakulu *et al.*, 2023; Terefe *et al.*, 2022). Fermentation detoxifies proteins and crude fibre by microbially digesting them (Mukherjee *et al.*, 2015). Spontaneous fermentation improves the nutritive value of feed offered to ruminants and herbivores by free amino acids and peptides and by improving protein digestibility and absorption (Anyiam *et al.*, 2023). Beyond detoxification through free amino acids and peptides, and improving the protein's digestibility and absorption, 'spontaneous fermentation improves the nutritive value of the feed given to ruminants and herbivores' (Anyiam *et al.*, 2023). It also alters the odour and taste of the substrate through the action of esters, alcohols, aldehydes, and other volatile substances. Fermented cassava products have the added advantage of improved microbial safety and shelf life, together with the acids produced during fermentation which inhibit spoilage organisms and enhance overall safety (Aryee *et al.*, 2025).

CHAPTER THREE
**EFFECTS OF FERMENTATION AND ENZYME TREATMENT ON NUTRITIONAL
COMPOSITION, *IN-VITRO* DIGESTIBILITY, AND CYANIDE REDUCTION IN
CASSAVA (*MANIHOT ESCULENTA*) ROOT LEAF MEAL**

Abstract

Cassava root and leaf meals are readily available sources of energy, protein, and vitamins for livestock feeding. However, their utilization in pig diets is limited by high cyanide and crude fiber levels, low crude protein content, and poor digestibility. Therefore, reducing cyanide concentration and enhancing the nutritional value and digestibility of cassava root-leaf meal are essential for its effective use as an alternative to maize based feed. This study investigated the effects of *Aspergillus niger*, spontaneous fermentation, the exogenous enzyme Natuzyme®, and *Lactobacillus brevis* treatments on the nutritional composition, *in-vitro* dry matter digestibility (IVDMD), and cyanide reduction of cassava root-leaf meal. A 4×4 Factorial Completely Randomized experimental design was used to evaluate these parameters in KME 01 cassava variety. Samples of Cassava root-leaf meal (CRLM in the ratio of 1:0.3, Root: 1, leaf :0.3) 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 spontaneously fermented (T3); Cassava root-leaf meal treated with exogenous enzyme (Natuzyme ®) (T4); and 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 rate and the extent of gas produced in inoculated and spontaneous fermentation were significantly reduced as a result of fermentation. The outcome indicated that the spontaneous fermentation enhanced the digestibility of cassava root-leaf meal by a margin of 40.54%, while HCN decreased by 37.00 ppm from 45.00 ppm. Furthermore, the concentration of CF decreased by 1.29%, while the concentration of DM increased by 4.95%. In addition, the concentrations of EE and CP increased by 0.27% and 3.62% respectively, while pecking order and leadership behaviour decreased by 40.54% and 37.00 ppm respectively. 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, reduce hydrogen cyanide content, increase CP, DM and EE contents. Therefore, this study provided evidence that

FCRLM is locally available alternative to maize in pig feed, whose use will promote sustainable feeding strategies for pig production.

3.1 Introduction

Cassava (*Manihot esculenta* Crantz) ranks as the second most significant root crop in Kenya after the Irish potato. Its remarkable drought tolerance makes it a vital staple for many rural households, contributing substantially to food security and poverty reduction (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). 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 produce more than 70 tons of fresh roots per hectare, making it a highly productive crop for smallholder farmers (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). Moreover, the presence of toxic compounds such as hydrogen cyanide, phytates, flavonoids, oxalates, and tannins limits the efficient utilization of essential nutrients like proteins, vitamins, and minerals when cassava is used in animal feed. These compounds form complexes with nutrients, thereby reducing their availability and 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®, cultures of *Aspergillus Niger* and *Lactobacillus brevis* and spontaneous fermentation

on the Nutritional composition, *In-vitro* dry matter Digestibility (IVDMD), fermentation kinetics, and cyanide reduction in cassava root leaf meal for use in pig diets.

3.2 Materials and Methods

3.2.1 Study site

An *in-vitro* experiment was carried out at the Animal Nutrition Laboratory, Egerton University. It is located at 182 km northwest of Nairobi at an altitude of 2,238 m above sea level. The area experiences bimodal rainfall averaging 900–1,200 mm annually, with long rains from April to August and short rains from October to December. Daily temperatures range between 17°C and 22°C, conditions that are generally suitable for cassava growth and development (Egerton University Department of Agriculture Engineering Metrological Station, 2019).

3.2.2 Preparation of cassava root-leaf meal

Fresh cassava roots and leaves (12-month-old, high cyanide variety KME-01) were sourced from Kenya Agricultural and Livestock Research Organization (KALRO), Njoro, Nakuru. The roots were peeled, washed thoroughly, and sliced into approximately 1 cm pieces using a slicer (Lips® model 3601150, Zurich, Switzerland). The leaves were pre-screened by chopping them into smaller sections with a kitchen knife. Both the grated roots and chopped leaves were oven-dried at 60°C until a constant weight was attained. The dried samples were then milled using a Willy mill fitted with a 5 mm sieve, and the resulting flour was packed in airtight containers and stored for subsequent proximate composition analysis.

3.2.3 Preparation of experimental treatments

There were five (5) treatments (T1-T5) with three (3) replicates each.

T1 Untreated cassava root- leaf meal

T2 Cassava root-leaf meal fermented with *Aspergillus niger*

T3 Cassava root-leaf meal treated using spontaneous fermentation

T4 Cassava root-leaf meal treated with an enzyme (Natuzyme®)

T5 Cassava root-leaf meal fermented with *Lactobacillus brevis*

3.2.4 Preparation of *Aspergillus niger*-treated cassava root-leaf meal

3.2.4.1 Preparation of Inoculum

The *Aspergillus niger* inoculum was developed from fresh, mature cultures aged between three and five days, previously grown on Potato Dextrose Agar (PDA) slants. A spore suspension intended for inoculation was then obtained by introducing 10 ml of sterile distilled water onto the agar surface 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.

3.2.4.2 Solid state fermentation

A mixture of fresh cassava root-leaf meal and distilled water was prepared in a 250 ml conical flask to achieve a moisture content of 60%. The contents were then sterilized at 121°C for 15 minutes and subsequently allowed to cool to room temperature before inoculation. Inoculation was done aseptically with 3 ml of spores suspension mixed well and incubated at 37°C, pH of 5.2, for 96 hours. Under these optimized conditions, the inoculum concentration for *Aspergillus niger* was 5% of 10⁶ cfu/ml (Muralikandhan & Dhanasekaran, 2020). After incubation, the fermented substrates were dried in an oven at 60°C for two days.

3.2.5 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°C for four days under anaerobic conditions (Muremera *et al.*, 2022). After a four-day incubation period, the pH of the sample was determined using a portable pH meter. The fermented material was then analyzed for its proximate composition, *in-vitro* dry matter digestibility, and hydrogen cyanide (HCN) content.

3.2.6 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 (Natuzyne ®), 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 as 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.

3.2.7 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 10⁶ cfu/ml of the *Lactobacillus brevis*. The inoculated cassava root-leaf meal was then incubated at 37°C in airtight 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.

3.2.8 Proximate analysis

The proximate composition of the samples was determined following the standard procedures described by AOAC (1990). Dry matter content was measured by oven-drying the samples at 105°C for 24 hours (Method 934.01). Ash content was obtained by incinerating the samples in a muffle furnace at 550°C for 8 hours (Method 942.05). Ether extract was determined by using the Soxhlet extraction method with diethyl ether as the solvent (Method 920.39). Total nitrogen content, used to estimate crude protein ($N \times 6.25$), was analyzed using the micro-Kjeldahl method (Method 954.01).

3.2.9 Determination of cyanide

The remaining analysis was conducted at the Kenya Agricultural and Livestock Research Organisation (KALRO) based in Njoro, Nakuru. Levels of Hydrogen Cyanide (HCN) were analysed with the help of the adapted edition of the paper test procedure as outlined by Ndung'u et al. (2012) and Ojiambo et al. (2017). Fresh cassava roots and leaves (100 mg each) were accurately weighed and placed into glass vials measuring 250 mm in diameter and 84 mm in height using sterilized forceps. To promote the liberation of hydrogen cyanide, 3-5 drops of toluene were added to each vial. Small filter paper strips (0.7× 1.0 cm) were immersed in a sodium picrate solution prepared by dissolving 25 g of anhydrous sodium carbonate and 5 g of moist picric acid in one liter of distilled water. The strips were carefully suspended from the caps of the vials so that they were not submerged within the sample itself. Each vial was capped with a rubber stopper and, with a sample blank used for cross validation, incubated at ambient conditions for 24 hours. During the 24 hours, the supplied hydrogen cyanide from the cassava sample was able to completely saturate the filter paper, resulting in a complete colour change from yellow to red at the end of the incubation. After 24 hours, the vials were opened and the concentration of red colour was recorded using a standard picrate chart from 0 to 800 ppm.

3.2.10 Dry matter digestibility determination

The *in-vitro* digestibility (IVDMD) of dry matter (DM) was computed using the following formulae (Boisen & Fernández, 1997).

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

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

3.3 Statistical analysis

All data from proximate analysis, HCN content, and *in-vitro* dry matter digestibility were analyzed using SAS (version 9.4 M8, 2023). Data normality was checked using the Shapiro-Wilk and Levene's tests, assuming normality at $p > 0.05$. The pH data were analyzed using an independent t-test, and mean differences were separated using Tukey's HSD test at a 5% significance level. The experimental design was a 4×4 completely randomized factorial design using the statistical model:

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijkl}$$

Where, X_{ijk} is the response on the dependent variables (digestibility of DM, OM, and Fermentation kinetics), μ = the overall mean, α_i = the effect of i th type of the microbial inoculation, β_j = the effect of j th level of fermentation times, $(\alpha\beta)_{ij}$ = the interaction effect of microbial inoculation and time, and ε_{ijk} = the random error term for replication k .

3.4 Results

3.4.1 The pH of fermented CRLM

The method of fermentation did not affect the pH of the CRLM (Table 3). Spontaneous and Inoculated showed the pH of 4.7 ± 0.09 and 4.5 ± 0.07 , respectively.

Table 2: The pH of fermented CRLM

Treatments	pH	P value
Spontaneous	4.7 ± 0.09	0.1706
Inoculated	4.5 ± 0.07	

Means in the same column with no superscript letters are similar at 0.05 level of significance.

CRLM=Cassava Root-Leaf Meal.

3.4.2 Nutrient composition of CRLM untreated, fermentation with *Aspergillus niger*, spontaneously fermented, enzyme treated, and fermented with *Lactobacillus brevis*

The nutrient composition of the untreated, enzyme-treated, and fermented CRLM is presented in Table 3.

Table 3 : Nutrient composition of untreated, enzyme-treated, and fermented CRLM

Nutrient composition (%)	T1	T2	T3	T4	T5	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	<0.0001
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	<0.0001
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	0.0013
HCN (ppm)	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	<0.0001
%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	<0.0001

^{a,b,c} means in the same row with different superscripts are different ($p < 0.05$). DM =Dry matter, CP =Crude protein, EE = Ether extract, CF = Crude Fiber, HCN = Hydrogen Cyanide, IVDMD = *In Vitro* Dry Matter Digestibility, T1= Untreated Cassava Root-Leaf Meal, T2= Cassava Root-Leaf Meal fermented with *Aspergillus niger*, T3= Cassava root-leaf meal spontaneously fermented, T4= Cassava root-leaf meal treated with Enzyme, T5= Cassava root-leaf meal fermented with *Lactobacillus brevis*.

3.4.3 The *in-vitro* digestibility of untreated, enzyme-treated, and fermented CRLM

T3 resulted in the highest digestibility (55.56±0.31) compared to the rest, while untreated cassava root-leaf meal T1 had the least (38.73±0.31). T3 significantly differed from T1 ($p < 0.05$). Spontaneous fermentation (T3) resulted to a higher IVDMD compared to untreated CRLM (T1) and other treatment methods. There was a 16.83% increase in IVDMD (Fig.1) after spontaneous fermentation (T3) (55.56±0.31), compared to untreated (T1) (38.73±0.31).

