REDUCTION OF ANTI-NUTRITIVE COMPOUNDS IN GROUND MATURE *Prosopis juliflora* PODS IN RABBIT DIETS USING FERMENTATION TECHNOLOGY

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EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This Thesis is my original work and has not, wholly or in part been presented for an award of a degree in any other University.

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Recommendation

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DEDICATION

To my biological parents, the late Odero Nyambogo and the late Awino Onyango, for their genetic contribution; my adopted parents, the late Ongadi Odero and the late Anyango Odero for provision of a favourable environment. They shaped my destiny morally, socially, academically and financially.

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ABSTRACT

Scarcity and seasonality of supply of cereals (for human and livestock feed) causes fluctuation in quantity, quality and increase in feed cost. This results in poor livestock growth, low reproductive performance and high production cost. *Prosopis juliflora* pods have been used to mitigate this, but success has been hampered by their high content of anti-nutritive compounds (ANC); tannins, crude fibre (CF) phytic acid and aflatoxins. Fermentation has been reported to reduce ANC concentrations. This study investigated the effects of fermentation technology in enhancement of the pods' nutritional value for rabbit diets. Mature pods from Marigat, Baringo County were fermented; spontaneously, with cultures of *Lactobacillus salivarius* (SL), *Saccharomyces cerevisiae* (SC) and a mixture of LS/SC for 24, 48 and 2 hours. Amino acid profile, acid detergent fibre (ADF), neutral detergent fibre (NDF), proximate and ANC analyses of maize, fermented ground mature Prosopis pods (FGMPP) and unfermented ground mature Prosopis pods (UGMPP) were conducted. In a completely randomized design (CRD), a digestibility experiment was conducted using 15 adult bucks. In a randomized complete block design (RCBD) a feeding trial was conducted using 60, 42-week-old growers and a lactation experiment using 15 primiparous does in a CRD. Different statistical softwares were used in data analyses; SPSS for descriptive and inferential statistics; probabilities, quantiles and random sample (PQRS) for randomization and descriptive statistics and SAS for data analysis in experimental designs. Tukey's HSD was used to separate significant means at ($p<0.05$). Except for aflatoxin, all fermentation methods reduced the ANC and improved ($p<0.05$) the crude protein (CP) and amino acid profiles. Fermentation and addition of cornstarch affected ($p<0.05$) dry matter intake (DMI) and digestibilities of dry matter (DM), CF, CP, ash and ether extract (EE). Grower rabbits offered diet with 30% FGMPP exhibited higher ($p<0.05$) average daily gain (ADG), lower mortality, and there was treatment effect ($p<0.05$) on blood haematological and metabolite indices. There was no treatment effect ($p>0.05$) for carcass characteristics except dressing % and caecal pH ($p<0.05$). Inclusion of FGMPP and UGMPP had no effect ($p>0.05$) on overall rating of descriptive sensory attributes of meat. There was economic benefit ($p<0.05$) of incorporating 30% FGMPP in grower rabbit diets. There was no treatment effect ($p>0.05$) on lactation performance. The study recommended the inclusion of 30% FGMPP in diets of grower and lactating rabbits.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACTH	Adeno-corticotropic Hormone
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
ADP	Animal Production Division
ADWG	Average daily weight gain
ANC	Anti-nutritive compounds
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ASALs	Arid and semi-arid lands
ATP	Adenosine triphosphate
Ca	Calcium
CESAAM	Centre of Excellence in Agriculture and Agribusiness management
CF	Crude fibre
CP	Crude protein
CRD	Completely randomized design
DCW	Dressed carcass weight
DE	Digestible energy
DM	Dry matter
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
FGMPP	Fermented ground mature Prosopis pods
FI	Feed intake
GDP	Gross Domestic Product
GE	Gross energy
GIT	Gastro intestinal tract
GLM	General Linear Model
GMPP	Ground mature Prosopis pods
GoK	Government of Kenya
H_2SO_4	Sulfuric acid
HB	Haemoglobin

HCl	Hydrochloric acid
HDL	High density lipoprotein
HSD	Highly significant difference
ISAPP	International Scientific Association on Probiotics and Prebiotics
KALRO	Kenya Agriculture and Livestock Research Organization
KNBS	Kenya National Bureau of Statistics
LAB	Lactic acid bacteria
LDL	Low density lipoprotein
LS	*Lactobacillus salivarius*
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
ME	Metabolizable energy
MG	Maize germ
MJ	Mega joules
µl	Microlitre
MoAL&F	Ministry of Agriculture Livestock and Fisheries
MRS	Ma, rogosa and sharpe
NaOH	Sodium hydroxide
NDF	Neutral detergent fibre
NPR	Normal physiological range
NRC	National Research Council
P	Phosphorus
PCA	Principal Component Analysis
PQRS	Probabilities, Quantiles and Random Sample
PVC	Packed Cell Volume
RBC	Red Blood Cell
RCBD	Randomised complete block design
RH	Rice husks
SAS	Statistical Analysis Systems
SC	*Saccharomyces cerevisiae*
SCFAs	Short chain fatty acids
SE	Standard errors
SEM	Standard error of mean

SFC	Sunflower cake
SPSS	Statistical Package for Social Sciences
TAP	Tatton Agriculture Park
UGMPP	Unfermented ground mature Prosopis pods
VFAs	Volatile fatty acids
VST	Vicam Science Technology
WB	Wheat bran
WHO	World Health Organisation
WWG	Weekly weight gain
YPA	Yeast Peptone Agar
YPD	Yeast extract peptone dextrose
YPM	Yeast peptone media

CHAPTER ONE

INTRODUCTION

1.1 Background information

Demand for food in developing countries continues to rise due to increase in human population (Cheeke, 1986). It is estimated that Kenyan demand for livestock meat will double by 2030 (Mutsami *et al.,* 2019) due to the increasing population estimated at 2-3% per annum (KNBS, 2009). The 2019 human population was at 47.6 million with an estimated 2.2 % annual growth rate (KNBS, 2019). Increasing human population has led to extensive land subdivisions for human settlement reducing the amount of land available per household. However, 75% of Kenya's land mass is classified as arid and semi-arid suitable only for extensive livestock production systems as ranching and pastoralism (GoK, 2007). This has put pressure on the available land in high rainfall areas for intensification of production to ensure more food supply. According to Cheeke (1986) economic reality for developing countries is to produce instead of import food, which must be done by use of livestock species that rearing is easy and cost effective given the available land and resources. Among the available options, rabbit meat has been identified as a suitable sustainable alternative for animal protein (Mutsami *et al.,* 2019). Rabbit production is therefore expected to contribute to improvement of food and nutritional security in Kenya.

