# PERFORMANCE OF GROWER RABBITS (*Oryctolagus cuniculus*) FED ON *Prosopis juliflora* PODS FERMENTED WITH *Aspergillus braziliensis* -BASED DIET

**MAUD BOAKYEWAA ACHEAMPONG**

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## DECLARATION AND RECOMMENDATION

**Declaration**

This thesis is my original work and has not been submitted or presented to any institution for the award of any degree.

Signature  Date: 31/03/2024

**Maud Boakyewaa Acheampong**

**KM113/13031/21**

**Recommendation**

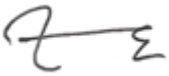
This thesis has been Prepared and submitted with our approval as university supervisors.

Signature…  ……. Date……2/4/2024…….

**Prof. Anthony M. King'ori, PhD**

Department of Animal Sciences,

Egerton University

Signature…  …… Date 03/04/2024 …….

**Prof. Joseph W. Matofari, PhD**

Department of Dairy and Food Science and Technology,

Egerton University

## DEDICATION

To alpha and omega God for His protection and guidance throughout my studies. Lastly, my parents (Harrison Asiedu and Catherine Darkwa) and siblings (Bill Acheampong, Princess Acheampong, Christiana Acheampong and Gabriel Acheampong) for constantly encouraging and supporting me in prayers.

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## ABSTRACT

Commercial rabbit feed is mainly composed of cereals that are also human foods. Usually, there is a scarcity of cereals. *Prosopis juliflora* pods (prosopis pods) are locally available and have been identified as a possible substitute for cereals in animal feed production. However, utilisation of the pods in feed production has been hindered by anti-nutritive factors (ANF) content, which decreases the bioavailability of nutrients. This study evaluated the effects of incorporating prosopis pods fermented with *Aspergillus braziliensis,* a probiotic with potential to break down ANFs rapidly and enhancing production of more nutritive functional products in grower rabbit diet for better performance. The feeding trial was conducted for fifty-six (56) days at Tatton Agriculture Park using forty-eight (48) New Zealand White grower rabbits (24 bucks and 24 does), 42 days old. Four dietary treatments were formulated containing of 0%, 20%, 40% and 60% fermented ground mature prosopis pods (FGMPP)-based diets for eight weeks. The results showed that the ANFs reduced by 10.71% (pectin to 10.5%, Phytate to 0.2% and condensed tannins to 0.01%). The proximate composition increased by 38.4% (protein 16.3%, total fibre 14.2%, total Ash 5.9% and crude fat 2.0%). Rabbits fed on a diet with 60% FGMPP had the highest average daily feed intake, while it was similar in diets with 20 and 40% FGMPP. The diet with 0% FGMPP had the lowest average daily feed intake. The diet with 60% FGMPP had the highest weight gain. The diet with 60% FGMPP had the lowest feed conversion ratio. The growth rate of grower rabbits did not significantly differ between the diet, with 40% and 60% FGMPP. There were no significant differences ($P>0.05$) in the dressing percentages among all prosopis pod inclusion levels. There was no significant difference between the intramuscular fat in all the prosopis pods inclusion levels. The 20, 40, and 60% FGMPP inclusion levels in the diet had no significant effect on the colour and pH of the carcass. From the results *Prosopis Juliflora* pods improved the performance (feed intake, weight gain, feed conversion ratio and growth rate) of grower rabbits. It is recommended that rabbit farmers incorporate up to 60% FGMPP in the diets of grower rabbits.

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| | |
|---|---|
| ADG | Average daily gain |
| ANF | Anti-nutritive factors |
| APD | Animal production division |
| ASALs | Arid and semi-arid lands |
| CF | Crude fibre |
| CP | Crude protein |
| FCR | Feed conversion ratio |
| GDP | Gross domestic product |
| GIT | Gastrointestinal tract |
| GoK | Government of Kenya |
| IMF | Intramuscular fat |
| KNBS | Kenya National Bureau of Statistics |
| LAB | Lactic acid bacteria |
| LS | *Lactobacillus salivarius* |
| ME | Metabolizable energy |
| SC | *Saccharomyces cerevisiae* |
| SFC | Sunflower cake |
| VFAs | Volatile fatty acids |

# CHAPTER ONE
# INTRODUCTION

## 1.1 Background Information

Due to population growth, the need for food in developing nations continues to rise (Fróna *et al*., 2019). The demand for beef in Kenya is anticipated to double by 2030 due to the country's population growth, which is predicted at 2-2.5% per year (Mutsami *et al*., 2019). In Kenya, the human population in 2019 was predicted to be 47.6 million, with an annual growth rate of 2.2% (KNBS, 2019). Population growth has led to high land subdivisions for human settlement, lowering the quantity of land accessible per household. However, 83% of Kenya's land mass is classified as arid or semi-arid and is only suitable for sustainable animal production methods like ranching and pastoralism (Kimani, 2022). This has pressured the available land in high-rainfall regions to intensify agricultural production to ensure a more sustainable food supply. According to Weishaupt *et al*. (2020), the economic reality of developing nations is producing food instead of importing it. Given the available acreage and resources, this must be accomplished by producing livestock species that are easier and inexpensive to raise.

Among the available alternatives for animal protein, rabbit meat has been highlighted as a viable and sustainable option (Mutsami *et al*., 2019). Therefore, rabbit production is anticipated to contribute to developing Kenya's food and nutritional security. Rabbit (*Oryctolagus cuniculus*) meat is considered a Mediterranean food, especially in France, Italy and Spain, because it is lower in fat, cholesterol, saturated fats, sodium and calories than beef, chicken and pork and higher in protein. Essential attributes of rabbit meat to consumers are colour, texture and flavour (Cullere & Zotte, 2018). Lipid oxidation is a significant problem in rabbit meat due to the high content of polyunsaturated fatty acids (PUFA), which can lead to oxidation, reducing the shelf life due to rancidity and colour deterioration. Therefore, effective, safe, low-cost methods for controlling rabbit product stability are critical to the meat industry. Rabbit meat is in high demand for its high nutritional and dietetic properties: its lean and lipids are highly unsaturated (60% of total F.A.), is rich in proteins (20-21%), and its amino acids are of high biological value, it is low in cholesterol and sodium and rich in potassium, phosphorus and magnesium ( Dalle Zotte, 2000), which will help prevent cardiovascular illnesses.

Meat quality not only includes nutritional properties such as appropriate proportions of bioactive compounds, proteins, lipids and their essential sub-constituents, sensory characteristics

such as tenderness, flavour and colour, healthiness such as fat and saturated fatty acids (F.A.), technological factors such as aptitude to be processed, but also views or perceptions about the conditions of animal production concerning animal welfare, the impact of animal production on the environment and, of course, food safety (Hernández & Zotte, 2020). Rabbits are livestock from the order *Lagomorpha* and the *Leporidae* and *Ochotonae* families. The males are known as bucks, the females as doe, and the young as kits. Their weight ranges from 0.5 to 5 kg, depending on breed and age. In most Kenyan regions, rabbit farming has been considered subsistence for young boys (Mbutu, 2013). However, this has altered over time, resulting in a shift in rabbit ownership on Kenyan farms, where 75% of rabbits belong to household heads or spouses, most of whom have attended tertiary institutions (Serem *et al*., 2013). Kenya does not produce enough to feed its human population, and cereals and their by-products are the predominant feed ingredients used in the production of animal feeds, particularly for non-ruminant animals; hence, feed will be scarce for livestock production. Consuming cereals creates rivalry between humans and livestock (Odero-Waitituh, 2015). *Prosopis juliflora* is abundant in arid and semi-arid areas of Kenya, including Tana River, Baringo, Isiolo, Marsabit, and Garissa Counties. Sensory studies on various recipes demonstrated that foods containing 20% prosopis flour have a pleasant flavour (Choge *et al*., 2007). Choge *et al*. (2007) and Odero-Waitituh (2015) observed that the pods contained 69 per cent carbohydrates and an ME value of 12.8MJ/kg, respectively. This makes it a viable energy source in rabbit diets. King'ori *et al*. (2011), in a review article, and Wanjohi *et al*. (2017) did a trial to investigate the growth performance of Kenyan indigenous grower chickens that fed on diets containing prosopis pods and observed a positive growth performance at 20% dietary inclusion. According to King'ori *et al.* (2011), Odero-Waitituh *et al*. (2015), and Wanjohi *et al.* (2017), it has been found that prosopis pods can be used to formulate animal feed in Kenyan ASALs.

Anti-nutritive substances such as high amounts of tannins, CF, phytate and trypsin inhibitor decreases livestock growth and reproductive performance (King'ori *et al*., 2011; Odero-Waitituh *et al*., 2016). These anti-nutrient chemicals decrease the bioavailability of nutrients. According to Odero-Waitituh (2021), fermentation technology, using spontaneous fermentation reduced anti-nutritive factors in animal feedstuffs. Fermentation of sorghum grains with lactic acid bacteria (LABs) decreased tannin levels from 3.34mg/g to 0.57mg/g. It increased essential amino acid content from 91.07mg/100g to 236.88 mg/100g. This study used probiotic fermentation as it promotes gut health by creating a balance between good and bad bacteria. They provide several

benefits for gut health by reducing the adverse effects of harmful bacteria. According to Odero-Waitituh (2021), *in vitro*, microbial fermentation breaks down the linear (1-4)-the linked backbone of D-mannose molecules to which single units of D-galactose are bound by (1-6) linkage. Solid-state fermentation is a process that occurs in a solid matrix, such as a substrate, with or without a certain level of water activity (aw) or free water to initiate metabolic activities. The study focused on the evaluation of incorporating fermented *Prosopis juliflora* (pods) in grower rabbit diet performance.

### 1.2 Statement of the Problem

Commercial rabbit feed is mainly compounded from cereals that humans also consume, creating competition between humans and livestock. Usually, the cereals supply is scarce due to climate change and drought. This makes the quantity of cereals available for livestock feed production low and cost of feed high. This negatively impacts on the productivity of the rabbits leading to food and nutrition insecurity. *Prosopis juliflora* pods are a locally available alternative for cereals in rabbit feed production. However, they have high amounts of anti-nutritive factors including pectin, condensed tannins and phytates that reduce nutrient bioavailability for animal nutrition. Microbial fermentation has been used to reduce the anti-nutrients and resulted in an increase in the nutritional value of rabbit diets. However, the fermentations have been based on spontaneous methods where starter culture is environmental microorganisms whose product quality and safety are not certain. This study determined the effect of incorporating a probiotic-fermented *Prosopis Juliflora* (pods)- based diet on performance (feed intake, weight gain, feed conversion ratio and growth rate) in grower rabbits.

### 1.3 Objectives

#### 1.3.1 Broad Objective

To contribute to sustainable rabbit production by utilising prosopis pods as a feed resource to enhance food and nutritional security.

### 1.3.2 Specific Objectives

i. To determine the effect of fermentation of prosopis pods with *Aspergillus braziliensis* in a controlled fermentation on the proximate composition and pectin, condensed tannins and phytates contents.

ii. To determine the effect of fermented prosopis pods-based diets on feed intake, ADG and feed conversion ratio of rabbits.

iii. To evaluate the effect of fermented prosopis pods-based diets on carcass quality characteristics of rabbits.

## 1.4 Hypotheses

i. Controlled fermentation of prosopis pods using *Aspergillus braziliensis* has no significant effect on the proximate composition and pectin, phytates, and condensed tannins contents.

ii. Fermented prosopis pod-based diets has no significant effect on feed intake, ADG and feed conversion ratio of rabbits.

iii. Fermented prosopis pods-based diets has no significant effect on rabbit carcass quality characteristics.

## 1.5 Justification of the Study

In developing countries rabbit (*Oryctolagus cuniculus*) production is critical in closing the protein malnutrition gap. Commercial rabbit feed is mainly composed of grains, that are usually in scarce supply. Prosopis pods, are a locally available alternative to grains but have anti-nutritive factors (ANFS). There are various traditional methods and technologies, which can be used to reduce the levels of anti-nutritive factors. Several processing techniques and methods such as fermentation (which can reduce the levels of anti-nutritive factors in *Prosopis juliflora* pods), germination, debranning, autoclaving and soaking have been used for reducing these anti-nutritional components in plant materials. This research contributed information on feeding rabbits on prosopis pods-based diet fermented with *Aspergillus braziliensis* to reduce the anti-nutritive factors so as to make the nutrients more available to the rabbit and improve performance. Consequently, this will contribute to achieving sustainable development goals (SDG) 1 and 2 of ending poverty and zero hunger. Therefore, this research has contributed vital information to rabbit

nutrition. Further, the information will contribute to environmental conservation by mitigating against prosopis invasions in ASALs and job creation.

### 1.6 Scope and Limitation of the Study

Since *Prosopis juliflora* grows under different environmental and management conditions, the proximate composition and anti-nutritional compounds may vary (Odero-Waitituh, 2021). The pods for this study were collected from a site with identical climatic and management conditions. Therefore, the pods' chemical composition and anti-nutritional contents were hypothesised to be similar. Results may vary for pods from sites with different conditions. The study limitation is pods from a different environment, hence different composition.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 Overview of the Livestock Industry in Kenya

Up to 10% of the gross domestic product (GDP) (Bulle, 2020) and 6% of agricultural exports (Kogo *et al*., 2021) are attributable to the livestock sector, which also accounts for approximately 30% of the farm gate value of agricultural commodities (Bulle, 2020). Ninety-five per cent of family incomes in ASALs are derived from this sector (MoLD-Strategic Plan, 2008). In addition, the Kenyan government proposed allocating Ksh 249 million to the State Department of Livestock for the fiscal year 2018/19 to implement the 'Big Four' Agenda of ensuring food and nutrition security, industrialisation, manufacturing, and Agro-processing to achieve broad-based economic growth by 2022 (Mungayo, 2018). The industry is comprised of a combination of large-scale and small-scale farmers as well as self-help groups (Odero-Waitituh, 2021), maintaining dairy cattle, beef cattle, rabbits, goats and sheep, fish, rabbits, and emerging livestock (shrimps, quails, and snails), with 3.2% of livestock keepers producing rabbits (Kale *et al*., 2016).

## 2.2 Rabbit Production in Kenya

Scientifically known as *Oryctolagus cuniculus,* Rabbits are considered livestock and belong to the order Lagomorpha and the Leporidae and Ochotonae families. *Oryctolagus cuniculus*, the domesticated rabbit of today, is a descendant of the European rabbit. Six to seven years is the usual lifespan of the domestic rabbit. The males are known as bucks, the females as does, and the young as kits. Their weight ranges from 0.5 to 5 kg, depending on breed and age. Due to the reduction in land size holdings and its contribution to income and nutrition among smallholder farming groups, it is one of the fastest-expanding livestock operations (Kale *et al*., 2016). The Kenyan government has consistently provided free extension and subsidised veterinary services at the County and National levels to support rabbit farming (Borter & Mwanzia, 2011). The Ngong National Rabbit Breeding Centre at Veterinary farm, Ngong, was founded to supply breeding stock to farmers and multiplication centres around the country (Kemose Sheep and Goat Station in Baringo County; Matuga Sheep and Goat Station in Kwale County; Marimba Livestock Government Farm in Meru County and Witiu Livestock Government Farm in Lamu County). According to Mungayo (2018), a budget allocation of Ksh 10 million was made to promote rabbit production in the provisional fiscal year 2018/19 budget. It was designated as one of the sectors

that would drive Kenya's "Big Four Agenda" of ensuring 100 per cent food and nutritional security. The government of Kenya finances and encourages rabbit production. New Zealand White, California White, and their hybrids are the most prevalent rabbit breeds produced by Kenyan farmers (Hungu *et al*., 2013; Ogolla *et al*., 2017; Serem, 2013). Other breeds are the Chinchilla, Dutch, Flemish Giant, French Ear lop, and Kenya White (Hungu *et al*., 2013).

Nonetheless, research conducted by Kale *et al*. (2016) revealed that Chinchilla was the predominant breed in the western areas of Kenya. Compared to the 17.8 million cattle, 17.4 sheep, and 25.4 goats that produced 348.4 metric tonnes of meat for Ksh 139.4 million in 2014, the rabbit population in Kenya is estimated to be 0.9 million (Appendix H). FAO (2017) data showed that the rabbit population increased from 534,000 in 2010 to 961,000 in 2015. Rabbit meat production has also shown an upward trend. Meat production was estimated at 348 Mt in 2015, valued at Ksh 139 million (US$ 1.39 million) (FAO, 2017).

### 2.2.1 Rabbit Production Systems in Kenya

Due to the limited land area in Kenya, the rabbit production system is primarily a small-scale system geared toward family consumption and income generation (Serem *et al*., 2013). Approximately 87.4 per cent of Kenyan rabbit farmers use a smallholder style of production in which 10 to 20 rabbits are raised in cages in the backyard for income generation and domestic use (Odero-Waitituh, 2021). Small-scale rabbit production is dominated by ultra-small and small-scale producers with minimal investment in housing, feeding, and other management practices (Borter & Mwanza, 2011). Unstructured production prevents farmers from accurately predicting the number of rabbits they can offer to the market at any time (Serem *et al*. (2013) classified the Kenyan rabbit production system into ultra-small-scale systems with 0-2 does and a few growers, small-scale systems with 10-30 does, medium-scale systems with 11-50 does, and large-scale systems with more than 50 does, with respective shares of 40.2%, 44.6%, 12.4%, and 2.4%. According to Kale *et al*. (2016), most small-scale farmers employed home-accessible feeds consisting primarily of wild green forage available during the rainy season, tree leaves or bananas during the dry season, and commercial meals at 92.3% and 7.7%, respectively.

### 2.2.2 Commercial Rabbit Diets: The Current Situation

Cereals and their by-products are the predominant feed ingredients used in the production of animal feeds, particularly for non-ruminant animals. Consuming cereals creates competition

between people and livestock (Odero-Waitituh, 2015). These cereals are produced chiefly through rain-fed agriculture. In addition, climate change and drought have contributed to the scarcity of cereals and their derivatives. In 2013, maize production in Kenya was assessed at 3 million tonnes, whereas national consumption was estimated at 4 million tonnes. Almost half of the produced maize produced is estimated to be consumed by non-ruminant animals, resulting in shortages. The 70 kg bag of animal feed in Kenya increased from 2,200 Ksh to 3,100 Ksh in 2019 due to a lack of maize. This was caused by using maize as a feed ingredient, even though it is also a human food (Andae, 2019). This has led to fluctuations in the quantity, quality, and cost of feeds, leading to poor development and breeding performance, which threatens the profitability of rabbit-rearing farmers and protein food security. According to Mburu (2015), most crops used as livestock feed are rain-fed which causes fluctuations in production during the rainy season, as a result dry matter (DM) production is high with higher CP and lower CF percentages and higher digestibility.

In contrast, DM production is lower with lower CP, higher CF percentages, and lower digestibility during the short rains and dry seasons. There is a correlation between the nutritional values of feeds, metabolizable energy (ME) value, and feed utilisation efficiency. There was a 22.75% shortfall between annual feed production and animal demand when rain-fed crops were used to feed animals (Odero-Waitituh, 2021).

### 2.2.3 Challenges of Rabbit Production in Kenya

Poor management practices, inadequate amount and quality of feed, pests and diseases, uncertain markets, lack of quality breeding stock, and local extension services (Hungu *et al*., 2013; Serem *et al*., 2013) have been identified as significant obstacles to rabbit production. According to Okumu *et al*. (2015), the most prevalent diseases in rabbits are diarrhoea, sudden death, and bloat at 81.97 per cent, 73.78 per cent, and 68.85 per cent, respectively and ear canker and pneumonia are other illnesses. Ogolla *et al.* (2017) showed that coccidiosis is the most prevalent disease in smallholder rabbit farms, with a prevalence rate of 49%. In addition, Hungu *et al*. (2013) showed that predation posed a limitation 29% of the time. Other obstacles to rabbit production include susceptibility to heat stress, particularly in the tropics, the requirement of expertise in rabbit rearing and market access (Hungu *et al*., 2013; Kale *et al*., 2016). Rabbits are regarded as pets and are consequently not crucial as food in the traditional structure of the communities (Odero-Waitituh, 2021). Selecting a feeding strategy for rabbit production must ensure a decrease in digestive diseases, greater nutrient utilisation, enhanced growth and reproductive performance,

and cost-effectiveness (Xiccato & Trocino, 2013). In Kenya, rabbits are fed weeds and grass hays, which inhibits their development and reproduction. In addition, Kibugu *et al*. (2019) showed that mycotoxin levels were considerably elevated in commercial feeds and feed additives sold on the Kenyan market, exposing rabbits to chronic levels of mycotoxin. This impairs nutrient absorption, utilisation, and metabolism while predisposing the rabbit to nutritional and metabolic problems. Due to their availability, mature prosopis pods may be utilised in commercial animal feed production. Fermenting the pods for rabbit diets will increase nutrient utilization and decrease anti-nutritional chemicals, particularly mycotoxins.

