PERFORMANCE OF IMPROVED INDIGENOUS LAYER CHICKEN IN KENYA FED ON PROCESSED CASSAVA *(Manihot esculenta* Crantz) ROOT MEAL-BASED DIET

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

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DECLARATION AND RECOMMENDATION

Declaration

I hereby declare that this thesis is my original work and has not been presented in this or any other university for the award of a degree.

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Recommendation

This thesis has been presented with our approval as the university supervisors.

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DEDICATION

This thesis is dedicated to my family whose support, encouragement, and love have been my strength in my entire academic life. Thanks to my parents, I believe that you have faith in me that you have sacrificed and made me always want to do something excellent. Your support and friendship have been priceless to me, my friends, mentors, and colleagues, who have made the difficult, yet rewarding journey one that is enjoyable. I am also dedicating this work to people who believe in power of knowledge and education and more so to positive change in the world. I am encouraged to work harder every day because of your perseverance and determination that you make a difference. Lastly, I dedicate this thesis to myself due to the amount of stamina, perseverance and efforts which I have invested to achieve this milestone.

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) root meal (CRM) is a readily available and inexpensive feed ingredient in tropical areas; it limits its use in poultry food due to low protein content and hydrogen cyanide (HCN) content. This study compared the impact of enzyme-treated cassava root meal (CRM) on nutrient profiles, productive traits, egg quality, and economic returns in enhanced indigenous layer hens. To determine the impact of the processing technique-fermentation (spontaneous, *Saccharomyces cerevisiae*), and the enzyme treatment on the nutritive value of CRM and the level of hydrogen cyanide, the pre-test was carried out. An experimental feeding was done over 12 weeks in a deep litter house where there were thirty-six chickens, sixteen weeks old. The cage was used to separate the experimental unit with three chickens per square meter, and each treatment was repeated three times. Four iso-nitrogenous and iso-caloric experimental diets were formulated containing 0, 25, 50 and 75% enzyme-treated CRM. Data on feed intake, feed conversion ratio, and egg production were collected and subjected to the Analysis of variance (ANOVA) in a completely randomized design (CRD) using the General Linear Model procedure of Statistical Analysis System (SAS) version 9.4 at a 5% significance level. There was no significant difference ($p>0.05$) in feed intake among the treatments, but the FCR was significantly lower ($p<0.05$) in the 75% CRM. There was a significant difference ($p< 0.05$) in egg production among treatments, with a significantly low egg production at 75% CRM. The highest egg shape index was recorded in the 75% CRM diet (73.385, $p<0.05$), while egg shell thickness was highest in the 50% CRM (0.594 mm, $p<0.05$). Yolk-to-albumen ratio was highest in the 50% CRM diet (57.420, $p<0.05$). Economic analysis showed that the 50% CRM inclusion gave the highest economic returns (ROI of 47.7%, CBR of 1.48, and profit of KES 3,681.74 per treatment (3 chicken), $p<0.05$). In comparison, the 75% CRM inclusion showed the lowest economic returns. It is concluded that a CRM inclusion level of up to 50% is optimal based on feed conversion ratio, percentage egg production, egg quality, and ROI. The study recommended inclusion of up to 50% of enzyme-treated CRM for better sustainability and profitability in improved indigenous layer chicken production.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADF	Acid detergent fibre
CBR	Cost-benefit ratio
CF	Crude fibre
CP	Crude protein
CRM	Cassava root meal
DDGS	Dried distiller's grains with solubles
DCP	Di-calcium phosphate
DM	Dry matter
ESI	Egg shape index
ESR	Eggshell ratio
FCR	Feed conversion ratio
FI	Feed intake
GLM	General linear model
HCN	Hydrogen cyanide
HDEP	Hen day egg production
IVDMD	*in vitro* dry matter digestibility
ME	Metabolizable energy
NDF	Neutral detergent fibre
NSP	non-starch polysaccharides
ROI	Return on investment
Y: A	Yolk to albumen ratio
YI	Yolk index

CHAPTER ONE
INTRODUCTION

1.1 Background Information

The global poultry industry faces significant challenges, primarily due to the volatility in feed resource availability and pricing. Maize (4-10 months maturity) is a conventional ingredient in poultry diets and is facing competing demands for the human food industry and biofuel, which results in a shortage of production and high prices (Govoni *et al*., 2021). This situation could be devastating for small-scale farmers and rural communities in which indigenous layer chickens are an important source of income and nutrition. Searching for alternative indigenous feed ingredients would not only provide an economic benefit but also enable more sustainable and resilient agricultural practices (Mitra, 2020).

Research into indigenous feed ingredients as an alternative to conventional feed ingredients for poultry production is not a recent development. In the past, the instability in the price of grains and other conventional feed components forced researchers and farmers to search for other available alternative feed resources. Cassava, being a high carbohydrate-yielding crop and its adaptability to diverse climatic and soil conditions, becomes a promising alternative indigenous crop (Fawole & Kolapo, 2022). Cassava's annual production in Kenya is 1,112,000 metric tons and takes 8-24 months to maturity (FAO, 2021).

Cassava meals have different nutritional contents (Diarra & Devi, 2015). This is a result of plant cultivar, climatic circumstances, edaphic factors, and management factors (Morgan & Choct, 2016). Notable nutritional compositions of cassava include: starch within a range of 60 to 80% (Chisenga *et al*. 2019), lipids in a range of 0.1 to 2.5% (Bhuiyan & Iji, 2015), proteins vary between a range of 1.17 to 5.13% (Omede *et al.* 2018), non-starch polysaccharides (NSP), water-soluble NSP (0.078 to 1.64%) and water-insoluble NSP 2.58 to 4.33% (Uthumporn *et al.* 2017), mineral content in cassava roots is low compared to cereals and legumes except for potassium, which amounts to about 2.50 g/kg DM (Omede *et al*. 2018) however, cassava leaves are a good source of calcium, magnesium, iron, manganese and zinc (Bayata, 2019). Cassava meals also contain anti-nutritional elements.

Due to the presence of anti-nutritional factors, primarily hydrogen cyanide (HCN), the use of cassava-based diets in chicken production has been restricted (Ngiki *et al*., 2014). In the form of cyanogenic glycosides, the cyanogen found in cassava is bonded to sugar molecules. Lotaustralin makes around 5% of the cyanogenic glycoside in cassava; the remainder is linamarin (Guerre, 2016). The enzyme linamarase converts linamarin and lotaustralin to HCN (Cardoso *et al*. 2005). On a DM basis, the HCN content of sweet cassava roots is approximately 75 mg/kg, although bitter varieties have been found to contain up to 1000 mg/kg (Guerre, 2016). Ngiki *et al*. (2014) found that compared to meals based only on cassava roots, poultry diets with cassava leaves had an HCN concentration that was six times greater. Boiling reduces HCN by 50-70%, soaking and fermenting by 80-90%, and sun drying by 80-90%. HCN level might drop by 95% during *gari* production operations like grating and fermenting. Bitter cassava with 400 mg/kg HCN can be lowered to 120-200 mg/kg by boiling, 40-80 mg/kg by soaking and fermenting, and less than 20 mg/kg by grating and fermenting. The HCN levels should be reduced to below 20 mg/kg to ensure safety for poultry. These procedures drastically reduce HCN in cassava, making it safe to eat (Nambisan, 2011). Therefore, hens raised on diets based on cassava should have methionine, an amino acid that contains sulphur, which is more necessary in diets because it helps the liver convert HCN into less toxic thiocyanate (Rakangtong & Bunchasak, 2011). Cassava-based diets also contain other small quantities of anti-nutritional factors such as phytate, oxalates, and tannins, which can impair nutrient absorption and lead to health issues in chickens (Morgan & Choct, 2016).

The high moisture, high fiber, low protein, and presence of HCN in cassava-based diets restrict their effective use as poultry feed. Various approaches have been employed to enhance the nutritional content of cassava, which include sun drying, boiling, and addition of fat, methionine supplementation, and fermentation /ensiling. However, fermentation and ensiling are successful techniques for enhancing the use of diets based on cassava because they increase digestibility and lower HCN concentrations (Diarra & Devi, 2015). Fermentation of cassava pulp with yeast (*Saccharomyces cerevisiae*) had a greater concentration of crude protein (13.4%) according to HieuLe and Khammeng (2014). Supplementing with microbial enzymes has also been shown to lower cassava's anti-nutritive factors (Morgan & Choct, 2016).

To assess the effectiveness of using fermented and enzyme-supplemented cassava root meal in place of maize to feed indigenous layers, fermentation procedures and enzyme supplementation have been used to reduce the naturally occurring components in cassava that inhibit animal nutrition, thereby improving the nourishing potential and profile for poultry meals (Zekarias *et al*., 2019). Firstly, enzyme supplementation entails the administration of specific exogenous enzymes to selectively break down the anti-nutritive components in cassava, which consequently transforms cassava to be much more digestible and enhances the nutrient availability. Through this, it attains a calculated and achievable strategy of improving the use of cassava as poultry feed.

Moreover, yeast fermentation involves fermentation of cassava with the selected strains of yeast, which is used to reduce the anti-nutrient properties of cassava, and, in the process, to enrich the cassava with a source of added nutrients, including proteins and vitamins produced by the yeast, hence enhancing the nutritional value of the feed. Finally, natural fermentation involves the use of microorganisms naturally available in cassava and the surrounding environment (Sanda, 2018). This kind of fermentation does not require expensive machinery and can be done inexpensively and with little difficulty, even though its effects are unpredictable and vary between batches of cassava, detoxifying it and enhancing its nutritional value. All these methods have specific benefits in cassava refinement to a better, more digestible, and better-balanced nutrient-enriched feedstuff, a sustainable alternative to traditional, maize-based poultry foodstuffs.

The objective of the study was to determine the impact of fermented cassava root meal on the growth performance, egg production, egg quality, and the economic analysis parameters of improved indigenous layer chicken. The findings of the research arrived at a conclusion that cassava root meal is relevant in the search for an inexpensive and widely available alternative to maize as a component of poultry food in the areas where maize is not excessively produced or is prohibitively expensive (Zekarias *et al*., 2019). An improvement in their role in improving the performance of chickens, sampled in terms of growth, production of eggs, and other health-related parameters, when fed on cassava root meal, is one of the confirmations of the feasibility of utilizing this product of cassava, rather than a maize-based product, as an alternative in the poultry diets. This, in its turn, can contribute to the reduction of the cost of the feed among the smallholder farmers as well as facilitate production sustainability. It may also be a move towards food security and sustainable agriculture (George, 2024). Through the problem statement, the significance of the research and the potential implications of the research to sustainable and cost-effective poultry production were captured.

1.2 Statement of the Problem

The threat of substitution by the human food and biofuel sectors, coupled with expensive prices and scarcity of maize are serious threat to the poultry industry in the world. Cassava would be a feasible alternative due to its high carbohydrate value and its adaptable climatic conditions, but the high content of cyanogenic glycoside in it that emits poisonous hydrogen cyanide (HCN) limits its use. Much research had been done before that examined numerous methods, including fermentation, enzyme treatment, and ensiling, to reduce the levels of HCN and improve the nutritional value of cassava. However, no detailed evaluations of these methods and their overall effect on the safety and nutritional quality of cassava root exist. Moreover, there is a lack of adequate data about the long-term effects of including processed cassava root meal in chicken diets on performance metrics and egg quality characteristics. This study looked at how different processing methods affected the nutritive value and cyanide content of cassava root meal. It also evaluated the effect of incorporating fermented CRM in an improved indigenous layer chicken diet on performance and egg quality.

1.3 Objectives

1.3.1 General objective

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

1.3.2 Specific objectives

i. To evaluate the effect of processing cassava root meal with Natuzyme® enzyme, *Saccharomyces cerevisiae,* and spontaneous fermentation on proximate composition, *in-vitro* dry matter digestibility, and HCN content.

ii. To determine the effect of the inclusion of enzyme-treated cassava root meal in an improved indigenous layer chicken diet on feed intake, feed conversion ratio, and Hen Day egg production.

iii. To determine the effect of the inclusion of enzyme-treated cassava root meal in an improved indigenous layer chicken diet on external and internal egg quality parameters

iv. To conduct an economic analysis of replacing maize with enzyme-treated cassava root meal in improved indigenous layer chicken diets.

1.4 Hypotheses

i. There is no significant difference in the effect of processing cassava root meal with Natuzyme® enzyme, *Saccharomyces cerevisiae,* and spontaneous fermentation on proximate composition, *in-vitro* dry matter digestibility, and HCN content.

ii. There is no significant difference in the effect of the inclusion of enzyme-treated cassava root meal in improved indigenous layer chicken diet on feed intake, FCR, and egg production (HDEP).

iii. There is no significant difference in the effect of the inclusion of enzyme-treated cassava root meal in the improved indigenous layer chicken diet on external and internal egg quality parameters.

iv. There is no significant difference in the economic analysis of replacing maize with enzyme-treated cassava root meal as feed for improved indigenous layer chickens.

1.5 Justification

The high price and unstable supply of maize, essential for human consumption, starch production, and the biofuel sector, significantly influence Kenya's poultry farmers and have resulted in the high cost of production in poultry farming. Kenya imported more than 519,611 metric tons of maize in 2022, a significant increase from the previous year, demonstrating that imports meet Kenya's maize needs (Omondi, 2023). The dependence on imports makes the poultry industry vulnerable to changes in world prices and supply chain disruptions (Omondi, 2023). The prices for poultry feeds are so high that it is hard for poultry farmers to make a profit, especially when feed is a big part of the production costs.

Cassava possesses high carbohydrate content; it is a climate-resilient crop and a locally available feed option for poultry diets (Diarra & Devi, 2015). Cassava yields from 10-40 tons /ha, whereas maize, producing between 5-10 tons/hectare, needs fertile soil and is frequently associated with water, thus not as flexible to climatic changes (Diarra & Devi 2015). These challenges have prompted cassava to be a potential alternative to maize in poultry feed. Although it has a high fiber content and contains anti-nutritional factors, its digestibility and nutritional profile can be improved through processing techniques such as fermentation and enzyme treatment; thus, it can be used as a feed option. This study supports SDG 2 (Zero Hunger) and, in particular, Vision 2030, aiming to ensure sustainable food production systems that increase productivity and production. Through the investigation of locally available resources such as cassava, the research may present a sustainable and cheap solution to the problems of maize dependency, and the food and nutrition security of Kenya will be enhanced.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Poultry Production in Kenya

Poultry production in Kenya is part of the agricultural industry, which, as a result, plays a vital role in food security and economic development. The conventional free-range methods, in rural areas, mostly rear local breeds of indigenous chickens (Padhi, 2016). People like these breeds because they can adapt to the local environment, are not susceptible to diseases, and can find food for themselves (Mujyambere, 2022). The growing demand for chicken products has led to the spread of modern intensive production systems, both semi-intensive and intensive, in urban and peri-urban regions. Kamau *et al.* (2023) proved that these systems are mainly for growing commercial poultry breeds of broilers and layers to meet the rising demand for poultry meat and dairy products. The reasons why the production system and the breed are chosen are several, with market dynamics, availability of resources, and cultural preferences being the main reasons. Small-scale farmers tend to use native breeds of chicken due to their low input levels and extra revenue they provide to the family (Kamau et al., 2023). Magothe *et al.* (2012) observed that commercial operations on a large scale are into commercial breeds due to their rapidness rate of development, high production of eggs, and size and quality uniformity. Commercial breeds, on the other hand, are also more prolific. Although production and breed of preference are different, poultry farming is a significant source of income to many in Kenya, and hence, it serves in poverty reduction and development of the rural regions.