Kenyas land mass comprise 75% arid and semi arid lands (ASALs) and *Prosopis juliflora* is readily available in ASAL areas of Tana River and Baringo Counties (King'ori *et al.,* 2011). The Prosopis weed is spreading and in areas where it spreads, it reduces available natural pasture, displaces native trees and reduces grazing potential of rangelands (Farm-Africa, 2008). According to Choge *et al*. (2007) and Wakie *et al.* (2012), sustainable control of the weed is by harvesting the pods. Prosopis plant matures within 3-4 years and is able to produce 10-50 kgs of pods annually with variations in acreage production from 2.04 tonnes/acre to 17.65 tonnes/acre depending on density of plants, rainfall, management and elevation (Kyuma *et al.,* 2018; Sawal *et al.,* 2004). Choge *et al.* (2006) reported efforts to control the weed by incorporating it into human food when baking and making beverages. Sensory tests on various recipes indicated a pleasant taste in food made with 20% inclusion of Prosopis flour (Choge *et al.,* 2007). Choge *et al.* (2007) and Odero-Waitituh (2015) reported that the pods had 69 % carbohydrates and 12.8MJ/kg ME value respectively. This

could possibly make it an energy source in rabbit diets. King'ori *et al*. (2011) in a review paper and Wanjohi *et al.* (2017a) in experiments to investigate growth performance of Kenyan indigenous grower chicken fed on diets with Prosopis pods, reported positive growth performance on 20% dietary inclusion.

Prosopis pods are available in ASALs of Kenya and can be used in compounding livestock feeds (King'ori *et al.,* 2011; Odero-Waitituh *et al.,* 2015; Wanjohi *et al.,* 2017a). However, presence of anti-nutritive compounds such as high levels of tannins; CF; phytic acid; trypsin inhibitor and aflatoxin reduce livestock growth and breeding performance (King'ori *et al.,* 2011; Mariam *et al.,* 2013; Odero-Waitituh *et al.,* 2016; Yusuf *et al.,* 2008). Also, studies by Cruz-Alcedo (1999), reported the pods to be composed of non-starch polysaccharide galactomannans. They are structurally formed by a linear β (1-4)-linked backbone of D-mannose molecules to which single units of D-galactose are attached through α (1-6) linkage reducing enzymatic break-down. These anti-nutritive compounds reduce nutrient bioavailability. Tannic acid forms a 0.46:1 complex with glucosidase enzyme; it also forms insoluble protein complexes with lipase, trypsin, α-amylase reducing the activity of these enzymes' activity and efficiency of feed digestion (Griffiths, 1986; Goldstein & Swain, 1965). Animals with low plane of nutrition are predisposed to stress due to micronutrient malnutrition and mineral deficiencies. These animals present abnormal blood haematology and metabolite indices, an indication of immunosuppression (Giammarco *et al.,* 2012; Nakyinsige *et al.,* 2013; Marzo *et al.,* 1990; Ogunsipe *et al.,* 2014; Samtiya *et al.,* 2020).

Several researchers have reported the use of fermentation technology in reduction of anti-nutritive compounds in livestock feedstuffs. For example, breakdown of the galactomannan linkages in non starch polysaccharides (NSP), reduction of phytic acid, tannins and aflatoxins and enhancement of nutritive value of different types of feeds and feed ingredients (Adeyeye *et al.,* 2020; Cruz-Alcedo, 1999; Samtiya *et al.,* 2020). Fermentation involves catabolism of organic compounds by anaerobic or facultative anaerobic microorganisms by an internal balance of enzymatic oxidative-reductive reactions. The fermenting substrate is used as reductant and oxidant, where the oxidation reaction is coupled to substrate level phosphorylation. The result being production of the metabolites or end products. These oxidative-reductive reactions result in breakdown of the structural linkages that form the chemical structures and linkages of anti-nutritive compounds thereby reducing their concentrations in the fermented substrates (Cruz-Alcedo, 1999; Müller, 2001). Fermentation using lactic acid bacteria (LABs) in sorghum grains reduced tannins from 0.57mg/g to

0.34mg/g and enhanced essential amino acid content from 91.07mg/100g to 236.88 mg/100g. Similar findings of the enhancement and reduction of anti-nutritive compounds using LABs was reported when red and yellow lentils, white and black beans, chickpeas and peas flours were fermented (De Pasquale *et al.,* 2020). Also, Shang *et al.* (2019) reported a 78% reduction in tannin content in Xuan Mugua fruits on fermentation. When fermenting different parts of Prosopis pods and different species of Prosopis pods spontaneously and with microbial inoculums, several researchers reported enhancement of nutritional value and reduction of anti-nutritive compounds (Aremu *et al*., 2015; Cruz-Alcedo, 1999; Sarasvati *et al.,* 2014; Yusuf *et al.,* 2008). According to Cruz-Alcedo (1999), the linear β (1-4)-linked backbone of D-mannose molecules to which single units of D-galactose are attached through α (1-6) linkage were broken down on microbial fermentation *in vitro*. Also, Sarasvati *et al.* (2014) reported that a multi-strain probiotic of *B. subtilis, B. circulans and S. cerevisiae* reduced tannins and crude fibre in Prosopis pods.