### 2.2.4 Rabbit Meat Consumption in Kenya

Recent research revealed that consumer food preferences for meat and meat products are impacted by their knowledge of the health consequences of the meats, the descriptive sensory characteristics of the goods, and fair costs (Xue *et al.,* 2009). As a result, rabbit production and consumption are viable for meeting consumer demands and ensuring animal protein supply, hence preventing malnutrition. However, this has not been accomplished since only 42% of the Kenyan population consumes rabbit meat due to marketing difficulties and a lack of understanding regarding the benefits of eating rabbit meat (Borter and Mwanzia, 2011). The Kenyan annual per capita meat consumption was projected to be 10.8 kilograms of red meat and 1.1 kilograms of white meat (MoAL&F, 2015), a decrease of three kilograms for red meat since independence (FAO, 2013). This is significantly lower than the global mean of 34. This must be addressed to boost animal protein availability (Mbutu, 2013). Large numbers of Kenyans, particularly in metropolitan areas, consume red and white meat, with different types regarded as both a luxury and a need. Among the meat items, mutton and chevon were considered necessities, whereas bone beef and chicken were considered luxuries (Shibia *et al*., 2017). However, as per capita income increased and knowledge of the health effects of meat intake grew, white meat consumption increased relative to red meat consumption (Moreno *et al*., 2019). Due to the popularity of the rabbit that produces white meats and the health benefits associated with white meat eating, the urban population of Kenya began replacing red meats with white meats (Magothe *et al*., 2012). Consequently, there are chances in rabbit farming, as there is a large market for their products due to the growing number of people who want white meat.

## 2.3 Influence of Diet on Rabbit Meat Quality

The continued demand for high-quality standards in meat production calls for developing new tools to meet such demands. The quality of rabbit meat largely depends upon the rabbit's Nutrition. Most research conducted on rabbit meat quality in recent years has focused on incorporating bioactive compounds in meat using different feeding strategies. An efficient chemo static appetite regulation mechanism makes daily energy intake constant, but this only occurs at >9.2 MJ digestible energy (DE) $kg^{-1}$. Feeding rabbits ad libitum with a DE concentration >10.45 MJ $kg^{-1}$ is the best meat production regime. Rabbits also require a certain fibre quantity, which limits high DE intake (Hernandez & Zotte, 2020). Nutrient requirements change as rabbits age, and feeding plans have been developed accordingly. The influence of feeding plans on carcass and meat quality has not been shown to have a significant effect. Meat quality consists of (i) nutritional properties, such as appropriate proportions of bioactive compounds, proteins, lipids and their essential sub-constituents; (ii) sensory characteristics, such as appearance, texture and flavour; (iii) health, which depends on fat and saturated fatty acid (SFA) content; and (iv) technological factors, such as processing. It also includes the consumer's perception of animal rearing conditions concerning animal welfare, the impact of animal production on the environment and food safety (Hernandez & Zotte, 2020). Rabbit meat consumption depends heavily on cultural, traditional and religious beliefs. Rabbit meat production is strongly developed in Mediterranean countries and the European Union. Meat sensory properties like palatability, tenderness and odour are crucial in the consumer's choice. Traditional consumers consider rabbit meat to have favourable sensory properties, being tender, lean, and having a delicate flavour (Dalle Zotte, 2002).

## 2.4 Benefits of Keeping Rabbits

Rabbits are generally reared for meat, income generation, fur, and pets (Odero-Waitituh, 2021). Rabbit production is feasible for farmers as it requires less space and low capital for investment; rabbit manure can be used to fertilise crops, and rabbits use crop residues as feed in smallholder systems (Kale *et al*., 2016). Rabbit urine has several merits when used as a fertiliser and pesticide. It can be cheaply sourced in sufficient volumes, contains a high level of nitrates, phosphorus and potassium, which the plant needs to grow, and is environmentally friendly and non-toxic. Rabbits can be reared on diets consisting wholly of roughages. They can utilise herbage biomass more efficiently than ruminants, posing minimal competition with humans for food

(MoLD, 2012). They are highly prolific, producing up to 40 kits a year in three to four kindling. Small-scale backyard rabbit units can be established in the backyard with little start-up capital and land. Other non-marketed benefits of rabbits are the provision of manure by urine and faeces (Mbutu, 2013). Rabbit meat production is, therefore, expected to reduce animal protein malnutrition, create employment and increase incomes in rural and urban populations (Mbutu, 2013).

### 2.5 Benefits of Rabbit Meat to Human Health

The composition of rabbit meat is 21% CP, 7.1% crude fat, and 71.2% water (Odero-Waitituh, 2021). It has the largest concentration of linolenic acid, which decreases inflammation (Alagawany *et al*., 2019). High protein, calcium, phosphorus, low calories and sodium (Nelson, 2011), and less cholesterol than red meat (Petrescu *et al*., 2018). This is beneficial for reducing the incidence of lifestyle disorders caused by dietary cholesterol and high blood pressure. Compared to other meats, the nutritional content of rabbit meat is higher (Nelson, 2011).

### 2.6 The Digestive System of the Rabbit

An animal's digestive system determines the type of feed it can digest. The rabbit's digestive system (Appendix J) differs from that of other domesticated animals in that it is non-ruminant but capable of digesting fibrous foods (Odero-Waitituh, 2021). It has digestive physiology adapted to a sizeable dietary fibre intake, fermented in the hindgut, and a high intake of high-concentrate foods efficiently digested in the upper GIT segment (Odero-Waitituh, 2021). This is facilitated by their feeding and digestive techniques, which include enzymatic digestion in the stomach and small intestine, followed by fermentation of feed leftovers in the caecum and large intestines (Odero-Waitituh, 2021). A rabbit's alimentary canal is 5 meters long, with a short oesophagus, a 3-meter-long small intestine, a 45-centimetre-long caecum, and a 1.5-meter-long colon. Except for the functional caecum, the digestive physiology of the rabbit resembles that of a non-ruminant, as it depends on adrenal secretions (Odero-Waitituh, 2021).

#### 2.6.1 The Caecum of the Rabbit

The caecum accounts for 49% of the overall capacity of a rabbit's digestive tract (Appendix J). The rabbit's digestive system can dig 40% to 50% of digestible fibre and non-starch

polysaccharides pre-caecally. The caecum is the site of microbial fermentation that converts the remaining fibre components into VFAs (acetic, propionic, and butyric acids), the quantities of which are diet-dependent (Chaucheyras-Durand *et al*., 2012). According to Villamide *et al*. (2020), the VFAs absorbed in the caecum and colon meet forty per cent of rabbits' energy needs. The fibre in a rabbit's diet also avoids digestive issues by limiting the expansion of toxin-producing bacteria in the caecum due to excess carbohydrates (Hassan *et al*., 2020). Franck *et al*. (2016) showed high morbidity and mortality in rabbits on low-fibre diets; this was remedied when the level of dietary fibre was raised, resulting in high digestibility due to improved intestinal function and enhanced growth rate.

**2.7 Feeds and Feeding in Rabbits**

Rabbits have a unique digestive system and are omnivorous. Therefore, they can eat a wide variety of non-conventional feed sources. This makes feeding them less expensive, which reduces the expense of raising them. However, the level of inclusion of these non-conventional feed resources should be high enough to assure optimal performance but low enough to minimise toxicity. These include, but are not limited to, citrus pulp, sunflower cake, cotton seed meal, fruit industry by-products, apple pomace, neem seed meal, cow pea hulm, and peanut hulm (Odero-Waitituh, 2021). Rabbits can simultaneously digest fibrous and soluble carbohydrate-containing diets. Liu *et al*. (2018) determined that approximately one-third of rabbit diets comprise fibrous materials such as ground Lucerne hay used in commercial concentrate feed formulations. Therefore, rabbit food ingredients range from readily digestible corn to fibrous wheat straw and sunflower meal (Odero-Waitituh, 2021). Their architecture and physiology permit the digestion of highly digestible foods in the stomach and absorption in the small intestine. The fibrous materials are fermented in the caecum, where volatile fatty acids (VFAs) are released and absorbed in the caecal and colon walls. Cecotropes are also known as caecal pellets or night faeces are material forming in the fermentation of food in the part of the digestive system called the cecum. Cecotropes are rich in nutrients and they are eaten by the animals as they exit the anus to help them absorb the proteins.

In contrast, amino acids formed from the microbial proteins are re-ingested as Cecotropes and absorbed in the small intestines (Odero-Waitituh, 2021). In most tropical places where rabbit production is practised for subsistence by young boys, weeds and grass hays are fed. In large-scale

commercial rabbit farming, commercial rabbit pellets are used as feed. In the tropics, there are numerous weeds and vegetables with varying digestibility, including *Amaranthus hybridus*, *Corchorus olitorius*, *Myrianthus arboreus*, *Ipomea batatas*, *Vigna unguiculata*, *Abelmoschus esculentus, Solanum melongena, Hibiscus sabdariffa, Celosia argentea, Basella alba, Manihot esculenta,* which when fed to rabbits with and without the addition of concentrates, the growth performance is varied. When rabbits were fed vegetables, there were variances in feed intake, feed conversion ratio, and growth rates. Nonetheless, in commercial rabbit feeding and production in many tropical production systems concentrates are crucial feed resources (Franck *et al*., 2016). Other feed resources include, but are not limited to, fruits, grasses, fodder trees, and kitchen wastes such as cabbages, carrots, pineapple, strawberries, broccoli, papaya, high-quality grass hays, fresh grass, mulberry leaves, Leucaena, and Gliricidia.

### 2.8 *Prosopis juliflora* in Kenya: An Overview

Prosopis *juliflora* belongs to the *Fabaceae* family, the *Mimosoideae* subfamily, and the prosopis genus. It is native to South America but was introduced to Africa and Asia as a measure of agroforestry for environmental conservation and desert restoration. The plant is thorny and has a broad, flat-topped crown and numerous genetic variants (Odero-Waitituh, 2021). *Prosopis juliflora* is a tiny, fast-growing, drought-resistant, evergreen plant whose root system (deep taproot) can reach depths of 35 meters. This makes it appropriate for arid regions with yearly precipitation between 150 and 700 mm (Odero-Waitituh, 2021). High economic, environmental, and societal costs have occurred from its invasive nature in numerous parts of the world (Odero-Waitituh, 2021). Prosopis is reported to have colonised more than 4 million hectares in Africa alone, affecting crop and rangeland production, depleting water resources, and displacing native flora and wildlife (Shiferaw *et al*., 2018).

In the early 1970s, it was introduced in Bamburi, a coastal town in Kenya; with the planting materials were from Brazil and Hawaii (Odero-Waitituh, 2021). The Fuelwood afforestation initiative and the National irrigation board planted prosopis on a large scale in the Rift Valley and coastal regions, respectively (Koech *et al*., 2021). Currently, in Kenya, it is widespread in the Rift Valley, particularly in Baringo County (Appendix K), the north-eastern region, and the coast (Appendix L). Prosopis are often fast-growing, drought-resistant, nitrogen-fixing trees and shrubs adapted to poor and saline soils in arid and semi-arid regions (Odero-Waitituh, 2021). Depending

on plant population, rainfall, management approaches, and elevation, the plant matures in three to four years. It produces 2.04 to 17.65 tonnes per hectare of biomass (Ruiz-Nieto *et al*., 2020). As it fixes nitrogen in soils, it can be utilised to improve soil fertility.

### 2.8.1 Nutritional Properties of Prosopis Pods

According to indigenous knowledge regarding the browse preferences of livestock, *Prosopis juliflora* is an excellent livestock feed since it enhances performance (Waitituh, 2021). Several researchers have reported that prosopis pods contain sufficient nutrients (Odero-Waitituh *et al*., 2015; Ruiz-Nieto *et al*., 2020). Odero-Waitituh *et al*. (2015) reported the ME concentration as (12.8MJ/kg). The ripe pods contained 46.35.18 mg/100g of vitamin C, 12% CP, of which only 7% was digestible (Ruiz-Nieto *et al*., 2020). Mohammadabadi & Chaji, (2018) investigated *in vitro* gas production and situ degradation of prosopis leaves and pods in Arabian camels in Iran. They highlighted the potential use of *Prosopis juliflora* pods and leaves as an alternative livestock feed resource. However, Odero-Waitituh (2015) found mineral content of 1.35mg/g and 2.43mg/g for P and Ca, respectively; this was recognised to be below the requirements for most animals, and supplementation was deemed essential. According to Odero-Waitituh *et al*. (2015), the purpose of the P and Ca supplementation is to meet livestock-specific requirements.

### 2.8.2 Anti-nutritive Compounds in Prosopis Pods

The mature prosopis pods are non-conventional feed resources that all classes of livestock can utilise; however, the presence of anti-nutritive chemicals limits their utilisation. Anti-nutritional substances discovered in the pods include alkaloids, tannins, phenolics, steroids, terpenes, and flavonoids (Odero-Waitituh *et al*., 2015; Ruiz-Nieto *et al*., 2020). Odero-Waitituh *et al*. (2015) reported a level of 8% tannins and 17% CF in prosopis pods that hindered the performance of animals, and they suggested that treatment could mitigate their effects. Galactomannans, a non-starch polysaccharide found in mature prosopis pods, are made up of mannose and galactose, which combine to form a linear (1-4) backbone of D-mannose molecules. Single units of D-galactose are attached to this backbone through (1-6) linkage, which prevents enzymatic digestion and reduces livestock performance (Odero-Waitituh *et al*., 2015). Odero-Waitituh (2021) found that these connections were dissolved after four hours of in vitro microbial

fermentation. It has been claimed that treatments such as enzyme fermentation, soaking, and the inclusion of probiotics reduce anti-nutritional chemicals and increase animal performance.

### 2.9 Fermentation in the Enhancement of the Nutritional Value of Feeds

Using bacteria, yeast, and fungi, fermentation converts carbohydrates into organic acids or alcohol. It involves oxidative-reactive processes in which organic substances function as electron donors and acceptors. Fermentation has been used for centuries to preserve foods before the invention of pasteurisation and sterilisation, and every culture consumes a range of fermented foods (Odero-Waitituh, 2021). The fundamental advantage of fermentation is the conversion of sugars and other carbohydrates into valuable end products, creating essential nutrients such as peptides and reduce anti-nutritional chemicals and mycotoxins (Mukandungutse *et al*., 2019). The benefits of fermentation include, but are not limited to, aroma generation, preservation, nutritional value enhancement, and the reduction of anti-nutritional chemicals (Odero-Waitituh, 2021). The principal metabolites and microorganisms involved in fermentation processes can be classified as follows: alcohol and carbon dioxide (yeast), lactic acid (LAB), acetic acid (Acetobacter), propionic acid (Propionibacterium), and ammonia and fatty acids (Bacillus and moulds) (Marco *et al*., 2017).

#### 2.9.1 Types of Fermentation

***Liquid Submerged State Fermentation* (SMF)**

Liquid-submerged state fermentation involves inoculating the required liquid substrate or media with a starter or microbial culture. The bioactive chemicals are secreted into a liquid medium with the use of substrates that are free-flowing. The relevant microorganisms need a high moisture content. However, the parameters that control fermentation will depend on the type of microbe employed as the starting culture and the products required after fermentation (Dawood *et al*., 2020). *Colletotrichum truncatum* with (particle size range = 180 gm to 425 gm) under a microscope was stable and effective in reducing hemp sesbania (*Sesbania exaltata*), a weed in soybean, cotton, and rice. Using propagules of *Colletotrichum truncatum* in liquid-submerged state fermentation, a specialised bioherbicide was created to suppress *Sesbania exaltata* (Odero-Waitituh, 2021).

***Solid-State Fermentation*** **(SSF)**

Solid state fermentation (SSF) is a biotechnological method in which organisms grow and ferment solid substrates with high water absorption in the absence or near absence of free water. It is a process that occurs in a solid matrix, such as a substrate, with or without a specific quantity of water activity (aw) or free water to initiate metabolic activities. This is the moisture required for microorganisms' growth and metabolic conditioning in solid substrates. The lignocellulosic structural characteristics of plant residues provide a strong base and serve as a substrate for microbial fermentation to produce a specific product with added value via SSF. The solid matrix may be inert or biodegradable, with sufficient moisture to sustain microbial development (Saleh *et al*., 2018). The starting culture is inoculated into the solid substrate with the requisite water, and fermentation can proceed. Solid state fermentation has uses in many biological domains, including the generation of biologically active secondary metabolites, the feed and food industry, the manufacture of fuels, and the manufacturing of pharmaceuticals and industrial chemicals. Bioprocesses such as bio beneficiation, bioleaching, bioremediation, and bio-pulping are utilised. Therefore, it is an excellent substitute for liquid-submerged fermentation (Mathur & Sadana, 2021).

Unlike most intensive biotechnological processes, solid-state fermentation has several advantages, including low production costs, stability of the end products, and a high proportion relative to the initial substrates. According to Viniegra-González *et al*. (2017), *Aspergillus niger* grown by solid-state fermentation produced significantly higher levels of the enzymes invertases, tanases, and pectinase. Other benefits of solid-state fermentation over liquid-submerged-state fermentation include, but are not limited to, lower demand on sterility conditions due to common water requirement and activity, lower catabolic repression, higher end concentration of products, cultivation of microorganisms for use in water-insoluble substrates, and cultivation of various fungi in mixed and single cultures (Xin *et al*., 2022). Compared to liquid submerged state fermentation, it has been used to improve animal performance through its application in animal nutrition in terms of the production of enzymes, bioactive components, organic acids, vitamins, and feed additives by bio-transforming products, biological degradation, and detoxification of plant residues/waste. Therefore, the inclusion of SSF biomass has a significant and positive effect on the nutritive composition of feed and the improvement of animal performance, health, carcass attributes, and environmental protection by modulating hemo-biochemical status, gut morphology,

gut microbiota, rumen fermentation, and enteric methane emission in animals (Odero-Waitituh, 2021).

### 2.9.2 Spontaneous Fermentation

This fermentation is spontaneous and uncontrolled, including mixed yeast, bacteria, and fungi cultures. Some microorganisms may function in parallel while others sequentially, with a shifting dominating microflora (Schwan *et al*., 2017). Microbial population and metabolic changes in spontaneously fermenting substrates depend on substrate type and the initial bacteria present, resulting in various microorganisms (Appendix F). High-protein substrates stimulate the development and activity of proteolytic enzymes, synthesising peptides, amino acids, and ammonia (Chukeatirote, 2015). This alkaline substrate pH provides selective conditions for the growth of desirable bacteria, such as *Bacillus* species, while inhibiting negative bacteria growth (Chukeatirote, 2015). During natural, uncontrolled fermentations of carbohydrate-rich substrates (dehulled maize), however, lactic acid bacteria (LAB) and yeast dominated the microbial community for the first 48 hours, after which LAB dominated as fermentation progressed and pH decreased further (Houngbédji *et al.,* 2020). Houngbédji *et al*. (2020) showed an increase in LAB from 3.2 x 106 c.f.u./g (wet wt) to 2 x 109 after 12 to 24 hours of fermentation in dehulled maize prepared at home. After 48 hours of fermentation, the yeast count in commercially produced dehulled maize decreased from 1.3 x 105 to 2.5 x 107 cfu/g. In grape juice-based wine production, *Candida krusei* was the most prevalent yeast, followed by *Candida kefyr*, *Candida glabrata*, and *Saccharomyces cerevisiae*, with *Saccharomyces cerevisiae* dominating after the fermentation process (Wade *et al*., 2019). After 24 to 48 hours, *Enterobacteriaceae* had dropped below the detection threshold. *Enterobacter cloacae* were predominant in commercially produced dehulled corn, whereas *Escherichia coli* was dominant in homemade dehulled maize (Houngbédji *et al*., 2020). The dominating microorganisms in a fermenting substrate may result from contamination or natural occurrence (Ojokoh *et al*., 2015).