2.2 Overview of the Feed Industry

The feed industry in Kenya plays a significant role in the satisfaction of the nutritional needs of the different poultry production systems. There is an increase in the cost of ingredients that are used to manufacture the feed. This trend can be explained by the increased demand for ingredients, the conflict between the food needs of animals and humans, and the industrial demands, including biofuel production, in the world market (Popp *et al.,* 2016). High dependence on rain-fed farming makes the agricultural industry more susceptible to climate change, which causes disruptions in supply chains when there is inadequate rainfall. The Kenyan feed industry has much competition because the barriers to entry are low, and thus, they are open to ordinary manufacturers and suppliers (Ncube *et al.,* 2016). These manufacturers and suppliers should trust the scientific data provided by the laboratories that identify the chemical composition and quality control of their products in real time to develop the feed products that correspond to the nutritional needs of animals. However, the use of similar raw materials makes it difficult for feed millers to differentiate their brands. According to Ncube *et al*. (2016), the absence of a strong regulatory framework poses a significant risk of counterfeiting and repackaging.

2.3 Nutrient Requirement for Indigenous Layer Chicken

The most important nutrients for layers to have for optimal laying performance are amino acids and energy (Mwai, 2021). Breeders design layer diets to optimize egg production and ensure the birds remain healthy and maintain a healthy weight. Different breeders prescribe a variety of feeding regimens for their birds, which include a number of different diets required during the laying stage. This is similar to the situation with layers. Eggshell development requires an increase in calcium; a balanced diet will offer various nutrients in the appropriate quantities according to the requirements for maintenance and the numerous productive functions that are required (Waheed *et al.,* 2019). It is necessary to provide poultry with the nutrients it needs in the form of diets, using components that are readily available in appropriate quantities and at an affordable price.

2.4 Conventional Sources of Energy in Poultry

In conventional chicken feed, cereal grains like corn, wheat, oats, and barley make up a large portion of the diet. Cereal by-products like wheat bran and protein are sources from plants and animals, such as fish, pork, soybean oil, and groundnut cake. Ghosh *et al.* (2019) stated that either chemically pure sources or items known to be rich in these nutrients supplement the entire ration with sufficient amounts of minerals and vitamins. As the cost of feed skyrockets and conventional ingredients become scarce, continuous and thorough attempts are underway to assess the nutritional value of agro-industrial by-products as potential substitutes for more expensive ingredients in poultry rations.

Maize is one of the most commonly used feedstuffs in poultry rations. The low fiber content and easy digestion characterize maize. It is a good source of energy but has very little protein, particularly the amino acid lysine and other sulphur-containing proteins. Xanthophyll and vitamin A are abundant in the yellow types (Sandeski *et al*., 2014). The high fiber level of barley makes it an unappealing grain that should not make up more than 15% of the ration (Izydorczyk & Edney, 2017). Its application would possibly suppress cannibalism, feather picking, as well as hock issues. Due to the content of fiber, manganese, and phosphorus, the wheat bran is not only bulky but also very laxative, which means that wheat can also be used as an alternative source of energy instead of maize (Tufail *et al*., 2022). Groundnut is the main protein feedstuff in chicken feeds, and pearl millet is a useful feedstuff due to its high protein and ash density (compared to wheat, pearl millet has the same nutritional value) (Oso & Ashafa, 2021). The protein content is approximately 40 percent of its weight. The fishmeal composition differs greatly when it comes to the source of fishmeal (either whole bony fish or fish cannery trash), although it is typically regarded as one of the best animal protein feedstuffs in poultry.

2.5 Non-Conventional Sources of Energy in Poultry

Over the past years, there has been a move towards the utilization of conventional and non-conventional sources of energy as a way of enhancing the efficiency of chicken feeds through diversification. Rice bran and copra meal are agro-industrial wastes that are commonly considered waste, whereas they can be used as a more affordable source of energy for the chicken industry. According to many experts, the supplement of nutrients in these products to the feed base may eventually lead to doubling of the feed input, which makes the use of traditional grains insignificant. They also oversee the recycling of crop by-products as well as environmental sustainability in poultry farming, in addition to these activities (Khempaka *et al*., 2016).

The application of cassava root meal instead of maize is creating issues in poultry nutrition, even though the traditional energy source is eliminated. The use of cassava has been quite acceptable in the human diet in recent times, as well as in the chicken diet, due to its richness in energy (Ogbuewu & Mbajiorgu, 2023a). Cassava root meal (CRM) is a highly valuable carbohydrate source that is rich in starch, which would be able to supply enough carbohydrate to the chicks when consumed. This is attributed to the fact that maize and other grains are substituted with this feed resource will restrict the quantity of grains that will be fed to animals, hence regulating the cost of feed and guaranteeing a harvest.

Similarly, it has been shown that the CRM may be used to substitute the conventional feedstuffs in the feed of chicken, and the feedstuff does not hurt the performance parameters such as body weight gain, feed ratio, and egg laying performance (Ngiki *et al*., 2014; Ogbuewu & Mbajiorgu, 2023b). However, the cassava needs to be processed and supplemented to remove the anti-nutritional factors present in the raw cassava roots (Ngiki *et al*., 2014). The right processing techniques, like drying, grinding, heat treatment, and enzyme supplementation or fermentation, can help in enhancing the safety and nutritional value.

Use of CRM in poultry feed formulation can have several benefits, like economic, high nutritious content of CRM, and can help promote the globalization of feed manufacturing. For instance, it doesn't only solve the problems related to the cassava trash disposal, but it also provides new market outlets for cassava farmers and low production cost for cassava processors. However, research needs to be conducted to improve the production characteristics and traditional management, and to change the local feeds used in poultry feedlot with CRM-based diet (Ngiki *et al*., 2014). Moreover, the potential economic feasibility of using CRM in poultry production systems and the potential environmental impact were also important aspects that industry players were considering to integrate into their value chain as sustainable feeding practices.

2.6 Cassava Production in Kenya

In Kenya, cassava is an important crop that is grown mainly in the coastal, eastern, and western regions of the country. Cassava is a crop that is well-suited to poor soils and areas prone to drought, and it plays an important role in providing food security and income for smallholder farmers in Kenya (Kidasi *et al.,* 2021). The demand for cassava and cassava products, including flour, starch, and animal food, in recent years has been rising at a rapid pace, and it is further escalating the role of the crop in the rural economy. Nevertheless, cassava cultivation in Kenya is characterized by multiple challenges, such as a low productivity index, diseases such as Cassava Brown Streak Disease (CBSD) and Cassava Mosaic Disease (CMD), and a lack of storage and processing facilities (Munguti *et al*., 2023). Kenya in 2021 produced about 711,261 metric tonnes of cassava on the land area of 61,592 hectares with an average yield of 11.5 tonnes per hectare. This compares to lower than the productivity rates of other nations within the region, like Uganda and Tanzania, which yield 4 million and 8 million metric tonnes of cassava per year, respectively (FAO, 2021). Poor productivity of cassava in Kenya has been blamed on a number of factors, including low availability of good planting materials, absence of extension services, and effects of pests and diseases. Research and development of better and disease-resistant cassava varieties and the promotion of good agricultural practices are efforts aimed at boosting the quantity of cassava produced in Kenya (Nabahungu *et al*., 2025).

The Kenyan government and other players have been aiding the production of cassava in Kenya by providing research and development of disease-resistant varieties, and other programs like the Cassava Development Strategy of Kenya, which is meant to enhance the production in terms of yield, market access, and value addition (Mulu-Mutuku *et al*., 2013). Also, cassava is being considered as an alternative feed source in the livestock and poultry industries in Kenya, which may further stimulate the production of cassava in the country (Wachira *et al*., 2023). There is a heightened interest in cassava as a food.

2.7 Nutritional Quality Cassava Root Meal (CRM)

Cassava root meal (CRM) is becoming a common non-conventional feed resource for local layer chicken farmers in Kenya. The main crop, which is the cassava root, is the primary produce of the tropical nations, which have contributed significantly to the provision of the base ingredient used in CRM, and can serve as a substitute for the relatively expensive energy boosters that constitute chicken feed. Nevertheless, the nutritional content and performance of this meal-feeding chicken could be affected by a number of variables. Consequently, one has to examine these factors thoroughly to make sure that the application of this ingredient in a layer production system is streamlined. The proximate composition comparison research findings on cassava meal have indicated a high level of heterogeneity across varieties, methods of processing as well and storage conditions. CRM has a protein content that is less than 1.5% and 3.5% on Dry Matter Basis (DMB) (Ogbuewu *et al*., 2023a). The primary nutritive factor is carbohydrates. CRM contains low-fat content, as indicated by Mwangi (2023), the fiber content is 1-3 percent. CRM can, but it can also contain anti-nutritional compounds like cyanogenic glycosides that could negatively affect the health of the chicken and hinder production when ingested in inappropriate proportions when the meal is not processed correctly and supplemented (Morgan & Choct, 2016). Table 2.1 compares the nutritional quality of Cassava root meal (CRM) and maize meal.

Table 2.1: Comparison of nutritional quality of cassava root meal (CRM) and maize meal

Components	CRM	Maize meal
Crude protein (%)	3.1	9
Crude fiber (%)	3.7	2
Ether Extract (%)	0.9	4.6
Ash (%)	3.9	5
Metabolizable Energy (Kcal/kgDM)	3145	3524

Source: Mwangi (2023)

2.8 Effect of CRM on Performance in Indigenous Layer Chicken

There has been research on the use of cassava root meal (CRM) as a dietary ingredient for native layer chickens. Its objective was to compare performance indices like feed consumption, body weight growth, and productivity. Because of its large carbohydrate volume and possible cost reduction, there has been interest in the viability of the replacement of traditional sources of energy, such as maize, with CRM in the chicken diet. The study revealed that CRM was capable of substituting maize in the diet of native layer chicken up to 50 percent in the diet without adversely affecting their performance (Mwangi, 2023). The experiment also found that birds fed diets containing CRM as feed also gained body weight and were fed at a rate that was not significantly different from that of the birds fed on maize-based diets. In addition, the research has had a substantial impact in lowering the price of feed, which can be an economic advantage to the owners of chickens.

Ogbuewu and Mbajiorgu (2023b) compared the impacts of varied CRM inclusion in the food of indigenous layers. They found that there was no significant difference in FCR or mortality between control diets and 30 percent CRM inclusion. The poorer performance at higher levels (above 50) was attributed by the research to be linked with the existence of anti-nutritional compounds such as cyanogenic glycosides, which might inhibit the absorption of nutrients and health in general. CRM in the indigenous layer chicken diet can improve performance as a result of maximization of the degree of inclusion and applying appropriate production methods to remove those that are anti-nutritional. Cassava root meal may serve as a possible alternative feedstock in a sustainable production system of poultry due to the financial incentives and the possibility of lessening the consumption of conventional sources of energy.

2.8.1 Effect of CRM on egg quality

Most of the experiments conducted to establish the effect of CRM on marketability and consumer preference were based on the quality measures of eggs. Parameters such as egg weight, shell thickness, yolk colour, and composition are important. Aderemi *et al*. (2012) measured the impact of CRM on the quality of eggs of indigenous layer chicken. The outcome was that CRM, to a maximum of 30 per cent of the diet, did not adversely impact the egg weight and shell thickness.

Nevertheless, the progressive decrease in the amount of some of the essential nutrients needed by eggs may be the cause of the minor decline in these characteristics with a rise in the degree of inclusion (Ogbuewu & Mbajiorgu, 2023b). The importance of carotenoids in the diet of a person cannot be overrated in this delicate characteristic. The experiment found that the birds placed on the CRM-based diet were found to possessed lighter yolks than the birds placed on the maize-based diet due to the low carotene concentration in the CRM. CRM usage can influence the preference for high CRM usage; hence, it is obliged to contain dietary supplements containing carotenoids. According to Feng *et al*. (2017), the application of enzyme supplementation can be used to improve the quality of eggs. The study established that the enzyme-treated CRM-fed birds had a higher albumen height as well as shell quality than untreated CRM-fed birds. The nutrient uptake and the decreased level of anti-nutritional elements in the system may be one of the causes of the observed improvements, and, in that manner, the quality of the egg may be enhanced.

2.9 Anti-Nutritional Factors Present in CRM

CRM is an ingredient that has anti-nutritive components such as cyanogenic glycosides, and when this is not handled correctly, it might be harmful to the health of poultry. Cyanogenic glycosides could directly yield cyanide following enzymatic hydrolysis (Cressey & Reeve, 2019). The toxic effects and alteration of bird performance were reported by Murugesan *et al*. (2015). Therefore, the focus should be on efficient processing and treatments that address the mentioned factors, enhance nutritional status, and ensure the safety of CRM-based diets. Several ways could be used to minimize the anti-nutritive factors in CRM, which include enzyme treatment, yeast supplementation, and fermentation.

2.9.1 Strategies to minimize anti-nutritive factors in poultry feed

Yadav and Jha (2019) found that certain feed ingredients and additives influence the gut microbiota and immune system of the host. Farmers employ antibiotics to alter the gut microbiota and highly regard them for their ability to enhance chicken growth performance. Nevertheless, apprehension regarding antibiotic resistance and other adverse consequences of using antibiotics as growth enhancers has compelled poultry farmers to discontinue or restrict their utilization of animal feed. Farmers employ feed additives and supplements, such as probiotics, prebiotics, organic acids, and exogenous enzymes, as substitutes for antibiotics to regulate the gut flora, resulting in positive outcomes. The CRM has been improved mainly by processes like heating, grinding, cooking, and physical and chemical processes (Vongsak *et al.,* 2013). Although some parts of the ANF can be removed with the help of these methods, the nutrient level can also be damaged. Therefore, a more suitable process is needed in order to improve the quality of the CRM feed.

2.9.2 Use of enzyme

The yeast of interest in recent years has been *Saccharomyces cerevisiae*. It is reported that the supplementation of feed with live yeast cells has enhanced feed efficiency, feed digestibility, and animal performance, decreased the incidence of pathogenic bacteria, improved animal health, and minimized negative environmental impact because of livestock production (Broadway et al., 2015). The application of yeast in cattle production led to the minimization of the adverse effect on the environment (Ogbuewu & Mbajiorgu, 2023b). It is also stated in studies that live yeast usage in a diet might assist in augmenting the digestion of fiber, lower the proliferation of harmful microorganisms, synthesize anti-bacterial chemicals, promote the immune system, and enhance the intestinal morphology. The use of S. *cerevisiae* in the diets of non-ruminants may lead to the increased intake of feed, the enhanced digestibility of the fiber, the decreased number of pathogenic bacteria, and improved health and performance of animals. The S. cerevisiae is thought to enhance the growth rate by enhancing the villi of the gut (jejunum) (Elghandour *et al.,* 2020).

2.9.3 Use of yeast

Over the recent years, yeast Saccharomyces cerevisiae has received significant attention. Research has demonstrated that adding live cells of yeast to feed may improve feed efficiency, feed digestibility, increase animal performance, reduce the occurrence of undesirable bacteria, improve animal health, and decrease the negative environmental impact of livestock production (Broadway *et al*., 2015). Moreover, the fact that the production of cattle involved the use of yeast contributed to the reduction of the negative environmental impact (Ogbuewu & Mbajiorgu, 2023b). It has been shown that by adding live yeast to a diet, it is possible to increase the digestion of dietary fiber, slow the growth of harmful microorganisms, produce compounds beneficial in combating bacteria, stimulate the immune system, and improve the physical makeup of the intestines. Adding S. *cerevisiae* to non-ruminant diets can induce feed consumption, augment fiber digestibility, reduce pathogenic bacteria, and enhance animal health as well as performance. Elghandour *et al*. (2020) state that S. cerevisiae increases its growth rate by inoculating the gut villi in the jejunum.