The objective of this study was to determine the effect of fermenting mature ground Prosopis pods spontaneously, where naturally occurring environmental microorganisms are used as starter culture and with pure selected starter culture of probiotic inoculums (*S. cerevisiae* and *L. salivarius*) on pod's proximate composition, nutritional value, anti-nutrient content plus growth and reproductive performance when included in rabbit diets. Use of Prosopis pods is expected to contribute to sustainable supply of rabbit feeds and rabbit meat at affordable prices, increase of rabbit farmer household incomes and improvement in animal protein in food security.

1.2 Statement of the problem

Commercial rabbit feeds are compounded from cereals which are also a source of human food. They are usually in short supply, and in seasons of scarcity, the quality available is low and of high cost. This impacts negatively on the quality, quantity, and increases the prices of commercial rabbit feed, leading to poor rabbit growth and breeding performance, animal protein deficit, food and nutrition insecurity. Mature Prosopis pods have good nutritional value and can replace maize in rabbit diets. However, they contain anti-nutritive compounds (ANC) (high CF, tannins, aflatoxin and phytic acid) that lower the pods' nutrient utilization by livestock. Fermentation technology has been proposed as an intervention to minimize the negative effects of the ANC on nutrient utilization. This study therefore investigated the use

fermentation technology on reduction of ANC in mature *Prosopis juliflora* pods and performance of rabbits fed on diets with mature ground fermented pods.

1.3 Objectives

1.3.1 Broad Objective

To contribute to improved food and nutrition security through sustainable rabbit production by optimum utilization of Prosopis pods as a feed resource in commercial rabbit diets through fermentation technology.

1.3.2 Specific objectives

i. To determine the effect of fermentation on the nutrient (CP, essential amino acids) and ANC (CF, tannins, phytic acid and aflatoxin) composition of ground mature *Prosopis juliflora* pods (GMPP).
ii. To determine the effect of fermentation on energy values, apparent and true digestibilities of nutrients in ground mature *Prosopis juliflora* pods (FGMPP).
iii. To determine the effect of incorporation of FGMPP in grower rabbits' diet on average daily feed intake (ADFI), average daily weight gain (ADWG), level of blood haematological and metabolite indices.
iv. To determine the effect of incorporation of FGMPP in grower rabbits' diet on carcass characteristic, pH of caecum contents, descriptive sensory characteristics of meat and economic benefit.
v. To determine the effect of inclusion of FGMPP in lactating does diet on kits growth and does' body weight change.

1.4 Hypotheses

i. Fermentation has no significant effect on the nutrient (CP, essential amino acids) and ANC (CF, aflatoxin, phytic acid, tannins) content of GMPP.
ii. Fermentation has no significant effect on energy values, apparent and true nutrient digestibilities of ground mature *Prosopis juliflora* pods.
iii. Inclusion of FGMPP in grower rabbits' diet has no significant effect on, ADFI, ADWG, level of blood haematological and metabolites indices.

iv. Inclusion of FGMPP in grower rabbits' diet has no significant effect on carcass characteristics, pH of caecum contents, descriptive sensory characteristics of meat and economic benefit.
v. Incorporation of FGMPP in a lactating doe's diet has no significant effect on kits' growth and doe's body weight change.

1.5 Justification

Feed cost accounts for about 70% of rabbit production cost (Oliveira *et al.,* 2008). About 70% of commercial feed is compounded from cereal and agro-industrial ingredients which are usually produced under rain-fed conditions. Effects of global warming have led to frequent drought, crop pests and sometimes excessive rainfall with some regions experiencing complete crop failure. This has led to scarcity of cereals and other agricultural products (Ochieng *et al.,* 2016; Tongruksawattana & Wainaina, 2019). About 87% of Kenyans consume maize as a dietary staple food at an average intake of 400g per person per day. However, 3 million tonnes of maize were produced in Kenya in 2013 which was far much below the estimated national consumption of 4 million tonnes (Chisholm, 2014; Mbithi & Huyleenbroeck, 2000; Shephard, 2008). The deficit between production and consumption has been experienced through the years necesitating annual maize importation to bridge the deficit gap of over 2 million tonnes (Kariuki *et al.,* 2020; Mbithi & Huyleenbroeck, 2000) The above factors have led to a scarcity of cereal ingredients for the production of rabbit feeds, resulting in low growth and breeding performance of rabbits. Therefore, stakeholders (producers and feed millers) in the rabbit industry are in need of locally available feed ingredients that are cost-effective and available throughout the year. Prosopis pods are locally available and have been reported to be high in CP 18.5% (Koech *et al.,* 2010), 13% and 69% sugars and carbohydrates, respectively (Choge *et al.,* 2007) and 12.8 MJ/kg ME (Odero-Waitituh, 2015). This chemical composition made the pods be identified as an alternative feed resource for livestock feed production. Prosopis thrives well in the ASALs (which comprise 70% of Kenya's land mass), waterlogged and saline soils and produces pods all year round (King'ori *et al.,* 2011). Several researchers have recommended treatment of mature pods to improve nutrient utilization and livestock performance. Pod fermentation using spontaneous, microbial and probiotic cultures, has given positive results on reduction of ANC, animal nutrient utilization and growth (Sarasvati *et al.,* 2014; Yusuf *et al.,* 2008).

However, there is scanty information available on the use of fermented Prosopis pods in feeding rabbits. This study evaluated the effect of fermenting the pods on the nutritional and feeding value in rabbits. The knowledge from this study on growth and breeding performance of rabbits fed on fermented Prosopis pods-based diets is useful in the utilization of the pods in the formulation of rabbit diets thereby ensuring improved, sustainable rabbit production and reduction of Prosopis bush encroachment in the ASALs. This will in turn contribute to food and nutrition security.