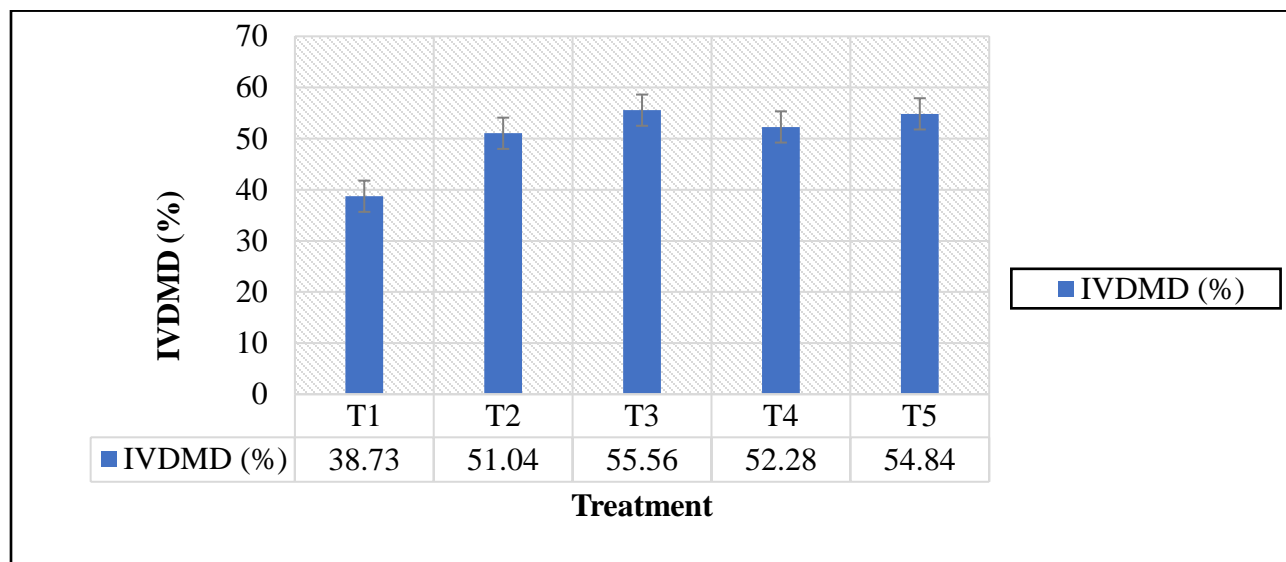


Figure 1: The *in-vitro* digestibility of untreated, enzyme-treated, and fermented cassava root-leaf meal. Error bars represent the Mean (\pm SE) IVDMD of CRLM. IVDMD = *In Vitro* Dry Matter Digestibility, T1= Untreated Cassava Root-Leaf Meal, T2= Cassava Root-Leaf Meal fermented with *Aspergillus niger*, T3= Cassava root-leaf meal spontaneously fermented, T4= Cassava root-leaf meal treated with Enzyme, T5= Cassava root-leaf meal fermented with *Lactobacillus brevis*.

3.5 Discussion

The results for pH recorded on the 4th day showed that the effects of spontaneous fermentation and other inoculum fermentation (*Aspergillus niger* and *Lactobacillus brevis*) were similar. This phenomenon can be attributed to the metabolic activities of the microorganisms involved, which can lead to comparable acidification processes under certain conditions (Hadinia *et al.*, 2022; Zheng *et al.*, 2021).

The untreated cassava root-leaf meal (T1) demonstrated the lowest amount of dry matter (DM) which is 93.67%. This is probably due to the lack of physical, microbial, or enzymatic processes which may lower the moisture content or break down certain structures which hold the water. The cassava leaves and roots are known to possess high water content and are prevalent with fibrous tissues which have the ability to retain water (Christine *et al.*, 2021). Whenever there is no participation of the fermentation or enzymatic processes, the moisture which is the water bound within the cellular structure remains unaltered which results in lower dry matter content and an increased water retention value. In contrast, microbial or enzymatic activity during the fermentation processes which results in moisture being bound within the cells, potentiates the increase in dry matter proportion as the processes aid in the breakdown of the cell structures. The

results from this study show that irrespective of the treatment methods used, there was an increase in dry matter content area which is an indicator of reduction of moisture. Most impressive is the result which was obtained from spontaneous fermentation and enzyme treatment which yielded the highest dry matter content of 98.61%. This indicates that within the 96 hour interval, the treatment groups consisting of natural microbes and added enzymes were effective.

Close behind were treatments with *Aspergillus niger* (97.84%) and *Lactobacillus brevis* (97.67%). These increments may be a result of the microbial metabolism and the particular enzymes breaking down the substrate which in turn enhances the moisture loss during processing. This finding corroborates those of Oboh *et al.* (2010), who revealed that microbial fermentation improves the physicochemical properties of moisture in cassava based feeds. *Aspergillus niger* is recognised for its high ability in massive enzyme production and moisture reduction is probably due to ease of hydrolysis of the fibrous and carbohydrate components, thus promoting easier release of water. The vigorous growth rapidly expands in a network of mycelia, which contributes to the dry matter content. The other aspect of the metabolic process is the production of heat which is sufficient enough to enhance the moisture loss thus increasing dry matter. In the same way, *Lactobacillus brevis* which is a lactic acid bacteria that produces lactic acid and reduces the pH and water activity in the feed and thus aids in the dry matter retention (Garcia *et al.*, 2024).

The metabolic breakdown of complex polysaccharides and the crude out of the more readily digestible, and fermentable polysaccharides also accounts for the dry fibrous residue and crude fibre content reduction of the cassava after fermentation. According to Ona *et al.* (2019) and Ortega *et al.* (2018) fermentation and enzyme application, are treatments which apply to cassava and its components for complex carbohydrate and fibre breakdown. With lipids making up less than 2% of total cassava composition, it is understandable that fermenting microorganisms, such as *Rhizopus oryzae* or lactic acid bacteria, would ingest protein and carbohydrates instead of lipids. These results corroborate previous findings that fermentation has minimal impact on the lipid content of cassava. Therefore, rather than raising fat levels, fermentation mainly improved the nutritional value of cassava by increasing its DM. It also decreased the HCN content, hence reduced toxicity (Okoth *et al.*, 2022).

The untreated sample had the lowest CP content (3.26%), which reflected 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 spontaneous fermentation. This agreed 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. In the process of controlled fermentation, the dominant biomass in the bioreactor is the protein-rich cells of the prolific fermenter *Levilactobacillus brevis*. Microbial enzymes also digest proteins, enhancing the protein anti-nutritional factors, thus improving the overall protein utilisation and bioavailability for the animal (Dhiman *et al.*, 2025; Okoth *et al.*, 2022).

The lactic acid bacteria, yeasts, various *Bacillus* species, and *Enterobacteriaceae* in every batch analogue microbial population confined in every batch exhibit imprecision and, thus, interfere with fermentation goals. This population also contributes to the fermentation's inaccuracy and the inconsistency in protein recovery (Okoth *et al.*, 2022). This claim lies in the low 3.26% CP of the samples (T1), which supports the findings of Zekarias *et al.* (2019), which indicated the low protein to carbohydrate ratio of cassava. The animal also confirms this. Particularly, the rapid growth of *Aspergillus niger* and the extracellular enzymes which facilitate the breakdown of complex substrates drive its protein production capacity (Cheriaparambil & Grossmann, 2025).

In lactate brevis and spontaneous fermentation, the microbial breakdown of complex fibres to proteins also enhances the diploid cells for-bound nitrogen of increased CP with *Lactobacillus brevis* and spontaneous fermentation. Fermentation, in addition, greatly reduces the anti-nutritional factors, such as the cyanogenic glycosides which generally inhibit protein digestion (Ram *et al.*, 2020). It is also likely that the enzymes increase crude protein concentrations as they cleave the fibrous cell walls and release protein fractions bound to the fibrous material. There has been the promotion of protein release as well as protein bioavailability from fermented cassava meal as a result of enzymes (Mukhtar *et al.*, 2023). Fermentation of cassava roots and leaves with distilled water at a 1.3:1 (w/v) ratio, and then anaerobically stored for 4 days at 37°C, and further diluted for microbial population densification, improves digested 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*, *Lactobacillus 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 *Lactobacillus brevis*-inoculated treatment.

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 (Adeyemi & Familade, 2003; Bakare *et al.*, 2022). The untreated cassava root-leaf meal of KME 01 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 denatured the cyanogenic glycosides. These treatments decreased the anti-nutrient factors in cassava and increased nutrient bioavailability (Ojiambo *et al.*, 2017).

Retto *et al* (2021) describes the innovative processes involving *Lactobacillus brevis*, Natuzyme®, and *Aspergillus niger* as the fermentation processes with the least efficiency regarding HCN reduction. 8ppm HCN was what these ‘advanced’ treatments achieved, and that level is an order of magnitude lower than the toxicity threshold of 10ppm. In contrast to the previously discussed treatment methods involving enzymes or inoculated microbes, the meal was greatly benefited from spontaneous fermentation. Its simplicity and system versatility made it sophisticated and efficient at the same time.

3.6 Conclusion

The outcomes of the investigation showed that the nutritional quality of a fermented, four-day cassava root-leaf meal improved significantly. It increased the dry matter content of the meal from 93.67% to 98.62%, increased the crude protein from 7.47% to 11.09%, improved the *in-vitro* dry matter digestibility from 15.02% to 55.56%, and decreased the hydrogen cyanide (HCN) concentration from 45.00 ppm to 8.00 ppm. Such improvements demonstrated the value of

fermented cassava root-leaf meal as a safe and nutritious alternative ingredient for grower pig diets.

CHAPTER FOUR

EFFECT OF FEEDING SPONTANEOUSLY FERMENTED CASSAVA ROOT-LEAF MEAL-BASED DIETS ON THE GROWTH PERFORMANCE OF GROWER PIGS

Abstract

An experiment was conducted to assess what effect the inclusion of spontaneously fermented cassava root leaf meal (FCRLM) would have on the growth performance of grower pigs (spontaneously fermented cassava root leaf meal pig grower pigs). A total of 18 pigs comprising Landrace x Large White crossbred pigs with an average initial weight of 22 ± 3 kg and balanced sex were randomly allocated to three diet treatments in a completely randomised block design with 6 pigs per treatment per block. The experimental diets consisted of three treatments : T1 - 0% FCRLM, T2 - 20% FCRLM, and T3 - 40% FCRLM. All experimental diets were analyzed for their proximate composition before feeding. The results showed that pigs fed diet T2 recorded a significantly higher final body weight compared to other dietary treatments ; T2 (77.98 ± 0.61 kg), T1 (77.52 ± 0.61 kg), and T3 (75.48 ± 0.61 kg ; $p < 0.05$). Weight gain was 52.30 ± 0.16 kg (T1), 52.86 ± 0.16 kg (T2), and 50.38 ± 0.16 kg (T3) ($p < 0.05$). Average daily gain (ADG) was 0.67 ± 0.09 kg/day (T1), 0.68 ± 0.09 kg/day (T2), and 0.65 ± 0.07 kg/day (T3) ($p < 0.05$). Daily feed intake (DFI) decreased with increasing FCRLM inclusion: 2.87 ± 0.02 kg (T1), 2.80 ± 0.04 kg (T2), and 2.70 ± 0.11 kg (T3) ($p < 0.05$). Feed conversion ratio (FCR) was 4.31 ± 0.58 (T1), 4.16 ± 0.55 (T2), and 4.18 ± 0.39 (T3) ($p < 0.05$). As shown in Table 3, growth performance was best with the treatment of 20% inclusion, which supports the claim that moderate inclusion levels are optimal. Furthermore, the results with 0% inclusion are statistically different from the 20% inclusion, indicating that 20% inclusion is optimal. Thus, it can be said that part of the conventional feed ingredients can be replaced in a grower pig diet with a moderate level (20%) of inclusion of the FCRLM, without significantly affecting feed efficiency.

4.1 Introduction

Prior to 2012, FAO reported that pig farming in Kenya is mostly conducted by smallholder farmers, with each herd consisting of 10 to 100 pigs. Some of these smallholders engage in extensive, semi-intensive, or intensive small-scale pig farming. Pigs are considered to be economically beneficial livestock due to their relative availability, short generation intervals, superior feed efficiency, and economically feasible returns (Schild *et al.*, 2020). All of these economic returns, coupled with improved farming practices, have provided impetus for the diversification of pig farming (Muthui *et al.*, 2019). However, the rapid growth of this sector is

impeded by the costly seasonal deficits and the traditional feed consisting of maize and soybeans (Mugumaarhahama *et al.*, 2020).

For seasonal deficits, smallholder pig farmers have increasingly explored unconventional feed alternatives, particularly cassava roots and leaves (Mugumaarhahama *et al.*, 2020). Some of the dryland perennial crops are staples for most rural households; cassava roots and leaves are excellent sources of carbohydrates along with some protein, vitamins, and minerals, and help in alleviating food insecurity ((Gatto *et al.*, 2021; Kipyego & Mugalavai, 2019).

Leaves do contain considerable anti-nutritional factors (ANFs) which can hinder absorption, lower bioavailability, and utilisation in monogastric animals, which lack the enzymes to digest fibre efficiently (Lambebo & Deme, 2022; Latif & Müller, 2015). Tannins, crude fibre, Phytate, and trypsin inhibitors, which are anti-nutritive substances, are reported to hinder the growth and reproduction of livestock (Kemboi *et al.*, 2023; Samtiya *et al.*, 2020).