### 2.9.3 Fermentation Using Microbial Inoculums (Controlled)

Microbial fermentation entails inoculating or introducing microorganisms into the fermenting substrate to promote fermentation, achieving a quicker and even fermentation rate and a more uniform quality output (Ciani *et al*., 2019). However, the idea that injected microorganisms

suppress naturally occurring microorganisms is not precisely accurate, particularly in wine production, where diverse strains of *S. cerevisiae* were found in the final product (Littleson, 2021).

***Probiotic Fermentation***

According to Lopez-Santamarina *et al*. (2021), probiotics means "for life" and is currently used to refer to bacteria with beneficial effects on humans and animals. The International Scientific Association on Probiotics and Prebiotics (ISAPP) reaffirmed the FAO/WHO definition of probiotics with slight modifications: "live microorganisms that bestow a health benefit on the host when provided in suitable proportions" (Hill *et al*., 2014). Most probiotic microorganisms are bacterial strains of *Lactobacillus, Bifidobacterium, Lactococcus, Bacillus, and Streptococcus,* together with yeast strains of *Saccharomyces*. The food and feed industry has utilised them for decades (Yirga, 2015). According to Amin *et al*. (2021), viable and biologically active microorganisms are typically required at the target site in the host; therefore, the probiotic must be able to withstand the host's natural barriers against ingested bacteria by resisting gastric acid and bile salts, and pancreatic enzymes to adhere to the intestinal mucosa and readily colonise the intestinal tract and exert their beneficial properties (Djezzar *et al*., 2019). When *Saccharomyces cerevisiae* and *Pediococcus acidilactici* were incorporated into broiler feeds, Djezzar *et al*. (2019) showed an improvement in broiler gut health and, consequently, a boost in performance. Common probiotic microorganisms include species of *Lactobacillus, Bifidobacterium* and *Streptococcu*s that are Gram-positive (Amin *et al*., 2021). Probiotic fermentation requires starting cultures with certain desirable features (the physiologic state of the probiotic organisms added, the physical conditions of product storage and the chemical composition of the product to which the probiotics are added) to maintain consistency and economic viability (Hill *et al*., 2014). Lactic acid bacteria (LAB) including the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus,* and *Streptococcus* are the most prevalent and play a vital role in the feed and food industries, fermenting raw vegetables, milk, meat, and cereals because they are associated with several health benefits, including improved gut health and increased nutrient absorption. *Aspergillus niger* is one of the most important microorganisms used in biotechnology. It has been already used to produce extracellular enzymes such as glucose oxidase, pectinase, α-amylase and glucoamylase, organic acids (citric acid), and recombinant proteins. In fact, citric acid and many *Aspergillus niger* enzymes are generally recognized as safe by the United States Food and Drug

Administration. In addition, *Aspergillus niger* is used for biotransformation and waste treatment. In the last two decades, *Aspergillus niger* has been developed as an important transformation host to over-express food enzymes.

# CHAPTER THREE

# THE EFFECT OF *ASPERGILLUS BRAZILIENSIS* FERMENTATION ON BIOCHEMICAL AND NUTRITIONAL COMPOSITION OF *PROSOPIS JULIFLORA* PODS


## Abstract

Fermentation is a technology used to enhance digestibility and improve nutritional value of plant-based animal feeds. Microorganisms break down complex substances producing metabolites with functional properties. *Aspergillus braziliensis,* a probiotic produces a variety of enzymes in large quantities including; tannase, phytases and pectinolytic enzymes such as polygalacturonases, pectin methyl esterase and pectin lyases. It was therefore chosen as a pure culture for *Prosopis juliflora* pods fermentation. This study investigated the effect of *Aspergillus braziliensis* fermentation on biochemical and nutritional composition of *Prosopis julifora* pods as an ingredient in rabbit diet and effects on growth performance and carcass characteristics. The pods without blackening or discolouration were harvested, dried under shade, milled into flour and preserved. Initial proximate analysis and anti-nutritional factors were determined before fermentation. The levels of ANFs were; pectin 23.686%, condensed tannins 0.0238% and phytates 0.473% while the biochemical composition was 16.3% protein, 14.2% total fibre, 5.9% total Ash and 2.0% crude fat. To ferment the flour, optimal growth conditions of *Aspergillus braziliensis* were determined using Box–Behnken Design (BBD) method of Response surface methodology (RSM). The optimum fermentation conditions for *Aspergillus braziliensis* were 34.7 $^{\circ}$ C for 72 hours at pH 5.2. After fermentation of the prosopis flour, the levels of ANFs reduced to; pectin 10.5%, condensed tannins 0.01% and phytates 0.16.%. and the biochemical composition was 18.2% protein, 28.7% total fibre, 6.4% total Ash and 3.7% crude fat as compared to the initial composition before fermentation. These findings indicated that fermentation of *Prosopis juliflora* pods using *Aspergillus braziliensis* as a pure starter culture at optimal conditions established (34.7 $^{\circ C}$ for 72 hours at pH 5.2) increased nutrient bioavailability.


## 3.1 Introduction

Fermentation is the conversion of carbohydrates to organic acids and alcohols using microorganisms. It enriches the diet through development of diversity of food nutrients in food substrates. In animal nutrition, feeds can be more palatable by modifying their chemical

composition by pre-digesting them, and breaking down complex protein structures into peptides and amino acids or complex polysaccharides to simple sugars. This can lead to increased digestibility, especially for fibrous and anti-nutritive materials. Fermentation is an alternative that can improve palatability, reduce ANFs, and provide functional properties to plant-based ingredients. In particular, several studies have shown that fermentation of plant-based protein sources can improve their nutritional and biochemical quality by reducing ANFs.

Plant based animal diets have high amounts of anti-nutritive factors (ANFs) including pectin, condensed tannins and phytates that reduce nutrient bioavailability in animal nutrition. The commonest animal feeds are from cereals which are also staple foods for human diets. This creates a completion and impacts on food security for humans. Alternative animal feeds have been sort for and *Prosopis juliflora* has been identified due to its abundance and richness in biochemical composition as an animal feed. However, it has high amounts of anti-nutritive factors (ANFs). Reduction of these ANFs has been tried using spontaneous fermentation with satisfactory results. Good examples include; Fermenting decorticated *Prosopis africana* seed meal increased its CP% from 22.62 to 42.52, resulting in better broiler growth (Yusuf *et al.* 2008), Fermenting Mesquite bean's decreased CF% from 11.35±0.44 to 8.22±0.03, while the CP% increased from 11.77±0.26 to 15.19±0.02 (Aremu *et al.*, 2015) and after fermentation of. *Prosopis juliflora* pods with *Bacillus subtilis, Bacillus circulans,* and *Saccharomyces cerevisiae* improved CP% and reduced tannin and phytic content (Sarasvati *et al.*, 2014). However, using these environmental microorganisms poses a quality and safety concern because the culture is of mixed environmental organisms whose metabolic pathways are not the same and toxic compounds can form as secondary metabolites in feed hence the safety and quality of the feed is not guaranteed.

This study focused on use of selected and purified probiotic culture, *Aspergillus braziliensis* to ferment *Prosopis juliflora* pods to form part of the plant-based diet for rabbits. *Aspergillus braziliensis* is a probiotic fungus that produces antimicrobial compounds at the same time create more health benefiting compounds from feeds like peptides and amino acids that have functional properties that enhance growth and health performance of animals. *A. braziliensis* also produces a variety of enzymes including; tannase, phytases and pectinolytic enzymes such as polygalacturonases, pectin methyl esterase and pectin lyases. These enzymes are suitable for the breakdown of the ANFs in *Prosopis juliflora*. The fungus also has high multiplication rate, under

optimal conditions and this increases biomass, hence high protein content of the feed. This makes it the choice for the fermentation of plant-based animal feeds to breakdown the ANFs.

## 3.2 Materials and Methods

### 3.2.1 Study site

This study was divided into two distinct phases. The first phase consisted of the pilot study to optimize the fermentation conditions of *Aspergillus braziliensis* including the pH, Temperature and Time. The second phase consisted of fermentation of the *Prosopis juliflora* pods using *Aspergillus braziliensis* to determine the reduction of anti-nutritional factors (ANFs) and enhancement of the biochemical composition of the fermented feed were carried out at Egerton University.

Prosopis pods were obtained from Marigat Sub County in Baringo County, about 130 km from Egerton University. Marigat is located at latitude 0º28'10.1"N and longitude 35º58'59.79"E with a semi-arid area situated 1067 metres above sea level. They were transported to Egerton University's food microbiology laboratory for substrate preparation. Proximate composition analysis and anti-nutritive factors such as phytate, pectin, and tannin (condensed tannins) content determination were conducted at the Animal Science laboratory at Egerton University. Egerton University is located at latitude 0º 23' S and longitude 35º 57' E with an altitude of 2,238m above sea level with an average temperature between 17–22$^{0C}$ but can drop to 11$^{0C}$ during the cold season (July-August). The average annual rainfall in two short and long seasons is 1,200±100 mm. The long rain starts in March and ends in July, while the short rain starts in October and ends in December (Egerton University Meteorological Station, 2019).

**General Sample Acquisition, Preparation and Analysis**

### 3.2.2 Prosopis Pods Harvesting, Drying, Storage and Grinding

Dry, ripened, mature brown pods were collected from the ground after shaking the trees. Pods with any blackening or discolouration or evidence of browsing by livestock or attack by insects or moulds were discarded to avoid infection with aflatoxin. Pods were dried under shade until a constant moisture content of 11% was achieved. The first grinding of the pods was done without passing through a sieve. Second grinding was done according to the procedure described by Choge *et al.* (2006), and flour passed through a 5 mm sieve. The flour was kept in airtight containers to prevent moisture attraction.

***Substrate Preparation*:** About 50 Kg sorted pods were dried under shade to reduce the moisture content to about 11%. It was ground at first without sieving and on second grinding it was sieved through a 5mm pore size sieve. Choge *et al*. (2006). The flour of ground pods was kept in an airtight container to prevent attracting moisture. The flour was then analysed for initial proximate composition (biochemical composition) and determination of levels of pectin, condensed tannins, and phytates.

### 3.2.3 Preparation of Inoculum

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

### 3.2.4 Solid State Fermentation

Thirty grams (30g) of prosopis flour was placed in each of the three (3) 250 ml conical flasks and their moisture content was adjusted to 60% using distilled water. The flour was sterilized at 121$^{\circ C}$ for 15 minutes and cooled to room temperature.

Inoculation was done aseptically with 3 ml of spore suspension and mixed well. The flour was incubated at 30$^{\circ C}$, 38$^{\circ C}$ and 45$^{\circ C}$. In each substrate, the initial pH of the substrate was adjusted to 4.0, 5.0 and 6.0 using 0.1 M NaOH and 0.1 M HCl. Each treatment was monitored for 24, 48 and 72 hours. After incubation, the fermented substrates were dried in an oven at 60$^{\circ C}$ for two days.

### 3.2.5 Proximate Analysis and determination of anti-nutritive Compound Content

Dried samples of fermented ground matured prosopis pods (FGMPP) and unfermented ground matured prosopis pods (UFGMPP) were analysed for proximate composition using the standard procedures of the Association of Official Analytical Chemists (AOAC, 2006). Moisture, Crude protein, crude fibre, Ether extract and Ash were determined using AOAC, (2006) method 950.46, 992.15, 962.09, 923.03 and 920.153 respectively.

***Determination of dry matter***

Equation 1. Calculate dry matter

% Dry matter = 100 - {((Dish + wet sample) - (Dish +dry sample))/weight of sample) *100}

Determination of crude protein

Equation 2. Calculation of % C.P.

% CP = %N * 6.25 *100

***Determination of crude fibre***

Equation 3. Calculation of % CF

Wt of crude fibre (g) = (wt of dish+ residue) - (wt of dish + ash)

Wt of crude fibre (g/) = (wt of crude fibre (g) *1000)/ wt of sample D.M. basis

Determination of ether extract

Equation 4. Calculate the % E.E.

(Wt of lipids /wt of sample D.M. basis) * 100

***Determination of ash***

Equation 5. Calculation of % organic matter

Organic matter % = {((Dish +wet sample) - (Dish +residue))/weight of sample} *100

**Determination of Condensed Tannins, Pectin and Phytate**

***Extraction of Tannins:***

Aqueous acetone (70%) was used in the extraction process. Each dried (finely ground) sample (200 mg) was taken in a glass beaker of approximately 25ml capacity. Ten (10) ml of aqueous acetone (70%) was added, and the beaker suspended in an ultrasonic water bath for 20 minutes at room temperature. The contents of the beaker were then transferred to centrifuge tubes and subjected to centrifugation for 10 min at approximately 3000g at $4^{\circ C}$ using a refrigerated centrifuge (Rahman & Lamara, 2023). The supernatant was collected and kept on ice. The pellet left in the tube was transferred to the beaker using two portions of 5 mL each of 70% aqueous acetone and again subjected the contents to ultrasonic treatment for 20 min. The supernatant was again collected as described above.

***Determination of Condensed Tannins***

The method described by Porter *et al.* (1986) was used to determine condensed tannins in the extracts. The Butanol–HCl reagent (butanol–HCI 95:5 v/v) was prepared by mixing 950 mL of n–butanol with 50 mL concentrated HCl (37%). Ferric reagent (2% ferric ammonium sulfate in 2N HCl) was prepared by dissolving 2.0 g of ferric ammonium sulfate in 2N HCl (16.6 mL of concentrated HCl was made up to 100 mL with distilled water to make 2N HCl). The reagents

were stored in dark bottles. In a 100 mm x 12 mm glass test tube, 0.5 mL of the tannin extract diluted with 70% acetone was pipetted. The quantity of acetone was large enough to prevent the absorbance (550 nm) in the assay from exceeding 0.6. Three mL of the butanol–HCl reagent and 0.1 mL of the ferric reagent were added to the tubes. The tubes capped with a glass marble were shaken using a Vortex and then placed on a heating block adjusted at 97 to 100°C for 60 min. After cooling the tubes, Absorbance was recorded at 550 nm. The absorbance of the unheated mixture (considered a suitable blank) was subtracted from the absorbance of the heated mixture, which was the actual reading at 550 nm, to calculate condensed tannins.

The development of pink colour without heating the sample indicates the presence of flavanols. If this happened, one heated blank for each sample, comprising 0.5 mL of the extract, 3 mL of butanol and 0.1 mL of the ferric reagent, was used. Condensed tannins (% in dry matter) as leucocyanidin equivalent was calculated by the formula: (A 550 nm x 78.26 x Dilution factor)/ (% dry matter). This formula assumes the effective El%, 1 cm, 550 nm of leucocyanidin is 460 (Porter, 1986). Here, the dilution factor equals 1 if no 70% acetone was added and the extract was made from a 200 mg sample in 10 mL solvent. Where 70% acetone was added (for example, to prevent the absorbance from exceeding 0.6), the dilution factor was 0.5 mL/ (volume of extract taken) in the current.

### 3.2.6 Determination of Phytates Content

Phytates content was determined according to Wheeler and Ferrel (1971). Phytates were extracted with 50 ml of 3% trichloroacetic acid and precipitated as the ferric salt, from which Fe was estimated. Phytates was calculated assuming a constant molecular ratio of 4 fe:6 P in the precipitate.

### 3.2.7 Determination of Pectin Content

A blended sample of 50g were put-into a 1L beaker and 300mL 0.01N HCl added. It was boiled for 30 minutes and filtered under suction. The residue was washed with hot water and the filtrate collected. To the residue, 100mL 0.05N HCl was added, boiled for 20 minutes filtered, washed and collected the filtrate. 100mL 0.3N HCl was added to the residue, boiled for 10 minutes, and filtered, washed, and collected the filtrate. The filtrates were pooled, cooled, and made it to a volume of 500mL. Aliquots of 100 to 200mL were pipetted out into 1L beakers. 250mL water was

added and neutralized the acid with 1N NaOH using a phenolphthalein indicator. An excess of 10mL of 1N NaOH was added with constant stirring and allowed to stand overnight. 50mL 1N acetic acid was added; after 5 minutes, 25mL was added in calcium chloride solution with stirring. It was allowed to stand for 1hour, boiled for 1 to 2 minutes and filtered through a pre-weighed Whatman No. 1 filter paper. The precipitate was washed with water that was almost boiling until the filtrate was chloride-free. The filtrate was tested with silver nitrate for chloride. The filter paper with the calcium pectate was transferred, dried overnight at $100^{\circ C}$ in a weighing dish, cooled in a desiccator and weighed.

## Phase One

## Pilot study to optimize the fermentation conditions of *Aspergillus braziliensis* including the pH, Temperature and Time

### 3.3 Experimental Design and Statistical Analysis

To identify the optimal fermentation conditions for *Aspergillus braziliensis* that significantly reduces the anti-nutrient factors in *Prosopis juliflora* pods, a range of fermentation temperatures, pH, and incubation time, as outlined in Table 1, were investigated. There was a total of 30 treatments from the combinations of temperatures, pH, and incubation time within the set ranges using a polynomial Response Surface Methodology (RSM) model.

The minimum condensed tannins, phytates and pectin were optimized; Response Surface Methodology (RSM) with Box-Behnken Design (BBD) with their respective ranges are shown in Table 1.

Table 1: Generation of Coded Levels Using Box-Behnken Design of Response Surface Methodology

| Independent variable | Symbol | Coded levels | | |
|---|---|---|---|---|
| | | -1 | 0 | 1 |
| Temperature ($^{o}$C) | $X_1$ | 30 | 37.5 | 45 |
| pH | $X_2$ | 4.0 | 5.0 | 6.0 |
| Time | $X_3$ | 24 | 48 | 72 |

The runs obtained from Box–Behnken Design (BBD) used for optimization were replicated two times. The statistical model used for optimization is as follows:

$$Y = \beta_o + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_{12} + \beta_{22}X_{22} + \beta_{33}X_{33} + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \varepsilon$$

Where;

Y is the response (cell yield) $\beta_o$ is

the model intercept coefficient

$X_1$, $X_2$, $X_3$ are the independent factors; temperature, time and pH

$\beta_1$, $\beta_2$, $\beta_3$ are linear coefficients

$\beta_{11}X_1^2$, $\beta_{22}X_2^2$, $\beta_{33}X_3^2$ are quadratic coefficients

$\beta_{12}X_1X_2$, $\beta_{13}X_1X_3$, $\beta_{23}X_2X_3$ are interaction coefficients of the independent factors

$\varepsilon$ is the error term.