1.6 Scope and limitation of the study

Since *Prosopis juliflora* plants are not kept under the same environment and management conditions, the proximate and anti-nutritive compounds content might be variable. The pods were, therefore, collected from regions with similar management practices. Pods used for the experiment were therefore harvested from the same region with similar environmental and management conditions. It was assumed that the pods exhibited similar proximate composition and content of anti-nutritive compounds.

1.7 Definition of terms

Post-Weaning phase: Solid feeding period immediately after weaning (5-14) weeks in rabbits.
Doe: Mature female rabbit
Anti-nutritive compounds: Substances which when ingested, interfere with absorption or metabolism of other nutrients.
Probiotics: Live microorganisms which when administered in adequate amounts confer a health benefit on the host.
Primiparous: Adult female ready for first or second service.
Kit: Young rabbit before weaning
Pre-weaning phase: Milk feeding period from birth to weaning (35-42) days in rabbits
Spontaneous fermentation: Fermentation under natural conditions/ without inoculant
ad-libitum: Without restriction

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of livestock industry in Kenya

Livestock sector accounts for up to 10% of the gross domestic product (GDP) (Kiptarus, 2005) and 6% of Agricultural exports (GoK, 2007) and makes over 30% of farm gate value of agricultural commodities (Kiptarus, 2005). The sector accounts for 95% of family incomes in the ASALs, (MoLD-Strategic Plan, 2008). Also, there was a proposed provisional budget allocation of Ksh 249 million for the fiscal year 2018/19 by Kenyan government to State Department of Livestock to drive the '**Big Four'** Agenda of ensuring food and nutrition security, industrialization, manufacturing, and agro-processing to ensure broad-based economic growth by 2022 (Mungayo, 2018). The sector comprises of a mixture of large and small-scale farmers as well as self-help groups (Radull, 2000) keeping dairy cattle, beef cattle, rabbits, goats and sheep, fish, rabbits and emerging livestock (shrimps, quails, and snails) with 3.2% of livestock keepers rearing rabbits (Kale *et al.,* 2016).

2.2 Rabbit production in Kenya

Rabbits are livestock in the order *Lagomorpha* with two families namely *Leporidae* and *Ochotonidae*. The modern domesticated rabbit *Oryctolagus cuniculus* is a descendant of the European rabbit. The domestic rabbit has an average lifespan of six to seven years. Males are called bucks; females are does and yound ones are kits. Their weight is in the range of 0.5-5 kgs depending on breed and age (Das *et al.,* 2014). Rabbit production has been considered an enterprise for small boys and subsistence in most areas of Kenya (Owen *et al.,* 1977). However, this has changed over the years (Hungu *et al.,* 2013) with resulting change in rabbit ownership in Kenyan farms, where 75% of rabbits belong to household heads or their spouses, most of who have attended tertiary colleges (Serem *et al.,* 2013). It is one of the fastest growing livestock enterprises due to the reduction in land size holdings (Borter & Mwanzia, 2011) and its contribution to income and nutrition among the smallholder farming communities (Kale *et al.,* 2016).

There have been consistent efforts by the Kenyan government to promote rabbit production in provision of free extension and subsidized veterinary services at the county and national levels (Borter & Mwanzia, 2011). The Ngong National Rabbit Breeding Centre at Veterinary

Farm, Ngong was established with an objective of country wide provision of breeding material to farmers and multiplication centres (Kemose Sheep and Goat Station in Baringo County; Matuga Sheep and Goat Station in Kwale County; Marimba Livestock Government Farm in Meru County and Witi Livestock Government Farm in Lamu County). According to Mungayo (2018), rabbit production promotion had a budget allocation of Ksh 10 million in the provisional budget for the fiscal year 2018/19 and it was ear marked as one of the sectors that was to drive Kenyan '**Big four Agenda'** of ensuring 100% food and nutritional security. The government of Kenya finances and supports rabbit procud

The common rabbit breeds kept by Kenyan farmers are New Zealand White, California White and their crosses (Hungu *et al.*, 2013; Ogolla *et al.*, 2017; Rangoma, 2012; Serem, 2013). Others, are Chinchilla, Dutch, Flemish Giant, French Ear lop and Kenya White (Hungu *et al.*, 2013). However, studies by Kale *et al.* (2016) indicated that Chinchilla was the common breed found in the western regions of Kenya. Kenyan rabbit population is estimated at 0.9 million rabbits as compared to 17.8 million cattle, 17.4 sheep and 25.4 goats having produced, 348.4 metric tonnes of meat valued at Ksh 139.4 million in 2014 (Figure 1) (GoK, 2015).

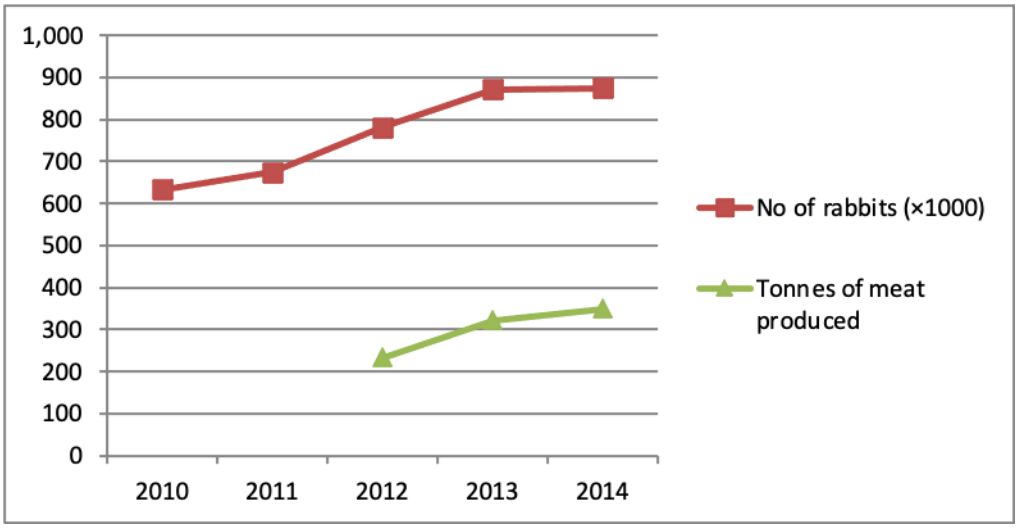

Figure 1. Number of rabbits and amount of meat produced

Source: GoK (2015)