As reported in the literature, fermentation and ensiling are valuable methods to enhance the digestibility of and decrease HCN levels in cassava-based diets (Williams *et al.*, 2023). This research study aimed to assess the growth performance of grower pigs fed diets that included spontaneously fermented cassava root-leaf meals.

4.2 Materials and Methods

4.2.1 Experimental location

This was as described in Section 3.2.1

4.2.2 Preparation of cassava root-leaf meal and experimental diets

Diets were carefully prepared to supply the nutrients required for optimal growth of pigs, following the recommendations of NRC (2012). The formulations consisted of maize germ, wheat pollard, soybean meal, fish meal (omena), and FCRLM, with their specific compositions summarized in Table 4.

4.2.3 Preparation of CRLM

Preparation of CRLM was as described in Section 3.2.5

4.2.4 Management of experimental pigs

The experimental pigs were procured from a reputable commercial pig breeding farm where they were tagged and logged into the farm database. Subsequently, they were placed in concrete floored pens where each pen measured (3m x 3m). Automation was established in all records whereby pigs were marked for deworming and administered subcutaneous Ivermectin® and subsequently given (Multivitamin B-complex injections) in preparation for the experiments.

For a week, the pigs were placed on experimental diets prior to data collection. Feeding was done using concrete feeding troughs, and all pigs had unrestricted access to water through nipple drinkers. Over the next 90 days, all pigs were continuously monitored for illness and disease, and if any were detected, they were treated immediately as all proper biosecurity precautions were followed.

4.2.5 Experimental design

The experiment utilised eighteen pigs, consisting of 9 barrows and 9 gilts. The pigs, of Landrace and Large White crossbreed, had an average initial weight of 22 ± 3 kg. The pigs were randomly assigned to three dietary treatments using a randomised complete block design, the pigs' sex being the blocking factor. Each treatment had two pens, three pigs per pen. The barrows and gilts were kept in separate pens for the entire experimental duration. The pigs' initial weight was used as a covariate in the statistical analysis.

Table 4: Composition of the experimental diets (g/100g).

Ingredients	Treatments		
	T1	T2	T3
Maize meal	58.00	49.00	28.80
CRLM	0.00	20.00	40.00
Wheat bran	2.00	1.00	1.00
Rice germ	4.50	1.20	1.00
Maize germ	5.00	1.50	0.50
Soybean meal	19.00	16.40	16.00
Vegetable oil	2.60	3.00	5.00
Fish meal (Omena)	6.00	6.00	4.50
Lime	1.00	1.00	0.30
DCP (granular 24%)	1.00	1.00	1.00
Lysine	0.100	0.100	0.100
Methionine	0.20	0.20	0.20
Iodized salt	0.30	0.30	0.30
Vitamin and mineral Premix*	0.30	0.30	0.30
Mycotoxin binder	0.10	0.10	0.10
Chemical analysis			
Dry matter	97.24	96.52	96.49
Crude Protein (CP)	18.63	18.80	18.85
Ether Extracts	7.95	8.79	8.89
Ash	9.82	10.30	11.10
Crude Fiber (CF)	5.35	4.56	4.72
GE (MJ/Kg DM)	11.76	11.89	12.88

T1=0% FCRLM; T2=20% FCRLM;T3=40% FCRLM *Vitamin premix: Vitamin and mineral premix: Vitamin A 8,000 IU; vitamin D3 2,000 IU; vitamin E 37.5 mg; vitamin K-3 0.925 mg; vitamin B2 8.43 mg; vitamin B12 0.04 mg; nicotinic acid 34.5 mg; pantothenic acid 26 mg; 450 mg Fe; 400 mg Cu; 250 mg Zn;150 mg Mn; 0.5 mg I; 0.25 mg; GE= Gross energy.

4.2.6 Data collection

Each pig's body weight was recorded on a weekly basis with a portable hanging scale (50 kg max capacity) accurate to the nearest gram, which recorded weekly weight gain to calculate average pig weight gain. Pigs were fed at 0800 hours and 1500 hours; they also had the refusals weighed at the next feeding, which was the following morning. This was done to calculate the daily intake, which was the weight of refusals measured on a digital scale, and they were also used to calculate the pig disposal in the order of the daily intake. Weight gain and daily intake were the parameters used to calculate the feed conversion ratio.

Feed Intake (FI)

Feed intake was determined by calculating the difference between the amount of feed offered and the leftovers (refusals) after a 24-hour period (Madsen *et al.*, 2020).

Feed Intake (FI) = Feed offered (kg) - Feed left over (kg)

Average Daily Gain (ADG)

Each pig was weighed weekly before the morning feeding. The average daily gain (ADG) was calculated by taking the difference between the body weight recorded after seven days and the initial weight at the start of that period, then dividing the result by seven days, following the procedure described by Zumbach *et al.* (2010).

Average daily gain (ADG) in kg /day = $\frac{\text{Weight after 7 days} - \text{Weight at start of 7 days}}{7 \text{ days}}$

Feed Conversion Ratio (FCR)

The feed conversion ratio was determined as the ratio of the average feed intake (kg) to the average body weight gain (kg) per pig during each week, following the procedure described by Chantziaras *et al.* (2020). This was computed using the formula:

Feed conversion ratio (FCR) = $\frac{\text{Feed consumed per pig (kg)}}{\text{Average weight gain per pig (kg)}}$

Weight Gain (WG)

Weight gain was determined by subtracting the body weight recorded in the previous week from that of the current week, following the approach described by Chehade *et al.* (2022) and Ostafichuk (2017).

Growth Rate (GR)

Growth rate was calculated as the difference between the final and initial body weights, divided by the duration of the weighing period, following the method described by Deputy *et al.* (2015).

4.3 Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS, 2023) with a two-way analysis of variance (ANOVA). Mean separation was performed using Tukey's Honest Significant Difference (HSD) test at a 0.05 level of significance, and differences were considered statistically significant at $p < 0.05$. The initial body weight of the pigs was included as a covariate, while sex was treated as a blocking factor. The statistical model used was as follows:

$Y_{ijk} = \mu + T_i + \beta_k + S_f + \epsilon_{ijk}$ where;

Y_{ijk} = Response variable of interest (e.g; ADG, FCR)

μ = population mean

T_i = fixed i^{th} treatment effect (T1, T2, and T3)

β_k = fixed effect of sex (barrows and gilts)

S_f = fixed effect of initial weight used as a covariate

ϵ_{ijk} = random error.

4.4 Results

4.4.1 Nutrient Composition of the Experimental Diets and Cassava Root-Leaf Meal

The experimental diets were designed to be isonitrogenous and isocaloric, following the nutrient requirements recommended by NRC (2012). The chemical composition of both the diets and cassava root -leaf meal (CRLM) was determined in triplicate to ensure accuracy. The analyzed parameters, including dry matter (DM), ash, crude protein (CP), crude fiber (CF), and ether extract (EE), are summarized in Tables 5 and 6.

Table 5: Nutrient Composition of Cassava Root-Leaf Meal

Nutrient Composition (%)	Cassava Root-Leaf Meal
Dry matter	93.67
Ash	7.8
Crude Protein	3.26
Crude Fiber	4.17
Ether Extracts	1.02
Metabolizable energy (ME, MJ/Kg)	10.6

Table 6: Nutrient Composition of the Experimental Diets

Nutrients (%)	T1	T2	T3	SEM	P Value
Dry matter	97.24 ^a	96.52 ^b	96.49 ^b	0.09	0.0027
Ash	9.81 ^b	10.29 ^b	11.11 ^a	0.14	0.0018
Crude protein	18.64	18.80	18.85	0.04	0.0607
Crude Fiber	5.35 ^a	4.56 ^b	4.72 ^b	0.04	<0.0001
Ether Extracts	7.95 ^b	8.79 ^a	8.89 ^a	0.17	0.0140
ME (MJ/Kg)	11.75	11.89	12.88	0.90	0.6503

^{a,b} means within the same row bearing different superscripts are significantly different at $p < 0.05$.

T1 = 0% FCRLM, T2 = 20% FCRLM; T3 = 40% FCRLM, SEM = Standard error of the mean.

Proximate analysis of the experimental diets revealed a significant increase ($p < 0.05$) in ash, crude protein (CP), crude fiber (CF), ether extract (EE), and metabolizable energy (ME) with increasing levels of FCRLM inclusion in the diets.

4.4.2 Performance of the growing pigs

Growth performance parameters varied significantly ($p < 0.05$) among the dietary treatments. Pigs fed diets containing FCRLM exhibited significantly lower feed intake and average daily gain ($p < 0.05$) compared to those receiving the control diet.

Table 7: Effect of Diets on Feed Intake, Average Daily Gain, and Feed Conversion Ratio of Growing Pigs

Parameters	Dietary Treatments			P value	
	T1	T2	T3	Diet	Sex
Initial Weight (kg)	25.21±0.53	25.11±0.53	25.10±0.53	0.98	<0.0001
Final Weight (kg)	77.52 ^a ±0.61	77.98 ^a ±0.61	75.48 ^b ±0.61	0.03	<0.0001
Weight Gain (kg)	52.30 ^a ±0.16	52.86 ^a ±0.16	50.38 ^b ±0.16	<0.0001	<0.0001
Average Daily Gain (kg/Day)	0.67 ^b ±0.09	0.68 ^a ±0.09	0.65 ^c ±0.07	<0.0001	<0.0001
Daily Feed intake (kg)	2.87 ^a ±0.02	2.80 ^b ±0.04	2.70 ^c ±0.11	<0.0001	<0.0001
Feed conversion ratio (FCR)	4.31 ^a ±0.58	4.16 ^b ±0.55	4.18 ^b ±0.39	<0.0001	<0.0001

^{a,b,c} means within the same row bearing different superscripts are significantly different at $p < 0.05$.

T1 = 0% FCRLM; T2 = 20% FCRLM; T3 = 40% FCRLM.

4.4.3 Daily feed intake (DFI)

Pigs fed on Treatment 1 had higher daily feed intake (2.87±0.02) in comparison to Treatment 2 (2.80±0.04) and Treatment 3 (2.70±0.11). Daily feed intake decreased significantly ($p < 0.05$) as the inclusion level of FCRLM in the diet increased, compared to the control.

4.4.4 Feed conversion ratio (FCR)

Feed conversion ratio (FCR) differed highly significantly ($p < 0.05$) among pigs across all dietary treatments. Treatment 1 (T1) had higher feed conversion ratio (4.31±0.58) while treatment 2 (4.16±0.55) and treatment 3 (4.18±0.39) had the lowest.

4.4.5 Average daily gain (ADG)

The ADG was lower for T3 (40% FCRLM) diet in comparison to T2 (20% FCRLM). Increasing levels of FCRLM inclusion in the diets resulted in a reduction of average daily gain (ADG) in the pigs, with T2 (0.68 kg/day) being significantly higher than T3 (0.65 kg/day).

4.5 Discussion

4.5.1 Daily feed intake (DFI)

Daily feed intake declined significantly ($p < 0.05$) as the inclusion level of FCRLM in the diet increased, with pigs fed on T1 (2.87 kg/day) consuming more than those on T2 (2.80 kg/day) and T3 (2.70 kg/day). The decline in daily feed intake (DFI) in T3 may be attributed to improved nutrient utilization and higher dietary energy density, allowing pigs to meet energy needs with less feed. Similar observations were reported by Adebayo *et al.* (2021) and Oluwatosin *et al.* (2023), who noted that increasing the inclusion of cassava-based ingredients in pig resulted in reduced feed intake.

While some fiber is lost during fermentation, the remaining crude fiber contributed to increased gut fill, which slowed gastric emptying and limited voluntary feed intake (Sampath *et al.*, 2023; Williams *et al.*, 2023). Similarly, Hamid *et al.* (2017); Villacrés *et al.* (2020) reported decrease in feed intake due to some residual anti-nutritional compounds such as tannins, saponins, and cyanogenic glycosides which may persist and impact bitter flavors thus the decrease of feed acceptability.

Cassava leaves are comparatively lower in energy than conventional ingredients such as maize or rice bran. Consequently, pigs may have experienced early satiety due to bulkiness before meeting their energy requirements, thus limiting overall feed intake (Akinola *et al.*, 2013).

4.5.2 Average Daily Gain (ADG)

The initial body weight of the pigs did not differ significantly ($p > 0.05$) among the dietary treatments. However, the initial weight was strongly influenced by sex ($p < 0.05$), which may be explained by the biological differences between male and female pigs. Although the diets had no significant effect on the initial body weight of the pigs, they significantly influenced ($p < 0.05$) the final body weight, as shown in Table 9.

As the level of fermented cassava root-leaf meal (FCRLM) inclusion increased in the diets, the average daily gain (ADG) of the pigs decreased significantly ($P < 0.05$), with values of 0.68 kg and 0.65 kg observed for T2 (20% FCRLM) and T3 (40% FCRLM), respectively.