### 3.4 Results

The optimum temperature, time and pH for fermentation of *Prosopis Juliflora* pods in the reduction of anti-nutritive factors are shown in Figure 1. It was established that optimum fermentation temperature was 34.5$^{oC}$, time 72 hours and pH 5.2 with a composite desirability of 95.56% (D= 0.9556). Under these optimum conditions, pectin was reduced to 10.5% with a desirability of 99.4% (d=0.99399), phytate was reduced to 0.16% with a desirebility of 96.3% (d=0.96288) and condensed tannins reduced to 0.01% with a desirebility of 91.2% (d=0.91178)

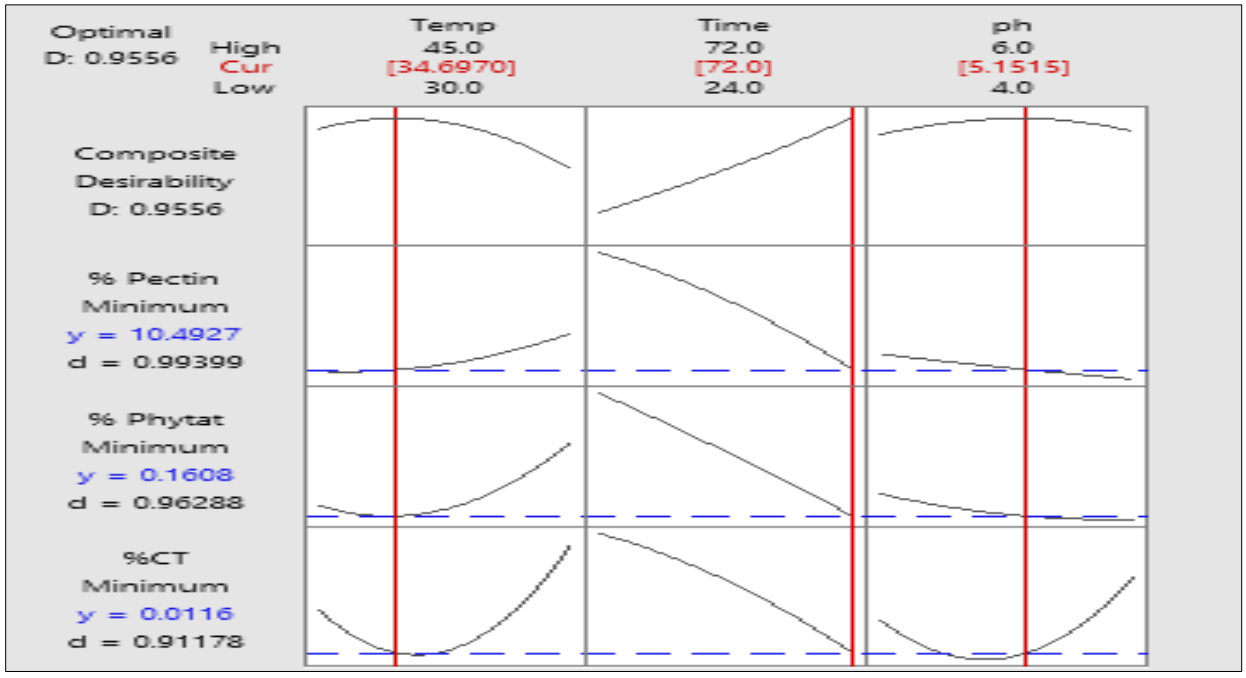


Figure 1: Optimum Temperature, Time and pH for Fermentation of *Prosopis juliflora* Pods in the Reduction of Anti-Nutritive Factors

**Key:** D- desirability.

### 3.5 Discussion

To identify the optimal fermentation conditions for *Aspergillus braziliensis* that significantly reduces the anti-nutrient factors in *Prosopis juliflora* pods, a range of fermentation temperatures, pH, and incubation time, as outlined in Table 1, were investigated. There was a total of thirty (30) treatments from the combinations of temperatures, pH, and incubation time within the set ranges. The RSM analysis successfully predicted the reduction rate of anti-nutritive factors concentration by *Aspergillus braziliensis* in fermenting *Prosopis juliflora* pods among the thirty (30) fermentation treatments. Figure 1 showed that the optimum conditions for *Aspergillus braziliensis* in fermenting *Prosopis Juliflora* pods was a temperature of $34.7^{0C}$, time of 72 hours and pH of 5.2. It reduced pectin from 23.7% to 10.5%, phytate from 0.5% to 0.16% and condensed tannins from 0.02% to 0.01%. This finding agrees with earlier studies which showed that the optimum temperature for *Aspergillus braziliensis* is 35- $37^{oC}$ and pH 5.48 (Chang *et al*., 2018; Pera *et al*., 2006; Srivastava & Kar, 2009).

Das *et al.* (2020) reported that condensed tannins survived temperatures of $120^{oC}$ during extraction. However, higher temperatures than the ones used will destroy *Aspergillus braziliensis* itself and fermentation could not have occurred. pH range used in this study did not significantly influence phytates because phytases are active within pH range 4.5–6.0, and stability decreases dramatically when the pH value is less than 3.0 and greater than 7.5. Optimum pH for fungal-origin phytases is between 4.5 and 5.5 and 6.5 and 7.5 for bacterial origin (Gupta *et al*., 2015). In contrast, extending the incubation period allows for more time to produce metabolites and enzymes responsible for the breakdown of anti-nutritive factors.

## Phase Two

## Fermentation of the *Prosopis juliflora* pods to determine the reduction of anti-nutritional factors (ANFs) and enhancement of the biochemical composition of the fermented feed

### 3.1 Introduction

Alternative animal feeds have been sort for to reduce completion with humans and *Prosopis juliflora* has been identified due to its abundance and richness in biochemical composition as an animal feed. However, it has high amounts of anti-nutritive factors (ANFs). Reduction of these ANFs has been tried using spontaneous (mixed culture from the environment) fermentation with satisfactory results. However, using these environmental microorganisms pose

a quality and safety concern because the culture is of non-selected organisms whose metabolic pathways are not the same and toxic compounds may form as secondary metabolites in feed hence the safety and quality of the feed is not guaranteed.

This study focused on use of selected and purified probiotic culture, *Aspergillus braziliensis* to ferment *Prosopis juliflora* pods to form part of the plant-based diet for rabbits. *Aspergillus braziliensis* is a probiotic fungus that produces antimicrobial compounds at the same time create more health benefiting compounds from feeds like peptides and amino acids that have functional properties that enhance growth and health performance of animals. *Aspergillus braziliensis* also produces a variety of enzymes including; tannase, phytases and pectinolytic enzymes such as polygalacturonases, pectin methyl esterase and pectin lyases. These enzymes are suitable for the breakdown of the ANFs in *Prosopis juliflora*. The fungus also has high multiplication rate, under optimal conditions and this increases biomass, hence high protein content of the feed. This makes it the choice for the fermentation of plant-based animal feeds to breakdown the ANFs,

Using the recommended optimal conditions from the pilot study of $34.7^{0C}$ ($35^{0C}$), pH of 5.2 and time of 72hrs as optimal conditions for *Aspergillus braziliensis* to ferment *Prosopis juliflora* pods, the prosopis pods flour was fermented as per the procedure in the materials and methods and the results and discussion are here below:

## 3.4 Results

### 3.4.1 Proximate Composition and Anti-Nutritive Factors content of *Prosopis juliflora* Pods

The proximate composition (of both unfermented and fermented *Prosopis juliflora* pods is shown in Figure 2. The raw Prosopis *juliflora* pods had 16.3% protein, 14.2% total fibre, 5.9% total Ash and 2.0% crude fat while the fermented one had 18.2% protein, 28.7% total fibre, 6.4% total Ash and 3.7% crude fat.

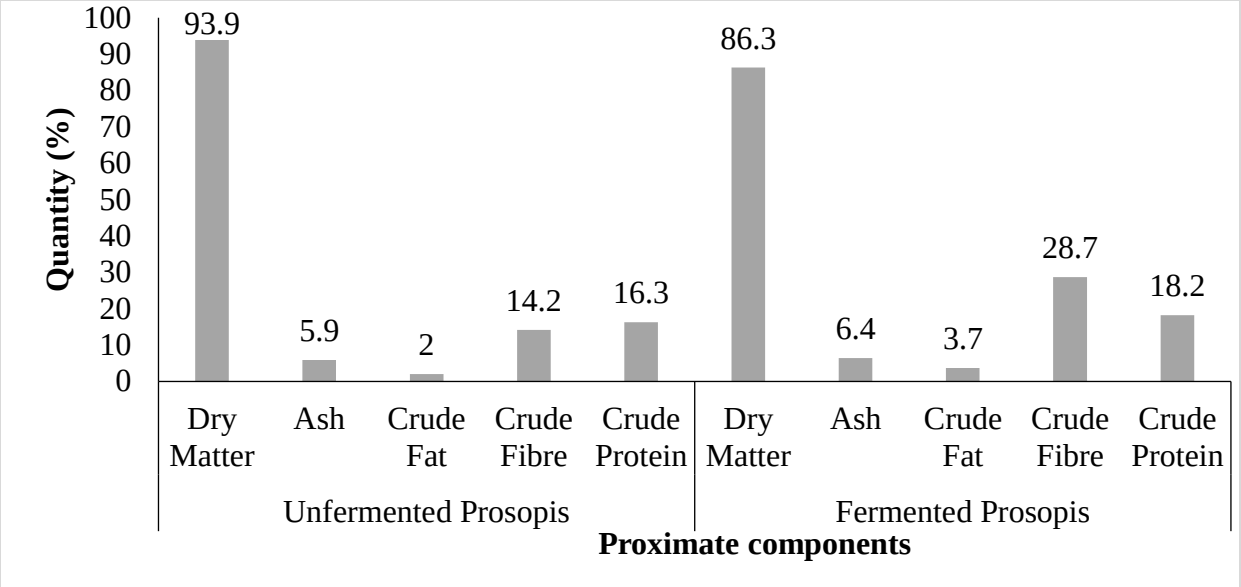


Figure 2: Proximate Composition of Both Unfermented and Fermented *Prosopis juliflora* Pods

The anti-nutritive factors concentration of unfermented *Prosopis juliflora* pods is shown in Figure 3. Unfermented *Prosopis juliflora* pods contained about 23.7% pectin, 0.5% phytates and 0.02% condensed tannins.

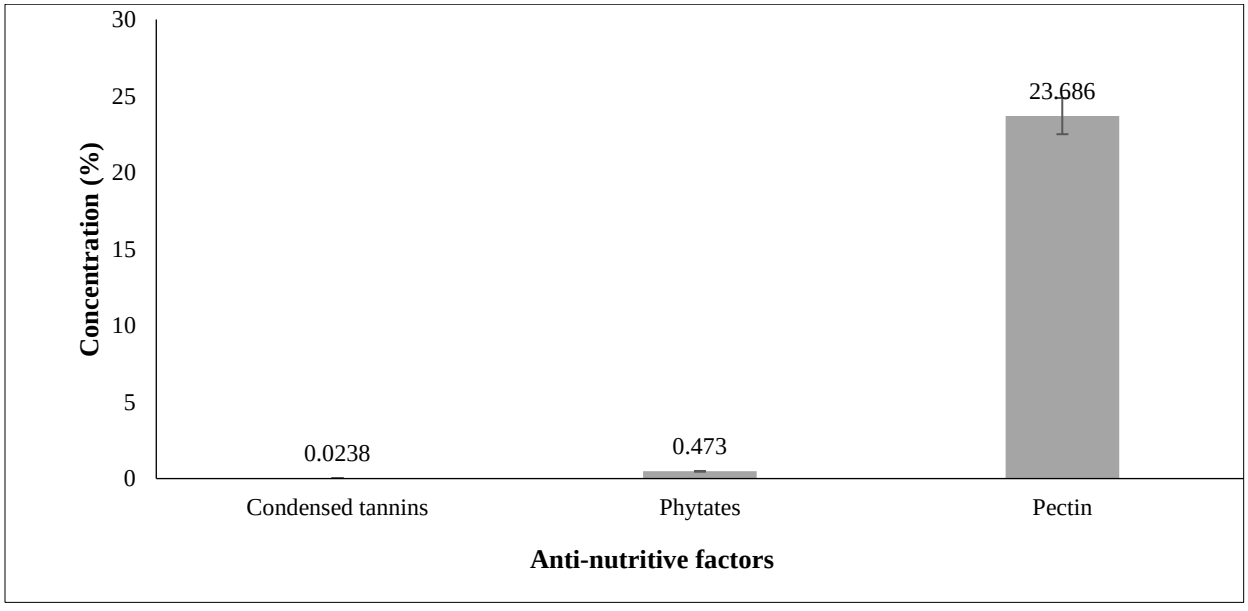


Figure 3: Anti-Nutritive Factors Concentration of Unfermented Prosopis juliflora Pods

**3.5 Discussion**

Fermentation has been used to effectively reduce anti-nutritional factors in feeds for animals and food for humans (Arbab-Sakandar *et al*., 2023). The optimal conditions for microbial

fermentation, including temperature, pH, redox potential, nutrient availability, water activity, and the presence of inhibitors and stimulators vary depending on the specific substrate being fermented and the type of the organism (Mayo *et al*., 2021). With the piloted optimal conditions for *Aspergillus braziliensis* at 34.5 ° C for 72 hours at pH 5, fermentation of *Prosopis juliflora* pods (substrate) was enhanced. Figure 2 shows the difference in biochemical composition (proximate composition) before and after fermentation. The crude fibre and crude protein content significantly increased on fermentation.

The breakdown of anti-nutrients in *Prosopis juliflora* pods by *Aspergillus braziliensis* is by degrading condensed tannins to Gallic acid and flavan-3-ol units, phytates to inositol and free inorganic phosphate as well as pectin to oligogalacturonides and simple sugars (Bhandari *et al*., 2023; Jjaz et al., 2020; Yadav, 2004). The most probable enzymes responsible include xylanases and glucanases that are produced by this organism. *Aspergillus braziliensis* produces an array of enzymes, particularly tannase which specifically target condensed tannins, phytases that specifically target phytates and pectinolytic enzymes such as polygalacturonases, pectin methyl esterase and pectin lyases that target pectin to cleave the intermolecular bonds between their sub units of anti-nutritive factors (Dharmakar *et al*., 2023; Gupta *et al*., 2015; Riaz *et al*., 2022). Its glycolytic pathway converts sugars to pyruvate, entering the TCA (Tri-Carboxylic Acid cycle) cycle for energy production (Wang *et al*., 2019). With a well-developed pentose phosphate pathway, *Aspergillus braziliensis* breaks down pentose sugars from plant biomass (Li *et al*., 2022). The organism has specialized routes of metabolism that enable it to degrade complex polysaccharides and the utilization of organic acids. The fungus adapts its metabolism based on type of nutrient available and optimizing growth and resource utilization (Jo *et al*., 2023; Watkinson, 2016). *Aspergillus braziliensis* synthesizes a diverse range of other biochemical compounds including citric, itaconic, oxalic, gluconic, and kojic acids which are distributed during the fermentation of feed and makes the feed digestible and constitute a source of energy, enhance improving the bioavailability of the minerals by forming complexes, in addition to producing a variety of enzymes (Park *et al*., 2017; Rodriques, 2016). The multiplication rate under solid state fermentation of this organism is about 0.323- per h and this increases its biomass and eventually increases the biochemical composition of the fermented feeds (Aguilar-Zárate *et al.,* 2023), especially crude proteins as shown in Figure 2.

As illustrated in Figure 3, *Prosopis juliflora* pods also contain anti-nutritional factors, which can reduce the bioavailability of nutrients like proteins and minerals. The nutritional profile of *Prosopis juliflora* pods varies depending on the cultivar, growing conditions, and maturity stage. Previous studies reported that *Prosopis juliflora* pods contain approximately 0.5-3.0% condensed tannins, 0.5-2.0% phytate, and 1.0-3.0% pectin (Gayathri & Uppuluri, 2022; Odero-Waitituh *et al*., 2015; Rizwanuddin *et al*., 2023). However, from this study, the pods contain about 23.7% pectin, 0.5% phytates and 0.02% condensed tannins. The difference could be the cultivar, growing conditions, and maturity stage.

Phytates are a storage form of phosphorus that is found in many plants while pectin is a type of soluble fibre and condensed tannins are high molar mass polyphenols biopolymers (Muñoz-Almagro *et al.,* 2021; Rizwanuddin *et al*., 2023). Anti-nutritional factors such as tannins and phytates in cereals have been found to negatively affect the bioavailability of minerals such as iron when consumed in large quantities. Pectin has been shown to decrease non-heme iron absorption. Tannins can bind to protein, reducing its digestibility. Tannins can also inhibit the activity of digestive enzymes. Anti-nutrients bind to proteins and minerals like iron, zinc, calcium, and magnesium, rendering them less accessible for absorption, thereby diminishing their bioavailability (Alemayehu *et al.,* 2021). Therefore, for the preparation of *Prosopis juliflora* pods to be used as feed, fermentation technology should be used to reduce these anti-nutritive factors.

### 3.6 Conclusion

Fermentation of *Prosopis juliflora* pods using *Aspergillus braziliensis* significantly reduced the anti-nutritive factors (condensed tannins, phytate, and pectin) and improved the proximate composition (Ash, Crude fat, Crude fibre and Crude protein). These findings indicated that fermentation of *Prosopis juliflora* pods using *Aspergillus braziliensis* as a pure starter culture at optimal conditions established ($34.7^{\circ C}$ for 72 hours at pH 5.2) increased nutrient bioavailability.

### 3.7 Recommendation

This study recommends a temperature of $34.7^{0C}$ ($35^{0C}$), pH of 5.2 and time of 72hrs as optimal conditions for *Aspergillus braziliensis* in fermentation of *Prosopis juliflora* pods**.**

# CHAPTER FOUR

# EFFECT OF INCORPORATION OF FERMENTED PROSOPIS PODS IN RABBIT DIETS- ON FEED INTAKE, FEED CONVERSION RATIO AND GROWTH RATE


## Abstract

Rabbit feed for commercial use is primarily compounded from cereals that are also human food. It is proposed that incorporating *Prosopis juliflora* pods meal, a locally available substitute for cereals, in rabbit feed production will make rabbit production sustainable. This study examined the effects of incorporating prosopis pods fermented with *Aspergillus braziliensis* in the grower rabbit diet on performance. The feeding trial was conducted at Egerton University, Tatton Agriculture Park using forty-eight New Zealand White grower rabbits. Four dietary treatments containing 0, 20, 40, and 60% fermented ground mature prosopis pods (FGMPP) were formulated. A randomised complete block design was used. From the result of the study, diet with the 60% FGMPP had the highest average daily feed intake, while there was no significant difference between 20 and 40%, and the diet with 0% had the lowest intake. There was an improvement in Average Daily Gain with 60% FGMPP diet. The results on weight gain showed that 60% FGMPP diet had the highest, while the lowest was recorded in the one with 0%. For the feed conversion ratio, diet containing 60% FGMPP had the lowest; there was no significant difference between the diet with 20 and 40%, while the diet with 0% had the highest. There was no significant difference in the growth rate between 40 and 60% FGMPP diet, while the one with 60% differed from the 0% diet. Growth rate did not differ in the rabbits fed on the diets containing 0 and 20% FGMPP. It was concluded that including 60% FGMPP in grower rabbit diet improved performance. Mature pods should be harvested, fermented with *Aspergillus braziliensis* at 34.7$^{\circ C}$ for 72 hours at pH 5.2 and incorporated at 60% in grower rabbit diet to improve performance.


## 4.1 Introduction

Raising rabbits for meat is a rapidly expanding livestock industry worldwide (Borter & Mwanza, 2011). It is believed that the decrease in land-size ownership has prompted farmers to select livestock businesses such as rabbit farming, which require less land and feed resources (Borter & Mwanza, 2011). This is why rabbit farming is particularly suitable for peri-urban farmers who may want to raise their rabbits without worrying about disturbing their neighbours since they are quiet (Omole, 1988). Some of the advantages of rabbit farming include high productivity, early maturity, and a fast growth rate. Rabbits can convert low-cost high-fibre into high-quality meat,

making them a cost-effective choice for meat production. Livestock farming can provide multiple revenue streams, such as the sale of meat, fur, or breeding stock, which can significantly contribute to the farmers' overall income. Their meat is lean, low in cholesterol, and high in protein, making it an appealing option for the health-conscious people (Lukefahr *et al.,* 2004).