2.2.1 Rabbit production systems in Kenya

Rabbit production system in Kenya is mainly small-scale production system directed towards home consumption and income generation (Serem *et al.*, 2013) due to small land space

(Hungu *et al.*, 2013). About 87.4 % of Kenyan farmers who keep rabbits practice smallholder system of production where (10-20) rabbits are kept in cages in the backyard for income generation and home consumption (Bergevoet & Van-Engelen, 2014; Mailu *et al.*, 2014; Mbutu, 2013; Serem *et al.*, 2013). Small scale rabbit production is dominated by ultra-small- and small-scale producers having minimal investment in housing, feeding and other management practices (APD, 2010) with unstructured production preventing farmers from clearly projecting the number of rabbits they are able to avail to the market at any given time. Furthermore, Serem *et al.* (2013), classified the Kenyan rabbit production system using criteria by Oseni *et al.* (2008), into ultra-small-scale having 0-2 does and a few growers, small scale having 10-30 does, medium scale having 11-50 does and large scale having over 50 does each system with a share of 40.2 %, 44.6%, 12.8% and 2.4% respectively. According to Kale *et al.* (2016) most small-scale farmers used home accessible feeds comprising of wild green forage that is available mostly in the rainy season and leaves of trees or bananas during the dry season and commercial feeds at 92.3% and 7.7%, respectively.

2.2.2 Commercial rabbit diets: The current situation

Feed ingredients used in the production of livestock feeds especially non-ruminant livestock are mostly cereals and their by-products. The use of cereals creates competition between livestock and humans (Odero-Waitituh, 2015). The production of these cereals is majorly dependent on rain-fed agriculture. Also, effect of climate change and drought has resulted in scarcity of cereals and their by-products (Mbithi & Huyleenbroeck, 2000). Also, maize production in Kenya was estimated at 3 million tonnes in 2013 versus the estimated national consumption of 4 million tonnes. Of the produced maize, it was estimated that about half is consumed by non-ruminant livestock resulting in shortages (Chisholm, 2014; Smith *et al.*, 2013). Maize shortage in Kenya in 2019 pushed up the price of livestock feeds from 2,200 Ksh to 3,200 Ksh for the 70 kg bag. This was due to use of maize as a feed ingredient while it's also food for man (Andae, 2019). This has resulted in fluctuation in quantity, quality and cost of feeds resulting in poor growth and breeding performance which compromises profits for the farmers who keep rabbits and protein food security. According to Mburu (2015) most crops used as livestock are rain fed leading to fluctuation in production; in rainy season, there is high dry matter (DM) production with higher %CP and lower %CF and higher digestibilities while during the short rains and dry season, there is lower DM production with lower %CP, higher %CF and lower digestibilities. There is a direct relationship between

nutritional value of feeds, metabolizable energy (ME) value and efficiency of feed utilization. A 22.75% deficit in annual feed production and animal requirements was reported when rain fed crops were used in feeding livestock (Stevens *et al.,* 2002). In Kenya, 22 % of commercial rabbit farms reported that they changed their rabbit feed supplier due to seasonal fluctuation in quality, quantity and price of rabbit feeds. This deficit resulted in undernutrition, reduced reproductive and growth performance of animals (Hungu, 2011; Mburu, 2015; Stevens *et al.,* 2002).

2.2.3 Challenges of rabbit production in Kenya

Poor management practices, inadequate quantity, and quality of feeds, pests and diseases, unstable markets, lack of quality breeding stock and limited extension services (Hungu *et al.,* 2013; Rangoma, 2012; Serem *et al.,* 2013) are reported to be major challenges to rabbit production. According to Okumu *et al.* (2015), the main diseases in rabbits are diarrhoea, sudden death, and bloat at 81.97%, 73.78%, and 68.85% respectively. Other diseases are ear canker and pneumonia. However, Ogolla *et al*. (2017), reported coccidiosis to be the major rabbit disease in smallholder rabbit farms, at a prevalence rate of 49%. Also, Hungu *et al.* (2013) reported predation a constraint at 29%. There are other challenges to rabbit production as susceptibility to heat stress especially in the tropics (Cheeke, 1986); requirement of skills in rearing rabbits (Cheeke, 1986; Hungu *et al.,* 2013; Kale *et al.,* 2016) and market access (Kale *et al.,* 2016). According to Mailu *et al.* (2014), 38% of rabbit keepers who consumed rabbit meat did so at most once every year. Rabbits are considered as pets (Lukefahr & Cheeke, 1990; Oseni, 2016) and therefore not important as food in the traditional set up of the communities.

The choice of a feeding strategy to be used in rabbit production must ensure reduction of digestive disorders, increased nutrient utilization leading to improved growth and reproductive performance and must be cost effective (Xiccato & Trocino, 2010). Rabbit production in Kenya is based on feeding of weeds and grass hays which compromises growth and reproductive performance of rabbits (Sergon *et al.,* 2020). Also, Kibugu *et al.* (2019) reported that mycotoxin level was significantly high ($p<0.01$) in commercial feeds and feed ingredients in the Kenyan market, exposing rabbits to chronic levels in feeds. This interferes with nutrient absorption, utilization and metabolism at the same time predisposing the rabbit to metabolic and nutritional disorders. This creates an opportunity for use of mature Prosopis pods in commercial feed manufacture due to their availability. Fermentation of the pods in

rabbit diets will provide enhanced nutrients and ensure reduction in anti-nutritive compounds especially mycotoxins.