The findings described differ from those of Aro *et al.* (2015) and Zendrato *et al.* (2020) because of the explanation by Aro *et al.* (2015) and Zendrato *et al.* (2020) findings wherein incorporating cassava by-products at a 20% inclusion rate into starter and grower pig diets significantly ($p < 0.05$) improved weight gain compared to commercial control diets. In this study, pigs fed diets that included 20% FCRLM showed significantly ($p < 0.05$) greater weight gain than

those fed diets with a 40% inclusion of FCRLM. This gain at moderate inclusion levels could be related to nutrient availability and digestibility, as noted by the improved fermentation which increases protein levels and decreases anti-nutritional factors, especially hydrogen cyanide, that improve gut health as well as nutrient absorption (Quintieri *et al.*, 2023). On the other hand, the 40% inclusion FCRLM may contribute to an excess of dietary fibre and remaining anti-nutritional factors which may lead to problems with nutrient digestion, energy use, and feed efficiency. High levels of dietary fibre also lead to increased gut fill and passage rate which decreases absorption of nutrients, especially in pigs and other monogastric animals (Aro *et al.*, 2015; Montagnac *et al.*, 2009).

4.5.3 Feed Conversion Ratio (FCR)

The different diets had a significant effect on feed conversion ratios (FCR) ($p < 0.05$). Pigs on diets containing 20% and 40% fermented cassava root-leaf meal (FCRLM) were more efficient at converting feed into weight, registering FCRs of 4.16 and 4.18, respectively, while the control group had an FCR of 4.31. This suggests that feed conversion improved with the addition of low levels of FCRLM. The improvements in feed conversion efficiency can be attributed to the fermentation process described by Zhang *et al.* (2024), whereby nutrients become more accessible and the reduction of anti-nutritional factors improves nutrient absorption and utilization.

The feed conversion ratio (FCR) for the 40% inclusion treatment (T3) was similar to that of the 20% inclusion group (T2) in the previous section. However, T2 showed better overall performance as a result of lower feed intake, even though T2 had greater weight gains. Maximizing the benefits of fermentation through increased microbial growth and improved digestibility, does not appear to be the case with the inclusion of 40% FCRLM (Olayemi *et al.*, 2020; Promkot *et al.*, 2017). In this instance, it seems that higher FCRLM inclusion levels may lead to reduced feed intake and weight gain due to excess fiber. Excess fiber can reduce the palatability of the diet, resulting in imbalances, particularly regarding the energy component of the diet.

The poor performance of pigs on diet T3 could be associated with anti-nutritional elements in the feed, which, among other things, could have negatively affected feed digestibility and nutrient metabolism (Clasadonte & Der Poel, 2009; Jacob Nte *et al.*, 2023; Woyengo *et al.*, 2017). This is consistent with observations made by Torhemen *et al.* (2017), on the test diets, where pigs experienced a considerable decrease in both feed intake and weight gain and had a virtually unchanged feed conversion ratio ($p > 0.05$) compared to the control diet.

4.6 Conclusion

It was concluded that including 20% spontaneously-fermented CRLM in grower pig diet improved feed intake. There was a decline in average daily gain and feed conversion ratio as the levels of FCRLM in the diet increased up to 40% inclusion level.

CHAPTER FIVE

EVALUATION OF MEAT QUALITY AND SENSORY CHARACTERISTICS OF PORK FROM PIGS FED ON FERMENTED CASSAVA ROOT-LEAF MEAL-BASED DIETS

Abstract

The quality of pork, as well as its sensory characteristics, is significantly influenced by diet. With traditional feed ingredients, primarily corn and soybean meal, becoming more expensive and less available, studies are investigating more sustainable feed options for pigs. This study examined the effects of incorporating fermented cassava root leaf meal (FCRLM) into the diets of growing and finishing pigs on the quality and sensory characteristics of the resulting pork. Eighteen crossbred pigs were assigned to different diets for 12 weeks, with FCRLM at 0% (control), 20%, and 40%. This allowed for the evaluation of carcass quality, texture, and sensory attributes of ham and shoulder cuts. The different feeding treatments did not significantly impact the initial and final pH of the meat ($p > 0.05$), although they did affect the percentage of carcass yield, ranging from 55.62% with the 40% FCRLM group (T3) to 60.21% with the 20% FCRLM group (T2), indicating better carcass yield at moderate inclusion levels. Pigs fed 20% FCRLM produced meat that retained good sensory qualities for both ham and shoulder cuts. The 40% FCRLM group showed better qualities in attributes such as color intensity (4.40), aroma, flavor (4.00), and fat mouthfeel (3.95 ± 0.15), but slightly reduced tenderness (3.85) and juiciness.

There were no discernible differences between the treatments applied in the sensory evaluations of the shoulder cuts. Overall, these findings indicate that the use of up to 20% FCRLM in pig diets supports good carcass performance and quality in pork, underscoring the value of FCRLM as a more economical and locally available substitute compared to conventional feed components.

5.1 Introduction

Due to their rapid reproduction rates and efficient feed-to-meat conversion, pigs also contribute to the food and nutritional security of smallholder farmers (Muthui *et al.*, 2019; Okello *et al.*, 2021). More than one billion pigs are raised annually worldwide, with China raising nearly half of them and producing 118 million metric tons of pork in 2022 (Kim *et al.*, 2024; Pius *et al.*, 2024; Zhao *et al.*, 2022). In Africa, the pig population has also seen tremendous growth, increasing from 6 million in 2001 to 43.5 million and producing 2.1 million metric tons of pork by 2024, with East Africa being the fastest-growing region (Adesehinwa *et al.*, 2024; Kim *et al.*, 2024). Kenya

has more than 1.2 million pigs and produces 25,800 tons of pork annually (Carter *et al.*, 2016). Economical and reliable sources such as corn and soybean meal do not achieve optimal growth and high productivity.

Because kitchen scraps and other waste are composed of large, fibrous materials that are nutritionally poor (Muthui *et al.*, 2019), small-scale farmers turn to other fuels. This has resulted in a greater focus on cassava root and leaf flour, which is locally available, abundant, and energy-dense. Benson *et al.* (2023) and Chisoro *et al.* (2023) refer to high levels of poisonous HCN and other fibers as limiting factors for its use.

The use of fermentation improves the nutritional value of cassava and reduces its fiber and HCN content to levels suitable for pig feed (Chisoro *et al.*, 2023). However, there is limited information on the use of fermented cassava root and leaf meal (FCRLM) in pig feed and its associated meat quality, specifically the essential sensory attributes of flavor, tenderness, and color, which determine consumer preference and market value (Ojediran *et al.*, 2024; Warner *et al.*, 2024). Therefore, this study focused on how various grades of FCRLM in pig diets affect the quality and sensory attributes of pork, concentrating on ham and shoulder cuts.

5.2 Study Site

This observation is consistent with the description provided in Section 3.2.1

5.3 Data Collection

The pigs used in this study were the same as in the previous feeding trial, where they were fed diets containing 0%, 20%, and 40% fermented cassava root and leaf meal (FCRLM) for twelve weeks. One pig per treatment was selected and kept overnight fasted, with ad libitum access to water, before slaughter. Pigs were weighed to determine their slaughter weight (SW) and were subsequently slaughtered humanely in accordance with standard procedures. Stunning was done using a stun gun to render the pigs unconscious before exsanguination by severing the jugular veins (Njoga *et al.*, 2023). Carcasses were scalded in hot water (60–65 °C) for about five minutes to facilitate hair removal, then eviscerated and thoroughly rinsed with clean water (Isbrandt *et al.*, 2022). Evisceration was done carefully to avoid puncturing internal organs and prevent contamination. The cleaned carcasses were subsequently chilled at 4 °C and weighed to determine the hot carcass weight. Dressing percentage was calculated following the method described by Ekeocha *et al.* (2023).

$$\text{Dressing percentage} = \frac{\text{weight of dressed pig (kg)}}{\text{Weight of live pig (kg)}} \times 100$$

Measurements of pH

Measurements of pH ($\text{pH}_{0\text{h}}$) were taken within 15 minutes after slaughter. After chilling the carcasses for 24 hours at 4°C, the muscle pH ($\text{pH}_{24\text{h}}$) was measured in the *Longissimus dorsi* at the head of the last rib. Measurements were taken using a portable pH meter (HI99163, Hanna Instruments, Romania) equipped with a stainless-steel probe, which was inserted directly into the muscle to a depth of 3 mm.

5.4 Sensory Evaluation

5.4.1 Descriptive sensory analysis

5.4.1.1 Selection of the panelists

Eight panelists were selected from a consumer panel using pre-screening questions about color availability and discrimination. Training was conducted according to ISO standards (2012), where the evaluators were trained to define the sensory qualities and descriptors developed for the pre-screening tests. The objective was to select panelists who could verbally articulate the sensory characteristics of pork. In the orientation session, the panelists reached a consensus on the primary characteristics to be evaluated and assessed meat samples (pork, beef, and chicken) to determine intensity levels and create a sensory lexicon for color, following the methods of Meilgaard *et al.* (2006). Eight trained panelists were selected and instructed in both qualitative and quantitative descriptive methods that aided in the creation of the sensory lexicon for characterizing the color of pork.

5.4.1.2 Lexicon

A descriptive analysis was used in the sensory evaluation, focusing on 12 attributes classified as Appearance, Texture, Flavor, Aroma, and Overall Sensory Quality. Feelings and emotions regarding pork depend largely on each of these attributes. The first impression formed depends on color and appearance (Millán & Retamosa, 2025). The texture of the meat, especially its tenderness and juiciness, will imprint a satisfying meat experience in the memory. Aroma, flavor, and aftertaste are integrated and define the final subjective rating for the pork (Jeon *et al.*, 2025; O'Quinn *et al.*, 2018). The fatty mouthfeel adds to the richness of the meat, but too much oiliness can turn off some consumers, reducing their overall liking (Guichard *et al.*, 2018). Overall, pork with balanced colour, texture, and flavor attributes achieves higher sensory ratings and consumer

acceptability. Each attribute was defined clearly and rated on a 9-point intensity scale, where 1 indicated very mild or absence and 9 represented very intense or high presence of the attribute.

Table 8: The attributes lexicon analysis

Attribute	Definition	Reference / Anchor Example	Rating Scale (1 = low/mild, 9 = high/intense)
Colour	Degree of colour vividness and saturation in meat	9 = Deep reddish-pink fresh pork; 1 = Very pale, washed-out	1 = very mild colour, 9 = very intense vivid colour
Appearance	Visual appeal including surface gloss and uniformity	9 = Smooth, glossy fresh meat; 1 = Dull, patchy surface	1 = very poor appearance, 9 = excellent appearance
Fibrousness	Visibility and coarseness of muscle fibers	9 = Very coarse, distinct fibers; 1 = Smooth, fine texture	1 = very mild fibrousness, 9 = very intense fibrousness
Springiness	Elasticity; how well meat returns to original shape after pressure	9 = Very elastic, springs back quickly; 1 = Very soft, no bounce	1 = no springiness, 9 = very high springiness
Aroma Intensity	Strength of the characteristic pork smell	9 = Strong fresh pork aroma; 1 = Very weak or no aroma	1 = very mild aroma, 9 = very intense aroma
Taste	Combined flavor perception including taste and aroma	9 = Rich, full pork flavour; 1 = Bland or off-flavor	1 = very mild flavour, 9 = very intense flavour
After Taste	Flavor lingering after swallowing	9 = Strong lingering savory taste; 1 = No aftertaste	1 = no aftertaste, 9 = very intense aftertaste
Tenderness	Ease of chewing; softness of the meat	9 = Very tender, breaks easily; 1 =	1 = very tough, 9 = very tender

		Tough and hard to chew	
Chewiness	Amount of effort/time needed to chew before swallowing	9 = Requires prolonged chewing; 1 = Soft and easy to chew	1 = very soft, 9 = very chewy
Juiciness	Perceived moisture release during chewing	9 = Very juicy and moist; 1 = Dry and lacking moisture	1 = very dry, 9 = very juicy
Cohesiveness	Degree meat holds together during chewing	9 = Meat holds shape very well; 1 = Falls apart easily	1 = very low cohesiveness, 9 = very high cohesiveness
Fatty Mouthfeel	Perception of fat richness or oily sensation in the mouth	9 = Very rich, oily sensation; 1 = Lean and dry mouthfeel	1 = very mild fatty mouthfeel, 9 = very intense fatty mouthfeel

5.4.2 Consumer acceptability test

5.4.2.1 Panel recruitment and training

A total of 40 untrained consumers balanced males and females aged for 21 to 42 years old were randomly selected from among students and staff who consume pork. Participants signed a consent form and confirmed they were not allergic to pork.