In Kenya's rabbit production, small-scale producers invest minimally in housing, feeding, and management practices (Borter & Mwanza, 2011). The high cost of feed resources in livestock production is a major challenge to meeting the demand for animal protein, especially in developing nations. *Prosopis juliflora* pod meal has been identified as the feed resource for livestock. Mature prosopis pods, whether they contain seeds or not, are highly palatable (Wilson *et al*., 2017). These plants produce pods twice a year, resulting in an annual yield of 10-50 kg per plant, according to Sawal *et al.* (2004). Due to the widespread presence of *Prosopis Juliflora* plants in tropical and subtropical regions and their fruiting cycle, harvesting large quantities of pods from forested areas and roadsides is feasible and can provide a source of income for low-income individuals. The pods can be used as an affordable feed alternative by substituting up to 50% of the grain component and bran in rabbits' diets with no adverse effects on their health, as reported by Odero-Waitituh (2015). *Prosopis juliflora* pods have a high protein content and are rich in essential amino acids, including lysine, tyrosine, phenylalanine, and isoleucine, which are necessary to maintain rabbit production (Zhong *et al.,* 2022). *Prosopis juliflora* pods contain high levels of Sodium, Potassium, Calcium, Magnesium, Phosphorus, Iron, Copper, Zinc, and Manganese. *Prosopis juliflora* is a highly versatile plant that has been used in rabbit production for many years. These pods are an excellent source of protein for rabbits due to its high nutritional value (Bhat & Karim, 2009). It is also a good energy source and contains vitamins that are vital for rabbits' overall health and well-being. Therefore, prosopis pods can be used as a feed ingredient for rabbits to promote their health and meet their nutrient requirements.

## 4.2 Materials and Methods

### 4.2.1 Study Site

The study was conducted at Egerton University, Kenya, Tatton Agriculture Park (TAP), Rabbit Research Unit. The University is in Nakuru County, Njoro sub-County. Egerton University is located at latitude 0º 23' S and longitude 35º 57' E with an altitude of 2,238m above sea level with an average temperature between 17–22$^{0C}$ but can drop to 11$^{0C}$ during the cold season (July-August). The average annual rainfall in two short and long seasons is 1,200±100 mm. The long rain starts in March and ends in July, while the short rain starts in October and ends in December (Egerton University Meteorological Station, 2019).

### 4.2.2 Pod Collection and Substrate Preparation

As described in section 3.2.2

### 4.2.3 Experimental Animals and Management

Forty-eight (48) 42-day-old New Zealand White grower rabbits: 24 bucks and 24 does were used. They were housed in flat deck system (cages:75×55×40 cm) suspended 1m above the ground. Before introducing the rabbits to the rabbit house and experimental cages, the watering and feeding troughs were thoroughly cleaned and disinfected with kupacide®. (5mls into 10 litres of water), and dusted with Sevin® to control external parasites.

They were dewormed with ascarex® (2gm into 50mls of water) and dusted with Sevin® (425 mg/kg) as per the manufacturer's prescription to control internal and external parasites, respectively. The rabbits were fed on a standard diet for 3 days (Deblas & Mateo, 2010) as they adapted to the housing.

### 4.2.4 Experimental Diets

Ingredients for formulating the experimental diets were ground and mixed at the Tatton Agriculture Park feed mill, Egerton University. Four dietary treatments comprised 0, 20, 40, and 60% FGMPP (Table 2). The diets were iso-caloric and iso-nitrogenous, formulated to meet the nutrient requirement for grower rabbits (Deblas & Mateo, 2010).

Table 2: Composition of Experimental Diets

| | T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| **Ingredients kg** | | | | |
| Maize grain | 44.0 | 39.0 | 29.0 | 21.9 |
| Wheat bran | 42.0 | 23.5 | 12.5 | 0.0 |
| Vegetable oil | 0.0 | 2.5 | 4.5 | 6.5 |
| FGMPP | 0.0 | 20.0 | 40.0 | 60.0 |
| Fish meal | 3.0 | 4.9 | 4.9 | 8.5 |
| Cotton seed meal | 9.9 | 9.0 | 8.0 | 2.0 |
| Iodised salt | 0.5 | 0.5 | 0.5 | 0.5 |
| pig premix | 0.5 | 0.5 | 0.5 | 0.5 |
| Binder | 0.1 | 0.1 | 0.1 | 0.1 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 |
| *Calculated analysis* | | | | |
| CP% | 16.5 | 16.5 | 16.3 | 16.1 |
| ME (MJ/Kg) | 12.8 | 12.5 | 12.0 | 11.7 |
| CF% | 7.5 | 10.4 | 14.1 | 16.9 |

Treatment 1(T1 Contained 0% Prosopis) Treatment 2 (T2 contained 20% Prosopis) Treatment 3 (T3 contained 40% Prosopis) Treatment 4 (T4 contained 60% prosopis)

### 4.2.5 Data Collection

The following parameters were recorded: feed offered, feed refusal, and live and carcass weights. The following were calculated from the data collected: feed intake, feed conversion ratio (FCR), weight gain (W.G.), Average daily gain (ADG), and dressing percentage (D%). Feed offered and refusal was recorded daily, while live weight was recorded weekly. Feed intake was

calculated daily, while FCR, W.G. and growth rate were calculated weekly. The dressing percentage was calculated at the end of the feeding trial.

***Feed intake***: It was calculated as the difference between feed offered and feed refusal (Abebe & Tamir, 2016).

***Feed conversion ratio***: It was calculated as the average feed intake (g) divided by the average weight gained during each week (Knudsen *et al*., 2014).

***Weight gain*** was obtained by subtracting the previous week's weight from the current week's weight (Chehade *et al*., 2022).

***Growth rate:*** was determined as the difference between final body weight and initial body weight divided by the period between the weighing (Christiansen *et al*., 1996).

### 4.2.6 Experimental Design

A randomised complete block design (RCBD) with sex as the blocking factor and initial weight as a covariate was used. The model was;

$Y_{ijk} = \mu + \alpha i + \beta j + Sk + \varepsilon_{ijk}$

where;

$Y_{ijk}$= response variable of interest

$\mu$= overall mean

$\alpha_i$=effect due to the $i^{th}$ treatment, fermented Prosopis juliflora (diet 1, 2, 3, and 4…. $i^{th}$)

$\beta_j$=fixed effect of the block (sex)

$S_k$ = fixed effect of initial weight to be used as a covariate

$\varepsilon_{ijk}$= random error term.

### 4.2.7 Data Analysis

The data from the experiment was analysed using the GLM (General Linear Model) procedure of the Statistical Analysis System (SAS, 2009) software. Overall data was analysed using a one-way ANOVA test. The mean separation was done using Tukey's HSD test at a significant level of 0.05.

**4.3 Results**

The analysed chemical composition of the diets is shown in table 3. The highest CP was recorded in T4, but there was no significant difference among T3, T2 and T4. There was a significant difference in CF among the treatments with the highest recorded in T4. There was no significant difference in Moisture content (MC) in T2, T3 and T4. Treatment 4 had the highest Ash and Ether extract content (EE).

Table 3: Analysed Chemical Composition of the Diets (D.M. Basis %)

| Diet | **MC** | **ASH** | **EE** | **CF** | **CP** |
|---|---|---|---|---|---|
| **T1** | 10.711±0.16$^{b}$ | 8.087±0.58$^{b}$ | 6.266±0.04$^{c}$ | 12.462±0.04$^{d}$ | 16.329±0.18$^{b}$ |
| **T2** | 11.5272±0.11$^{a}$ | 7.844±0.12$^{b}$ | 4.595±0.06$^{d}$ | 13.993±0.19$^{c}$ | 16.410±0.15$^{b}$ |
| **T3** | 11.818±0.06$^{a}$ | 7.911±0.09$^{b}$ | 7.788±0.03$^{b}$ | 17.982±0.08$^{b}$ | 16.511±0.20$^{b}$ |
| **T4** | 11.753±0.05$^{a}$ | 11.570±0.21$^{a}$ | 8.388±0.03$^{a}$ | 21.613±0.04$^{a}$ | 16.538±0.13$^{a}$ |

$^{abdc}$ means within a column with different superscripts letter are statically different p≤0.05 T1 0% *Prosopis juliflora* pods, T2 20% *Prosopis juliflora* pods, T3 40% *Prosopis juliflora* pods T4 60% *Prosopis juliflora* pods, MC (moisture), E.E. (ether Extract) C.F. (crude fibre), C.P. (crude protein).

Effect of the incorporation of fermented prosopis pods in diets-on feed intake, feed conversion ratio, weight gain and growth rate of rabbit is shown in table 4. Diets T2, T3 and T4 had the highest average feed intake (ADFI)and were similar. Treatment 4 had the highest weight gain and lowest feed conversion ratio (FCR), Treatment 3 and 4 had similar average daily gain (ADG) that was different from T1 and T2. However, T1 and T2 had similar ADG. Feed conversion ratio (FCR) was similar in T2 and T3, was lowest in T1 and highest in T4.

Table 4: Effects of the Incorporation of Fermented Prosopis Pods in Diets- on Feed Intake, Feed Conversion Ratio, Weight Gain and Growth Rate of Rabbit

| Diets | ADFI (g) | Weight gain (kg) | FCR (g) | ADG (kg) |
|---|---|---|---|---|
| **T1** | 109.90±3.60$^{c}$ | 0.100±0.009$^{d}$ | 3.65±0.13$^{a}$ | 1.06±0.01$^{b}$ |
| **T2** | 115.42±2.03$^{b}$ | 0.100±0.010$^{c}$ | 3.01±0.21$^{b}$ | 1.25±0.02$^{b}$ |
| **T3** | 116.96±2.31$^{b}$ | 0.103±0.008$^{b}$ | 2.56±0.18$^{b}$ | 1.59±0.05$^{a}$ |
| **T4** | 124.73±2.34$^{b}$ | 0.105±0.007$^{a}$ | 1.55±0.15$^{c}$ | 1.70±0.08$^{a}$ |
| **P. value** | <.0001 | <.0001 | <.0001 | 0.0003 |

$^{abdc}$ means within a column with different superscripts letter are statically different p≤0.05 T1 Contained 0% Prosopis T2 contained 20% Prosopis T3 contained 40% Prosopis T4 contained 60% Prosopis ADFI: average daily feed intake, FCR: feed conversion ratio.

### 4.4 Discussion

The highest ADFI was recorded in the rabbits fed on the diet with 60% FGMPP because it had high fibre content which affects the rate of digestion of the feed, absorption of nutrients, and the movement of waste products (faeces) through the colon compared to the rest. This result agrees with the findings of Chaudhury *et al.* (1995), who reported that rabbits given higher fibre consumed more feed and gained more weight than rabbits on lower-fibre diets. Rabbits are hind-gut fermenters, which means that they have a huge organ called the caecum that contains lots of microbes to break down the tough fibre in their diet. This is vital for their overall gut health, mobility, caecotrophy, and appetite stimulation. (Tissue, 2020). They have a high feed intake, consuming 65-80 g of feed per kg of body weight, and produces Cecotropes. This allows them to consume more feeds while still meeting their nutritional requirements (De-Blas & Wiseman, 2003). According to Attia *et al.* (2021), the digestibility and intake of fibre residues with high lignin content can be enhanced through physical and biological treatment. Several studies have been conducted on using exogenous microorganisms to boost nutritional usage (Falcao-Cunha *et al.,* 2007), increase growth rate, and improve feed conversion ratio (Eiben *et al.,* 2004).

The treatment of the prosopis pods with *Aspergillus braziliensis* in this study also improved utilisation by the grower rabbits to increase growth rate. According to a study conducted by Gado *et al.* (2009), the inclusion of *Aspergillus braziliensis* in the diet of rabbits increased caecal fermentation. This, in turn led to improved feed utilisation Gutierrez *et al.* (2002) also observed

that supplementing enzymes like lysozyme in a rabbit diet resulted in increased nutrient digestibility and improved feed conversion ratio. From the results, 60% of FGMPP-based diets had the highest weight gain, while the lowest weight gain was recorded in diets with 0%. The diet with 40% resulted in higher weight gain than 20% FGMPP. The improved weight gain in rabbits fed on 60% FGMPP could be a result of the increased efficiency of feed utilisation by the rabbits due to the improved nutritional value from fermentation, which reduced the anti-nutritive factors (condensed tannins, phytate and pectin) in the diets. Fermentation improves the nutritional quality of non-conventional feed resources (Yao *et al.,* 2018). Wang *et al.* (2018) reported that the quality and digestibility were noticeably enhanced via solid-state fermentation (SSF) using lactic acid bacteria. These increments in weight gain of rabbits may be attributed to the effect of fibre, which may increase caecal length, decrease pH, NH3-N (mmol/l), and increase the digestible protein (Battaa *et al.,* 2013). Concentrating on converting ammonia-N into microbial protein benefits rabbits characterized by pseudo-rumination caused by delayed return of the fibrous silage particles into the reticulum. It may also be due to the increase of volatile fatty acids (VFA) in the cecum (Bakr, 2019). Prosopis pods are high in protein, fibre, and minerals (Sawal *et al.,*2004). According to Ruiz-Nieto *et al.* (2020), rabbits fed *Prosopis juliflora* pods exhibited better weight gain and feed conversion ratio than those not fed the same. Jiwuba *et al.* (2018) found that rabbits fed with *Prosopis juliflora* pods did not experience any negative effects on their haematological and serum indices, even with a 30% inclusion rate. Based on this, the researchers suggested that including *Prosopis juliflora* pods up to 30% in rabbit diets is safe without any harmful consequences. However, in this study, prosopis pods were included up to 60% without negatively affecting the performance of the rabbits. The feed conversion ratio measures how efficiently the rabbits convert their feed into meat or other desired products. The results for the feed conversion ratio showed that the rabbits fed on the diet with 60% FGMPP had the lowest because they utilised their feed more and were able to produce more meat per unit of feed consumed, this agreed with a study by Adamu *et al.* (2013) who reported that feed conversion ratio (FCR) was lower with increasing levels of prosopis pods, where lowest value was recorded in 40% fermented prosopis pods inclusion in diets. Monitoring and improving the feed conversion ratio can also help prevent overfeeding (Knudsen *et al*., 2014).

The increased growth rate was attributed to the fermentation of *prosopis juliflora* pods with *Aspergillus braziliensis* that reduced the anti-nutritive factors that hinder nutrient utilisation.

However, *Prosopis juliflora* pods also have a growth-promoting activity such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, siderophore, and indole acetic acid (IAA) and produce compounds that can potentially act as manipulators of hindgut fermentation. These compounds exhibit antibacterial, antioxidant, antifungal, anthelminthic, and antitumor activity. (William & Jafri, 2015). These fermentations activities result in optimised propionate production, decreased deamination of dietary amino acids, reduced methanogenic bacteria, increased protein flow to the small intestine, and improved diet digestibility, Tapia *et al.* (2000) which agree with the results of this study. Adequate dietary fibre intake reduces digestive issues (intestinal infection), enhances intestinal motility, and promotes growth in rabbits (Johnson-Delaney, 2006). Rabbits fed on high fibre diets have been reported to gain weight better than those on low fibre. This is because the total production of acetate and propionate is slightly greater for the high fibre diet, because of the larger caecal volume. For butyrate, the increase due to volume is offset by the higher production rate in the rabbits fed the low fibre diet. Adamu *et al.* (2013) reported that better performance was obtained by replacing 50% of the maize with fermented *Prosopis juliflora* pods (20% inclusion) in the diets of growing rabbits. Therefore, grower rabbits' performance was not compromised when fed diets with 60% *Prosopis juliflora* pods (fermented with *Aspergillus braziliensis*).

### 4.5 Conclusion

From the results, it was concluded that incorporating 60% *Prosopis juliflora* pods fermented with *Aspergillus braziliensis* (FGMPP) at 34.7$^{\circ C}$ for 72 hours at pH 5.2 in a grower rabbit diet improved average daily feed intake, average daily gain and feed conversion ratio.

### 4.6 Recommendation

It was therefore recommended that to reduce the competition for cereals between humans and livestock for feed production and manage the rapid spread of prosopis trees in the ASALs of Kenya, prosopis pods should be harvested, fermented with *Aspergillus braziliensis* and incorporated at 60% into the grower rabbit diet.

# CHAPTER FIVE
# EVALUATION OF CARCASS QUALITY CHARACTERISTICS OF RABBITS FED FERMENTED PROSOPIS PODS-BASED DIETS


## Abstract

The basis for estimating the nutritional requirements of growing animals can be found in understanding the changes in the body composition that occur throughout growth. This study evaluated the carcass quality characteristics of rabbits fed fermented prosopis pods-based diets. A feeding trial using *Prosopis juliflora* pods fermented with *Aspergillus braziliensis* at a temperature of $34.7^{0C}$ ($35^{0C}$), pH of 5.2 and time of 72hrs was incorporated at (0%, 20%, 40% and 60%) of diet was conducted at Egerton University, Tatton Agriculture Park. It used forty-eight New Zealand White grower rabbits, forty-two days old. At the end of eight-week study, one rabbit from each treatment was selected randomly, identified and fasted overnight (12 hours) with *ad-libitum* provision of drinking. They were slaughtered according to the welfare law and carcass pH ($pH_{0\,h}$), colour (ranging from light brown to dark brown) and intramuscular fat (IMF) were taken. The descriptive organoleptic evaluation was conducted in the sensory room at Egerton University, Department of Dairy and Food Science and Technology. Pre-screening questionnaires (Appendix D) were administered to select eight (8) panellists. The study results showed no significant differences ($P > 0.05$) in the dressing percentages among all the treatments. The panellists did not find a significant difference between the intramuscular fat in all the treatments. There was a significant difference between before and after of chilling rabbit carcass of diet with 0% FGMPP in the pH. The panellist did not find any significant effect of diet with 20, 40, and 60% FGMPP on colour and pH. This study showed that fermented ground mature prosopis pods (FGMPP) levels in diet did not affect the meat quality attributes. Therefore, 60% *Prosopis juliflora* pods fermented with *Aspergillus braziliensis* incorporated in grower rabbits' diets did not compromise their carcass quality characteristics.


## 5.1 Introduction

Rabbit farming is becoming a significant agricultural enterprise in Africa (Steven *et al.,* 2022). This is particularly evident due to society's growing interest in knowing their food's origin and production methods. Rabbit meat is the main product of the rabbit industry and are also raised for wool and exported to other countries to produce high-quality luxury garments. In addition to being raised commercially for their meat, wool, and fur, rabbits are also bred in large numbers for

laboratory use (Steven *et al.,* 2022). Rabbits have become famous for poverty reduction initiatives due to their low investment cost. They can subsist on available feed resources for feeding, housing, and general management. As such, rabbit farming has been identified as a potential avenue for low-income individuals to improve their circumstances (Lukefahr, 1999). The sustainability of rabbit production hinges on two key factors: nutrition and on-farm feed security. Developing non-conventional feed resources is essential, and rabbit nutrition is an internal factor that plays a significant role in this process (Lukefahr, 2004). In Africa, there is an abundance of plant protein that can be utilised to compound rabbit feed. To ensure year-round feed availability, implementing a forage security plan within a farm is recommended, according to Lukefahr (2022). Forages, cereals, and their by-products are the predominant feed ingredients used in the production of animal feeds, particularly for non-ruminant animals, rabbits, and poultry. Consuming cereals creates competition between humans and livestock (Odero-Waitituh, 2015). Exploring non-conventional feed sources that could be used in rabbit feed is therefore crucial. *Prosopis juliflora* has been identified as a non-conventional feed resource in rabbit production since it enhances performance (Odero-Waitituh, 2021). Odero-Waitituh *et al*. (2015) and Ruiz-Nieto *et al*. (2020) reported that prosopis pods contain sufficient nutrients for livestock performance Odero-Waitituh *et al*. (2015) reported the ME concentration as 12.8MJ/kg. The ripe pods contained 46.35.18 mg/100g of vitamin C, 12% CP, of which only 7% was digestible (Ruiz-Nieto *et al*., 2020). The protein content of rabbit meat is around 21%, and the total mineral content is 1.5%. The sodium content of rabbit meat is relatively low (49 mg/100 g), whereas the phosphorus level is high (277 mg/100 g). The cholesterol content is also in the beneficial range of 59 mg/100 g with a better omega 6/omega 3 ratio of 5.9, which makes rabbit meat attractive for health purposes (Combes, 2004). The meat significantly addresses food scarcity in many countries (Khalil *et al.,* 2016). Recently, the quality features of food products, including rabbit meat, have garnered increasing interest. Meat products from rabbits are known for their high nutritive value, better taste, and quality attributes, as indicated by Horsted *et al.* (2010).