2.2.4 Opportunities for rabbit production in Kenya

Kenyan population growth has been on the increase at 2-3% per year (KNBS, 2009). This is expected to cause a proportionate increase in demand for rabbit meat due to human nutritional demands for animal protein. According to Jayne *et al*. (2017), there is a steady subdivision of agricultural land in Kenya, resulting in the reduction of arable land size to 0-2 hectares as the total number of farms increased by 50% by 2006. This implies that small-scale intensive agriculture will be more feasible in food production and ensuring food security. Rabbit production can be integrated into small farming systems, with rabbits being fed on crop residues, weeds, waste fruits, vegetables and poultry droppings (Cheeke, 1986; Mailafia *et al.,* 2010; MoLD, 2012). Also, 51% of Kenyan rabbit farmers have been keeping rabbits for a period of 1-5 years and 29% are new farmers indicating that rabbit farming is gaining popularity (Hungu *et al.,* 2013). This trend is expected to create a linear relationship in demand and supply for rabbit meat.

2.2.5 Rabbit meat consumption in Kenya

Recent studies indicate that consumer food choices for meat and meat products are influenced by their understanding of the effects of the meats on their health, descriptive sensory attributes of the products and reasonable prices (Mermelstein, 2002). Rabbit production and meat consumption is therefore a feasible option to meet demands of consumers and ensure animal protein supply thereby preventing malnutrition (Mutsami *et al.,* 2019). However, this has not been realized as only 42% of Kenyan population consumes rabbit meat due to challenges in marketing and inadequate knowledge on the benefits of rabbit meat consumption (Borter & Mwanzia, 2011; Mailu *et al.,* 2017)

The Kenyan annual per capita meat consumption ws estimated at 10.8 and 1.1 for red and white meat respectively (MoAL&F, 2015), 3 less for red meat than at independence (FAO, 2013). This is far below the world's average of 34. This needs to be addressed to improve on supply of animal protein (Mbutu, 2013). A large population of Kenyans, especially in the urban areas consume both red and white meat with different kinds of meats considered as luxury and necessity. Mutton and chevon were considered a necessity good among the meat products while bone beef and chicken a luxury good (Shibia *et al.,* 2017). However, as

income improved and better understanding of health implications of meat consumption, the consumption of white meat increased as compared to red meat (Gamba, 2005). This change in consumption trend where the Kenyan urban population was substituting red meats with white meats was due to the popularity of the livestock that produce white meats plus the health benefits attributed to white meat consumption (Bett *et al.* 2012; Magothe *et al.*, 2012). Also, Lebas *et al.* (1997) reported that rabbit meat is the only white meat with high level of linolenic acid, one of the omega 3 fatty acids useful in reduction of inflammatory conditions thus reducing infections. Therefore, there exists opportunities in keeping rabbits as the market for their products is readily available due an increasing population that prefers white meat.

2.3 Benefits of keeping rabbits

Rabbits are generally reared for meat, cash generation, fur and as pets (Bergevoet & Van-Engelen, 2014; Hungu *et al.,* 2013; Serem *et al.,* 2013). Rabbit production is a feasible enterprise to farmers as it requires less space, low capital for investment; rabbit manure can be used fertilizing crops and rabbits use crop residues as feed-in smallholder systems (Cheeke, 1986; Kale *et al.,* 2016; Tembachako & Mrema, 2016). Rabbits can be reared on diets consisting wholly of roughages and can utilize herbage biomass more efficiently than ruminants posing minimal competition with humans for food (Cheeke, 1986; MoLD, 2012). They are highly prolific, producing up to 40 kits a year. Small-scale backyard rabbit units can be established in the backyard with little start-up capital and land (MoLD, 2012). Their small size makes them easy to raise and handle by vulnerable members of the household as women and children (MoLD, 2012). Other benefits attributed to the small size include; low feed consumption, ease of rearing and home consumption; can be eaten in one meal, presenting no conservation problems (Butcher *et al.,* 1981). Other non-marketed benefits of rabbits are the provision of manure by urine and faeces (Cheeke, 1986; Mbutu, 2013). Farmers can sell rabbits in case of a family need for finances (Serem, 2013). There are employment opportunities in meat marketing and services related to general rabbit production including, provision of feeds, veterinary care and extension services (GoK, 2007). Other attributes include; early maturity, fast growth and high genetic selection potential (Cheeke, 1986; MoLD, 2012). Rabbit meat production is therefore expected to reduce animal protein malnutrition, create employment and increase incomes in rural and urban populations (Cheeke, 1986; Mbutu, 2013).

2.4 Benefits of rabbit meat to human health

Rabbit meat is of high quality, with high protein and low-fat content (Cheeke, 1986). Rabbit meat is composed of 21% CP, 7.1% crude fat and 71.2% moisture (Pla *et al.,* 2004). Comparative to all other meats, it has the highest amount of linolenic acid which reduces inflammation (Lebas *et al.,* 1997), high protein, calcium and phosphorus, low calories and sodium (Nelson, 2011) with low cholesterol level compared to red meat (Chakrabarti *et al.,* 1999). This is good for reducing the occurrence of lifestyle diseases caused by high cholesterol in the diet (Shrivastava *et al.,* 2012) and high blood pressure (Nelson, 2011). The nutritional value of rabbit meat compared to other meats (Figure 2).