2.9.4 Fermentation treatment

A method of manufacturing a fermented diet is by soaking the compound feed with water. The fermented diets have a pH of 3.5-4.5 and contain high levels of lactic acid and, to a lesser extent, acetic acid and alcohol (Tefera *et al*., 2014). Fermented diets improve the growth performance of pigs compared to non-fermented diets. Conversion of carbohydrates into alcohol, carbon dioxide, and organic acids, known as fermentation, has been extensively studied in swine nutrition. Fermented-wet feeding can be a very useful technique for pigs because it uses different foods and fermentation. The presence of lactic acid bacteria in the stomach and small intestines, which create lactic acid, can lead to a decrease in gastric pH and a reduction in the number of coliform bacteria in the gastrointestinal tract (Ljungh & Wadstrom, 2006). Lactic acid bacteria and yeast, commonly found in fermented feed, can break down myo-inositol hexakisphosphate (IP6). This microbial degradation helps with nutritional issues in monogastric animals and prevents phosphate pollution in the environment, which means that cereal-based diets have more phosphorus available to them. The characteristics of wet feed are changed by fermentation in a way that depends on the activity and make-up of the microbes that are present. This can be altered by the addition of yeast cultures like *Saccharomyces cerevisiae* or other inoculants. Fermentation of feeds for indigenous layer chickens might be a better approach to increase nutrient utilization and productivity than wet feeding, according to previous studies.

2.9.5 Challenges and opportunities of CRM as a chicken feed ingredient

CRM may be a good source of indigenous feed, though its application still has several limitations and challenges, especially with its anti-nutritional properties. CRM naturally contains anti-nutritional factors, mainly cyanogenic glycosides, which can pose health risks to poultry if not properly managed. Cyanogenic glycosides can liberate cyanide through hydrolysis. This might lead to cyanide poisoning and could harm the performance of the birds (Ogbuewu & Mbajiorgu, 2023a). Prior studies have focused on mitigating the occurrence of nutritional impairing factors by utilising different processing and treatment approaches, such as adding enzymes, yeast fermentation, and heat processing. However, there is still a need for extensive studies to understand the effectiveness of these processing methods in reducing cyanide content and ensuring the safety of chicken feeds that incorporate CRM. Also, research should be conducted on alternative detoxification and processing, economically efficient and appropriate to small-scale poultry production systems that are dominated in Kenya.

Moreover, little is known about the chronic impacts of subclinical levels of cyanide in the diet of poultry. The effects of acute cyanide poisoning are well known, but little is known about the effects of long-term exposure to low levels of cyanide. It requires long-term research to establish the impact of subclinical exposure to cyanide in chickens on their health, performance, and quality. The chronic consequences of cyanide poisoning are significant to understand to set safe recommendations of feed and to ensure the production of safe feed healthy, and quality poultry products for the consumer's health and safety.

More studies should be conducted to understand the impact of low cyanide levels on the growth of indigenous layer chicken, their reproductive potential, and their immune system. It is necessary to conduct long-term feeding studies in controlled conditions to calculate the outcomes of cyanide intoxication and the possible health hazards of using CRM in poultry feed. Studies have to be conducted to analyze the connection between CRM and other dietary elements that is mainly in multi-ingredient diets that are typical in commercial poultry production. In order to develop balanced and nutritiously adequate diets, the joint or antagonistic effects of CRM with other feed constituents on the use of nutrients, intestinal health, and overall performance of indigenous layer chickens are most important. The solution of these gaps in research can help players to realize the full potential of CRM as a valuable feed element.

CHAPTER THREE
EFFECT OF FERMENTATION AND ENZYME TREATMENT ON NUTRITIONAL QUALITY, DIGESTIBILITY, AND HYDROGEN CYANIDE REDUCTION IN CASSAVA (*Manihot esculenta* Crantz) ROOT MEAL

Abstract

In the tropical areas, Cassava (*Manihot esculenta* Crantz) root meal is a commonly used poultry food rich in carbohydrate, namely starch. Nevertheless, it has low protein content, high fiber content, and hydrogen cyanide (HCN), which limits its application in animal feed. This study aimed to examine how fermentation and enzyme treatment (Natuzyme®) have impacted the chemical composition of cassava root meal, its digestibility, and HCN level. They were treated in four different treatments T1: untreated cassava root meal, T2: enzyme-treated, T3: Saccharomyces cerevisiae fermented and T4: Self-fermented cassava root meal. Proximate analyses of the nutrient compositions in each treatment were conducted in the laboratory using standard methods of AOAC (2005). Results showed that there was a significant difference ($p<0.005$) in all the nutrient composition (CP, DM, %DMD, EE, NDF, ADL, ADF, and HCN) among different treatments. However, both enzyme treatment and fermentation with *S. cerevisiae* significantly improved the crude protein content (7.80 and 8.2, respectively), *in-vitro* dry matter digestibility (82.30% and 75.20% respectively), and significantly reduced the fiber and HCN content to indeterminable levels in cassava root meal. These results indicated that the combination of enzyme treatment and fermentation with *S. cerevisiae* improved the nutritional quality of cassava root meal. The meal has potential as an alternative to maize for poultry feed production, providing a locally, more sustainable and cost-effective option. This will reduce competition for maize as food for human consumption and animal feed production.

3.1 Introduction

Cassava (*Manihot esculenta*) is a root crop native to tropical and subtropical regions and serves as a staple food for millions of people in sub-Saharan Africa, Southeast Asia, and Latin America. It is a significant source of carbohydrates, although it is not usually a nutritionally complete diet since its protein is poor, and it lacks the necessary levels of essential vitamins and minerals (Jaramillo *et al*., 2022). Nevertheless, cassava has one significant benefit, which is its ability to grow in harsh soils as well as endure droughts, and thus cassava is an essential food crop in most low-income countries (Da Costa *et al*., 2024). Nevertheless, cassava has potentially harmful cyanogenic glucosides, on the one hand, most notably, linamarin, which can emit lethal hydrogen cyanide (HCN) when consumed (Zidenga *et al*., 2017).

When cassava is added with higher HCN levels, severe poisoning, neurotoxicity, and even death may take place, particularly when the food is improperly cooked. The nutritional value and the risks to health mentioned above could be mitigated in a number of ways, including fermentation and enzymatic treatment. One of the oldest and most effective ways of adding nutritive value and safety to cassava is fermentation by microorganisms. Fermentative microorganisms and the subsequent breakdown of complex molecules of cassava into simpler, bioavailable molecules can make the food more digestible, higher in protein content, and lower in antinutritional and toxic compounds, including cyanogenic glucosides (Halake & Chinthapalli, 2019). S. cerevisiae, which is also known as baker's yeast, is a well-studied microorganism used to ferment cassava. Organic acids and alcohols are some of the metabolites that are produced by Saccharomyces cerevisiae because it breaks down starches and may enhance the texture and nutritional qualities of cassava (Pimpisai *et al*., 2024). Ideal growth conditions of S. cerevisiae include pH 4, dissolved oxygen level of 5 per cent, and temperature 30 °C to less than a temperature of 45^0C, a pH of 2.5 to 8 (Minden *et al*., 2022). The content of hydrogen cyanide and crude fiber was decreased through the enzymatic hydrolysis of cassava root meal by the S. cerevisiae using its detoxifying and enzymatic hydrolysis capabilities (Khurshida *et al*., 2025).

Besides the application of the fermentation of S. cerevisiae, spontaneous fermentation is based on the natural growth of the local microorganisms and can enhance the quality of cassava. Fermentation of cassava root meal can be done spontaneously to augment the crude protein content of the fermented feed by transforming the nitrogen feeds available in the feed source into protein and enhancing the nutritional value of the fermented feed (Egbune *et al*., 2023). Spontaneously fermented microbial diversity also could play a role in enhanced cyanogenic glucoside detoxification and as well as nutrient accessibility.

Conversely, Natuzyme is a multi-enzyme mix, which contains xylanase, phytase, betaglucanase, amylase, cellulase, and protease that was also studied to determine how to break down cassava complex starches, cellulose, and proteins (LN *et al*., 2018). The enzymes can enhance the digestibility of cassava by digesting its non-digestible parts and releasing the nutrients present in the cassava to be more bio-accessible (Oloruntola, 2020). Moreover, it has been demonstrated that the application of enzyme treatments would contribute to the detoxification of cyanogenic glucosides, decreasing the level of HCN, and the supply of a safe cassava to be consumed (Moses *et al*., 2024). The nutritional value of cassava is mainly dependent on the proximate composition of cassava, which consists of moisture, protein, fat, fiber, and carbohydrate of cassava. These components can be altered with enzyme treatments and fermentation; both of these processing methods involve enzymatic and microbiological reactions to change the chemical composition and structure of cassava that could result in higher protein concentrations, fiber reduction, and increased digestibility of the plant (Ona *et al*., 2019). Consequently, the enhanced proximate structure of cassava makes it a better-balanced food supply that may supply vital nutrients. In vitro dry matter digestibility (DMD) is also a critical parameter determining the potential nutritional value of cassava, and it determines the extent to which the nutrients can be digested and utilized by the human or animal body. Fermentation and enzyme treatments can increase DMD, which makes cassava a more promising and preferable source of food (Dagaew *et al.*, 2021). Nonetheless, cyanogenic toxicity, as the most important problem in cassava consumption, may also occur due to the consumption of raw or insufficiently cooked cassava food that may contain high concentrations of HCN, causing cyanogenic toxicity and health risks, especially in populations that consume cassava as a staple food (Nuwamanya *et al*., 2022).

HCN has been shown to be reduced through fermentation by Saccharomyces cerevisiae and enzyme treatment by Natuzyme (McMahon *et al*., 2021). Specifically, the fermentation of S. cerevisiae purifies Cassava and cyanogenic glucoside is degraded into a safe compound (Wang et al., 2024). On the same note, Natuzyme helps break down cyanogenic compounds, hence improving the nutritional value of cassava and safety (Bakare *et al*., 2021). This study set out to examine the effect of two fermentation processes, S. cerevisiae fermentation and spontaneous fermentation, and enzyme treatment with Natuzyme on the proximate composition, in-vitro dry matter digestibility (DMD), and the HCN level of cassava root Meal.

This study's primary objective was to evaluate the effect of different processing techniques on the safety, digestibility, and nutritional quality of cassava. The results of this study will be of significant value in creating new cassava processing methods that can improve the crop's potential as a safe and nutritious feed ingredient in chicken diets.

3.2 Materials and Methodology

3.2.1 Study site

The experiment was conducted at Egerton University, Animal Nutrition Laboratory, Department of Animal Sciences, located in the Njoro Sub-County, Nakuru County, 0° 23' S and 35° 55' N. It is 1800 meters above sea level with an average annual rainfall of 900-1,200 mm and temperatures ranging from 17°C to 22°C (Egerton University Department of Agriculture Engineering Metrological Station, 2024 unpublished).

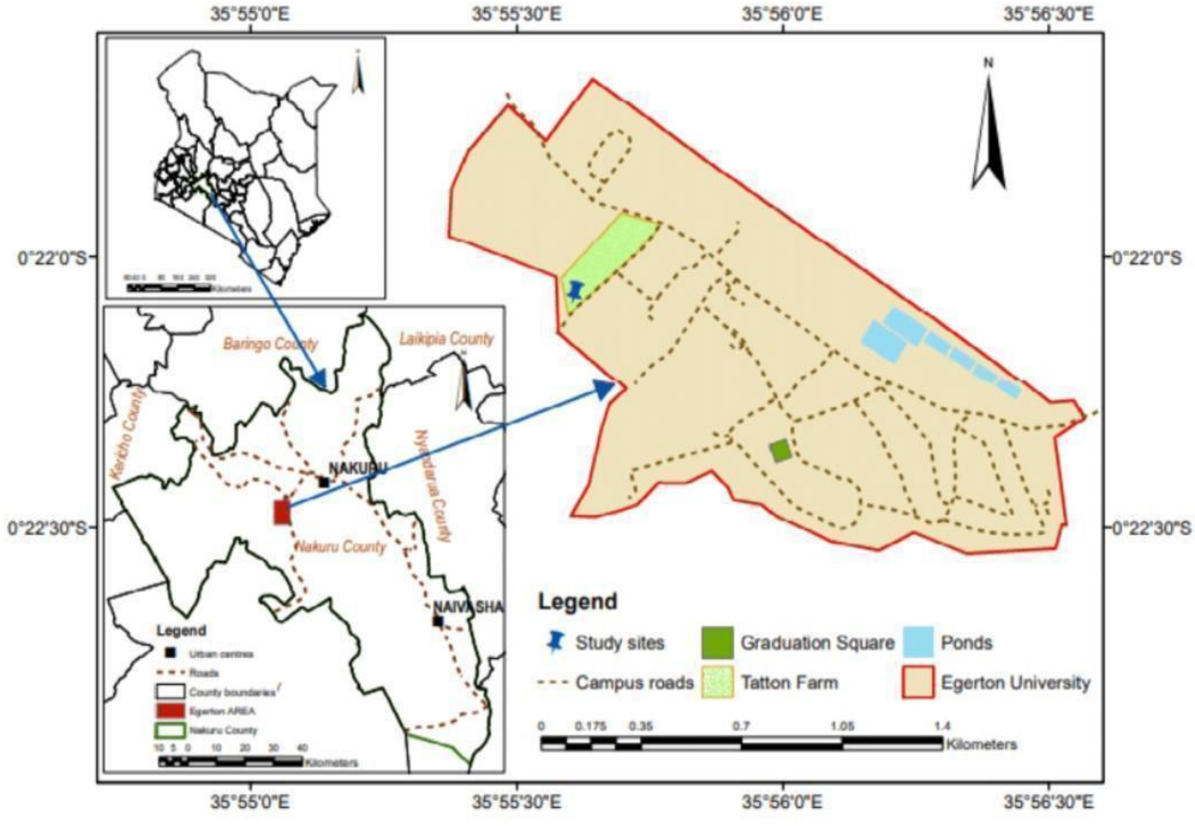

Figure 1: Map showing the study site

3.2.2 Collection and preparation of cassava root meal

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

3.2.3 Preparation of experimental treatments

There were four treatments, each of which was replicated three times. The treatments included:

T1: Untreated Cassava root meal (Control)

T2: Enzyme (Natuzyme®) treated cassava root meal,

T3: Yeast (*Saccharomyces cerevisiae)* fermented cassava root meal and

T4: Spontaneously/ Naturally fermented cassava root meal

3.2.4 Preparation of enzyme-treated cassava root meal

An enyme (Natuzyme®) was procured from Coopers® Kenya Brand Limited. It contained 12,000 parts per gram of xylanase, 6,000 parts of cellulase, 1,500 parts of phytase, 700 parts of beta-glucanase, 700 parts of protease, and 400 parts of alpha-amylase. The dried cassava root meal was appropriately mixed with the enzyme according to the manufacturer's directions and instructions (at a rate of 350 mg/kg) (Muremera *et al.,* 2022). Samples were collected and analyzed for proximate composition, *in vitro* dry matter digestibility, and HCN content.

3.2.5 The yeast powder was bought from Agro-chemical and Food Company Ltd, Kenya

Dry cassava root meal was blended with an equal water ratio of (1:1), 5% of the yeast powder was added, inoculated in triplicate at 30 °C, under anaerobic conditions, and left to ferment for 4 days. Samples were collected and analysed for proximate composition, *in vitro* dry matter digestibility, and HCN content.