## 5.2 Materials and Methods

### 5.2.1 Study Site

The descriptive organoleptic evaluation was carried out in Egerton University, Department of Dairy and Food Science and Technology sensory room. Egerton is located at latitude 0° 23'S and longitude 35° 57'E with an altitude of 2,238m above sea level with an average temperature between 17–22$^{0C}$ but can drop to 11$^{0C}$ during the cold season (July-August). The average annual rainfall in two short and long seasons is 1,200±100 mm. The long rain starts in March and ends in July, while the short rain starts in October and ends in December (Egerton University Meteorological Station, 2019).

### 5.2.2 Training and Selection of Panellists

The administration of pre-screening questionnaires narrowed down the selection of panellists to eight candidates. The pre-screening questionnaires included questions about panellist availability and the ability to differentiate colours (Appendix D). The panel was trained according to the ISO procedures (2012). In the pre-screening testing, the assessors were trained to develop sensory descriptors and define the sensory attributes. This was done to select candidates who were verbal about organoleptic properties and could participate in the rabbit meat organoleptic evaluation. During the orientation sessions, the panel agreed on the attributes to evaluate. They evaluated several meat samples from the rabbits offered (control diet, diet with 20, 40, and 60% FGMPP inclusion levels) and rated their intensities (agreed upon by the panellists). The samples were used as warm-up and were provided to the panellists to enable them to identify the intensities and develop the sensory lexicon for colour during the descriptive sessions. From the pre-screening questionnaires, eight of the candidates were selected according to the procedure by Meilgaard *et al.* (2000) as verbal concerning sensory properties. They were then trained in both qualitative and quantitative meta-descriptions. The organoleptic lexicon for describing the colour of the rabbit meat was developed during the training, Appendix F.

### 5.2.3 Data Collection

At the end of eight-week study, three rabbits from each treatment were randomly selected, fasted overnight (12 hours) with *ad-libitum* provision of drinking water and weighed before slaughter for carcass characteristics evaluation. Slaughtering was done according to welfare law (Lafuente & Lopez, 2014). The head of the rabbit was dislocated, and the rabbit was held firmly

by the rear legs and head; it was stretched full length. Then, with a hard, sharp pull, the head was bent backwards to dislocate the neck. One of the hind legs above the hock joint hanged the rabbit. The head was immediately removed to allow complete bleeding. The forefeet were then removed. The next step was to cut the skin around the hock joints of the legs and then cut between these points across the lower part of the body. The tail was removed, and the skin was pulled down and forward over the body as shown in figure 4. Hot carcasses were weighed.

Plate 1: Pictorial View of Slaughtering Rabbits

**Source**: Rabbit -SINT Tecnologie

***Retrieved date 05/11/ 2023***

***Dressing Percentages:*** As Maidala *et al*. (2020) described, dressing percentages were calculated as follows:

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

***Measurements of pH, Colour and Intramuscular fat (IMF)***

Measurements of pH ($pH_{0\ h}$) and colour (ranging from light brown to dark brown) were taken within 15 minutes after slaughter. After carcasses had been chilled for 24 hours at 4℃, measurements were taken for pH ($pH_{24\ h}$), colour (ranging from light brown to dark brown), and intramuscular fat (IMF). pH, intramuscular fat (IMF), and colour measurements were taken from

the *Biceps femoris* and *longissimus dorsi* muscles. The pH measurements were taken with a probe using a pH meter. The probe was inserted directly into the muscle to a depth of 3 mm. The colour was measured using a scale of 15cm long, ranging from light brown to dark brown. The intramuscular fat (IMF) was determined using the marbling reference standards shown in Appendix E.

### 5.2.4 Data Analysis

The data from the experiment was analysed using the GLM (General Linear Model) procedure of the Statistical Analysis System (SAS, 2009) software. Overall data was analysed using a one-way ANOVA test. The mean separation was done using Tukey's HSD test at a significant level of 0.05.

## 5.3 Results

Effect of the level of prosopis pods (FGMPP) inclusion in feed on dressing percentage is shown in figure 5. There were no significant differences in dressing percentage among treatments (T1, T2, T3 and T4).

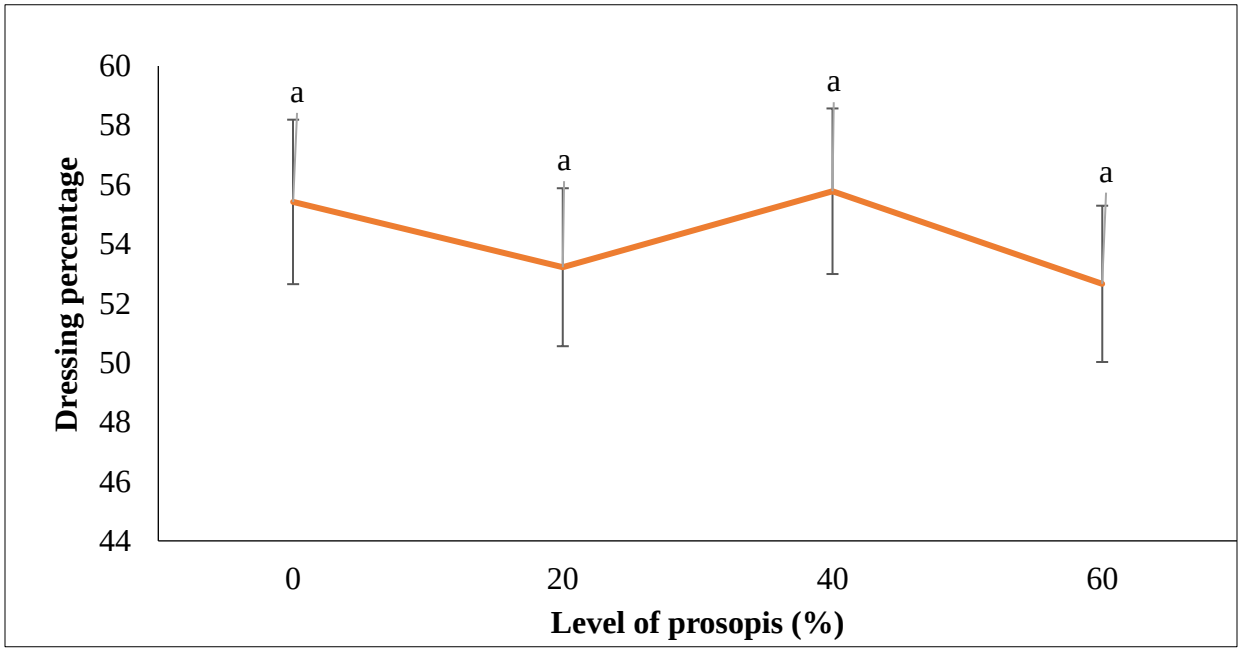


Figure 4: Effect of the Level of Prosopis Pods (FGMPP) Inclusion in Feed on Dressing Percentage

Effect of the level of prosopis pods (FGMPP) inclusion in feed on intramuscular fat is shown in figure 6. There were no significant differences among treatments (T1, T2, T3 and T4).

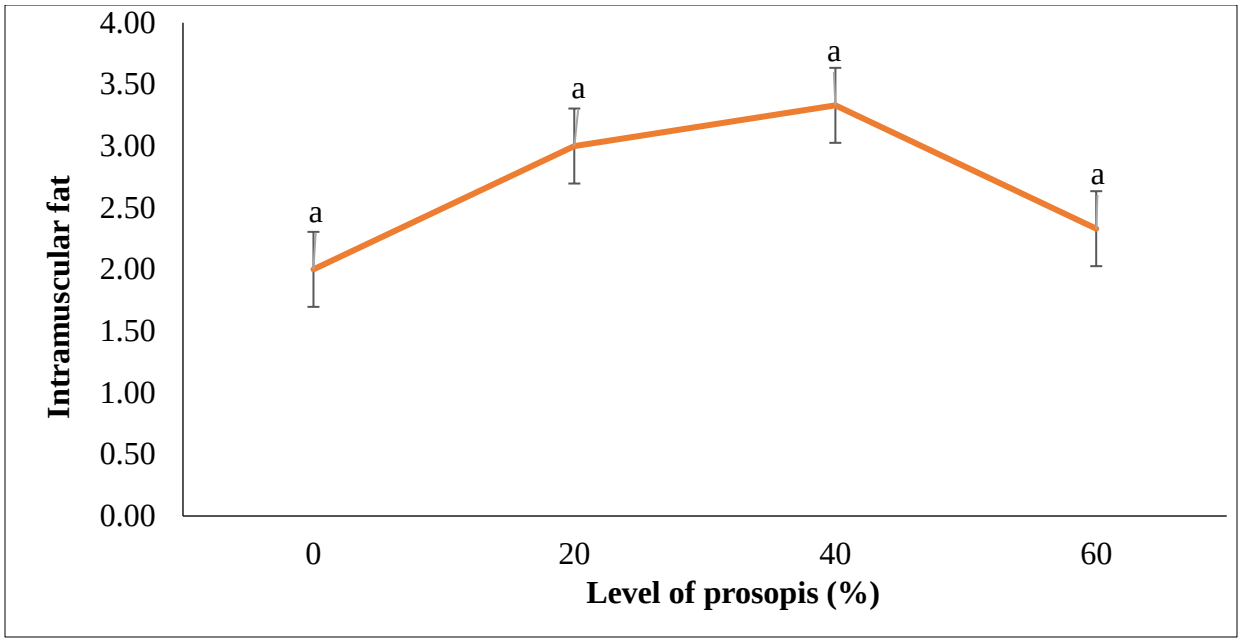


Figure 5: Effect of the level of Prosopis pods (FGMPP) inclusion in feed on intramuscular fat

Effect of chilling on carcass pH and colour is shown in table 5. There were no significant differences in pH and colour of carcass among T2, T3 and T4.

Table 5: Effect of Chilling on Carcass pH and Colour

| Prosopis level | | pH | | Colour | |
|---|---|---|---|---|---|
| | | Mean | (Confidence limit) | Mean | (Confidence limit) |
| T**1** 0% | Before chilling | 7.30[a] | (6.65- 7.95) | 2.25[b] | (1.73- 2.77) |
| | After chilling | 6.86[a] | (6.63- 7.08) | 4.71[a] | (3.44- 5.98) |
| | t-value | | -2.79 | | 3.70 |
| | p-value | | 0.08 | | 0.001 |
| T**2** 20% | Before chilling | 7.47[a] | (6.93- 8.06) | 3.21[a] | (1.83- 4.59) |
| | After chilling | 6.93[a] | (6.82- 7.03) | 4.67[a] | (3.12- 6.21) |
| | t-value | | -4.22 | | 1.46 |
| | p-value | | 0.05 | | 0.15 |
| T**3** 40% | Before chilling | 7.11[a] | (6.70- 7.53) | 3.17[a] | (1.50- 4.83) |
| | After chilling | 6.80[a] | (6.50- 7.10) | 4.21[a] | (2.43- 5.98) |
| | t-value | | -2.65 | | 0.89 |
| | p-value | | 0.06 | | 0.38 |
| T**4** 60% | Before chilling | 7.44[a] | (6.87- 8.02) | 4.04[a] | (2.64- 5.45) |
| | After chilling | 6.90[a] | (6.83- 6.96) | 5.92[a] | (4.08- 7.75) |
| | t-value | | -4.07 | | 1.68 |
| | p-value | | 0.05 | | 0.10 |

[a]Means with the same superscript letter are not significantly different

**5.4 Discussion**

Results on dressing percentage are presented in Figure 6. There were no significant differences (P> 0.05) in the dressing percentages among all the treatments. This is because decreasing anti-nutritional factors of prosopis pods increased bioavailability of the nutrients, hence supporting rabbits' growth. Other factors such as rabbits being of the same age, sex and breed may also play a role in dressing percentage. These findings are consistent with those of Igwebuike *et al*. (1995), who reported that the growth and development of the carcass were not negatively impacted by different levels (20%) of fermented prosopis pods in the diets of growing rabbits. This is supported by the works of Sankhyan *et al*. (1991). Ijaiya and Fasanya, (2004) who reported that

there were no significant differences in the average dressing percentages and weights of body parts of rabbits fed different levels (30%) of fermented prosopis pods.

This could indicate that the inclusion levels of FGMPP 20, 40 and 60% in the diet supplied adequate nutrients for rabbit growth. It was therefore concluded that the inclusion of prosopis pods fermented with *Aspergillus braziliensis* up to 60% in the diets of growing rabbits did not affect the dressing percentage. Animals fed high energy-based diets, grow rapidly and have high rates of protein and lipid accretion. Therefore, these animals are heavier with higher levels of subcutaneous, seam, and intramuscular fat and greater muscle mass. Intramuscular fat (IMF) is one of the main parameters affecting meat quality. From the result of the study, the panellists did not find a significant difference between the intramuscular fat in all the treatments. This could be because rabbit meat is lean compared with other species (Valsta *et al.*, 2005). Only a few experiments for IMF have been published in rabbits (Schwab *et al.,* 2009). This study agrees with the findings of Gašperlin *et al*. (2006) and Xicatto *et al.* (1994), who found no differences in rabbits or changes in sensory properties of IMF. Intramuscular fat (IMF) did not change meat sensory attributes in the experiment in pigs (Schwab *et al.,* 2009) but modified some instrumental texture traits in the experiment in chickens (Zhao *et al.,* 2007). Several meat quality attributes are affected by pH, including tenderness, water-holding capacity, colour and juiciness (Mir *et al.,* 2017). Husak *et al.* (2008) stated that meat with a higher pH maintains better colour and improves moisture retention. Ijaz *et al.* (2020) highlighted that post-mortem glycolysis reduces lactic acid in muscle, resulting in a substantial increase in meat pH. The results of this study showed a difference in pH between before and after chilling in treatment 1 (0% FGMMP) for rabbit carcasses. In this study, it was found that there was no significant difference in colour and pH levels of rabbit meat when 20, 40, and 60% FGMPP inclusion levels were compared. This could be that the rabbit from the treatments with fermented prosopis pods were handled well before slaughter, by ensuring that humane slaughtering was done-hence no stress and had similar protein composition and energy value which made the pH levels of *biceps femoris* and *longissimus dorsi* muscle to become lower resulting in the lack of significant difference in pH. The pH levels observed in the rabbit meat fell within the normal range of 5.6 and 7.85, which is accepted and an indication of good quality meat. A lower pH level can result in meat that is firm and dry due to the shrinkage of the myofibrillar network and reduced water-holding capacity (Morshdy *et al.,* 2022). This happens directly as a result of reduced sarcomere on the water-holding capacity. The colour of meat plays a vital role in

determining its acceptance by consumers and is a crucial factor in the meat purchasing process (Muchenje, 2009). The result showed no significant differences (P>0.05) in the colour of the rabbit meat, ranging from light brown to dark brown across the different dietary treatments. This was due to the fact that the muscle fibres of the leg did not perform any hard work (movements) as they were confined in their cages and had a lower myoglobin content. According to Ribarski and Genchev, (2013), the colour of meat indicates its freshness and tenderness, which vary with animal species. Freshness of meat is determined by the concentration and condition of the myoglobin in the muscles (Jacobb and Pethick, 2014). Red meats such as beef or lamb are usually bright red in colour as an indication of the freshest meat available. However, if the colour is slightly purple, the meat has been exposed to oxygen. Joo *et al.* (2013) found that the colour of meat varies significantly across different species, primarily due to the amount of myoglobin in the muscle. Rabbit myoglobin levels are high because they turn to be more physically active animals. These results are in agreement with the findings of Bidner *et al.* (2004), who found no differences in pork colour. Similarly, Tarasewicz *et al.* (2007) found no effect of protein levels on the colour coordinates of quail breast meat.

### 5.5 Conclusion

It was concluded that the level of incorporation of FGMMP in the diet did not affect the carcass quality characteristics.

### 5.6 Recommendation

Include up to 60% of *Prosopis juliflora* pods fermented with *Aspergillus braziliensis* for 72hrs, with a temperature of $34.7^{0C}$ ($35^{0C}$) and pH of 5.2 (FGMPP) in formulation of grower rabbit diets.

# CHAPTER SIX

# GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 6.1 General Discussion

Commercial rabbit production is known to be expensive, with feeding being the main cost. There is a scarcity of cereals and legumes available for feed production. Thus, finding alternatives to minimize dependency on these conventional sources is crucial for sustainable rabbit meat production. *Prosopis juliflora* is an invasive leguminous tree that was introduced as a way of combating desertification, deforestation, and improving soil fertility in Baringo County, Kenya. There are efforts directed to turn it into a valuable resource to provide the rural communities with a ready cash income. *Prosopis juliflora* pods are locally available and are a source of energy and protein. However, the pods are not widely utilised because of anti-nutritive factors, which limit the bioavailability of nutrients to livestock. This study focused on the use of biotechnology to enhance nutrient utilization of *Prosopis juliflora* pods as a livestock feed resource. *Prosopis juliflora* is a fast-growing, drought-resistant, evergreen plant whose root system (deep taproot) can reach depths of 35 meters. This makes it appropriate for arid regions with yearly precipitation between 150 and 700 mm (Odero-Waitituh, 2021). *Prosopis juliflora* is an excellent livestock feed because of its nutritive content but contains anti-nutritive factors. Anti-nutritional factors are mainly decreased by processing methods like blanching, drying, fermentation and de-fatting, which result in reduced or deactivated activity (Devisetti *et al.,* 2016).

Chapter three (3) of this thesis presented the results of treatment of prosopis pods using *Aspergillus braziliensis* as a controlled fermentation and determination of proximate composition and anti-nutritive factors content. The findings showed that fermentation significantly improved the nutrient utilization of prosopis pods, which is an important indicator of its nutritive value. This research suggested that biotechnological interventions, such as fermentation, can be a viable solution to overcome the limitation of poor digestibility associated with ANFs in prosopis pods. This study found that *Prosopis juliflora* pods contained 16.3% protein, 14.2% total fibre, 5.9% total Ash, and 2.0% crude fat, as well as about 23.7% pectin, 0.5% phytates, and 0.02% condensed tannins. The optimization model revealed that increasing fermentation temperature and time decreased condensed tannins, phytates, and pectin. Increasing fermentation pH decreased condensed tannins only. The optimum fermentation conditions were 34.5 $^{\circ C}$, 72 hours, and pH 5.2. At these conditions, pectin was reduced from 23.7% to 10.5%, phytate from 0.5% to 0.16%, and

condensed tannins from 0.02% to 0.01%. These findings showed that fermentation of *Prosopis juliflora* pods using *Aspergillus braziliensis* at 34.7$^{oC}$, 72 hours, and pH 5.2 increased nutrient bioavailability.

Chapter four determined the effect of incorporating fermented prosopis pods in diets- on feed intake, feed conversion ratio, weight gain and growth rate of grower rabbits. The highest ADFI was recorded in the rabbits fed on the diet with 60% FGMPP because it had high fibre content compared to the rest. Rabbits depend on the bacterial population in their hindgut to help them digest fibre, which is vital for their overall gut health, mobility, caecotrophy, and appetite stimulation. Caecotrophy enhances the rabbit's strategy of high feed intake (65-80 g/kg bodyweight) and rapid feed transit time (19 hours). This allows them to consume more feeds while still meeting their nutritional requirements (De Blas & Wiseman, 2003). The digestibility and intake of fibre residues with high lignin content can be enhanced through physical (corona treatment and cold plasma treatment) and biological treatment (fungi, bacteria and enzymes). Several studies have been conducted on using exogenous microorganisms to boost nutritional usage (Falcao-Cunha *et al.,* 2007), increase growth rate, and improve feed conversion ratio (Eiben *et al.,* 2004). The treatment of the prosopis pods with *Aspergillus braziliensis* in this study also improved utilisation by the grower rabbits to increase growth rate.

Chapter Five described determination of carcass quality characteristics of rabbits fed fermented prosopis pods-based diets. There were no significant differences ($P>0.05$) in the dressing percentages among all the treatments. This may be because fermented prosopis pods incorporated in the diets are good source of nutrients required for supporting rabbits' growth. From the results of the study, the panellists did not find a significant difference between the intramuscular fat in all the treatments due to the reason that the diets directly influence the physiological composition of the fat. Studies have shown that dietary macronutrient and energy content can influence the proportion of intramuscular fat (IMF), which mediates various metabolic and endocrine dysfunction. The study results showed a difference between before and after chilling in pH of rabbit carcass with diets containing 0% FGMPP which indicates that the acidity of the rabbit meat in these periods (before and after) were unstable. There was no significant difference in colour and pH in carcasses from rabbits fed on diets with 20, 40, and 60% FGMPP because humane slaughtering was done, hence no stress and these treatments also had similar nutrient value.