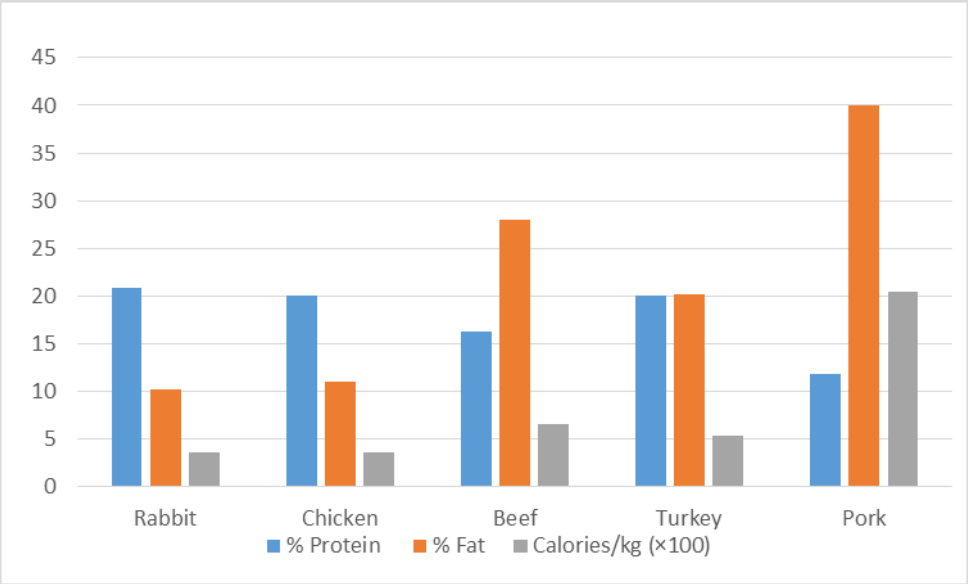

Figure 2. Comparative nutritional value of rabbit meat

Source: Nelson (2011)

2.5 The digestive system of the rabbit

The nature of an animal's digestive system dictates the type of feed it's able to digest. The rabbit's digestive system is different from other domestic animals, being classified as a non-ruminant but able to digest fibrous feeds (Cheeke, 1987). It exhibits a digestive physiology adapted to high intake of dietary fibre, fermented in the hindgut but also adopted to intake of high concentrate diets efficiently digested in the upper segment of the GIT (Carabano *et al.,* 1998). This is possible due to their feeding and digestive strategies comprising enzymatic digestion, in the stomach and small intestine, followed by fermentation of feed residue in the caecum and large intestines (Cheeke, 1987; Leng, 2008). The total length of the alimentary

canal of a rabbit is 5 m having; a short oesophagus, a 3 m long small intestine, 45 cm long caecum and a 1.5 m long colon. The physiology of digestion in the rabbit depends on adrenal secretions and except for the functional caecum; its digestive physiology is of a non-ruminant (Lebas *et al.*, 1997).

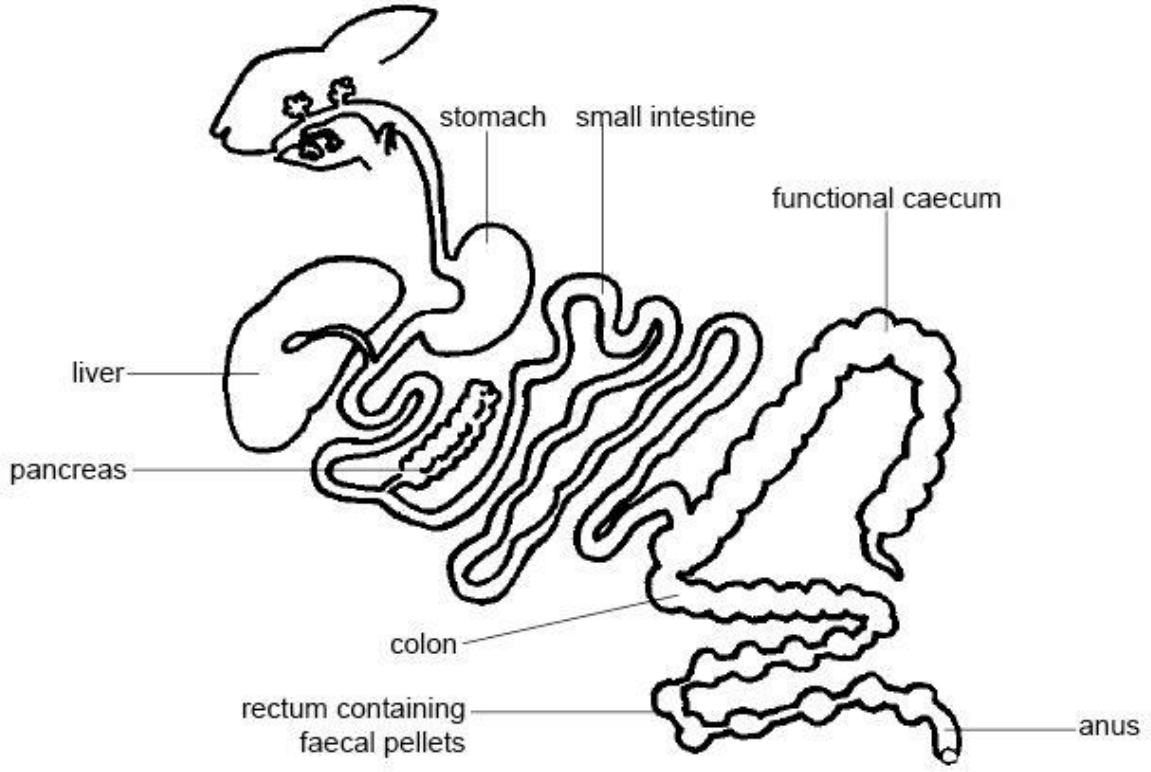

Figure 3. Anatomy and physiology of animals/the gut and digestion in the rabbit

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

2.5.1 The caecum of the rabbit

The caecum makes up 49% of the total capacity of the digestive tract of a rabbit (Figure 3). The digestive system of the rabbit is capable of 40-50% pre-caecal digestion of digestible fibre and non-starch polysaccharides. The caecum is the site of microbial fermentation (Szendro *et al.,* 2011) converting the rest of the fibre components leading to the production of VFAs (acetic, propionic and butyric acids), concentrations depending on the diet (Guedes *et al.,* 2009). According to Leng (2008), 40% of rabbits' energy requirement is provided by the VFAs which are absorbed in the caecum and colon. The fibre in a rabbits' diet also prevents

digestive disturbances by preventing the proliferation of toxin-producing bacteria in the caecum (Marounek *et al.,* 1997) due to carbohydrate overload (Cheeke & Patton, 1980). Franck *et al.* (2016), reported high morbidity and mortality when rabbits were fed low fibre diets, this situation was rectified when the level of dietary fibre was increased in the diets resulting in high digestibility due better intestinal function and increased growth rate.