3.2.6 Spontaneous fermentation of cassava root meal

A 1:1 (wt/vol) mixture of cassava root meal and distilled water was incubated in triplicate at 30°C for four days in a 250 ml airtight sealed plastic bottle (Muremera *et al*., 2022). After four days, the pH of the sample was measured using a portable pH meter. Additionally, the sample was analyzed for proximate composition, *in vitro* dry matter digestibility, and HCN content.

3.2.7 Determination of nutrient composition

Proximate analysis was conducted using standard methods of AOAC (2005). The dry matter (DM), (AOAC, 2005: 934.01), organic matter (OM) (AOAC, 2005: 942.05), and crude protein (CP) (AOAC, 2005: 984.13). To determine the dry matter, samples were dried in a hot air oven at 105 °C for 24 hr, method 930.15. The ash content was determined by burning samples in a muffle furnace at 550°C for 8 hours, method 942.05, and ether extract by the Soxhlet method (using ether), method 920.39. Total nitrogen for crude protein (N x 6.25) by the Kjeldahl procedure method 984.13, Neutral detergent fibre (NDF), acid detergent lignin (ADL), and acid detergent fibre (ADF) were determined using the Van Soest method (Van Soest *et al.,* 1991).

3.2.8 Determination of *in vitro* digestibility

The experiment was conducted to mimic the digestion process in the chicken digestive system as described by (Zhao *et al.,* 2014). The treatments were T1: Untreated Cassava root meal, T2: Enzyme-treated Cassava root meal, T3: Yeast-fermented Cassava root meal, and T4: naturally treated Cassava root meal. A ground Cassava sample (0.4g) was weighed and put in a 100 ml digestibility test tube before simulated gastric fluid was added. To mimic the *in vivo* activity of pepsin in chicken stomach fluid, a fluid containing 1,550 U/mL of pepsin (Sigma 10070; Sigma-Aldrich Co., St. Louis, MO) was used. The gastric buffer solution contained 16.9 mmol/L of NaCl, 9.6 mmol/L of KCl, and 10 mmol/L of HCl to match the *in vivo* ionic concentration of gastric fluid from roosters. The pH was raised to 2.0 at 41°C by adding 200 mmol/L of HCl. Each digestibility test tube was put in 2ml Chloramphenicol C-0378; Sigma-Aldrich Corp., St. Louis, MO, USA (0.5 g/100 mL 19 ethanol) to prevent bacterial growth. The test tubes were then sealed and incubated in a water bath at 39°C with continuous stirring for 2 hours. The first step's mixture was combined with 20 ml of 0.6MNaOH and 80 ml of phosphate buffer (0.2M, pH 6.8). The pH was brought down to 6.8 using 1M HCl or 1M NaOH to establish a stable environment for intestinal enzymes to perform well. To the mixture, 10.6 ml of artificial pancreatin P-1750, Sigma-Aldrich Corp, St. Louis, MO, USA, porcine grade enzyme with 3 x USP activities) containing 100 mg/1 litre buffer was added and incubated at 39°C with constant stirring for 4 hours. The remains were put in 1.5ml centrifuge tubes and centrifuged (12700×g) for 2 minutes. The mixture was carefully withdrawn, washed twice with 20 ml of 95% ethanol and 99.5% acetone, and then rinsed with distilled water. Those that remained were then dried for 12 hours at 70°C in the oven before weighing.

Calculation of *in vitro* dry matter digestibility

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

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

3.3 Statistical Analysis

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

$Y_{ij} = \mu + ti + \varepsilon_{ij}$

where;

Y_{ij} = observation of the response variable

μ = overall mean

ti = effect of the i'^{th} treatment (fermentation and enzyme treatment)

ε_{ij} = random error

3.4 Results

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

Table 3.1 presents the effects of enzyme treatment, *Saccharomyces cerevisiae* fermentation, natural fermentation, and untreated cassava root meal on nutrient composition and HCN levels.

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

Nutrient (%)	Enzyme treated	Naturally fermented	Untreated	*S. cerevisiae* fermented	*P value*
DM	95.46^{a}±0.12	94.74^{b}±0.12	94.11^{c}±0.12	94.59^{bc}±0.12	0.0003
CP	7.80^{a}±0.16	4.09^{b}± 0.16	2.30^{c}±0.16	8.20^{a}±0.16	<.0001
EE	1.84^{a}±0.06	1.53^{bc}±0.06	1.36^{c}±0.06	1.82^{ab}±0.06	<.0001
NDF	13.66^{c}±0.19	15.26^{b}±0.19	19.40^{a}±0.19	13.52^{c}±0.19	<.0001
ADF	6.45^{b}±0.21	9.24^{a}±0.21	8.41^{a}±0.21	7.09^{b}± 0.21	<.0001
ADL	1.56^{c}±0.07	2.52^{b}±0.07	3.33^{a}±0.07	1.82^{c}±0.07	<.0001
HCN (ppm)	Indeterminable	10^{b}	100^{a}	Indeterminable	<.0001
%DMD	82.30^{a}	64.67^{b}	44.67^{c}	75.20^{a}	<.0001

^{a,b,c} Means within a row, with different superscripts, are significantly different ($p<0.05$)

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

3.4.2 Dry matter digestibility

Enzyme treatment had the highest dry matter and ether extract, the lowest ADF, NDF, and ADL, and noticeably lower levels of HCN contents. Saccharomyces cerevisiae fermentation significantly increased crude protein and also decreased HCN levels. Untreated samples had the highest levels of indigestible fiber and HCN, indicating lower safety and nutrient quality. Naturally fermented samples exhibited intermediate results in all the parameters. Enzyme-treated CRM had the highest dry matter digestibility (DMD) of 82.30%, followed by S. cerevisiae fermented CRM with 75.20%, which were significantly different from the untreated and natural fermentation with 44.67% and 64.67%, respectively. The dry matter digestibility of cassava root meal is represented in Figure 2.

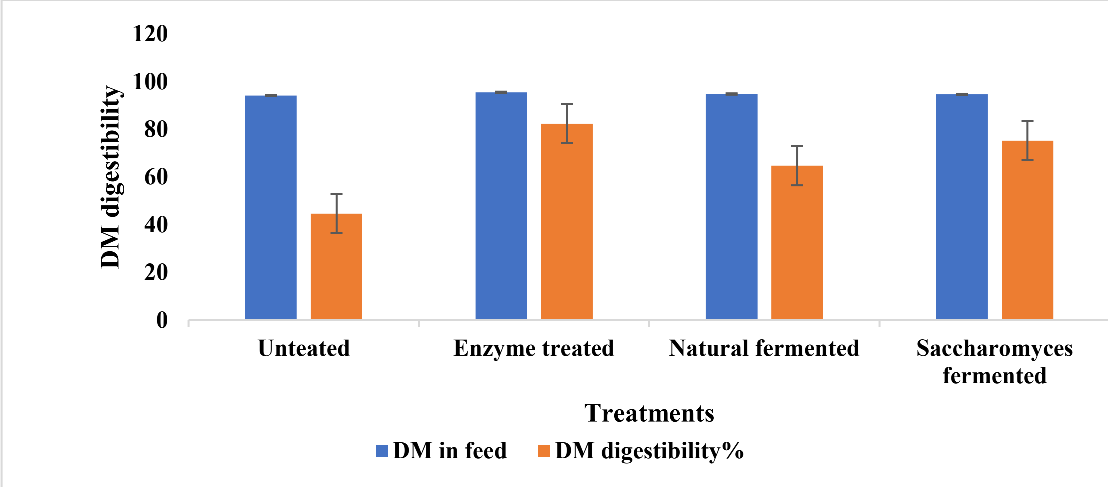

Figure 2: Dry matter digestibility of cassava root meal

3.5 Discussion

3.5.1 Chemical composition of treated and untreated cassava root meal

There was a significant difference in the crude protein among treatments. This high increment of crude protein in both fermented and enzyme-treated CRM may be due to their metabolic activities, which produce extracellular enzymes and absorb nitrogenous substances during fermentation, both of which contribute to protein enrichment (Khurshida *et al*., 2025). This coincided with the views expressed by Khejornsart *et al*. (2024), who said that both S. cerevisiae and enzyme treatment use the carbohydrates in cassava pulp as energy sources, and the results of the cassava root are an increase in the protein content in the microbial biomass. The protein content of cassava flour increased to 5.54 percent after fermentation of the cassava flour with S. cerevisiae at 35 o C down the cell wall to 96 hours (Khurshida *et al*., 2025). It was a consequence of the metabolic activities of S. cerevisiae, which involve the secretion of extracellular enzymes and the assimilation of nitrogenous compounds during fermentation processes, which has been known to enhance protein enrichment.

The NDF and ADF content of the enzyme-treated cassava root meal was very low as compared to the other treatments, possibly due to the presence of cellulases, hemicellulases, and pectinases in Natuzyme enzyme that were likely to hydrolyze complex polysaccharides and fibrous materials, which form the hydrolysis of the structural carbohydrates in the cell wall, including cellulose, hemicellulose, and lignin. The fiber content of the cassava root meal dropped due to this process (De Souza & Kawaguti, 2021). Huang *et al*. (2018) also mentioned that the enzymatic treatment of cassava residue has augmented its water-conferring capacity and general quality by decreasing its crystallinity and enhancing the soluble dietary fiber part. This conformed to the findings of Bunterngsook *et al*. (2017), who indicated that ADF and

NDF of enzymatic-treated cassava root meal and other starchy plants were reduced significantly as a result of hydrolysis of cellulase and hemicellulase on the structural carbohydrates. The decrease in the fiber was attributed to the enzymatic hydrolysis of the cell wall polysaccharides. Another study done by Aruwajoye *et al.* (2019) regarding enzyme-assisted processing of cassava gave the same result that is, the fiber content of the cassava root meal decreased due to the enzyme treatments, which broke down the structural polysaccharides of the root. Cellulose and hemicellulose content degradation was the main factor in the decrease in the NDF and ADF.

It was not possible to determine the HCN content of fermented and enzyme-treated cassava root meal of S. *cerevisiae*. This may have been because in the course of fermentation, microorganisms like S. cerevisiae species release enzymes like linamarase and beta-glucosidase, known to break down cyanogenic glycosides and hence decrease the level of HCN (Zidenga *et al*., 2017). This is also consistent with the results of Obi *et al*. (2019), who cited that fermentation lowered the pH, which in turn aided in breaking down the cyanogenic compounds and, as a result, reduced the production of cyanides. The acidic pH favoured the hydrolysis of linamarin and other cyanogenic glycosides. The decrease in HCN content of CRM treated with enzymes was attributed to the presence of exogenous enzymes that are known to reduce cyanogenic glycosides that increase the amount of HCN content (Easson *et al*., 2021).

3.5.2 Dry matter digestibility of cassava root meal

Treatments differed significantly (P<0.05) in DMD, and the DMD of the enzyme-treated and S. cerevisiae-fermented CRM significantly increased. It aligns with what Emmanuel *et al*. (2024) found, who reasoned that fermentation and enzymatic treatments decreased the concentrations of adverse anti-nutritional factors and tripled the feeding value and digestibility of cassava peels as an animal feed supplement. In their study, Huang *et al*. (2018) found that fermentation and enzymatic treatment of cassava residue decreased the crystallinity of cassava residue and enhanced the ratio of soluble dietary fiber, which enhanced the water-binding ability and their digestibility.

Finally, the findings of this research indicated that both Natuzyme enzyme treatments were positive.

Moreover, fermentation technologies enhanced the nutritional value of cassava root meal and can be applied as Effect of the processing of cassava root meal in chicken diets.

3.6 Conclusion and Recommendations

The findings indicated that the treatment of cassava root meal with enzymes enhanced protein level (7.80), digestibility (82.30), and a low amount of hydrogen cyanide, which was not visible. S. cerevisiae cassava root meal fermented well (digestibility was 75.2%), contained high amounts of crude protein 8.2, crude fiber (2.49), and contained no detectable amounts of hydrogen cyanide.

Fermentation with autochthonous fermentation had a crude protein of 4.09, digestibility of 64.67, crude fiber of 3.27, and hydrogen cyanide (HCN) was 10 ppm. From the results, enzyme treatment and fermentation of CRM with *S. cerevisiae* were the best processing methods, with no significant difference between the two processing methods. However, Natuzyme® enzyme was a more cost-effective and readily available option compared to *S. cerevisiae*. They are inexpensive and easy to source, making them a practical choice for use in processing cassava root meal.

CHAPTER FOUR

EFFECTS OF ENZYME-TREATED CASSAVA ROOT MEAL (*Manihot esculenta* Crantz*)* ON EGG PRODUCTION AND FEED EFFICIENCY IN IMPROVED INDIGENOUS LAYER CHICKENS

Abstract

The rising prices of energy ingredients for poultry feed production have triggered investigations into locally available feed resources for sustainable feeding options. These feed resources should be adaptable to various tropical weather conditions and soil types. One such feed is cassava root, but the bitter type varieties contain linamarin, which converts to hydrogen cyanide once the root tissues are damaged. Processing techniques such as sun-drying, fermentation, and enzymatic treatment have been used to lower cyanide concentration. This research assessed the performance of improved indigenous layer chickens fed on enzyme-treated cassava root meal as a partial energy substitute for maize in the diet. Thirty-six improved indigenous layer chickens were randomly allocated to four dietary treatments with 0% (T1), 25% (T2) (T3), 50% and 75% (T4) cassava root meal, with three replicates per treatment. The study evaluated feed intake, feed conversion ratio (FCR), and hen-day egg production. The results revealed that feed intake was statistically similar across all treatments. Feed Conversion Ratio was similar in T1, T2, and T3, but different in T4. This indicated that the feed utilization efficiency was lower at T4. Egg production was similar in

T1, T2, and T3, but there was a decrease in egg production in T4. The results indicated that enzyme-treated cassava root meal can be used to replace maize up to 50% in improved indigenous layer chickens feed without loss of productivity. It is, therefore, a locally available alternative for maize as an energy ingredient in poultry feeds.

4.1 Introduction

The ever-increasing global demand for poultry products has increased demand for poultry feeds. This has made farmers seek low-cost and environmentally friendly feed alternative (Khalifah *et al*., 2023). However, the increased cost of Conventional imported feed inputs like maize and soybean prompted the scientists to evaluate locally available and cheaper alternatives. These alternatives should have the capability of maintaining the efficiency of poultry production at the lowest cost possible (Dury *et al*., 2019). The cassava plant produces a starchy cassava root meal that can serve as an alternative ingredient in poultry feed because it provides an abundant source of energy and is very adaptable to various tropical weather conditions and soil types (Abouelezz *et al*., 2022).

Scientific findings have proven that cassava root meal (CRM) can be used as a suitable Replacement for regular feed inputs because of its economic advantage and ready availability (Bakare *et al*., 2021). However, cyanogenic compounds present in high-cyanide cassava root (bitter types) are the reason why it is not used for animal feed purposes since linamarin converts to hydrogen cyanide once the root tissues are damaged.

Livestock, including poultry, face substantial dangers from cyanide toxicity when management practices fail to address it (Tshala-Katumbay *et al*., 2016). Sweet cassava roots contain around 75 mg/kg, while bitter types can reach 1000 mg/kg (Guerre 2016). According to Kim (2023), hydrogen cyanide concentrations must be lowered to under 20 mg/kg to maintain poultry safety.