## 6.2 Conclusions

i. The treatment of prosopis pods using *Aspergillus braziliensis* as a controlled fermentation significantly improved the proximate composition and reduced all the anti-nutritive factors (pectin, condensed tannins and phytates).

ii. Incorporating 60% FGMPP in a grower rabbit diet increased average daily feed intake, weight gain, growth rate and reduced feed conversion ratio.

iii. The incorporation of FGMPP up to 60% level in diet had no significant effect on the carcass quality characteristics of rabbits.

## 6.3 Recommendations

i. Sort pods by examining any blackening or discolouration or evidence of browsing by livestock or attack by insects or moulds and discard, thereafter dry well under shade to reduce moisture before grinding.

ii. Ferment prosopis pods to be incorporated into rabbits' diets with *Aspergillus braziliensis* at temperature of $35^{oC}$, pH of 5.2 and time of 72hrs to improve nutrient utilization.

iii. Incorporate up to 60% *Prosopis juliflora* pods fermented with *Aspergillus braziliensis* (FGMPP) in grower rabbit diet.

## 6.4 Areas of Further Studies

i. Determine the cost-benefit of feeding *Prosopis Juliflora* pods fermented with *Aspergillus braziliensis* to grower rabbits.

ii. Determine optimal conditions for fermenting *Prosopis Juliflora* pods using other probiotic microorganisms (*Lactobacillus, Bifidobacterium, Bacillus and Pediococcus*,).

iii. Determine the effect of FGMPP on occurrence of digestive disorders in grower rabbits and their effects on gastrointestinal tract morphology and function.

iv. Determine the effect of increased fermentation time on the content of anti-nutritive factors, level of incorporation in diet and performance of grower rabbits.

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# APPENDICES

## APPENDIX A: NASCOTI PERMIT

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **124632**

Date of Issue: **13/August/2023**

**RESEARCH LICENSE**

**This is to Certify that Ms.. Maud Boakyewaa Acheampong 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 GROWER RABBITS (Oryctolagus cuniculus) FED ON Prosopis juliflora PODS TREATED WITH Aspergillus niger-BASED DIET for the period ending : 13/August/2024.**

License No: **NACOSTI/P/23/28140**

**124632**

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NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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# APPENDIX B: ETHICAL CLEARANCE


**EGERTON UNIVERSITY**

TEL: (051) 2217808
FAX: 051-2217942

P. O. BOX 536
EGERTON


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

**EU/RE/DIR/009**
*Approval No. EUISERC/APP/243/2023* *26th May 2023*


Maud Boakyewaa Acheampong
Department of Animal Sciences
P.O. Box 536
Egerton,
Telephone: 07961795480
E-mail: Maudacheampong552@gmail.com


Dear Maud,

**RE: ETHICAL APPROVAL: PERFORMANCE OF GROWER RABBITS FED ON *Prosopis juliflora* PODS TREATED WITH *Aspergillus niger*-BASED DIET**

This is to inform you that ***Egerton University Institutional Scientific and Ethics Review Committee*** has reviewed and approved your above research proposal. Your application approval number is ***EUISERC/APP/243/2023***. The approval period is *26th May, 2023 –27th May, 2024*

This approval is subject to compliance with the following requirements;

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



vi. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.
vii. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
viii. Submission of an executive summary report within 90 days upon completion of the study to ***Egerton University Institutional Scientific and Ethics Review Committee***.

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

Yours sincerely.

EGERTON UNIVERSITY RESEARCH ETHICS Date. 26/05/2023

Prof. Raphael M. Ngure
**CHAIRMAN, EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW CTTEE**
*RM/VBK/*


*"Transforming Lives through Quality Education"*

# APPENDIX C: ANOVA OUTPUT

## CT (Condensed tannins)

### Model Summary

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.0022807 | 69.47% | 55.74% | 21.85% |

## PHYTATE

### Model Summary

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.0269944 | 89.74% | 85.12% | 73.74% |

## Pectin

### Model Summary

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.253224 | 99.24% | 98.90% | 98.06% |

The GLM Procedure

Bonferroni (Dunn) t Tests for CF

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type I error rate than REGWQ.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 8 |
| Error Mean Square | 0.033895 |
| Critical Value of t | 3.47888 |
| Minimum Significant Difference | 0.523 |

Means with the same letter are not significantly different.

| Bon Grouping | Mean | N | trt |
|---|---|---|---|
| A | 21.6133 | 3 | 4 |
| B | 17.9817 | 3 | 3 |
| C | 13.9930 | 3 | 2 |
| D | 12.4623 | 3 | 1 |

The GLM Procedure

Bonferroni (Dunn) t Tests for CP

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type I error rate than REGWQ.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 8 |
| Error Mean Square | 0.082684 |
| Critical Value of t | 3.47888 |
| Minimum Significant Difference | 0.8168 |

Means with the same letter are not significantly different.

| Bon Grouping | Mean | N | trt |
|---|---|---|---|
| A | 18.9820 | 3 | 4 |
| B | 17.5383 | 3 | 3 |
| B B | 17.0290 | 3 | 1 |
| B | 16.9100 | 3 | 2 |

The GLM Procedure
Dependent Variable: ADFI

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 60 | 634791.2964 | 10579.8549 | 38.52 | <.0001 |
| **Error** | 611 | 167826.2037 | 274.6746 | | |
| **Corrected Total** | 671 | 802617.5001 | | | |

| R-Square | Coeff Var | Root MSE | ADFI Mean |
|---|---|---|---|
| 0.790901 | 14.19565 | 16.57331 | 116.7492 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TRT** | 3 | 18887.5348 | 6295.8449 | 22.92 | <.0001 |
| **REPS** | 2 | 2586.2436 | 1293.1218 | 4.71 | 0.0094 |
| **DAYS** | 55 | 613317.5180 | 11151.2276 | 40.60 | <.0001 |

| | |
|---|---|
| **Ha** | 0.05 |
| **Error Degrees of Freedom** | 611 |
| **Error Mean Square** | 274.6746 |
| **Critical Value of Studentized Range** | 3.64309 |
| **Minimum Significant Difference** | 4.6583 |

| **Means with the same letter are not significantly different.** | | | |
|---|---|---|---|
| **Tukey Grouping** | **Mean** | **N** | **TRT** |
| A | 124.726 | 168 | T4 |
| | | | |
| B | 116.960 | 168 | T3 |
| B | | | |
| B | 115.415 | 168 | T2 |
| | | | |
| C | 109.896 | 168 | T1 |

The GLM Procedure
Dependent Variable: GR

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 4 | 0.79574583 | 0.19893646 | 47.33 | <.0001 |
| **Error** | 7 | 0.02942083 | 0.00420298 | | |
| **Corrected Total** | 11 | 0.82516667 | | | |

| R-Square | Coeff Var | Root MSE | GR Mean |
|---|---|---|---|
| 0.964346 | 4.625234 | 0.064830 | 1.401667 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TRT** | 3 | 0.79563333 | 0.26521111 | 63.10 | <.0001 |
| **REPS** | 1 | 0.00011250 | 0.00011250 | 0.03 | 0.8747 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TRT** | 3 | 0.79563333 | 0.26521111 | 63.10 | <.0001 |
| **REPS** | 1 | 0.00011250 | 0.00011250 | 0.03 | 0.8747 |

Tukey's Studentized Range (HSD) Test for GR

| | |
|---|---|
| **Alpha** | 0.05 |
| **Error Degrees of Freedom** | 7 |
| **Error Mean Square** | 0.004203 |
| **Critical Value of Studentized Range** | 4.68121 |
| **Minimum Significant Difference** | 0.1752 |

| **Means with the same letter are not significantly different.** | | | |
|---|---|---|---|
| **Tukey Grouping** | **Mean** | **N** | **TRT** |
| A | 1.70333 | 3 | T4 |
| A | | | |
| A | 1.59000 | 3 | T3 |
| | | | |
| B | 1.25333 | 3 | T2 |
| | | | |
| C | 1.06000 | 3 | T1 |

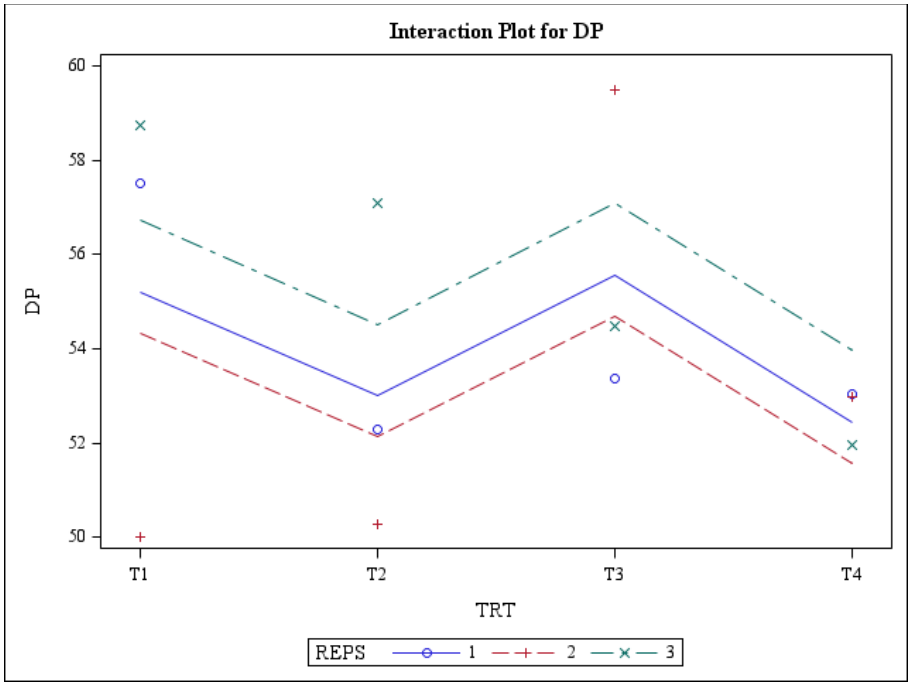
The GLM Procedure

Dependent Variable: DP

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 5 | 33.4741500 | 6.6948300 | 0.50 | 0.7651 |
| **Error** | 6 | 79.6316167 | 13.2719361 | | |
| **Corrected Total** | 11 | 113.1057667 | | | |

| R-Square | Coeff Var | Root MSE | DP Mean |
|---|---|---|---|
| 0.295954 | 6.713062 | 3.643067 | 54.26833 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TRT** | 3 | 21.88963333 | 7.29654444 | 0.55 | 0.6666 |
| **REPS** | 2 | 11.58451667 | 5.79225833 | 0.44 | 0.6653 |




The GLM Procedure

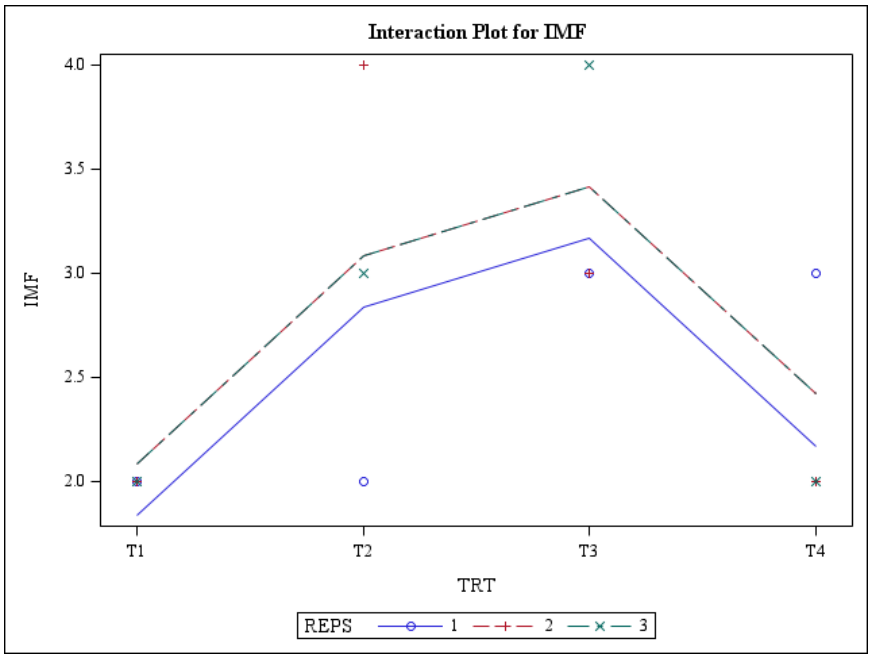
Dependent Variable: IMF

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 5 | 3.50000000 | 0.70000000 | 1.33 | 0.3660 |
| **Error** | 6 | 3.16666667 | 0.52777778 | | |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Corrected Total | 11 | 6.66666667 | | | |

| R-Square | Coeff Var | Root MSE | IMF Mean |
|---|---|---|---|
| 0.525000 | 27.24312 | 0.726483 | 2.666667 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TRT | 3 | 3.33333333 | 1.11111111 | 2.11 | 0.2010 |
| REPS | 2 | 0.16666667 | 0.08333333 | 0.16 | 0.8574 |

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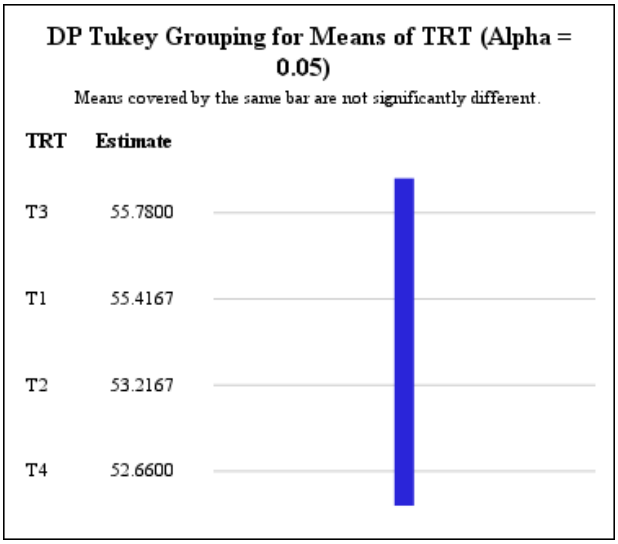
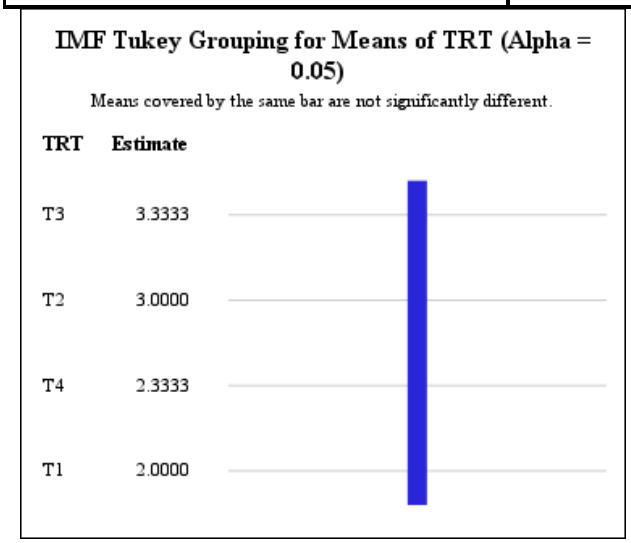
Note: This test controls the Type I experiment wise error rate, but it generally has a higher Type II error rate than REGWQ.

| Alpha | 0.05 |
|---|---|
| Error Degrees of Freedom | 6 |
| Error Mean Square | 13.27194 |
| Critical Value of Studentized Range | 4.89559 |
| Minimum Significant Difference | 10.297 |




Note: This test controls the Type I experiment wise error rate, but it generally has a higher Type II error rate than REGWQ.

| | |
|---|---|
| **Alpha** | 0.05 |
| **Error Degrees of Freedom** | 6 |
| **Error Mean Square** | 0.527778 |
| **Critical Value of Studentized Range** | 4.89559 |
| **Minimum Significant Difference** | 2.0534 |



The TTEST Procedure

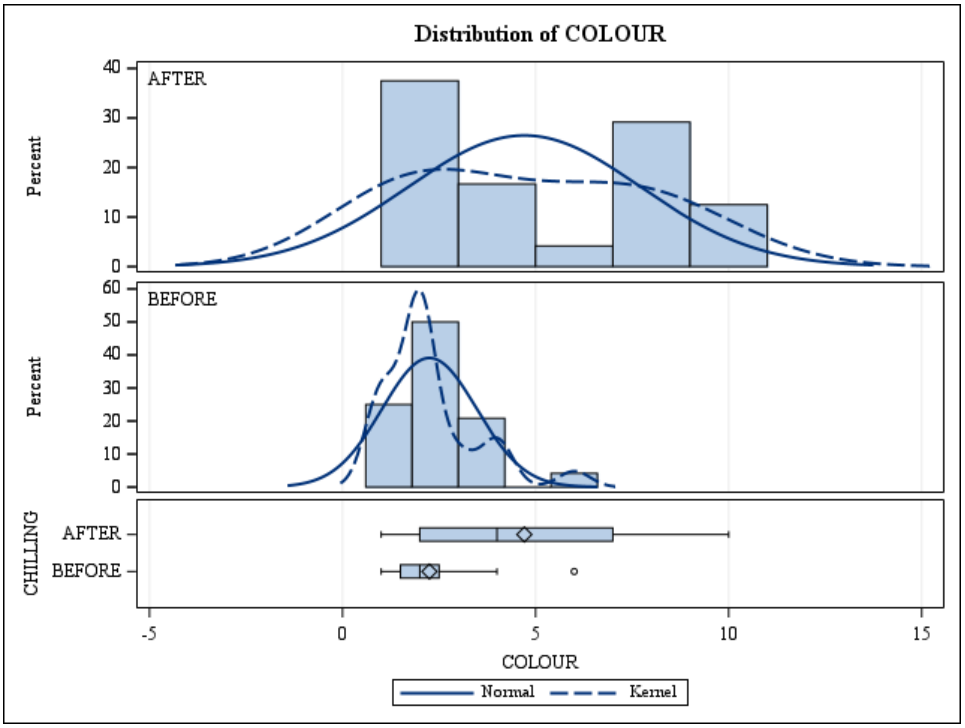
Variable: COLOUR

| CHILLING | Method | N | Mean | Std Dev | Std Err | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| **AFTER** | | 24 | 4.7083 | 3.0142 | 0.6153 | 1.0000 | 10.0000 |
| **BEFORE** | | 24 | 2.2500 | 1.2247 | 0.2500 | 1.0000 | 6.0000 |
| **Diff (1-2)** | **Pooled** | | 2.4583 | 2.3006 | 0.6641 | | |
| **Diff (1-2)** | **Satterthwaite** | | 2.4583 | | 0.6641 | | |

| CHILLING | Method | Mean | 95% CL Mean | | Std Dev | 95% CL Std Dev | |
|---|---|---|---|---|---|---|---|
| **AFTER** | | 4.7083 | 3.4356 | 5.9811 | 3.0142 | 2.3426 | 4.2281 |
| **BEFORE** | | 2.2500 | 1.7328 | 2.7672 | 1.2247 | 0.9519 | 1.7180 |
| **Diff (1-2)** | **Pooled** | 2.4583 | 1.1215 | 3.7951 | 2.3006 | 1.9117 | 2.8895 |
| **Diff (1-2)** | **Satterthwaite** | 2.4583 | 1.1028 | 3.8139 | | | |

| Method | Variances | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|
| **Pooled** | Equal | 46 | 3.70 | 0.0006 |
| **Satterthwaite** | Unequal | 30.393 | 3.70 | 0.0008 |

| Equality of Variances | | | | |
|---|---|---|---|---|
| **Method** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Folded F** | 23 | 23 | 6.06 | <.0001 |