5.4.2.2 Evaluation procedure

A 5-point hedonic scale (5= like extremely, 4= like slightly, 3= neither like nor dislike, 2= dislike slightly, and 1= dislike extremely) was used to assess consumer acceptability of the following attributes: color, texture, taste, juiciness, tenderness, and overall liking. The evaluations were conducted in individual booths under controlled lighting and ventilation to ensure consistent and unbiased assessments.

5.4.3 Sample preparation

Pork samples (ham and shoulder) from each dietary treatment (Diet 0%, Diet 20%, and Diet 40% FCRLM) were thawed, trimmed, and cooked using a standardized method of oven-roasted at 150°C until reaching an internal temperature of 75°C. The cooked samples were then cut into

uniform cubes approximately 2cm³, coded with random three-digit numbers, and served warm at 60°C in a randomized order. Water and unsalted crackers were provided to panelists for palate cleansing between samples.

5.4.4 Data analysis

Data on meat quality and sensory intensity scores were analyzed using the General Linear Model (GLM) procedure in SAS (2023). A t-test assessed the effect of chilling on carcass pH, while a two-way ANOVA evaluated treatment effects. Correlation analysis was performed for consumer acceptability and lexicon descriptors, and mean differences were compared using Tukey's test at a 0.05 significance level.

5.5 Results

5.5.1 Dressing Percentage of Pigs Fed on Fermented Cassava Root-Leaf Meal-Based Diets

Figure 2 presented the dressing percentage of pigs fed diets with different inclusion levels of fermented cassava root-leaf meal (FCRLM) was evaluated. Pigs fed the diet containing 20% FCRLM (T2) recorded the significantly highest dressing percentage for 60.21% meat dressing percentage, followed by T1(58.50%), and T3 (55.62) the lowest.

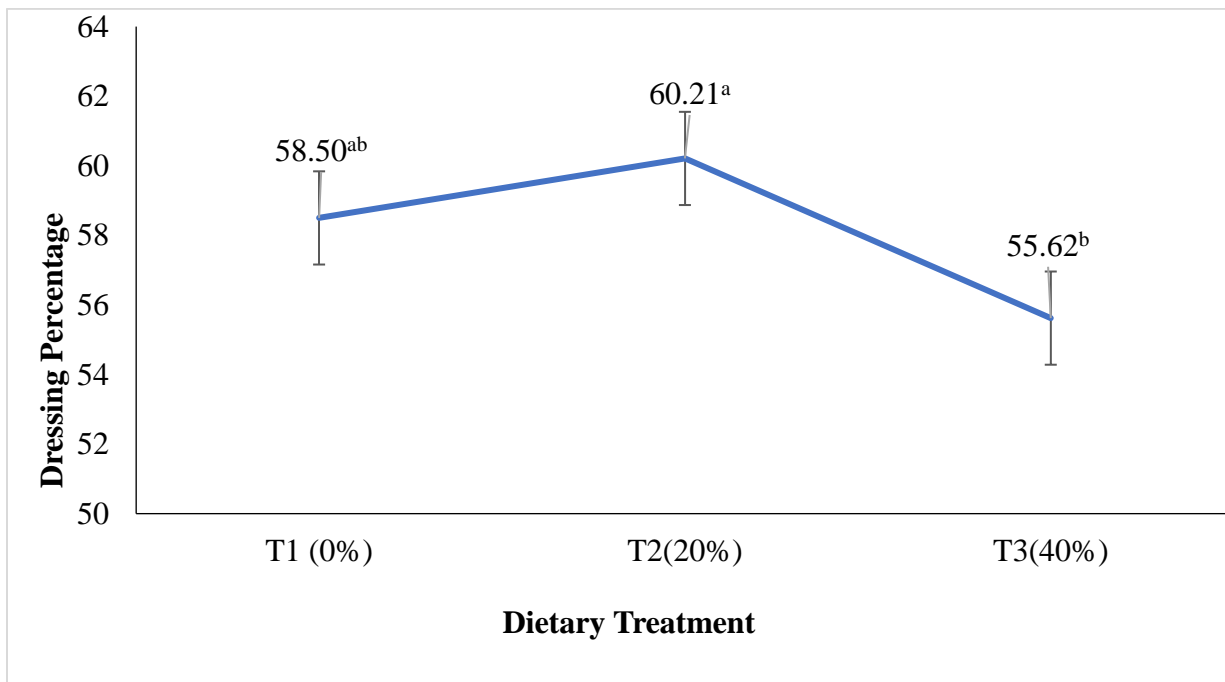


Figure 2: Dressing Percentage of Pigs Fed Fermented Cassava Root-Leaf Meal -Based Diets.

T1=Diet containing 0 % Fermented cassava root-leaf meal, T2= Diet containing 20 % Fermented cassava root-leaf meal, and T3= Diet containing 40 % Fermented cassava root-leaf meal.

5.5.2 Effect of chilling on carcass pH

Table 9 presented the effect of before chilling and after chilling on the carcass pH. The pH values decreased from pre-chilling to post-chilling across all dietary treatments, although the reduction in pH was not statistically significant ($p > 0.05$).

Table 9: Effect of chilling on carcass pH

Dietary Treatment	pH	
	Mean	(Confidence limit)
T1 0% Before chilling	6.02	(5.40-6.65)
After chilling	5.55	(4.93-6.18)
t-value		-2.80
p-value		0.09
T2 20% Before chilling	5.92	(5.30-6.55)
After chilling	5.45	(4.83-6.08)
t-value		-4.21
p-value		0.07
T3 40% Before chilling	5.73	(5.11-6.36)
After chilling	5.36	(4.74-5.99)
t-value		-2.78
p-value		0.06

Means within the same column with no superscript-letter are not significantly different. T1=Diet containing 0 % Fermented cassava root-leaf meal, T2= Diet containing 20 % Fermented cassava root-leaf meal, and T3=Diet containing 40 % Fermented cassava root-leaf meal.

5.5.3 Lexicon of descriptors for Ham and Shoulder from Pigs Fed Fermented Cassava Root- Leaf Meal-Based Diets

Pork descriptors from pigs fed diets partially substituted with FCRLM are presented in Tables 10 and 11.

Table 10: Intensity scores for flavor, and appearance characteristics of pork from pigs fed on fermented cassava root-leaf meal-based diets

Body part	Diet	Colour	Appearance	Aroma	Overall flavour	After Taste	Fatty mouthfeel
Ham	T1	1.89 ^c ±0.35	7.22±0.49	7.56±0.47	8.11±0.31	7.00±0.53	7.33±0.53
	T2	3.44 ^b ±0.44	7.00±0.44	7.33±0.37	7.78±0.32	7.56±0.38	8.11±0.31
	T3	6.00 ^a ±0.41	7.22±0.40	7.78±0.32	7.67±0.29	7.33±0.29	8.00±0.44
Shoulder	T1	6.00±0.53	7.44±0.24	6.89±0.54	7.22±0.32	5.78 ^{ab} ±0.22	7.22 ^b ±0.28
	T2	7.67±0.41	7.78±0.22	7.33±0.33	7.78±0.32	4.44 ^b ±0.63	7.56 ^b ±0.34
	T3	7.00±0.53	7.11±0.39	7.78±0.36	6.78±0.28	7.33 ^a ±0.50	8.56 ^a ±0.18

^{a,b,c} means within the same column with different superscripts are significantly different

($p < 0.05$). T1=Diet containing 0 % FCRLM, T2= Diet containing 20 % FCRLM and T3= Diet containing 40 % FCRLM, FCRLM=Fermented Cassava Root Leaf Meal.

Table 11: Intensity scores for texture characteristics of pork fed on fermented cassava root-leaf meal based diets.

Body part	Diet	Fibrousness	Springiness	Tenderness	Chewiness	Juiciness	Cohesiveness
Ham	T1	2.78 ^c ±0.43	7.44 ^a ±0.53	8.33 ^a ±0.29	8.33±0.24	7.78 ^a ±0.28	7.89±0.26
	T2	5.56 ^b ±0.38	5.00 ^b ±0.37	8.00 ^{ab} ±0.24	7.89±0.31	7.11 ^a ±0.31	8.22±0.28
	T3	7.22 ^a ±0.43	4.33 ^b ±0.33	7.00 ^b ±0.33	7.89±0.26	3.33 ^b ±0.44	8.56±0.18
Shoulder	T1	7.89 ^a ±0.31	7.44 ^a ±0.47	8.11 ^a ±0.39	8.00±0.29	7.44±0.41	6.11 ^b ±0.31
	T2	3.11 ^b ±0.48	5.67 ^b ±0.50	7.56 ^a ±0.38	8.56±0.18	6.22±0.22	8.33 ^a ±0.29
	T3	3.56 ^b ±0.60	8.00 ^a ±0.41	4.78 ^b ±0.52	7.78±0.28	6.56±0.56	8.33 ^a ±0.24

^{a,b,c} means within the same column with different superscripts are significantly different at $p < 0.05$.

T1=Diet containing 0 % Fermented Cassava Root-Leaf Meal, T2= Diet containing 20 % Fermented Cassava Root-Leaf Meal, and T3= Diet containing 40 % Fermented Cassava Root-Leaf Meal.

Taste intensities were highest for the 0% (8.11) and 20% (7.78) diet respectively. Aroma and fatty mouthfeel were also consistently high across all diets (above 7.3). Colour increased significantly with FCRLM inclusion (from 1.89 to 6.00), but darker colour at (40%) may have negatively impacted flavour perception and tenderness. Tenderness was highest at 0% (8.33) and

decreased at 40% (7.00), while juiciness dropped drastically at 40% (3.33). Fibrousness increased at higher inclusion (from 2.78 to 7.22).

The Colour brownness increased (6.00 to 7.67) with increase in level of FCRLM inclusion. Fatty mouthfeel increased with inclusion level (up to 8.56 at 40%), which have enhanced palatability. However, aftertaste at 20% (4.44) was lower than at 0% and 40%. The degree of tenderness remained high at 0% and 20%, but dropped at 40% (4.78), cohesiveness was low at 0% (6.11) but improved with inclusion, up to 8.33 (40%). Fibrousness decreased at 20% and 40%, which could be positive, but springiness and juiciness varied less distinctly.

Data on Pearson correlation analysis are presented in Table 6. Results revealed both positive and negative associations among pork sensory descriptors. A significant negative correlation was observed between colour and aftertaste ($r = -0.324, p < 0.05$), as well as between fibrousness and cohesiveness ($r = -0.343, p < 0.05$). Juiciness was negatively correlated with cohesiveness ($r = -0.378, p < 0.01$). In contrast, springiness showed a significant positive correlation with juiciness ($r = 0.394, p < 0.05$), while aroma was positively associated with aftertaste ($r = 0.278, p < 0.05$). Additionally, both taste ($r = 0.287, p < 0.05$) and aftertaste ($r = 0.326, p < 0.05$) were positively correlated with cohesiveness.

Table 12: Correlation coefficients between the intensity ratings of pork descriptors from pigs fed diets partially substituted with fermented cassava

	Colour	Appearance	Fibrousness	Springiness	Aroma	Taste	Aftertaste	Tenderness	Chewiness	Juiciness	Cohesiveness	FM
Colour	1.000	0.054 ^{ns}	0.012 ^{ns}	-0.061 ^{ns}	-0.039 ^{ns}	-0.135 ^{ns}	-0.324 [*]	-0.332 ^{ns}	-0.092 ^{ns}	-0.207 ^{ns}	0.005 ^{ns}	0.094 ^{ns}
Appearance		1.000	0.062 ^{ns}	0.045 ^{ns}	0.083 ^{ns}	0.054 ^{ns}	-0.192 ^{ns}	0.108 ^{ns}	0.060 ^{ns}	0.000 ^{ns}	0.083 ^{ns}	0.108 ^{ns}
Fibrousness			1.000	-0.177 ^{ns}	0.062 ^{ns}	-0.068 ^{ns}	0.067 ^{ns}	0.140 ^{ns}	-0.257 ^{ns}	-0.172 ^{ns}	-0.343 [*]	0.001 ^{ns}
Springiness				1.000	0.012 ^{ns}	-0.045 ^{ns}	0.108 ^{ns}	-0.112 ^{ns}	-0.041 ^{ns}	0.394 [*]	-0.254 ^{ns}	0.189 ^{ns}
Aroma					1.000	0.122 ^{ns}	0.278 [*]	-0.088 ^{ns}	0.083 ^{ns}	-0.144 ^{ns}	0.212 ^{ns}	0.190 ^{ns}
Taste						1.000	0.246 ^{ns}	0.26 ^{ns}	-0.077 ^{ns}	0.102 ^{ns}	0.287 [*]	0.019 ^{ns}
Aftertaste							1.000	-0.125 ^{ns}	-0.227 ^{ns}	-0.060 ^{ns}	0.326 [*]	0.169 ^{ns}
Tenderness								1.000	0.240 ^{ns}	0.084 ^{ns}	-0.257 ^{ns}	-0.141 ^{ns}
Chewiness									1.000	-0.030 ^{ns}	-0.034 ^{ns}	-0.025 ^{ns}
Juiciness										1.000	-0.378 ^{**}	-0.042 ^{ns}
Cohesiveness											1.000	0.103 ^{ns}
FM												1.000

Key: FM= Fatty Mouth feel; ns= Not significant at $p \geq 0.05$; *= Significant at $p \leq 0.05$; ***= Significant at $p < 0.01$

5.5.4 Consumer liking of pork from pigs fed on fermented cassava root-leaf meal-based diets

Mean scores of the sensory attributes of pork from pigs fed on fermented cassava root-leaf meal-based diets are presented in Table 13.