Processing techniques such as sun-drying have been used to lower cyanide concentrations, but it is not effective at eliminating the toxin; therefore, there is still the potential for animals to suffer from cyanide toxicity (Diarra & Devi, 2015). Enzymatic treatments have been developed to break down the toxic components, which makes cassava root meal (CRM) available as a safe poultry feed ingredient (Oloruntola *et al*., 2018). Enzymatic processing of CRM enhanced its digestibility, and it provided a choice of feed that could be utilized by poultry farmers as a local source of feed. A better breed of indigenous layer chickens is the preferred breed in developing and tropical regions because it can survive in extreme environmental factors (Kamau *et al*., 2019). Smallholder farmers have enhanced indigenous layer chickens, which assist in regulating the production cost, and the price of conventional poultry feed is one of the key setbacks that smallholder farmers are currently facing. It has become the purpose of the researchers to find a sustainable solution by search for alternative feed ingredients such as CRM that are locally available and cost-effective to find (El-Deek *et al*., 2020). This experiment examined the feed consumption and feed ratio (FCR), and egg production of the improved indigenous layer chicken given diets with enzyme-treated cassava root meal as a 0, 25, 50, and 75% replacement of maize. These values served as performance indicators because they provided an understanding of the efficiency of the feeds in generating energy and eggs, which are the key products in the production of layer chickens (Akinola & Nwanochi, 2021). The growth and the egg rate of production, as well as being the determinant of the overall cost of feed, make feed intake an essential part of production (Simeneh, 2019). Birds should receive adequate feed so as to satisfy their nutritional needs. The feed conversion ratio (FCR) is a parameter that determines the performance of the animals to convert the feed into either weight gain or egg production (Chang'a *et al*., 2019).

The hen day egg production is an essential parameter in the production of layers since it directly interferes with the profitability of the business (Salvia & Valderrama, 2021). Researchers should increase the scope of the possible advantages of enzyme-treated CRM, as the impact of the CRM on poultry dietary inclusion rates is sparse. Research established that poultry feed with up to 25 percent CRM produced a proficient energy yield at constant feed.

Feed ratio and consumption rates, in addition to stable production of eggs (Yadav *et al*., 2019). The favorable results were followed by the need to carry out further research to investigate the effects of 50 per cent and 75 per cent CRM inclusion rates on egg production and the feed ratio (FCR) measures. By critically evaluating such interactions, this study was able to establish the optimal level of CRM inclusion that would produce optimum poultry performance with reduced exposure to risk. This research provided essential knowledge while offering practical information to poultry farmers who would want to use sustainable and cost-efficient feed alternatives for improved productivity and reduced costs, which promote sustainable farming practices.

4.2 Materials and Methods

4.2.1 Study site

The study was conducted at Egerton University, Tatton Agricultural Park (TAP). Egerton University is in Njoro sub-County, Nakuru County (latitude: -0.369734; longitude: 35.932779) in Kenya, with an average temperature between 17- 22 °C, with a drop to 11 °C during the cold season of July–August. In two distinct seasons, the yearly rainfall average is 1,200+/-100 mm, while the short rains begin in October and stop in December; the long rains start in March and last until July (Egerton University, Department of Agricultural Engineering, Meteorological Station, 2024 unpublished).

4.2.2 Experimental diet

The experimental diets were formulated with 0, 25, 50, and 75% CRM as a partial replacement for maize to meet the nutritional requirements of improved Indigenous chicken (King'ori *et al.,* 2014). The composition of feed ingredients in the diets is presented in Table 4.1.

Table 4.1: Composition of feed ingredients in the experimental diets.

Ingredients (kg)	T1	T2	T3	T4
Maize grain	47.8	27	18.5	3.5
rice germ	7.0	5.7	0.5	0.3
CRM	0	25.0	50.0	67.5
Soya bean meal	13.5	13.0	16.0	15.0
Freshwater shrimp/ *Ochong'a*	5.0	4.3	5.5	5.0
Maize germ	17.0	15.0	0.6	0.3
Vegetable oil	0.3	0.6	0.5	0.8
DCP (granular 24%)	2.0	2.0	0.6	0.3
Limestone	6.5	6.5	6.5	6.4
Salt	0.3	0.3	0.3	0.3
Premix	0.3	0.3	0.3	0.3
Toxin binder	0.1	0.1	0.1	0.1
Methionine	0.2	0.2	0.2	0.2
Total (kg)	100	100	100	100
CP (kg)	16.99	16.06	16.81	16.04
Calculated ME (Kcal/kg)	2680.67	2601.84	2646.77	2612.75

4.2.3 Proximate analysis

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

A combustion bomb calorimeter (Model e2k) was used to determine the gross energy of the feed materials. Metabolizable energy was calculated using the formula ME (MJ/kgDM) = GE X *k*, where GE is the gross energy, *k* is a correlation factor of 0.8 (NRC, 1994).

4.2.4 Management of experimental birds

Sixty (60), sixteen-week-old improved indigenous layer birds were purchased from Kenya Agricultural and Livestock Research Organization (KALRO), Naivasha, out of which 36 were randomly sampled for the study. The chickens were housed in a deep litter house with the floor covered with wood shavings. The litter was regularly turned to aerate and avoid caking. Wet litter was removed immediately to prevent the accumulation of microorganisms and parasites. The birds were given a two-week adaptation period. During the first week, they were fed a similar diet, and in the second week, they were fed an experimental diet before data collection. Vaccination and deworming were done according to the guidelines developed by KALRO. Birds were individually weighed and randomly assigned to dietary treatments to ensure similar body weights across the experimental groups. A cage with one square meter was provided with three birds; one round drinker (30.5cm by 28cm) and one rectangular feeder (90.06cm by 22.86cm). The routine cleaning of feeders and drinkers, fresh feed supplied daily at 08:00 hrs, after which water was provided at ad libitum, and litter changed was also part of routine management practices. This experiment was 12 weeks.

4.3 Data Collection

The sampled data included feed intake, feed ratio, and hen-day egg production.

a) Feed intake: This was estimated as the difference between the feed fed and leftovers (refusal) at the end of 24 hours, and it was divided by the number of chickens per treatment.

$$\text{F. I}= \frac{feed\ offered(g) - feed\ left(g)}{number\ of\ chickens}$$

b) Feed Conversion Ratio (FCR): This was determined as the ratio between the feed intake (g) and the average Egg weight (g) per week.

$$\text{FCR}= \frac{feed\ intake(g)}{average\ egg\ weight\ (g)\ per\ week}$$

c) Hen-Day Egg production (%)

Eggs laid in each treatment per day were physically counted, and the per day egg production was calculated as the number of eggs laid per treatment divided by the number of hens alive per treatment, multiplied by 100.

$$\text{Hen-Day Egg Production} = \frac{number\ of\ eggs\ laid}{number\ of\ hens\ alive\ per\ treatment\ per\ day} \times 100$$

4.4 Statistical Analysis

Data collected on feed intake, feed conversion ratio, and egg production were subjected to Analysis of variance (ANOVA) in a completely randomized design (CRD) using the General Linear Model procedure of Statistical Analysis System (SAS, 2023) version 9.4 at a 5% significance level. Before analysis, the data were tested for normality using Kolmogorov–Smirnov and Shapiro tests. Tukey's test at ($p<0.05$) was used to separate means that were significantly different. The model used for statistical Analysis was:

$Y_{ij} = \mu + ti + \varepsilon_{ij}$

where;

Y_{ij} = observation of the response variable

μ = overall mean

ti = effect of the i'th treatment at (0%, 25%, 50% and 75% CRM)

ε_{ij} = random error

4.5 Results and Discussion

4.5.1 Results

4.5.2 Chemical composition of experimental diets and cassava root meal

The chemical compositions of the diets and CRM are presented in Table 4.2.

Table 4.2: Chemical composition (%) and metabolizable energy (MJ/Kg) of cassava root meal and maize ingredients used in the diets

Nutrient component (%)	CRM	Maize
Ash	1.7	5.0
Crude protein	2.3	9.0
Ether extract	1.4	4.6
Dry matter	94.1	90.1
Metabolizable energy (MJ/Kg)	12.7	16.1

Crude fiber	19.4	2.0

4.5.3 Chemical composition of experimental diets

Table 4.3 presents the chemical composition of the diets.

Table 4.3 Chemical composition of experimental diets

Nutrients (%)	T1	T2	T3	T4	SEM	P Value
Dry matter	92.227[a]	92.612[a]	93.327[a]	94.361[a]	1.729	0.2673
Ash	6.424[c]	7.707[b]	7.674[b]	10.071[a]	0.127	<0.0001
Crude protein	16.705[a]	16.011[a]	16.667a	16.049[a]	0.346	0.4325
Crude fiber	4.722[b]	4.986[b]	4.346[b]	5.617[a]	0.399	<0.0001
Ether Extract	6.732[a]	8.754[a]	7.251[a]	6.956[a]	0.611	0.1264
HCN (ppm)	indeterminable	indeterminable	indeterminable	indeterminable		
Metabolizable Energy (MJ/Kg)	10.72[c]	10.89[c]	11.72[b]	12.37[a]	0.137	<0.0001

[a,b,c] Means within a row with different superscripts differ significantly ($p<0.05$)

4.6 Performance of chicken

4.6.1 Effect of diet and enzyme interaction on feed intake, feed conversion ratio, and hen day egg production

Table 4.4 presents the effect of enzyme-treated cassava root meal inclusion level in the diet on feed intake, feed conversion ratio, and egg production.

Table 4.4: Effect of enzyme-treated cassava root meal on feed intake, feed conversion ratio, and egg production

Parameters	T1	T2	T3	T4	SEM	P Value
Feed intake (g)	118.18[a]	119.54[a]	123.51[a]	115[a]	3.319	0.4256
Feed conversion ratio	2.252[b]	2.315[b]	2.287[b]	2.906[a]	0.065	0.0003
Egg production (%)	73.413[a]	72.090[a]	75.397[a]	55.210[b]	1.138	<0.0001

[a,b,c] Means within a row with different superscripts differ significantly ($p<0.05$)

There was no significant difference in the feed intake of chickens fed on diets with enzyme-treated cassava root meal. There was no significant difference in the feed conversion ratio in

T1, T2, and T3. However, T4 was significantly different, with the highest feed conversion ratio of 2.906.

Treatment T4 had a significantly lower percentage of egg production of 55.210% compared to T1, T2, and T3, which had no significant difference in egg production percentages with 73.413%, 72.090%, and 75.397%, respectively.

4.7 Discussion

4.7.1 Feed intake

The similar feed intake in all the treatments can be attributed to the fact that the cassava root meal used in the formulation of the diets was palatable and contained indeterminable levels of cyanogenic compounds. These results correspond with those of Yue *et al*. (2024), whose research findings demonstrated that there was no significant difference in feed intake of chickens whether they consumed diets with cassava pellets and enzyme supplements or traditional maize-based control diets because their diets were well balanced with similar levels of energy and nutrients. These results indicated that chicken diets can be formulated with maize replaced with well-processed cassava root meal without impacting feed consumption levels when enzyme supplementation is used.

Similarly, Bhuiyan and Iji (2015) found no significant difference in feed intake across all treatments of 0%, 20% and 30% cassava inclusion levels. The researchers attributed these findings to adequate microbial enzyme supplementation to cassava-based diets that enhanced cassava digestibility and palatability, which led to no significant differences in feed intake among all treatment groups.

Conversely, Ogbuewu and Mbajiorgu (2022b) in a meta-analysis have determined a Reduced CRM-based diets (significantly low feed intake of chickens). The sun-dried CRM Compared to the control diets, a considerably lower feed intake was given to the treatment group. This could have been due to alterations in the chemical and physical nature of cassava, which might have rendered it indigestible or less palatable for the birds.

4.7.2 Feed conversion ratio

The high increase in the FCR (feed ratio) at the 75 percent cassava meal addition was an indication of reduced efficiency with respect to nutrient utilization. High levels of fiber might have reduced the digestibility, resulting in poor performance (Ogundeji & Akinfala, 2020). The performance of laying hens under the influence of cassava root meal was of particular interest to the study in terms of the feed conversion ratio (FCR). The findings indicated that FCR was also substantially modified at the various levels of cassava root meal (CRM) since the FCR has a tendency to rise with the increase of the percentage of cassava root meal in the diet. This may be because of the large size of the high cassava diet and decreased palatability most likely led to higher FCR (Chauynarong *et al*., 2009). According to Chang *et al.* (2019), the FCR of CRM was significantly increased when enzyme-treated CRM was added to it with a high inclusion of 75 percent, which implies that the high level of feed inclusion decreased feed efficiency. This is perhaps because of the dilution of nutrients and perhaps other remaining anti-nutritional agents like phytates, tannins, etc.

This experiment proved enzyme-treated cassava root meal to be a viable feed ingredient to a 50% inclusion level, but beyond that, the feed efficiency becomes low, and this adds to the cost of production for poultry farmers.

4.7.3 Egg production percentage

The lowered egg production in the 75% CRM diet might have been due to reduced digestibility, dilution of dietary energy, and most likely other anti-nutritional effects, including phytates and tannins. The corresponding similar effect ($p>0.05$) in T1, T2, and T3 in the egg production determined the similar effects as Morgan and Choct (2016), who found that the maize could be substituted half by Cassava in the chicken feed, with the performance staying unaffected, with the processing and proper Nutrient balance planning.

A meta-analysis conducted by Ogbuewu and Mbajiorgu (2022) has provided evidence that the diets of laying hens containing a 50 percent cassava root meal did not adversely affect egg production, but further studies should be done with higher levels of inclusion of over 50 per cent. The significant decrease ($p< 0.05$) at 75% inclusion level of cassava root meal was in line with a study by Lei *et al*. (2017) established that the percentage of egg production was lower with an increase in the levels of cassava root meal.

4.8 Conclusion and Recommendation

In this research, the conclusion made was that an inclusion level of CRM of 50% resulted in positive FCR and egg production percentage. It is thus advised that poultry farmers need to include a portion of up to 50 per cent enzyme-treated cassava root meal in their feed recipes in order to reduce the portion of feed per pound and increase the production of eggs. Research is to be performed to find the ideal inclusion percentage of enzyme-treated CRM of 25-50% and 50-75.

CHAPTER FIVE

EFFECT OF INCLUSION OF ENZYME-TREATED CASSAVA ROOT MEAL IN DIET ON EGG QUALITY CHARACTERISTICS OF IMPROVED INDIGENOUS LAYER CHICKENS

Abstract

Feed quality affects egg quality, impacts consumer acceptance, market value, and the nutritional value to humans. In this study, Cassava Root Meal (CRM) was evaluated and found to be a promising ingredient for poultry feed production. The research investigated the effect of the inclusion of enzyme-treated cassava root meal in an improved indigenous layer chicken diet on egg quality characteristics. Thirty-six improved indigenous layer chickens were randomly allocated into four dietary treatments with 0% (T1), 25% (T2), 50% (T3), and 75% (T4) cassava root meal, with three replicates per treatment. Samples of eggs of the chicken fed on these treatments were examined for both external (egg weight, egg shape index, eggshell ratio, and eggshell thickness) and internal egg quality parameters (yolk index, yolk colour, and yolk to albumen ratio) using the samples. The hens that were fed with diet T4 (75% cassava root meal) gave eggs with the highest index value of 73.385, which was very high compared to the index values of T2 (71.630) and T3 (67.673). The eggs of the hens on diet T1 had the lowest index value. Eggs that hens on a diet hatched T4 had the highest value of the shell-to-egg weight ratio of 12.1708, and this was considerably different than the ones in T1 (9.4060), T2 (9.7528), and T3 (11.1425). T3 had the highest thickness of 0.594mm, which was far higher than T1 (0.448mm), T2 (0.512mm), and T4(0.349mm). There was no significant effect on the weight of the albumin, yolk diameter as well or yolk index in the diet. Hampered hens that were fed on T1 recorded the highest yolk colour index value of 10.167 and declined with the increase in the CRM levels ($p < 0.05$). The yolk-to-albumen ratio was highest in T3 (57.420), significantly different from T1 (52.610) and T4 (51.812) ($p < 0.05$). These results indicated that incorporation of 50 percent of enzyme-treated cassava root meal in the diet had a sizeable influence on the quality attributes of the eggs.