The TTEST Procedure

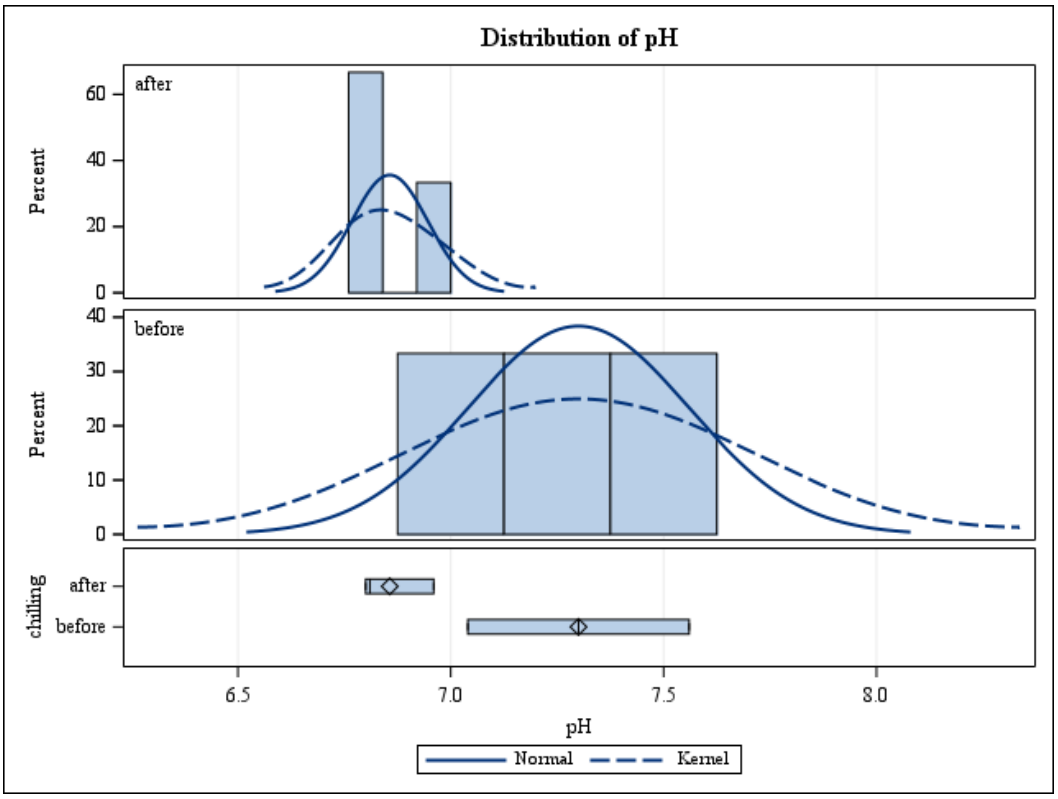
Variable: pH

| chilling | Method | N | Mean | Std Dev | Std Err | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| **After** | | 3 | 6.8567 | 0.0896 | 0.0517 | 6.8000 | 6.9600 |
| **before** | | 3 | 7.3000 | 0.2600 | 0.1501 | 7.0400 | 7.5600 |
| **Diff (1-2)** | **Pooled** | | -0.4433 | 0.1945 | 0.1588 | | |
| **Diff (1-2)** | **Satterthwaite** | | -0.4433 | | 0.1588 | | |

| chilling | Method | Mean | 95% CL Mean | | Std Dev | 95% CL Std Dev | |
|---|---|---|---|---|---|---|---|
| **After** | | 6.8567 | 6.6340 | 7.0793 | 0.0896 | 0.0467 | 0.5633 |
| **Before** | | 7.3000 | 6.6541 | 7.9459 | 0.2600 | 0.1354 | 1.6340 |
| **Diff (1-2)** | **Pooled** | -0.4433 | -0.8842 | -0.00249 | 0.1945 | 0.1165 | 0.5588 |
| **Diff (1-2)** | **Satterthwaite** | -0.4433 | -1.0161 | 0.1294 | | | |

| Method | Variances | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|
| **Pooled** | Equal | 4 | -2.79 | 0.0492 |
| **Satterthwaite** | Unequal | 2.4687 | -2.79 | 0.0853 |

| Equality of Variances | | | | |
|---|---|---|---|---|
| **Method** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Folded F** | 2 | 2 | 8.41 | 0.2124 |



## APPENDIX D: ORGANOLEPTIC PANEL RECRUITMENT FORM

I…… have voluntarily agreed to take part in this study. I understand that I will not directly benefit from this study. I have had the study explained to me, and I understand that it entails organoleptic evaluation of rabbits' meat. My participation in this study involves profiling the predetermined organoleptic properties according to my perception against the standards and availability and the ability to differentiate colours. I confirm that I am not allergic to the product. I understand that the results will be kept for 3 months after the date of examination and will be treated confidentially. My identity will remain anonymous after the study, and this will be done by coding my details.

…….

Signature of participant Date ……

……

Signature of researcher

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

## APPENDIX E: MEASUREMENT OF INTRAMUSCULAR FAT (IMF) USING AUS MEAT/ MSA MARBLING REFERENCE STANDARDS OF RABBIT MEAT

**MEASUREMENTS OF MARBLING**

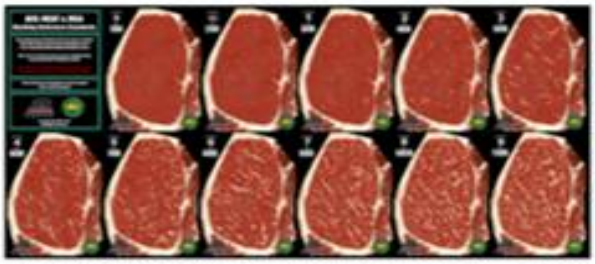
Marbling is the intramuscular fat (IMF) within the muscle and between the muscle fibres which appears as fine flecks of fat within a muscle. Marbling is a key specification of carcass value in many of our high quality beef markets, particularly grain fed carcasses for hospitality or export markets such as Japan. Marbling generally improves the eating quality of beef, particularly palatability traits such as juiciness and flavour.

Intramuscular fat is the last fat deposited in the animal so the finishing stages of an animal are most critical.

### Measuring Marbling

Generally, marbling is graded on a subjective visual basis, assessed and scored against marbling reference standards. Marbling is assessed on the eye muscle (M. longissumus dorsi) or at the quartering site (usually between the 10th and 11th ribs or 12th and 13thribs).

**MARBLING**



**Marbling is the fat that is deposited between muscle fibres of the M. longissimus dorsi muscle. Marbling is assessed and scored against the AUS-MEAT / MSA Marbling Reference Standards.**

**The AUS-MEAT Marbling system provides an indication of the amount of marbling in beef. The MSA Marbling System provides an additional indication of distribution and piece size.**

**Marbling is an assessment of the chilled carcase and scored by comparing the proportion of marble fat to meat at the surface of the assessment site which lies within the M. longissimus dorsi boundary.**

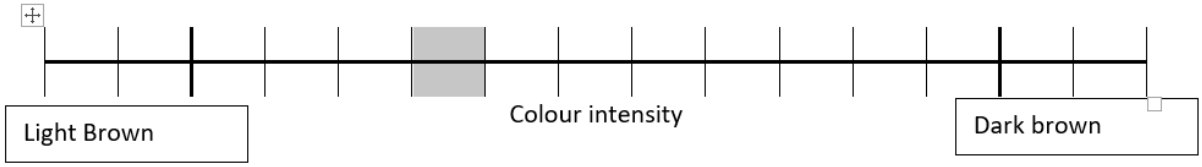
**Marbling may be assessed at any ribbing site from 5th-13th rib. The rib at which the measurement was performed must be nominated in company records.**

## APPENDIX F: ORGANOLEPTIC EVALUATION OF RABBIT MEAT COLOUR

**SENSORY ANALYSIS#-007-13-2023**

**Name** …… **Date**:……/……/……

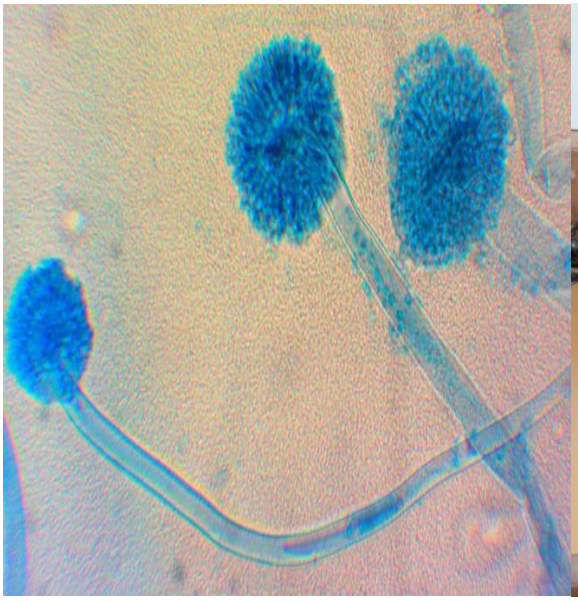
You are provided 4 rabbits meat samples WITH RANDOM LABELS XFZ, AFZ, YFY, YFA. Kindly score your judgement of the attribute listed on the left side of the table based on the score card provided for each samples.



## APPENDIX G: RESEARCH PICTORIAL

A: Dried *Prosopis juliflora* pods

B: Culturing of *Aspergillus niger*



C: *Aspergillus braziliensis* observed under a microscope


D: Setting up for Fermentation

E: Putting samples in an incubator at given temperature

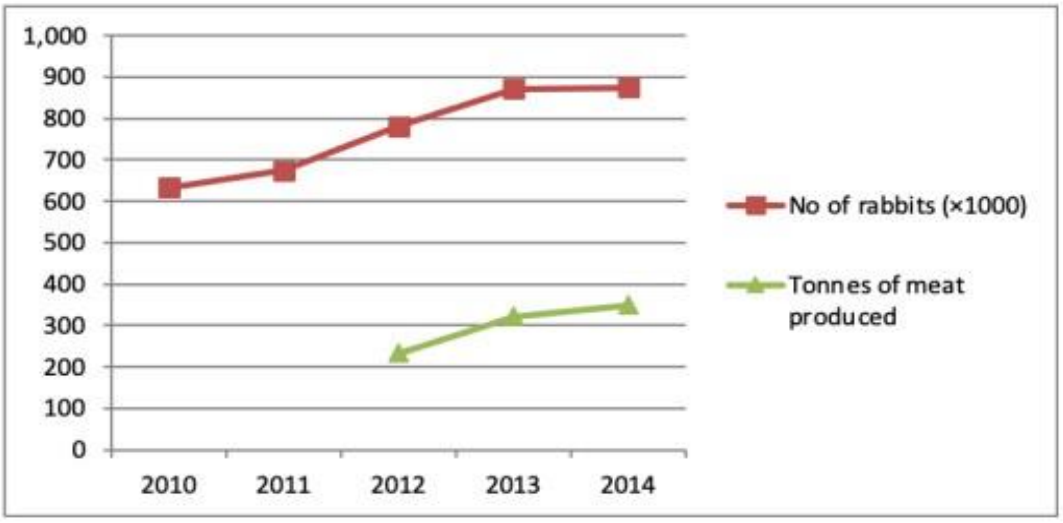
F: Feeding of New Zealand rabbits

G: Testing pH of rabbit meat

H: panellist scoring colour for rabbit meat

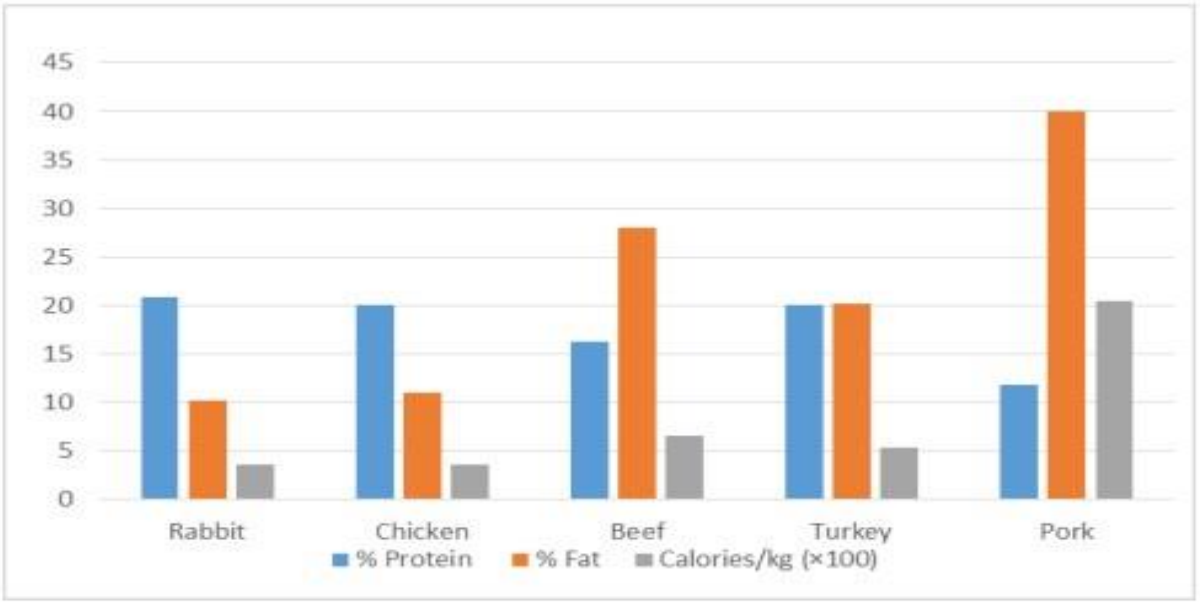
I: Scoring of intramuscular fat (IMF) of rabbit

## APPENDIX H: NUMBER OF RABBITS AND AMOUNT OF MEAT PRODUCED



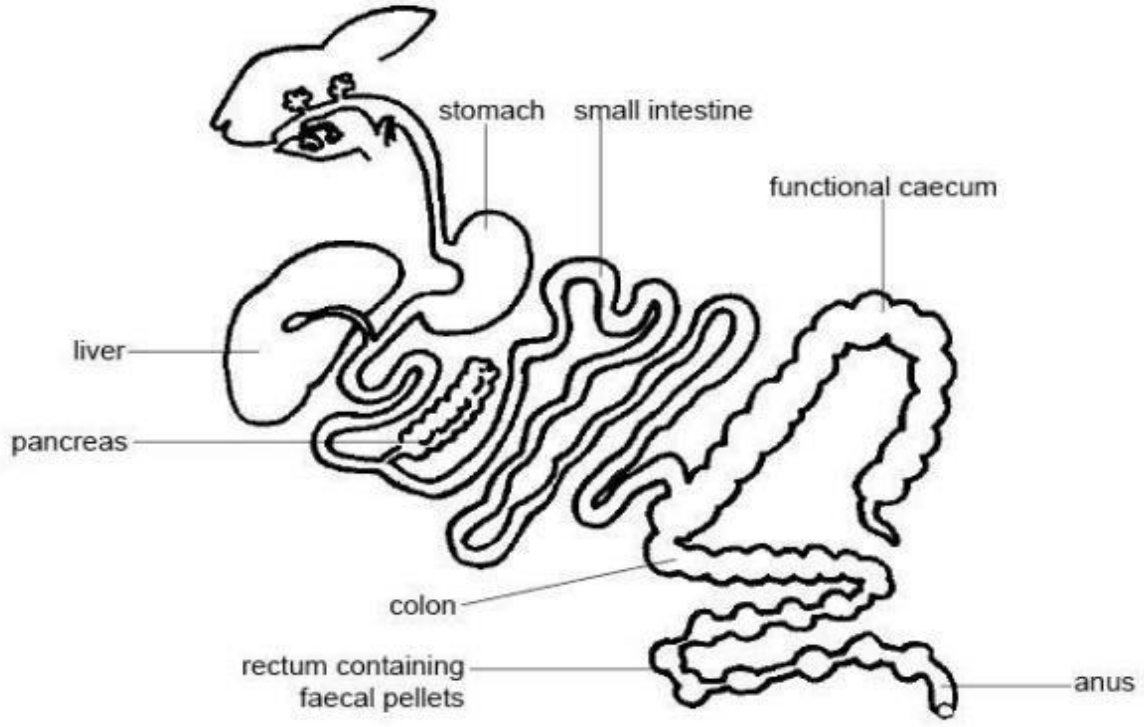

**SOURCE: GOK (2015)**

## APPENDIX I: COMPARATIVE NUTRITIONAL VALUE OF RABBIT MEAT




Source: Nelson (2011)

## APPENDIX J: ANATOMY AND PHYSIOLOGY OF ANIMALS/THE GUT AND DIGESTION IN THE RABBIT

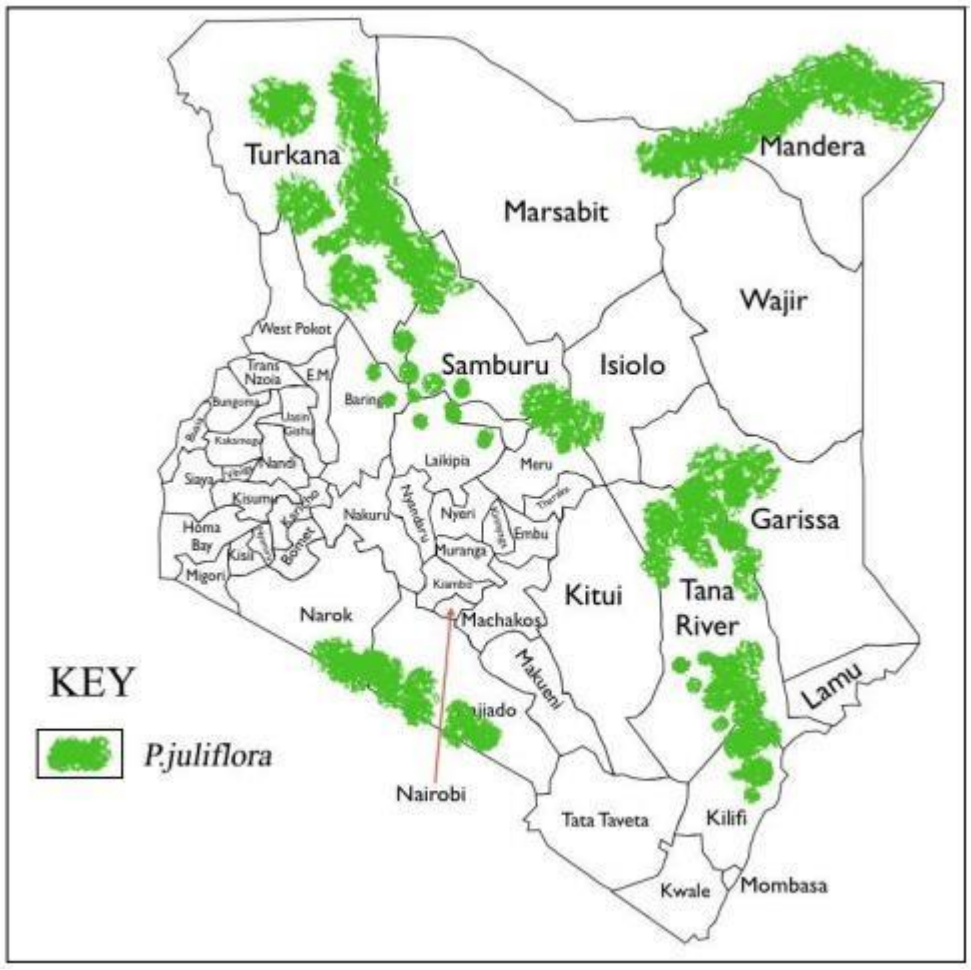



**Source :**

https://en.wikibooks.org/wiki/Anatomy_and_Physiology_of_Animals/The_Gut_and_Digestion

## APPENDIX K: PROSOPIS TREE IN A HOMESTEAD IN BARINGO, KENYA

## APPENDIX L: DISTRIBUTION OF *PROSOPIS JULIFLORA* IN KENYA




Source: Sirmah (2009)