Table 13: Mean scores of the sensory attributes of pork from pigs fed on fermented cassava root-leaf meal-based diets

Body part	Diet	Colour	Texture	Taste	Juiciness	Tenderness	Overall
Ham	T1	3.30 ^b ±0.17	3.30 ^b ±0.17	3.23 ^b ±0.16	3.13 ^b ±0.15	3.28 ^b ±0.15	3.38 ^b ±0.15
	T2	3.33 ^b ±0.14	3.90 ^a ±0.14	3.63 ^{ab} ±0.14	3.68 ^a ±0.14	3.80 ^{ab} ±0.16	3.78 ^{ab} ±0.12
	T3	4.40 ^a ±0.12	3.73 ^{ab} ±0.16	4.00 ^a ±0.17	3.30 ^{ab} ±0.19	3.85 ^a ±0.17	3.95 ^a ±0.15
Shoulder	T1	3.43±0.18	3.43±0.18	3.03±0.14	3.15±0.18	3.45±0.16	3.33±0.16
	T2	3.40±0.19	3.55±0.14	3.43±0.15	3.63±0.15	3.65±0.13	3.70±0.12
	T3	3.63±0.12	3.70±0.16	3.53±0.17	3.68±0.15	3.85±0.14	3.55±0.13

^{a,b} means within the same column with different superscripts are significantly different ($p < 0.05$).

T1=Diet containing 0 % Fermented cassava root-leaf meal, T2= Diet containing 20 % Fermented cassava root-leaf meal, and T3= Diet containing 40 % Fermented cassava root-leaf Meal.

Ham from pigs fed the diet containing 40% FCRLM had the highest scores for colour (4.40 ± 0.12), taste (4.00 ± 0.17), tenderness (3.85 ± 0.17), thus overall acceptability (3.95 ± 0.15). These scores were significantly higher compared to those from the control group (0% inclusion), which had lower ratings across all attributes. Notably, texture and juiciness were most improved at the 20% inclusion level, with scores of 3.90 ± 0.14 and 3.68 ± 0.14 , respectively.

In contrast to ham, no significant differences ($p > 0.05$) were observed in the sensory attributes of shoulder cuts across the three dietary treatments. All parameters, including colour, texture, taste, juiciness, tenderness, and overall acceptability, had similar scores irrespective of the level of FCRLM inclusion. This indicated that the dietary inclusion of FCRLM had minimal influence on the sensory quality of shoulder meat.

The Coefficients of correlation between sensory attributes of pork from Pigs Fed Fermented Cassava Root-Leaf Meal-Based Diets are presented in table 8. All sensory variables were positively and significantly correlated ($p < 0.05$). Pearson correlation analysis revealed significant positive associations among all sensory attributes of pork from pigs fed on FCRLM - based diets. Colour showed a strong positive correlation with overall acceptability ($r = 0.605$, $p <$

0.05), as well as with taste ($r = 0.481, p < 0.05$), texture ($r = 0.410, p < 0.05$), juiciness ($r = 0.349, p < 0.05$), and tenderness ($r = 0.286, p < 0.05$).

5.5.5 Coefficients of correlation between sensory attributes of pork from pigs fed on fermented cassava root-leaf meal-based diets

The Coefficients of correlation between sensory attributes of pork from pigs fed on fermented cassava root-leaf meal-based diets are presented in Table 14. All sensory variables were positively and significantly correlated ($p < 0.05$).

Table 14: Coefficients of correlation between sensory attributes of pork from pigs fed on fermented cassava root-leaf meal-based diets

	Colour	Texture	Taste	Juiciness	Tenderness	Overall
Colour	1.000	0.410***	0.481***	0.349***	0.286***	0.605***
Texture		1.000	0.457***	0.458***	0.596***	0.687***
Taste			1.000	0.381***	0.322***	0.699***
Juiciness				1.000	0.514***	0.581***
Tenderness					1.000	0.548***
Overall						1.000

Key: *** = Significant at $p < 0.05$.

5.6 Discussion

5.6.1 Dressing percentage

There were significant differences ($P < 0.05$) in the dressing percentages among the dietary treatments. Pigs on T2 (20% FCRLM) recorded the highest dressing percentage (60.21.00%), followed by T1 (0% FCRLM) at 58.50%, while T3 (40% FCRLM) had the lowest (55.35%). The improvements in nutrient use and carcass yield likely stem from fermentation boosting protein availability and making nutrients easier to digest, which also helps break down the fiber content in the feed (Chisoro *et al.*, 2023). The reduced lower carcass yield recorded in T3 (55.35%) could be due to the excessive fiber content in the diet, which may have restricted intake and the potential energy available for muscle deposition (Zhu *et al.*, 2023). In this respect, the yield of 58.50% in the control group (T1) was moderately satisfactory. This was in line with the moderate carcass characteristic results obtained with processed cassava leaves reported by Kumar *et al.* (2020) and Sittiya *et al.* (2020). As discussed in the introduction, very high levels

of cassava leaves likely negatively impact carcass yield as a result of reduced nutrient digestibility and excessive gut filling.

5.6.2 Carcass pH

No significant differences in meat pH were observed in pigs fed diets containing 0%, 20%, and 40% FCRLM. This was likely due to the effects of pre-slaughter handling, which mitigated stress during slaughter, and the balanced protein and energy provisions in the diets (Arsenoaia & Malancus, 2023). For the pH ranges observed (5.6–5.8), the pork obtained in this study was of good quality. Meat within this pH range is desirable: tender, with appropriate color, juicy, water-retaining, and less prone to myofibrillar water loss associated with low-pH lean meat (Aguirre *et al.*, 2024; Brewer, 2014; Warner *et al.*, 2022). Meat with a high pH is less dry and has greater myofibrillar water retention (Aguirre *et al.*, 2024; Brewer, 2014; Warner *et al.*, 2022). A high pH is also associated with better color and moisture retention (Kang *et al.*, 2019), due to the effects of postmortem glycolysis on lactic acid accumulation, pH, and post-slaughter moisture loss.

The increase in brownness colour of pork might have been attributed to the increased carotenoids and pigments in cassava leaves, which are known to deposit in animal tissues and through fermentation phytonutrients were released thus contributing to meat pigmentation (Alvarez & Fernández, 2012). However, at 40% inclusion, the darker colour might have resulted from oxidation of muscle pigments or stress-related pH elevation, affecting consumer perception negatively (Hughes *et al.*, 2020). Tenderness was highest at 0% (8.33) and decreased at 40% (7.00), while juiciness dropped drastically at 40% (3.33).

The Colour increased with FCRLM (6.00 to 7.67). The enhanced colour intensities were likely due to the presence of natural pigments (like carotenoids and chlorophyll derivatives) in cassava leaves. During fermentation, bioactive compounds become more bioavailable, and when absorbed, they accumulated in the muscle, affecting meat pigmentation (Chaiareekitwat *et al.*, 2022). Changes in meat color reflect increases in myoglobin content in muscle tissue, which, according to Jiang *et al.* (2025) may be attributable to the antioxidant properties of fermented cassava. In comparison, the inclusion of cassava root and leaf meal (CLRM) at 40% of the diet for growing pigs resulted in the meat beginning to lose its tenderness and juiciness. This is likely the result of the interaction of several factors, the most notable of which is the high fiber content of the diet due to the cassava root and leaf meal, which is also low in energy and poorly digestible compared to conventional feeds (Ojediran *et al.*, 2024). This may also be associated with poor muscle and fat accumulation, as well as low intramuscular fat deposition, which is crucial for meat

tenderness and juiciness, along with overall moisture. The meat also lacks fat, meaning it does not provide the necessary lubrication to soften the meat and aid in tenderization during chewing (Hedemann *et al.*, 2022; Ojediran *et al.*, 2024).

Fermented cassava root flour and leaf concentrates contain considerable amounts of crude fiber that can restrict muscle fiber growth, reduce fat deposition, glycogen storage within the muscle, and the energy density of the diet. Okrathok and Khempaka (2020) described the relationship between increased meat firmness and reduced juiciness, which is due to reduced proteolysis and elevated pH levels. The 40% reduction in juiciness (3.68) (3.33) is associated with intramuscular fat loss and WHC deporter (Zinina *et al.*, 2019).

Increased in fibrousness intensity was corresponding with the inclusion level of FCRLM (from 2.78 to 7.22), this was due to higher dietary fiber intake leading to leaner carcasses and tougher muscle structure. Lower fat content results in less lubrication, enhancing the perception of fibrousness during chewing (H. Cai *et al.*, 2024). Taste intensities were highest for the 0% (8.11) and 20% (7.78) but declined at 40%. This could have been due to high cassava leaf inclusion which introduced bitter tasting phytochemicals that negatively affected flavor (Anyiam *et al.*, 2023). Aroma and fatty mouthfeel were also consistently high across all diets (above 7.3) due to probable retention of sufficient lipid content and beneficial fermentation of byproducts that enhanced aroma (Zheng *et al.*, 2025).

Fatty mouthfeel increased with inclusion (up to 8.56 at 40%), which enhanced palatability. Despite cassava being a low fat ingredient, fermentation improves nutrient digestibility, allowing more efficient energy utilization and possibly better intramuscular fat deposition, especially in fatty tissues like the shoulder (Sugiharto *et al.*, 2017). The increased fatty mouthfeel significantly enhanced palatability, as intramuscular fat (IMF) contributed to a rich and lubricated texture in the mouth. This matched findings of Hirai *et al.* (2023). They reported that more IMF in beef, especially from Japanese Black cattle, intensified sweet and roasted flavors while reducing off-flavors, leading to higher palatability scores.

Aftertaste scores were lower (4.44) at a 20% concentration compared to 0% and 40%, perhaps because fermentation reduces some bitter phytochemicals and adds some volatile flavor phytochemicals (Huang *et al.*, 2024; Micheni *et al.*, 2020; Shi *et al.*, 2017). Seideman *et al.* (1988) captured this well when they stated that fermentation can change the volatile and non-volatile phytochemical composition of food. The lowest tenderness score was at 40% (4.78), and the lowest cohesion score at 0% was 6.11. At a total inclusion of 40%, these scores totaled 8.33, suggesting

that tenderness and cohesion improve with higher inclusion rates. This is likely due to differences in muscle structure and composition that arise from varied diets. At levels of zero and twenty percent, pork is considered more nutrient-dense and more readily promotes muscle and fat deposition, resulting in the production of more tender pork (Wellington *et al.*, 2020).

However, at 40%, the excess fiber content with a nutritional imbalance decreases fat deposition, resulting in denser, tougher muscle and, therefore, less tenderness (Doran *et al.*, 2006; Huang *et al.*, 2018). The increased cohesion associated with higher levels of FCRLM possibly results from the development of connective tissue or enhanced structural proteins in the muscle. The fermentation process may also have altered plant fibers and released bioactive compounds that facilitated muscle matrix formation, resulting in firmer, more uniform meat. The meat was less tender, but cohesion, or internal binding strength, was improved (Mienaltowski *et al.*, 2021).

The improvements observed as a result of FCRLM addition can be explained by the fermentation process, which yielded less toxic microbial proteins and free amino acids, thereby enhancing muscle protein synthesis (Cai *et al.*, 2025). The reduction in fiber content observed at the 20% and 40% levels suggests that fermentation and microbial action on structural carbohydrates in cassava leaves may also have resulted in lower indigestible residues and higher digestible energy, supporting lean tissue development (Chen *et al.*, 2024).

5.6.3 Consumer liking

Recording biochemical and physiological changes at a 40% level of feed intake affected sensory cassava and root meal effect while feeding FCRLM to pigs. This corroborates Ojediran *et al.* (2024) results, who observed the accumulation of bioactive compounds, especially carotenoids and flavonoids, in increased volumetric concentrations with cassava leaves. The pigmentation in the muscle is believed to change with the accumulation of carotenoids and flavonoids, cassava leaves improving the taste and the meat quality of the pork as observed by the sensory panelists. The breakdown of more complex phytonutrient structures through fermentation enhances their bioavailability, allowing them to be more readily absorbed, contributing to the deposition in muscle tissues, resulting in more richly coloured meat with added flavour complexity (Fernández *et al.*, 2024; Puspitasari & Riyanti, 2024; Sawant *et al.*, 2025).