5.1 Introduction

Enhanced native chicken in Kenya is vital to the world's food security due to their adaptability and resistance to diseases, and their products are highly preferred by consumers

(Padhi, 2016). Raising prices of maize have posed a substantial economic burden to smallholder poultry farmers, necessitating the need to consider alternative feed resources in the area (Wongnaa *et al*., 2023). Manihot esculenta (cassava) root meal is one of the energy ingredients available in the area. It is rich in carbohydrates, more adapted to the tropical climate and soil, and low cost of production (Abouelezz *et al*., 2022). However, it has significant constraints because it has anti-nutritional components that include cyanogenic glycoside and a large content of fiber. These compounds caused inefficiency in the digestion of the nutrients and a decrease in feed efficiency and potentially, the productive performance (Ngiki *et al*., 2014). In order to maintain the quality of eggs, the farmers will be required to maintain the nutritional value of the feed at times when there is a shortage of feed/food. The quality of the feed influences the quality of the egg, consumer acceptance, market price, and nutritional value to humans (Tian *et al.,* 2022). Studies have been conducted to use cassava root meal as an alternative to maize in the layer rations. To reduce the risk of chicken contamination, the level of hydrogen cyanide (HCN) should be reduced to less than 10 mg/kg (Kim, 2023). Enzyme supplementation is one such technique that has been utilized to overcome the anti-nutritional effects that are present in plant-based feed components. The nutrients are more accessible due to the use of exogenous enzymes, and this allows the use of more cassava root meal without lowering the egg quality (Bakare *et al*., 2021). Huang *et al*. (2024) discovered that multi-enzyme-containing supplements profoundly increase the egg shape index and albumen height and Haugh unit scores of laying hens, which reflect improved egg quality. Research by Asaniyan (2023) revealed that the substitution of maize by no more than 75 percent of the grain by another grain, which is less nutritious, is possible.

Cassava plant meal (leaves, stems, and tubers) in rations fed to laying hens did not reduce hen-day production compared to maize-based diets but had superior qualities in egg quality, such as eggshell weight and yolk height.

The cassava meal feed determines the quality results of eggs and also the genetic traits of the laying hens. It was found that Sasso chickens exhibited better quality of eggs with higher scores of egg weight, yolk width, yolk height, albumen height, yolk index, and Haugh unit compared to indigenous breeds (Assefa *et al*., 2023). Differences in the genetic makeup of the chicken breeds are fundamental in defining the quality attributes of eggs. There is an increasing number of developing nations that apply genetic improvement programs to native chicks, but there is a lack of research on their particular nutritional requirements and feeding methods. In their study, Kingori *et al*. (2014) found that indigenous breeds of chicken need different nutrients than the commercial hybrids because they are more efficient in protein usage and less tolerant to fiber.

The nutritional and consumer quality of eggs is mainly determined by such key indicators as physical (egg weight, shell thickness, shape index), internal (albumen height, yolk colour, Haugh unit), and chemical (protein, lipid, mineral content) indicators (Usturoi *et al*., 2025). Feed composition and egg-laying hens' genetic background interact to determine egg quality (Hailemariam *et al*., 2022). Research on the effects of enzyme-treated cassava root meal on egg quality indicators from improved indigenous laying hens will help develop feeding strategies that improve egg quality while lowering production expenses.

This study investigated the impact of enzyme-treated cassava root meal on egg quality characteristics produced by improved indigenous layer chickens. The research evaluated the effects of enzyme-treated cassava root meal inclusion levels on physical egg quality parameters (egg weight, shell thickness, shape index), internal egg quality attributes (albumen height, yolk colour, Haugh unit). The guiding hypothesis of this study proposed that enzyme-treated cassava root meal had no significant effect on both external and internal egg quality parameters of improved indigenous layer chicken.

5.2 Materials and Methods

5.2.1 Study site

The experiment was conducted in the study site as described in section 4.2.1

5.3 Data Collection

5.3.1 Egg quality parameters

The eggs were collected twice daily at 9.00 hrs and 16.00 hrs and placed in egg trays.

Twelve (12) eggs, three (3) per treatment, one egg per respective replicate, were randomly sampled per week for both external and internal egg quality analysis. The parameters included egg weight, egg shape index, eggshell weight, eggshell thickness, egg yolk: albumen ratio, and egg yolk colour.

5.3.2 External egg quality parameter analysis

a) Egg weight (g)

Eggs were accumulated weekly and weighed using a digital weighing balance to the nearest 0.01 g accuracy (Maurer *et al*., 2014).

b) Egg shape index

This was calculated as the ratio of egg length to egg breadth multiplied by 100 (Anderson *et al*., 2003).

E.S.I $=\frac{egg\ width\ (mm)}{egg\ length\ (mm)} \times 100$

Egg length and breadth were measured using a digital Vanier caliper

c) Eggshell ratio (%)

This was determined by carefully breaking the egg to separate the egg yolk and albumen from the shell. The shell was then dried in an oven at low temperatures and weighed using a digital balance, divided by the egg weight then multiplied by 100 (Nordstrom & Ousterhout 1982).

ESR$=\frac{shell\ weight}{egg\ weight} \times 100$

d) Eggshell thickness (mm)

This was determined by breaking the egg and immediately separating the egg membranes from the eggshell. The eggshell was wiped using a study towel, and then 3 measurements of the eggshell pieces from the broad, narrow end, and equator were measured using a precision micrometer calibrated in millimeters to the nearest 0.01mm. The mean thickness measurements were computed (Tyler & Geake, 1964).

5.3.3 Internal egg quality parameter analysis

a) Yolk index

This was determined as a ratio of yolk height to yolk diameter. The egg was broken into a stainless-steel plate and placed on a flat surface. The yolk height and diameter were measured using a Vanier calliper ruler calibrated in millimetres (Wesley & Stadelman, 1959).

b) Yolk colour

A Roche yolk colour fan was used to determine the colour score of the yolk. On a flat surface, samples were gathered carefully observed and compared against the colours on the Roche fan. The colour match for each sample corresponding with the fan's scale, which ranges from 1 to 12, was used to determine the yolk colour, was recorded. From the scale. 1 represents extremely pale colour and 12 represents intensely yellow colour (Galobart *et al.*, 2004).

c) **Yolk: Albumen ratio**

This is the ratio of yolk weight to albumen weight. The yolk and albumen weights were measured after the separation of the yolk from the albumen using a funnel. The albumen weight was obtained as the difference between total egg weight and the sum of yolk weight and shell weight, as shown below (Hussein & Harms, 1993).

Albumen weight= total egg weight - (yolk weight + shell weight)

5.4 Statistical Analysis

Data collected were subjected to the Analysis of variance (ANOVA) in a completely randomized design (CRD) using the General Linear Model procedure of Statistical Analysis System (SAS, 2023) at a 5% significance level. Before data analysis, the data were tested for normality using Kolmogorov–Smirnov and Shapiro tests. Tukey's test at ($p<0.05$) was used to separate means that were significantly different. The model used for statistical Analysis was:

$Y_{ij} = \mu + ti + \varepsilon_{ij}$

where;

Y_{ij} = observation of the response variable

μ = overall mean

ti = effect of the i'th treatment at (0%,25%,50% and 75% CRM)

ε_{ij} = random error

5.5 Results and Discussion

5.5.1 Effect of diet and enzyme interaction on egg quality characteristics

Table 5.1 shows the effect of the inclusion of enzyme-treated cassava root meal in the diet on external egg quality parameters.

Table 5.1: Effect of enzyme-treated cassava root meal on external egg quality parameters

Parameters	T1	T2	T3	T4	SEM	P value
Egg weight (g)	52.487^{b}	51.620^{b}	54.014^{a}	39.712^{c}	0.232	<0.0001
Egg height (mm)	64.058^{b}	60.171^{c}	65.245^{a}	55.042^{d}	0.059	<0.0001
Egg diameter (mm)	44.027^{b}	43.100^{c}	44.153^{a}	40.392^{d}	0.094	<0.0001
Eggshell weight (g)	4.937^{c}	5.034^{b}	6.018^{a}	4.833^{d}	0.0201	<0.0001
Eggshape index (%)	65.608^{d}	71.630^{b}	67.673^{c}	73.385^{a}	0.195	<0.0001
Eggshell ratio (%)	9.4060^{c}	9.7528^{c}	11.1425^{b}	12.1708^{a}	0.079	<0.0001

Eggshell thickness (mm)	0.448^{c}	0.512^{b}	0.594^{a}	0.349^{d}	0.0028	<0.0001

^{a,b,c,d} Means within a row with different superscripts differ significantly (p<0.05)

The diet had a significant effect on all the parameters. In the egg shape index, diet T4 had the highest value (73.385), significantly different from T2 (71.630) and T3 (67.673) compared to T1, which had the lowest value. Diet T4 had the highest eggshell ratio (12.1708a), significantly higher than T1 (9.4060), T2 9.7528), and T3 (11.1425), suggesting that the feed influenced the weight of the shell relative to egg weight ($p < 0.05$). Diet T3 had the thickest shell (0.594), significantly thicker than both T1 (0.448), T2 (0.512 and T4 (0.349), pointing to an effect of feed on shell thickness ($p < 0.05$). The improved eggshell thickness (0.594 mm in T3) indicated that 50% cassava meal inclusion optimally enhanced shell mineralization, most likely because of greater calcium bioavailability. Table 5.2 shows the effect of feed on the internal egg quality parameters.

Table 5.2: Internal egg quality

Parameters	T1	T2	T3	T4	SEM	P value
Yolk weight (g)	16.384^{b}	16.384^{b}	17.506^{a}	15.475^{c}	0.177	<0.0001
Albumen weight (g)	31.166	30.201	30.490	29.881	0.290	0.1456
Yolk height (mm)	14.759^{ab}	15.725^{ab}	17.847^{a}	12.525^{b}	0.916	<0.0001
Yolk diameter (mm)	39.173	37.593	37.508	38.066	0.688	0.2356
Yolk index (%)	47.775^{a}	48.106^{a}	48.529^{a}	48.692^{a}	2.514	0.1127
Yolk: albumen (%)	52.610^{b}	54.261^{ab}	57.420^{a}	51.812^{b}	1.027	0.0206
Yolk colour	10.167^{a}	7.167^{b}	4.833^{bc}	4.167^{c}	0.527	0.0002

^{a,b,c} Means within a row with different superscripts differ significantly (p<0.05)

There were no significant differences in albumen weight, yolk diameter, and Yolk index between the treatments ($p > 0.05$). Diet T3 (57.4195) had the highest ratio, significantly differing from T1 (52.6099) and T4 (51.8117b), suggesting that hens on T3 produced eggs with a higher proportion of yolk relative to albumen ($p < 0.05$). Hens on T1 (10.167) produced eggs with the most intense yolk colour, significantly different from T2 (7.167), T3 (4.833b), and T4 (4.167).

5.6 Discussion

5.6.1 Egg shape index (ESI)

There was a significant increase in ESI as the CRM inclusion level increased. This means that T4 produced eggs that had a consistent oval shape. These eggs are preferred by consumers because they look good and are easier to handle (Zhang *et al.,* 2023). Observed increase in egg shape index with increasing levels of cassava meal is in line with findings by Adeyemi and Asaniyan (2024), who reported similar improvements in egg shape index by layer chickens fed cassava-based diets. This was attributed to the high metabolizable energy content in T4, which promoted smoother egg formation, resulting in more oval egg shapes.

However, contrasting findings were reported by Lei *et al*. (2017) in diets supplemented with 8% dried distillers' grains with solubles (DDGS), who reported that egg shape index remained stable at 30% cassava root meal inclusion. They argued that the structural properties of eggs are not altered when moderate to slightly increased (30%) CRM levels were incorporated into nutritionally balanced diets.

5.6.2 Eggshell ratio

There was a significant difference among treatments with an increase in eggshell ratio as CRM levels increased, which was in agreement with a study by Gongruttananun (2018), whose research findings showed that hens given a 75% cassava root meal diet for four weeks improved eggshell ratio because cassava meal reinforced shell strength during induced moult periods. Similarly, a study by Adeyemi and Asaniyan (2024) found that the egg shell ratio significantly improved when laying chickens were fed on a diet containing unpeeled cassava tubers. This trend of the eggshell white being higher in the experimental group, in which the cassava root peel meal III was used, is explicable by the fact that the unpeeled cassava tubers contained more minerals and nutritional content, and consequently were able to develop better bone and shell in the laying chickens, and thus made heavier eggshells as compared to the actual weight of the eggs.

5.6.3 Eggshell thickness

The data was statistically analyzed and revealed that the data on the eggshell thickness significantly differed among treatments, with the eggshell thickness being maximum at 50% CRM. This can be attributed to the optimal mineralization of some of the crucial minerals, such as calcium, magnesium, and phosphorus, all of which were requisite in the development of the eggshell. This can also be compared to the results obtained by Gazi and Mnisi (2024), where a significant difference in eggshell thickness was found when using Jumbo quail.

Hens were given diets that were developed from cassava tuber meal. This was explained by the enhanced efficiency of digestion and absorption of dieters, especially at 50% CRM inclusiveness. The optimal calcium nutrition has a direct impact on the calcium deposition on the eggshell and causes shell thickness. The findings of this study were consistent with the findings of Almeida *et al*. (2020), who revealed that dried cassava meal was helpful in improving the thickness of the eggshells by enhancing the absorption of calcium that would facilitate shell development.

As it was revealed by a meta-analysis conducted by Ogbuewu and Mbajiorgu (2023a), the quality characteristics of eggs, including shell thickness and strength, were found to be enhanced when cassava-based diets were added to a minimum of 25% of the diet of laying hens. This was contributed to by the fact that more calcium is absorbed in the consumption of cassava, resulting in high eggshell quality due to an increase in shell formation. Abouelezz *et al*. (2022), however, also revealed the opposite results of their experiment that evaluated the impact of cassava starch extraction residue meal (CReM) on eggshell properties in laying ducks. It could not be seen that any form of improvements in either shell thickness or strength, and therefore, the impacts of cassava-based feed on eggshell quality might be influenced by processing and dietary inclusion rates. Also, according to a study carried out by Ghazalah and Abd-Elsame (2017) on feeding chickens with CRM, there was no significant difference in eggshell thickness. This meant that processed cassava root meal did not influence the thickness of the eggshell, and this indicated that the result of cassava diets was not dependent on their processing and inclusion quantity methods. The findings of the study in general indicated that further studies are necessary to identify the most efficient amounts of inclusion and processing techniques that could be steadily used to increase the eggshell thickness.

5.6.4 Yolk index

There was no significant difference in the yolk index of all treatments. This is because the composition of the yolk was consistent even in the presence of cassava root meal in the diet of the hen. This result was consistent with the findings of Kyawt *et al*. (2014), who concluded that the inclusion of CRM in the diets of laying hens did not have any remarkable impact on the index of egg yolk. This could be explained by the fact that the cassava root meal was an energy source, which could be provided by carbohydrates, but had no direct and substantial impact on particular nutrients that directly influence the yolk quality, e.g., some vitamins or fatty acids.