Moreover, the increased tenderness at the 40% inclusion level may be the result of improved nutrient digestibility and muscle development due to the fermentation-induced softening of dietary fibers. The aforementioned treatment may also have lower retention of juiciness which could be associated with low values of intra muscular fat as well as possible moisture evaporation triggered

by oxidative reactions commonly associated with high fiber diets (Haghi & Carvajal, 2014). The meat had the best texture and juiciness at 20% inclusion of fermented cassava leaf meal. This suggests that the moderate level of inclusion offers the best nutrient balance which enhances muscle hydration and fat retention without inducing oxidative damage or undue muscle fiber rigidity. This level likely maintains the favorable factors which promote uniform moisture and fat distribution within the meat, which is essential for palatability and pleasant overall mouthfeel (Brewer, 2014) This parallels the work of Xia *et al.* (2018) who discovered the water retention of meat increased with the concentration of myofibrillar proteins. This can be explained by stronger hydrophobic bonding and greater amounts of free water in the meat. The broader scope of literature demonstrated that, among consumers, flavor, texture, and appearance all rank within the top features that determine enjoyment (De Araújo *et al.*, 2022).

The investigation of pork meat and its sensory attributes offers pork bite numerous relationships that can be blamed on its external and internal physical-chemical states courtesy of the diet and muscular disposition. One such association is the negative correlation of color and aftertaste (0.324, $p < 0.05$). The connection of aftertaste fading with increment of color intensity is probably due to higher concentration of myoglobin (> myoglobin conj globally) or other oxidative phenomena, possibly dominated by unpleasant and overwhelming aftertaste oxidized lipids and pigments coloring meat (Bonny *et al.*, 2017).

Furthermore, muscle diets rich in antioxidants are known to improve muscle color and flavor retention. The fibrousness of meat also tended to be tougher and less cohesive and, just like with the negative correlation of fibrousness and cohesiveness (-0.343, $p < 0.05$), is explainable in that cohesive meat is easier to chew and therefore offers a less complex eating experience. Tough and fragmentable meat with low cohesiveness on the other hand is most likely the result of reduction in denaturation of protein structures (Warner *et al.*, 2022). Also, springiness with juiciness had a positive correlation ($r = 0.394$, $p < 0.05$). Meat that is more elastic is perceived to be juicier due to a greater retention of moisture and the ability to release more fluid during mastication. Elasticity and pH, intramuscular fat, and protein gelling during cooking all contribute to the perception of meat moistness and tenderness (Kang *et al.*, 2019).

There was also a correlation, although weak, between aroma and aftertaste ($r = 0.278$, $p < 0.05$), which suggests that the meat, to some extent, controlled the lingering flavor that followed its initial smell. This may be due to the presence of shared compounds, especially some volatile constituents formed via lipid breakdown and during the cooking process through the Maillard reaction, that

contribute to both aroma and lasting flavor (Bolkvadze *et al.*, 2024). Diets containing fermented cassava root-leaf meal appear to enhance the aroma and the flavor that persists after mastication through increasing these volatile compounds. Moreover, there was also a significant correlation between taste and the cohesiveness of the meat ($r = 0.287$, $p < 0.05$), as well as between aftertaste and cohesiveness ($r = 0.326$, $p < 0.05$), suggesting that firm, cohesive meat better retains and releases flavor compounds. While eating, cohesive meat is chewed more thoroughly, mixes better with saliva, and creates better taste perception, allowing for a gradual liberation of taste and aroma, which enhances the overall experience of eating even long after the meat has been chewed (Ma *et al.*, 2024; Shahidi & Hossain, 2022).

There also emerged a negative correlation of cohesiveness with juiciness ($r = -0.378$, $p < 0.01$), indicating that more cohesive pork is perceived to be less juicy. Excess moisture, especially moisture due to a high water-holding capacity or intramuscular fat, could facilitate the separation of meat particles during chewing where tessellated binding is perceived to be weak. This trade-off between moisture release and texture binding is articulated as juicy meat appearing less structured or 'mushy' (Watanabe *et al.*, 2018).

5.7 Conclusion

Including 20% of fermented cassava root-leaf meal (FCRLM) in swine feed improved meat quality and dressed carcass yield as indicated by improved dressing percentage, maintained optimal meat pH, and enhanced sensory attributes of several shoulder and ham cuts (colour, texture, taste, juiciness, tenderness, and overall acceptability) while 40% inclusion increased some attributes of ham (colour, aroma, fatty mouth feel) but decreased tenderness and juiciness.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

6.1 General Discussion

Pig farming in Kenya plays an important role in enhancing food security, employment, and income generation. Although it has historically been regarded as less important than other forms of livestock farming, it is gaining popularity in Central, Western and parts of Eastern Kenya due to a growing demand for pork and the potential profit to be made from pig farming (Micheni *et al.*, 2020; Muthui *et al.*, 2019). In the Kenyan context, pig farming is usually regarded as a more economically viable farming enterprise. This has resulted in increased separation production capacity; however, for the most part, the increased productivity has been economically driven. Most small-scale pig farmers rely on kitchen and other food scraps as supplementary pig feed. It is these feeds on this sort of feed that is the primary reason for decreased livestock productivity in this country (Muthui *et al.*, 2019).

Where there is maximal pig growth, there is maximal productivity, and this is only achievable if pigs are fed energetically and protein dense feeds. As the world's population grows and the costs of traditional feed ingredients rise, particularly in fish and soybean meals and maize, more farmers are beginning to feel the pinch of these more conventional feeds. This in itself sets the stage for a more systematic investigation and assessment of locally produced pig feed. To support optimal pig performance, it's essential that farmers adopt alternative feeds that can deliver similar benefits as conventional ones, helping improve productivity and sustain pig farming in Kenya.

Previous research has indicated that cassava has the potential to completely substitute maize as an energy source in pig diets (Bayata, 2019). Although cassava has been identified as a potential alternative to maize in monogastric animal diets, its utilization is limited by the presence of anti-nutritional factors (ANFs), particularly high levels of cyanogenic glycosides (HCN) and crude fiber (Jacob Nte *et al.*, 2023). However, limited information exists on whether treatments such as fermentation or the use of exogenous enzymes can enhance the nutritional composition, *in-vitro* digestibility, and cyanide reduction of cassava (*Manihot esculenta*) root-leaf meal, thereby improving the growth performance of pigs. This study was therefore conducted to :

- i. Determine the cyanogenic glycosides (HCN) levels, fiber content, and *in-vitro* digestibility of fermented, probiotic, enzyme-treated, and untreated cassava-based diets.

- ii. Evaluate the feed intake, feed conversion ratio, and growth rate of grower pigs fed treated cassava-based diets.
- iii. Assess the effect of treated cassava-based diets on carcass quality characteristics of grower pigs.

Processing methods such as sun-drying, fermentation, boiling, and enzyme treatment have been shown to reduce anti-nutritional factors (ANFs) in cassava, thereby improving its safety and nutritional quality for use in livestock feeds (Jeyakumar & Lawrence, 2022; Kemboi *et al.*, 2023b; Ngungulu *et al.*, 2024).

Chapter three of this thesis presented the results of enzyme treated, natural and cultured fermentation on the on nutritional composition, *in-vitro* digestibility, and cyanide reduction in cassava (*Manihot Esculenta*) Root-Leaf Meal. The findings demonstrated that spontaneous fermentation for four days (96 hrs) significantly improved the nutritional quality of cassava root-leaf meal, in terms of *in-vitro* dry matter digestibility 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 %.

Chapter four focused on determining the effect of incorporating fermented cassava root-leaf meal based diets on feed intake, feed conversion ratio, weight gain and growth rate of grower pigs. The highest average daily feed intake (ADFI) was recorded in the pigs fed the diet containing 0% FCRLM, followed by those fed the 20% FCRLM diet, while the lowest intake was observed in pigs fed the 40% FCRLM diet. The reduction in average daily feed intake (ADFI) observed in pigs fed the 40% FCRLM diet was likely due to the increased bulkiness associated with high fiber diets and the possible presence of residual anti-nutritional factors that persisted after fermentation. Additionally, there was a general increase in feed conversion ratio (FCR) with increasing FCRLM inclusion levels, with the 40% diet showing the highest FCR. However, despite the decline in feed intake, the growth rate of the pigs remained largely unaffected by increasing levels of FCRLM in the diet.

Chapter Five described the evaluation of carcass quality characteristics and sensory analysis of pigs fed on fermented cassava root-leaf meal-based diets. There were significant differences ($p < 0.05$) in the dressing percentages among the dietary treatments. Pigs fed the diet containing 20% FCRLM recorded the highest dressing percentage, whereas those fed the 40% FCRLM diet had the lowest. The decline in DP in T3 may have been caused by anti-nutritional

factors that persisted after fermentation, which led to low intake and thus reduced dressing percentage. The study results showed no differences between before and after chilling of pigs in carcass pH. This indicated that the post-mortem slaughter was conducted carefully and the acidity of the pork was stable. All sensory variables were positively and significantly correlated ($p < 0.05$). A strong positive correlation was observed between taste, texture, color, and overall acceptability of the pork.

6.2 Conclusions

- i. Pre-treatment of Cassava root-leaf meal, through spontaneous fermentation for four days (96 hrs) significantly improves the nutritional quality by increasing the dry matter from 15.02% to 55.56%, Crude protein content from 7.47 % to 11.09 %, and *in-vitro* dry matter digestibility from 15.02% to 55.56%, while effectively reducing cyanogenic glycosides (HCN) from 45.00 ppm to 8.00 ppm
- ii. Incorporation of up to 20% spontaneously-fermented cassava root-leaf meal (CRLM) in grower pig diets improved feed intake, average daily gain, final weight gain, and feed conversion ratio. However, average daily gain and feed efficiency declined as the inclusion level of FCRLM increased to 40%.
- iii. At the 20% FCRLM level, both ham and shoulder cuts maintained their sensory properties, while at the 40% inclusion level, they enhanced some ham attributes, colour, aroma, and fatty mouth feel, negatively impacting tenderness and juiciness.

6.3 Recommendations

The study recommends that:

- i. Use of spontaneous fermentation to improve nutritional quality of CRLM in terms of chemical composition, DM digestibility, and HCN reduction.
- ii. Use up to at 20% FCRLM inclusion level in grower pig diets to improve pig performance
- iii. Use up to at 20% spontaneously FCRLM to enhance meat quality, carcass parameters, and the overall acceptability.

6.4 Areas for Further Research

- i. Evaluate the effects of spontaneously fermented cassava root-leaf meal on the volatile fatty acid profile and methane production in pigs using an *in-vitro* study.
- ii. Assess the cost-benefit of feeding spontaneously fermented Cassava Root-Leaf Meal to grower pigs.

- iii. Investigate the microbial communities involved in spontaneous fermentation to identify beneficial strains that enhance detoxification and nutrient enrichment.
- iv. Evaluate how different pig breeds respond to diets containing FCRLM to optimize breed-diet matching for improved performance.
- v. Conduct a study to evaluate the effect of incubating cassava root-leaf meal (CRLM) for more than 96 hours on its nutritive value and HCN reduction.
- vi. Investigate the effect of incubation pH on enzyme production and fiber breakdown by *Aspergillus niger*, spontaneous fermentation, enzyme (Natuzyne®), and *Lactobacillus brevis* on the nutritional composition, *in-vitro* digestibility, and cyanide reduction in cassava root-leaf meal.

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APPENDICES

Appendix A. NACOSTI Permit

 REPUBLIC OF KENYA	 NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION
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RESEARCH LICENSE	
	
<p>This is to Certify that Ms. VALENTINE MUTUYIMANA of Egerton University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nakuru on the topic: REDUCTION OF HYDROGEN CYANIDE CONTENT IN CASSAVA ROOT-LEAF MEAL-BASED DIETS ON PERFORMANCE OF GROWER PIGS IN KENYA for the period ending : 29/October/2025.</p>	
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Appendix B. Ethical Clearance

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**EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW
COMMITTEE**

EU/RE/DIR/009

Approval No. EUISERC/APP/376/2024

17th December 2024

Valentine Mutuyimana
Telephone: 0704345760
Email: mutuye1989@gmail.com

Dear Valentine,

**RE: ETHICAL APPROVAL: REDUCTION OF HYDROGEN CYANIDE CONTENT IN
CASSAVA ROOT-LEAF MEAL-BASED DIETS ON PERFORMANCE OF GROWER
PIGS IN KENYA**

This is to inform you that the *Egerton University Institutional Scientific and Ethics Review Committee* has reviewed and approved your above research proposal. Your application approval number is *EUISERC/APP/376/2024*. The approval period is *17th December 2024 – 18th December 2025*

This approval is subject to compliance with the following requirements;

- i. Only approved documents including (informed consents, study instruments, MTA) will be used.
- ii. You are required to adhere Institutional Experimental Animals use and Care policy.
- iii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Egerton University Institutional Scientific and Ethics Review Committee*.
- iv. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Egerton University Institutional Scientific and Ethics Review Committee* within 72 hours of notification
- v. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to *Egerton University Institutional Scientific and Ethics Review Committee* within 72 hours.
- vi. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.