The addition of cassava meal would not most likely affect the yolk index unless the diet is changed with regard to these nutrients. Similarly, Aregay *et al*. (2021) found that cassava-based diets did not affect the yolk index of laying chickens significantly, implying that the carbohydrates in cassava did not affect the yolk index. Quite on the contrary, the research conducted by Ofioco *et al*. (2021) indicated that the index of egg yolk improved significantly when the diets contained a mixture of cassava root meal and moringa leaf meal, which implied that moringa leaf meal protein could increase the index of egg yolk.

The inconsistency of the research results sheds light on the need to conduct additional research on the role of various cassava processing procedures and blends of ingredients on the egg yolk quality in chickens.

5.6.5 Yolk: Albumen ratio

The yolk to albumin ratio was considerably different, and the highest yolk albumin ratio was at a 50 percent cassava root meal inclusion level. The yolk-to-albumen ratio is one of the crucial aspects of the quality analysis of eggs since it gives the data regarding the composition of the egg components of nutrients and part ratio (Rath *et al*., 2015). Some markets preferred eggs having higher yolk than albumin content since a high yolk: albumin ratio means superior nourishment, which is compared to an elevated amount of fat and proteins in the yolk, which enhances egg value (Zhang *et al*., 2024). The findings are in agreement with the study by Raphaelle *et al*. (2022) that found quails fed a diet with 50% CRM produced a better egg quality (high yolk: albumen ratios) than the control. The cause that was given was that the 50 percent CRM contained greater gross energy that could be converted to proteins and lipids, which are the primary constituents of yolk. The yolk-to-albumen ratio is high due to the large size of the yolk.

Ogunwole *et al*. (2017) noted a significant increase in the yolk and albumen ratio at 50% CRM of the study. The justification was that enhanced digestibility of cassava root meal (CRM), which is an essential precursor of enhanced nutrient absorption and utilisation in the reproductive processes, such as yolk formation. This boosted the size of the yolk as compared to the albumin, resulting in a high yolk-to-albumin ratio. Conversely, Ande *et al*. (2021) demonstrated contrary findings, and this indicated that no significant difference occurred in the yolk-to-albumen ratio. The reason adduced was that CRM contained low levels of crude protein, a significant proportion of the yolk, giving rise to a low yolk to albumin ratio.

5.6.6 Yolk colour

The difference in yolk colour was also high, and the lower the level of cassava root meal added, the lower the change in colour. This result was in line with a study by Omar (2018) who reported that yolk colour intensity decreased as cassava root meal inclusion level increased. The argument was that cassava has lower carotenoid levels, which resulted in yolks with decreased colour intensity. Ghazalah and Abd-Elsame (2017) showed that the colour of egg yolk was significantly lighter in birds fed a diet containing 50% CRM and without any additional pigmentation sources than in those fed the control diet. This showed that higher CRM levels of replacement would cause the yolk colour to be pale. Ogundeji and Akinfala (2020) reported a significant improvement in yolk colour intensity by 44.8% when cassava plant meal (the integration of cassava roots, cassava leaves, and tender stems) replaced maize completely during the feeding period. This was attributed to the fact that the carotenoid levels in the cassava plant meal, and in particular cassava, were higher leaves and tender stems have a high pigment content, such as lutein and 2-carotenes.

5.7 Conclusion and Recommendation

Diet (T4 treatment) that was made of 75 percent cassava root meal (CRM) resulted in good shell ratios and the shape of eggs. A diet including 50% cassava root meal (treatment T3) produced eggs with optimum shell thickness and acceptable yolk to albumen ratio. Finally, 50 percent cassava meal resulted in augmentation of eggshell as well as the ratio of yolk to albumin, indicating robust egg shells and a supplement of nutrient content, which is favorable to manufacturers and consumers. To achieve the best quality of eggs, the use of enzyme-treated cassava meal in a 50 percent ratio is advised. Research must be done to reveal the ideal amount of enzyme-treated CRM to be included between 25-50 to 50-75.

CHAPTER SIX

ECONOMIC EVALUATION OF ENZYME-TREATED CASSAVA (*Manihot esculenta*) ROOT MEAL IN DIETS OF IMPROVED INDIGENOUS LAYER CHICKENS

Abstract

The increasing price of the traditional poultry feeds, mainly of maize and soybean meal, keeps questioning the sustainability and profitability of rearing chicken in layers. This study examined the economics of the use of enzyme-treated cassava root meal (CRM) in the layers' chicken diets. The impact of four levels of inclusion (0, 25, 50, and 75) of CRM in the diet of layer chickens on performance was established in the study. Nine hens from each of the treatments were observed over a period of 12 weeks, during which there was an assessment of intake of feed, egg production, and economic returns. Treatment 3 (50% CRM) was most productive (570 eggs), the most profitable (KES 3,681.74), had a cost-benefit ratio (1.48), and paid back (ROI-47.7%). Turnover financial indicators that were used to determine the cost of production of eggs were the cost-benefit ratio (CBR) and the return on investment (ROI). It was confirmed in the analysis that T3 made the most significant profit of profit of KES 3,681.74 per treatment (3 chickens) and a cost-benefit ratio of 1.48 and ROI of 47.7, which is the highest as compared to all other treatments. Treatment 4 (75% CRM) had a KES 944.96 profit, a cost-benefit ratio of 1.13, and an ROI of 12.74%, which shows bad economic performance. The authors proved that the use of optimized feeding methods in which enzyme-treated cassava root meal was used was critical in enhancing the profitability and sustainability of egg production in indigenous layer chickens. The research provided practical suggestions to the poultry producers in order to attain improved payoffs in the form of cost-efficient production management. Implementing 50% cassava root meal enzyme-treated poultry food resulted in improved financial performance, which is a sustainable and more profitable poultry business.

6.1 Introduction

In developing countries, poultry production is very crucial in the provision of food security and the generation of income through the smallholder farmers who mainly produce better indigenous layer chickens (Birhanu *et al*., 2023). Even though these birds demonstrate high adaptability and disease resistance, their productivity remains restricted by the scarcity of affordable, high-quality feed. The rising costs and limited availability of typical feed ingredients like maize and soybean meal are due to their higher demand for both human food and industrial applications (Govoni *et al*., 2021). Although the nutritional advantages of using cassava root meal as an animal feed are well established, its cost-effectiveness in indigenous chicken layers has not been extensively researched. Findings showed that cassava root meal is of nutritional benefit for chickens. However, there is limited information on the analysis of economic evaluation, especially the effect of incorporation in the diet on the performance of improved indigenous layer chicken. Farmers who own small pieces of land and have limited capital as their input to their business have to consider the cost of feeding against the expected revenue to have a viable productivity level (Islam *et al*., 2015). Therefore, the actualization of the utilization of enzyme-treated cassava root meal in the production of poultry feed should be assessed for its economic value.

Cost-benefit ratio (CBR) and return on investment (ROI) are significant indicators. Cost consists of all the expenses incurred in the production and provision of feeds. Feed production costs thus take into account the price of raw materials and other operating expenses such as the enzyme treatment process, labour charges, overhead costs, and other value additions that might be needed (Adedokun *et al*., 2025). The financial burden a farmer may have when shifting his/her

feeding method lies in an accurate estimation of the cost.

Cost-benefit ratio displays the profitability of an investment by juxtaposing the monetary gains, which include factors like increased productivity of eggs and improved health of birds, to the total costs (Ezeoke *et al*., 2022). If the cost-benefit ratio is higher than one, it indicates that the feeding strategy is reaping more benefit relative to the cost it is running, hence, it is profitable. This helps the small-scale farmers to know if CRM is economical to substitute maize in the chicken diet (Ogunsipe *et al*., 2022).

Return on investment complements this evaluation as it shows the net positive or negative cash flow generated from an investment expressed as a percentage of the total amount of money invested in it (Aguihe *et al*., 2020). Positive ROI is an indicator that the feeding intervention will enhance profitability, but negative ROI means a loss of money; hence, re-evaluation of the intervention or adoption of alternative approaches is necessary. These metrics of the economy combined assist farmers, researchers, and policymakers in making decisions (Ojewola &

Okeke, 2016). In this study, the analysis of ROI results revealed that the introduction of enzyme-treated cassava root meal in the chicken meal is a sustainable intervention.

The impact of the addition of cassava root meal to poultry feed on their nutrition and performance has been studied in many research works. The studies showed that the nutrient digestibility and growth rates in broilers and layers were improved when treated with enzymes, but did not influence the productivity, and lowered the feed expenditure (Morgan & Choct, 2016). These studies were likely to give more emphasis on the biological outcome, yet they rarely conducted any economic analysis of the improved performance of indigenous layer chicken. The variety of the cassava processing methods and the involvement of various enzymes, as well as the specifics of the local market demand economic analyses with regard to the regional peculiarities in order to ensure proper outcomes (Aroh *et al*., 2024).

This determined the cost-effectiveness and the economic benefits of various levels of inclusion of enzyme-treated CRM in the diets of enhanced indigenous chicken layers. The study determined the financial feasibility of this method of feeding by quantifying its cost structure and its cost-benefit ratio and ROI. From the results, farmers will receive guidance to optimize feed formulations while adopting cost-effective feeding strategies, which will lead to increased poultry productivity and enhanced rural livelihoods.

6.2 Materials and Methods

6.2.1 Study site

The experiment was conducted in the study site described in section 4.2.1.

6.3 Data Collection

The data on feed intake, number of eggs collected, and costs of inputs were collected and used to calculate the profit, cost-benefit ratio, and return on investment.

6.3.1 Economic analysis

The two economic analysis methods used in this study were cost-benefit analysis (CBA) and return on Investment (ROI), and these were used to determine the most economical choice of treatment for the farmers.

6.3.2 Cost-benefit analysis (CBA)

The cost-benefit analysis (CBA) is a conceptual approach designed to help a decision-maker analysed the benefits and costs of decisions made at the level of a particular project. It is a means to judge the feasibility of an investment by comparing benefits and costs, by quantifying the extent of excess of benefits over costs, and by ascertaining the acceptability of the cost-benefit ratio (CBR) for alternative projects (diet) (Whitman *et al*., 2023). It helps the decision makers to select the option with the most financial return. The profit per treatment was determined by subtracting the cost of production from the revenue generated

Cost-Benefit Ratio (CBR) = $\frac{Total\ income}{Total\ cost}$

The CBR is the cost-to-benefit ratio. A value of CBR >1 indicates that the NPV of benefits for a project is greater than the NPV of the costs, in which case the project is a candidate for further consideration when the value exceeds 1 by a sufficient amount. When the CBR equals 1, it shows that the NPV of expected profits balances out against the costs. Projects with a cost-benefit ratio (CBR) below 1 have higher costs than benefits and should be rejected.

6.3.3 Return on investment (ROI)

Return on investment (ROI) is described as a measurement tool that assesses whether the investment generates extra profits. Return on investment functions as an effective tool to evaluate different investment possibilities by identifying which option maximizes profit and benefits for the project (Nicholson *et al*., 2024). Return on investment involves only two values: the total investment cost and the investment profit.

ROI (%) = $\frac{Gain\ from\ investiment - cost\ of\ investiment}{cost\ of\ investiment} * 100$

6.4 Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) in a completely randomized design (CRD) using the General Linear Model procedure of Statistical Analysis System (SAS, 2023) version 9.4 at a 5% significance level. Before data analysis, the data were tested for normality using Kolmogorov–Smirnov and Shapiro tests. Tukey's test at $p < 0.05$ was used to separate means where means are significantly different. The model used for statistical analysis was:

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

where,

Y_{ij} = observation of the response variable

μ = overall mean

t_i = effect of the $_i$'th treatment at (0%, 25%, 50%, and 75% CRM)

ε_{ij} = random error

6.5 Results and Discussion

6.5.1 Results

Table 6.1 shows the financial indicators of economic consideration.

Table 6.1: Financial indicators of economic analysis

INPUTS	T1	T2	T3	T4
Number of hens	9	9	9	9
Feed intake (Kg)	95.72	96.82	100.05	93.15
Feed cost/kg (KES)	45.74	45.1	45.16	45.25
Total feed cost (KES)	4378.23	4366.58	4518.26	4215.04
Labour (KES)	700	700	700	700
Transport (KES)	1000	1000	1000	1000
Drugs (KES)	500	500	500	500
Drinkers & Feeders (KES)	1000	1000	1000	1000
Total cost (KES)	7578.23	7566.58	7718.26	7415.04
Number of eggs	555	545	570	418
Price/egg (KES)	20	20	20	20
Income (KES)	11100	10900	11400	8360
Profit (KES)	3521.77	3333.42	3681.74	944.963
Cost-benefit ratio	1.46472	1.44054	1.47702	1.12744
Return on investment	46.4721	44.0545	47.7017	12.7439

The feed intake ranged between 93.15 kg and 100.05 kg, with a cost was KES 4,215 to 4,518. Chicken fed on diet T3 produced the highest number of eggs (570 eggs) with an income of KES 11,400, with a profit of KES 3,681, a cost-benefit ratio of 1.48, and an ROI of 47.7%, surpassing all the other treatments. Chicken fed diet T4 had the smallest profit of KES 944.96, the lowest cost-benefit ratio of 1.13, and an ROI of 12.74%, demonstrating poor economic returns.

6.6 Discussion

6.6.1 Cost-benefit ratio

The cost-benefit ratio for T3 was 1.48, while T4 had a lower ratio of 1.13. This implied that for each unit of money spent, T3 generated approximately KES 1.5 return, which demonstrated both resource efficiency and increased financial returns. The high cost-benefit ratio for T3 indicated that enzyme-treated cassava root meal at this level of inclusion offered the best balance between feed cost reductions and productivity enhancements, which led to better economic gains.

These findings are in agreement with a study by Ghazalah and Abd-Elsame (2017), who reported that feeding layer chickens on a diet with 50% cassava root meal supplemented with sodium thiosulphate increased economic efficiency. This was attributed to improved nutrient digestibility and superior overall laying performance. As a result, hens utilised more nutrients from the given feed to optimise production and make effective use of the CRM, and so, improved the economic efficiency of the diet.

Okosun and Eguaoje (2017) also found that 66.6% inclusion of graded cassava grit meal (CGM) supplemented with moringa leaf meal was the best economically, as it gave the best possible returns. They explained this by a balanced diet providing sufficient energy in the form of cassava and proteins in the Moringa leaf meal, increasing economic performance even with the high inclusion level.

Conversely, Nsa *et al*. (2019) carried out a bio-economic analysis to determine the impact of diets with various concentrations of sun-dried cassava root meal on chickens. The experiment discovered that a replacement of maize with cassava root meal above a 25 percent level led to lower growth outputs and low cost-saving, which lowered the economic feasibility of the feed.

6.6.2 Return on investment

Treatment 3 registered a high ROI of 47.7%. The investment received a payoff of nearly fifty percent of the invested amount as profit, giving the poultry producers an opportunity to increase their poultry feed quantities at a more affordable price. This was in line with the work of Aguihe *et al.* (2020), who examined the effects of exo-enzyme (Maxigrain, a multi-enzyme complex of 2 β-glucanases, xylanases, phytases, and arabinoxylanases) as a supplement in cassava peel meal (CPM) utilization. The experiment was that the addition of enzymes at 50% CPM augmented nutrient intake and intake of feed, leading to a decline in the cost of feed and a subsequent rise in profitability.