"Transforming Lives through Quality Education"

- vii. Submission of a request for renewal of approval at least 60 days prior to the expiry of the approval period. Attach a comprehensive progress report to support the renewal.
- viii. Submission of an executive summary report within 90 days upon completion of the study to *Egerton University Institutional Scientific and Ethics Review Committee*.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <https://oris.nacosti.go.ke> and also obtain other clearances needed.

Yours sincerely,

Prof. Kennedy N. Ondimu PhD
CHAIRMAN, EUISERC
KNO/BK/



"Transforming Lives through Quality Education"

Appendix C. Research Pictorial



C1. Cassava root-leaf plant



C2. HCN Determination from CRL



C3. Results of HCN





C4. Preparation of CRLM Samples



C5. Milling CRLM samples for Proximate Analysis



C6. Moisture and Ash Determination of CRLM



C7. Incubation of inoculated CRLM



C8. *In-Vitro* Dry matter Digestibility and Gas production



C9. Prepared Diets and arranged in the store per Dietary Treatments



C10. Experimental pigs and feeding process



C11. Preparation of Samples for Carcass Quality and Sensory Evaluation



C12. Training of the Panelists and Development of the Sensory Lexicon



C13. Sensory Evaluation by the Panelists

Appendix D. Sensory Panel Recruitment Form

I,, willingly agreed to participate in this study. I acknowledge that I will not receive any direct benefits from taking part. The purpose and procedures of the study, which involve the sensory evaluation of pork meat, have been clearly explained to me. My role will be to assess specific sensory attributes based on my own perception in comparison to set standards. I confirm that I have no allergies to the product. I understand that all results will be kept confidential, stored for a period of three months following the evaluation, and my identity will remain anonymous through the use of coded data.

.....

Signature of participant

Date

.....

Signature of researcher

I believe that the participant is giving informed consent to participate in this study.

SENSORY ANALYSIS

Name: Date...../...../.....

You are provided 3 pork samples with random labels.....

Kindly taste the samples and score your impression/judgement of the ATTRIBUTES listed below the score card on the score card provided for each sample.

	1	2	3	4	5	6	7	8	9	
Light brown	Colour intensity								Dark brown	
	1	2	3	4	5	6	7	8	9	
Rough/patched/inconsistent	Appearance								Smooth/Consistent	
	1	2	3	4	5	6	7	8	9	
Visible strands	Fibrousness								Extremely visible fibre	
	1	2	3	4	5	6	7	8	9	
Not springy	Springiness								Extremely springy	
		2	3	4	5	6	7	8	9	
	1	Aroma intensity								Extremely perceptible
	1	2	3	4	5	6	7	8	9	
Not perceivable	Overall flavour intensity								Highly perceivable	
		2	3	4	5	6	7	8	9	
	1	After Taste								Highly perceivable
	1	2	3	4	5	6	7	8	9	
Extremely tough	Tenderness								Extremely tender	
		2	3	4	5	6	7	8	9	
	1	Chewiness								Extremely chewy
	1	2	3	4	5	6	7	8	9	
Juicy	Juiciness								Extremely juicy	
	1	2	3	4	5	6	7	8	9	
Not cohesive	Cohesiveness of mass								Extremely cohesive	
	1	2	3	4	5	6	7	8	9	
Fatty	Fatty mouthfeel								Extremely Lean	

Appendix E. Anova Outputs

The GLM Procedure

Dependent Variable: Weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	45	48109.35526	1069.09678	589.69	<.0001
Error	152	275.57240	1.81298		
Corrected Total	197	48384.92766			

R-Square	Coeff Var	Root MSE	Weight Mean
0.994305	2.610423	1.346468	51.58045

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	1912.89942	1912.89942	1055.12	<.0001
Diet	2	46.34555	23.17277	12.78	<.0001
Sex*Diet	2	435.06582	217.53291	119.99	<.0001
Sex*week	20	45691.72520	2284.58626	1260.13	<.0001
Diet*week	20	23.31927	1.16596	0.64	0.8746

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	1912.899419	1912.899419	1055.12	<.0001
Diet	2	46.345548	23.172774	12.78	<.0001
Sex*Diet	2	435.065819	217.532910	119.99	<.0001
Sex*week	10	63.820609	6.382061	3.52	0.0003
Diet*week	20	23.319274	1.165964	0.64	0.8746

Least Squares Means for effect Sex*Diet

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Weight

i/j	1	2	3	4	5	6
1		0.8143	<.0001	<.0001	<.0001	<.0001
2	0.8143		<.0001	<.0001	<.0001	<.0001
3	<.0001	<.0001		<.0001	<.0001	<.0001
4	<.0001	<.0001	<.0001		0.0722	<.0001
5	<.0001	<.0001	<.0001	0.0722		<.0001
6	<.0001	<.0001	<.0001	<.0001	<.0001	

The GLM Procedure

Dependent Variable: Weight Gain

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	45	55.54698889	1.23437753	3.58	<.0001
Error	53	18.26671010	0.34465491		
Corrected Total	98	73.81369899			

R-Square	Coeff Var	Root MSE	WTGAIN Mean
0.752530	12.38525	0.587073	4.740101

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	1.40179596	1.40179596	4.07	0.0488
Diet	2	0.21346263	0.10673131	0.31	0.7350
Sex*Diet	2	0.72986768	0.36493384	1.06	0.3541
Sex*week	20	43.39883636	2.16994182	6.30	<.0001
Diet*week	20	9.80302626	0.49015131	1.42	0.1535

	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	1.40179596	1.40179596	4.07	0.0488
Diet	2	0.09357677	0.04678838	0.14	0.8734
Sex*Diet	2	0.72986768	0.36493384	1.06	0.3541
Sex*week	10	1.40475960	0.14047596	0.41	0.9371
Diet*week	20	9.80302626	0.49015131	1.42	0.1535

The GLM Procedure

Dependent Variable: FI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	45	2142.577586	47.612835	119.55	<.0001
Error	53	21.107513	0.398255		
Corrected Total	98	2163.685099			

R-Square	Coeff Var	Root MSE	FI Mean
0.990245	3.457961	0.631074	18.24990

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	3.166675	3.166675	7.95	0.0067
Diet	2	7.656057	3.828028	9.61	0.0003
Sex*Diet	2	3.694831	1.847416	4.64	0.0139
Sex*week	20	2111.831791	105.591590	265.14	<.0001
Diet*week	20	16.228232	0.811412	2.04	0.0203

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	3.16667475	3.16667475	7.95	0.0067
Diet	2	9.20957071	4.60478535	11.56	<.0001
Sex*Diet	2	3.69483131	1.84741566	4.64	0.0139
Sex*week	10	2.28098081	0.22809808	0.57	0.8287
Diet*week	20	16.22823232	0.81141162	2.04	0.0203

The GLM Procedure

Dependent Variable: ADG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	45	1.65594596	0.03679880	151.64	<.0001
Error	152	0.03688687	0.00024268		
Corrected Total	197	1.69283283			

R-Square	Coeff Var	Root MSE	ADG Mean
0.978210	2.313404	0.015578	0.673384

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	0.10181869	0.10181869	419.57	<.0001
Diet	2	0.03856465	0.01928232	79.46	<.0001
Sex*Diet	2	0.02567980	0.01283990	52.91	<.0001
Sex*week	20	1.47381414	0.07369071	303.66	<.0001

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Diet*week	20	0.01606869	0.00080343	3.31	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	0.10181869	0.10181869	419.57	<.0001
Diet	2	0.03856465	0.01928232	79.46	<.0001
Sex*Diet	2	0.02567980	0.01283990	52.91	<.0001
Sex*week	10	0.00989798	0.00098980	4.08	<.0001
Diet*week	20	0.01606869	0.00080343	3.31	<.0001

The GLM Procedure

Dependent Variable: DFI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	45	1.90608182	0.04235737	54.72	<.0001
Error	152	0.11764949	0.00077401		
Corrected Total	197	2.02373131			

R-Square	Coeff Var	Root MSE	DFI Mean
0.941865	0.994757	0.027821	2.796768

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	0.19289091	0.19289091	249.21	<.0001
Diet	2	0.97186162	0.48593081	627.81	<.0001
Sex*Diet	2	0.19703939	0.09851970	127.28	<.0001
Sex*week	20	0.40226263	0.02011313	25.99	<.0001
Diet*week	20	0.14202727	0.00710136	9.17	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	0.19289091	0.19289091	249.21	<.0001
Diet	2	0.97186162	0.48593081	627.81	<.0001
Sex*Diet	2	0.19703939	0.09851970	127.28	<.0001
Sex*week	10	0.02372020	0.00237202	3.06	0.0014
Diet*week	20	0.14202727	0.00710136	9.17	<.0001

The GLM Procedure

Dependent Variable: FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	45	51.11536970	1.13589710	100.17	<.0001
Error	152	1.72361212	0.01133955		
Corrected Total	197	52.83898182			

R-Square	Coeff Var	Root MSE	FCR Mean
0.967380	2.523215	0.106487	4.220303

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	1.82323232	1.82323232	160.79	<.0001
Diet	2	0.96560303	0.48280152	42.58	<.0001
Sex*Diet	2	2.40997677	1.20498838	106.26	<.0001
Sex*week	20	44.61679394	2.23083970	196.73	<.0001
Diet*week	20	1.29976364	0.06498818	5.73	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	1.82323232	1.82323232	160.79	<.0001
Diet	2	0.96560303	0.48280152	42.58	<.0001
Sex*Diet	2	2.40997677	1.20498838	106.26	<.0001
Sex*week	10	0.40544545	0.04054455	3.58	0.0003
Diet*week	20	1.29976364	0.06498818	5.73	<.0001

The GLM Procedure

Dependent Variable: Initial Weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	112.8911111	22.5782222	13.01	0.0002
Error	12	20.8333333	1.7361111		
Corrected Total	17	133.7244444			

R-Square	Coeff Var	Root MSE	INW Mean
0.844207	5.240186	1.317616	25.14444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	85.36888889	85.36888889	49.17	<.0001
Diet	2	0.04777778	0.02388889	0.01	0.9863
Sex*Diet	2	27.47444444	13.73722222	7.91	0.0064

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	85.36888889	85.36888889	49.17	<.0001
Diet	2	0.04777778	0.02388889	0.01	0.9863
Sex*Diet	2	27.47444444	13.73722222	7.91	0.0064

The GLM Procedure

Dependent Variable: Final Weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	363.0916444	72.6183289	32.14	<.0001
Error	12	27.1098000	2.2591500		
Corrected Total	17	390.2014444			

R-Square	Coeff Var	Root MSE	FW Mean
0.930524	1.952122	1.503047	76.99556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	281.3192000	281.3192000	124.52	<.0001
Diet	2	21.1662111	10.5831056	4.68	0.0314
Sex*Diet	2	60.6062333	30.3031167	13.41	0.0009

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	281.3192000	281.3192000	124.52	<.0001
Diet	2	21.1662111	10.5831056	4.68	0.0314
Sex*Diet	2	60.6062333	30.3031167	13.41	0.0009

The GLM Procedure

Dependent Variable: WEIGHTGAIN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	91.39697778	18.27939556	105.98	<.0001
Error	12	2.06980000	0.17248333		
Corrected Total	17	93.46677778			

R-Square	Coeff Var	Root MSE	WEIGHTGAIN Mean
0.977855	0.800969	0.415311	51.85111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	56.74675556	56.74675556	329.00	<.0001
Diet	2	20.28054444	10.14027222	58.79	<.0001
Sex*Diet	2	14.36967778	7.18483889	41.66	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	56.74675556	56.74675556	329.00	<.0001
Diet	2	20.28054444	10.14027222	58.79	<.0001
Sex*Diet	2	14.36967778	7.18483889	41.66	<.0001

Appendix F. Publication

ISRG Journal of Agriculture and Veterinary Sciences (ISRGJAVS)



ISRG PUBLISHERS
Abbreviated Key Title: ISRG. J. Agri. Vet. Sci.
ISSN: 3048-8869 (Online)
Journal homepage: <https://isrgpublishers.com/cjavs/>
Volume – II Issue- III (May-June) 2025
Frequency: Bimonthly



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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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