In the same way, Ezeoke *et al.* (2022) assessed the profitability potential of cassava peel meal used in the diet of laying hens and observed that ROI increased with an increase in the proportion of the blend of more than 20% cassava peel meal with palm oil sludge. The study has indicated that the decreasing cyanogenic compounds and the increased digestibility of the nutrients were due to enzyme treatment, and this lowered the cost of feeds and increased the profit margin. Enzyme treatment was crucial for unlocking cassava's economic potential in layer production.

6.7 Conclusion and Recommendation

Feed costs across treatments ranged between KES 4,215 and 4,518. Hens on 50% enzyme-treated cassava root meal produced the highest number of eggs (570) and achieved the highest profit (KES 3,681.74). Hens on a 50% CRM inclusion level achieved the highest cost-benefit ratio of 1.48 and return on investment of 47.7%; the 75% level had the lowest egg production (418 eggs), profit (944.96 units), cost-benefit ratio (1.13), and return on investment (12.74%), an indicator of poor economic efficiency.

The study concluded that the economic performance was maximized at 50% inclusion of enzyme-treated cassava root meal in the layer diets. It is, therefore, recommended that farmers should incorporate enzyme-treated cassava root meal at 50% in the improved indigenous chicken layer diet.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

7.1 General Discussion

Evaluation of chemical composition of cassava root meal (CRM), performance, egg quality characteristics, and economic analysis of feeding improved indigenous layer chickens. Enzyme-treated cassava root meal was the parameter assessed in this study. The outcomes of the study were then compared with other published works on similar studies in order to have a more grounded grasp of the entire discussion.

There was, however, a considerable decrease in the number of eggs produced at 75%. This study outcome is consistent with Lei *et al*. (2017), who also found a decrease in egg production in the laying hens with 75% CRM in their diets. The results are important as they can be used as a guideline in the optimum inclusion of CRM to prevent the adverse impact on egg production. Egg shape index and eggshell ratio improved significantly with increasing inclusion of CRM in the egg quality features of the laying hens, and were more noticeable at the level of inclusion of 75 percent. This study agrees with the findings of the study conducted by Adeyemi and Asaniyan (2024), who reported a high ESI among the laying hens fed on cassava-based diets. This may be attributed to the high metabolizable energy content of cassava, which enhances its production and deposition in the body, which consequently facilitates the egg formation process. The same was the case with the eggshell ratio, where the ratio also significantly rose as the cassava inclusion increased. The existence of minerals in cassava that also contributed to the enhancement of the bone development can also be attributed to the improved shell quality (Gongruttananun, 2018). Conversely, the findings regarding the impact of cassava on increasing the egg shell thickness were mixed across the research. As an example, Gazi and Mnisi (2024) and Almeida *et al*. (2020) have found a better egg shell thickness at 50 percent of the inclusion level, and Kyawt *et al*. (2014), Gongruttananun (2018), and Adeyemi and also Asaniyan (2024) failed to note any serious improvement. The differences in findings of these studies may be explained by the differences in the way of processing and the degree of inclusion. There was no significant difference between the various treatments on the yolk index. It coincides with the findings of Kyawt *et al*. (2014) and Aregay *et al*. (2021), who reported that the formation.

The presence of cassava did not influence of yolk and its characteristics. The researchers have explained an example of stability in the yolk composition by the fact that cassava is not a very high source of nutrients that can significantly affect the formation and properties of yolk, but is an energy source in the form of carbohydrates. On the other hand, Ofiço *et al*. (2021) found that there was an increase in the yolk index after feeding with cassava root meal when it was used with other feeds such as moringa leaf meal. The synergetic interaction of cassava and the other meal constituents can be attributed to the increase in the quality of the yolk in the present study. The economic analysis revealed that the enzyme-treated cassava with 50 percent inclusion (T3) had the best (high) cost-benefit ratio (1.48). This implied that it was an investment with high profitability. The findings were consistent with the research of Ghazalah and Abd-ElSame (2017), who determined the economic profitability of supplementation of cassava root meal with Enzymes. The reason may be that the nutrient digestibility and laying performance are improved after enzyme supplementation of cassava root meal. Also, the ROI of the diet that contained 50 percent CRM was the most significant (47.7 percent), and this may also serve to validate the potential of valuable financial gains should the enzyme-treated cassava be included. The fact that enzyme-treated cassava can result in valuable financial gains has been backed by the article by Aguihe *et al*. (2020) and Ezeoke *et al*. (2022), who also determined the economic value of enzyme supplementation in cassava diets. They claimed that the addition of enzymes to the cassava diet contributed to the fact that it increased the intake of nutrients and reduced the rate of feed used by the diet, and this contributed to the enhancement of financial gains. The excessive reliance on cassava and no processing, however, did affect the growth performance and egg production of the birds adversely and thus lower ROI, also reported by Ogunsipe *et al*. (2022).

7.2 Conclusions

i. The treatment of cassava root meal (CRM) with an enzyme, Natuzyme, yielded a final product with a higher protein content (7.80%), higher digestibility (82.30%), and lower hydrogen cyanide level than Saccharomyces cerevisiae fermentation (protein: 8.2, digestibility:75.2) and spontaneous fermentation (protein: 4.09, digestibility:64.67). These results indicated that enzyme treatment, as well as fermentation, increased the quality of CRM. Natuzyme® enzyme treatment would be more suitable for treating cassava for poultry feed in terms of the buying cost of the enzyme and its availability, other than fermentation with *Saccharomyces cerevisiae*.

ii. The treatments showed no notable difference in feed intake; thus, cassava root meal was also as palatable. At higher levels of CRM inclusion (75%), the FCR markedly went up, and the egg production declined as the CRM levels went up. The optimal balance of FCR and egg production was in T3 (50 percent enzyme-treated CRM), which indicated the possibility of its use in optimal feed efficiency and egg yield. Consequently, enzyme-treated CRM inclusion in enhanced indigenous layer chicken food should be 50% to get the maximum production.

iii. The addition of 75% CRM (T4) enhanced the quality of the egg shape and shell ratio, 50% CRM (T3) maximized eggshell thickness and yolk-to-albumen ratio, and internal egg quality was better. Nevertheless, yolk index and color did not show any substantial changes, indicating that enzyme-treated CRM had little effect on internal egg quality parameters. The best concentration of CRM has been found to be 50 percent to give the best quality of eggs, and it has been seen that supplementation of the green forages will enhance yolk coloration so that they can be more marketable.

iv. iv. The T3 (50% CRM enzyme-treated) registered the best cost-benefit ratio (1.48), return on investment (ROI) of 47.7, and profit (KES 3,681.74). It was found that the 50% enzyme-treated CRM maximised the economic efficiency, whereas the higher rates did not reduce profitability, and egg production due to inclusion rates (75) highlighted the need to balance the rates of CRM inclusion to achieve the most significant economic yields.

7.3 Recommendations

i. Cassava root enzyme-treatment increased protein content (7.80%) and digestibility (82.30%) of the meal significantly. This is a processing approach that must be applied in cassava root meal processing. It is cheaper, easy to use, and readily available, therefore increasing the nutritional quality of the diet provided and ultimately promoting better health and growth performance of the chicken.

ii. Fifty percent (50%) inclusion of enzyme-treated CRM should be incorporated in the diet as it resulted in the best FCR and percentage egg production values, balancing feed conversion efficiency and egg production, ensuring optimal feed utilization, and economic viability for poultry producers.

iii. Inclusion of 50% enzyme-treated CRM as it led to improved internal egg quality, including yolk-to-albumen ratio and eggshell strength. This enhanced the overall egg quality, benefiting both producers and consumers.

iv. Incorporate 50% enzyme-treated CRM, as this proportion resulted in the highest cost-benefit ratio (1.48) and ROI (47.7%), making it the most cost-effective and economically efficient dietary treatment for maximizing profitability in poultry production.

7.4 Further Research

i. Future studies to be conducted to compare other types of enzyme treatments to find the most optimal in terms of improving the nutritional quality of CRM.

ii. Supplementation of CRM with natural pigments to improve yolk colour for consumer preference, with other beneficial effects to the chickens. Lutein, zeaxanthin, or β-carotene, which are common in natural feed additives such as marigold petals, alfalfa, and corn.

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APPENDICES

APPENDIX 1: Research Ethics Clearance

EGERTON **UNIVERSITY**

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EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

EU/RE/DIR/009
Approval No. EUISERC/APP/399/2025 **10th March 2025**

Nasta Chelangat
Egerton University
P.O. Box 536-20115
Egerton
Telephone: 0703549130
Email: chelangatnasta5@gmail.com

Dear Nasta,
RE: ETHICAL APPROVAL: PERFORMANCE OF IMPROVED INDIGENOUS LAYER CHICKEN FED ON PROCESSED HIGH CYANIDE CASSAVA *(Manihot esculenta* Crantz) ROOT MEAL-BASED DIET

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/399/2025*. The approval period is ***10th March 2025 – 11thMarch 2026***

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.

"Transforming Lives through Quality Education"

APPENDIX 2: NACOSTI permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 604993

Date of Issue: 10/March/2025

RESEARCH LICENSE

This is to Certify that Ms.. CHELANGAT NASTA 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: PERFORMANCE OF IMPROVED INDIGENOUS LAYER CHICKEN FED ON PROCESSED HIGH CYANIDE CASSAVA (Manihot esculenta Crantz) ROOT MEAL-BASED DIET for the period ending : 10/March/2026.

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APPENDIX 3: Publication

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

Chelangat N, King'ori A and Kemboi F

DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i3c.2127

Abstract
Cassava (*Manihot esculenta*) root meal is a widely used feed in tropical regions due to its high carbohydrate content, primarily starch, and its dietary fiber. However, its low protein content and hydrogen cyanide (HCN) restrict its use in animal feed. This study investigated the effects of fermentation and enzyme treatment (Natuzyme®) on the chemical composition, digestibility, and HCN levels of cassava root meal. Four treatments were used. T_1: untreated cassava root meal, T_2: enzyme-treated, T_3: *Saccharomyces cerevisiae* fermented and T_4: spontaneously fermented cassava root meal. The study found that both enzyme treatment and fermentation with *Saccharomyces cerevisiae* significantly improved the crude protein content, in-vitro dry matter digestibility and significantly reduced the fiber and HCN content of cassava root meal. These results suggest that both enzyme treatment and fermentation with *Saccharomyces cerevisiae* improves the nutritional quality of cassava root meal, offering a sustainable and cost-effective alternative to maize for poultry feed, helping to alleviate competition for maize as food for human consumption and animal feed.

Keywords: Chicken, cassava root meal, enzyme, fermentation

1. Introduction

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

APPENDIX 4: Research Pictorial

Improved indigenous layer chicken and Cassava Plant (Google image)

Milling of CRM Ashing CRM samples

Ether Extract of CRM Egg Weight Determination

Yolk Index Determination using a Vanier calliper

Laboratory and egg quality analysis (Chelangat, 2025)

APPENDIX 4: ANOVA outputs

The SAS System

The GLM Procedure

Class Level Information		
Class	Levels	Values
TREAT	4	ET NF UT YT
Rep	3	1 2 3

Number of Observations Read	12
Number of Observations Used	12

The SAS System

The GLM Procedure

Dependent Variable: CP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	74.42006930	24.80668977	156.66	<.0001
Error	8	1.26679279	0.15834910		
Corrected Total	11	75.68686209			

R-Square	Coeff Var	Root MSE	CP Mean
0.983263	7.111171	0.397931	5.595858

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	74.42006930	24.80668977	156.66	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	74.42006930	24.80668977	156.66	<.0001

The SAS System

The GLM Procedure

Dependent Variable: DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2.79957419	0.93319140	22.71	0.0003
Error	8	0.32872868	0.04109109		
Corrected Total	11	3.12830288			

R-Square	Coeff Var	Root MSE	DM Mean
0.894918	0.214001	0.202709	94.72340

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	2.79957419	0.93319140	22.71	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	2.79957419	0.93319140	22.71	0.0003

The GLM Procedure

Dependent Variable: Intake

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	103.1479475	34.3826492	1.04	0.4256
Error	8	264.4070578	33.0508822		
Corrected Total	11	367.5550053			

R-Square	Coeff Var	Root MSE	Intake Mean
0.280633	4.824963	5.748990	119.1510

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treat	3	103.1479475	34.3826492	1.04	0.4256

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	3	103.1479475	34.3826492	1.04	0.4256

The GLM Procedure

Dependent Variable: FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.87389368	0.29129789	22.93	0.0003
Error	8	0.10161799	0.01270225		
Corrected Total	11	0.97551167			

R-Square	Coeff Var	Root MSE	FCR Mean
0.895831	4.619124	0.112704	2.439949

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treat	3	0.87389368	0.29129789	22.93	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	3	0.87389368	0.29129789	22.93	0.0003

The GLM Procedure

Dependent Variable: EGGP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	773.5995634	257.8665211	66.39	<.0001
Error	8	31.0741581	3.8842698		

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	11	804.6737215			

R-Square	Coeff Var	Root MSE	EGGP Mean
0.961383	2.854342	1.970855	69.04762

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treat	3	773.5995634	257.8665211	66.39	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	3	773.5995634	257.8665211	66.39	<.0001

The SAS System

The GLM Procedure

Dependent Variable: ESI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	114.2742690	38.0914230	334.25	<.0001
Error	8	0.9116896	0.1139612		
Corrected Total	11	115.1859587			

R-Square	Coeff Var	Root MSE	ESI Mean
0.992085	0.485213	0.337581	69.57385

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	114.2742690	38.0914230	334.25	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	114.2742690	38.0914230	334.25	<.0001

The GLM Procedure

Dependent Variable: ST

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.09650897	0.03216966	1372.57	<.0001
Error	8	0.00018750	0.00002344		
Corrected Total	11	0.09669647			

R-Square	Coeff Var	Root MSE	ST Mean
0.998061	1.017273	0.004841	0.475903

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	0.09650897	0.03216966	1372.57	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	0.09650897	0.03216966	1372.57	<.0001

The GLM Procedure

Dependent Variable: ESR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	14.71138549	4.90379516	262.81	<.0001
Error	8	0.14927359	0.01865920		
Corrected Total	11	14.86065908			

R-Square	Coeff Var	Root MSE	ESR Mean
0.989955	1.286482	0.136599	10.61800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	14.71138549	4.90379516	262.81	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	14.71138549	4.90379516	262.81	<.0001

The SAS System

The GLM Procedure

Dependent Variable: YI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	396.9405162	132.3135054	6.98	0.0127
Error	8	151.6533234	18.9566654		
Corrected Total	11	548.5938396			

R-Square	Coeff Var	Root MSE	YI Mean
0.723560	10.87786	4.353925	40.02559

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	396.9405162	132.3135054	6.98	0.1127

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	396.9405162	132.3135054	6.98	0.0127

The SAS System

The GLM Procedure

Dependent Variable: YA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	55.43854641	18.47951547	5.84	0.0206
Error	8	25.32216312	3.16527039		
Corrected Total	11	80.76070953			

R-Square	Coeff Var	Root MSE	YA Mean
0.686454	3.293107	1.779121	54.02559

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	55.43854641	18.47951547	5.84	0.0206

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	55.43854641	18.47951547	5.84	0.0206

The GLM Procedure

Dependent Variable: YC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	66.25000000	22.08333333	26.50	0.0002
Error	8	6.66666667	0.83333333		
Corrected Total	11	72.91666667			

R-Square	Coeff Var	Root MSE	YC Mean
0.908571	13.86639	0.912871	6.583333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	66.25000000	22.08333333	26.50	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	66.25000000	22.08333333	26.50	0.0002