Also, the caecum is important in protein reutilization, supplying about 18% of the total CP intake during caecotrophy, the highest values got when low digestible diets that increase the flow of undigested but digestible protein to the caecum (Szendro *et al.,* 2011). Caecotrophy contributes to recycling 36% of the total protein excreted, which is mainly of bacterial origin. This protein is a good source of the most frequently limiting amino acids (methionine, Lysine, and threonine). Microorganisms in the caecum also synthesize vitamins K and B complexes, therefore, no need for supplementation (Szendro *et al.,* 2011).

2.5.2 Feeds and feeding in rabbits

Rabbits are omnivorous with a unique digestive system. They are therefore able to feed on a wide range of unconventional feed resourses. This attribute makes rearing them less costly interms of feeding. However, level of inclusion of these unconventional feed resourses should be high enough to ensure optimum performance but at the same time low to prevent toxicity. These feed stuffs include but are not limited to citrus pulp, sunflower cake, cotton seed meal, fruit industry byproduct, apple pomace, neem seed meal, cow pea hulm and peanut hulm (Das & Khargharia, 2004). Rabbits are able to digest fibrous feeds at the same time feeds with soluble carbohydrates. About one third of rabbit feeds are composed of fibrous feed ingredients as Lucerne hay that is ground and in cooperated in to the commercial concentrate feed formulations (García *et al.,* 1995). Feed ingredients for rabbits therefore range from highly digestible ingredients as maize to fibrous materials as wheat straw and sunflower meal (Wiseman *et al.,* 1992). The anatomy and physiology of their digestive system allows for digestion of highly digestible feeds in the stomach and absorption in the small intestine. The fibrous materials are then fermented in the caecum, where volatile fatty acids (VFAs) are produced absorbed in the caecal and colon walls and amino acids produced from the microbial proteins re-ingested as ceacotrophs to be absorbed in the small intestines (Leng, 2008). In most tropical regions where rabbit production is practised by small boys for subsistence, weeds and grass hays are fed. However, in largescale commercial rabbit production commercial rabbit pellets are fed (Sergon *et al.,* 2020).

There exists a variety of weed and vegetables in the tropics, with different digestibilities; such as *Amaranthus hybridus, Corchorus olitorius, Myrianthus arboreus, Ipomea batatas, Vigna unguiculata, Abelmoschus esculentus, Solanum melongena, Hibiscus sabdariffa, Celosia argentea, Basella alba, Manihot esculenta, Talinum triangulare, Colocasia esculenta*. These weeds reported different growth performance when fed to rabbits with and without concentrate supplementation. There were variations in feed intake, feed convertion ratio and growth rates when the vegetables were fed to rabbits. However, in commercial rabbit feeding and production in different production systems in the tropics, they were important feed resources due to their availability (Franck *et al.*, 2016). Other feed resourses are but not limited to fruits, grasses, fodder trees and kitchen wastes such as cabbages, carrots, pineapple, strawberries, broccoli, papaya, grass hays which must be of good quality, fresh grass, mulberry leaves, Leucena and Gliricidia. Most of the fodder tress are used for supplementation (Das *et al.*, 2014).

2.6 An overview of stress in livestock

Stress is defined as a threat to an organism to which the body needs to adjust. The classification of stress involves psychological, physical and interoceptive aspects (Von-Borell, 2001). Stress denotes a real or perceived disturbance to an organism's physiological homeostasis, psychological well-being (NRC, 2008) or physical well-being and the state of each will affect the state of the other (McWilliams, 2001). These stressors include heat, cold, housing, handling, nutrition, light and dark cycle, and interaction or lack of interaction (McWilliams, 2001). Siegel (1980) classified stress sources into specific and non-specific, the body's response being short and long-term responses respectively. The response is by behavioral or physiological mechanisms or both to counter the disturbance (NRC, 2008). An animal's reaction to specific stress is by combating the stressor by behavioral changes (Siegel, 1980). However, the reaction to non-specific stress is by adapting to the stress (Siegel, 1980) through physiological mechanisms (Virden & Kidd, 2009). Stress, therefore, stimulates defense responses in animals (Scope *et al.*, 2002).

(ⅰ) Animals' physiological response to stress

An animal responds to stress by initiating adaptation responses which involves anatomical, physiological, morphological, blood biochemical, cellular and neuro-endocrine responses and behavioral changes to its environment geared towards promoting its welfare and ensuring its survival in the specific environment (Afsal *et al.,* 2018; Sejian, *et al.,* 2010). When animals are exposed to heat stress, they exhibit behavioural changes such as seeking the shade, high water consumption and increased standing time (Shaji *et al.,* 2017). An animals' physiological response to heat stress involves re-establishment of heat balance with their surroundings, reduction of activity to reduce heat production and reduction of feed intake to reduce heat production (Suganya *et al.,* 2015). According to NRC (2008), some of the physiological responses to long-term stress include changes in blood glucose levels, increased calcium and lactic acid levels and, low levels of lymphocytes (Nakyinsige *et al.,* 2013; Virden & Kidd, 2009) and, a double increase in serum corticosterone concentration (Giammarco *et al.,* 2012; Virden & Kidd, 2009). Suganya *et al*. (2015) suggested that nutritional manipulations could help alleviate stress due to high temperatures and therefore nutritional supplementation of amino acids, vitamins, minerals, higher energy and feed additives improved livestock performance. According to Isaac *et al*. (2013), haematological components are important in monitoring feed toxicity and therefore used in routine screening of health and physiological status of livestock (Aro *et al.,* 2013). It is, therefore, expected that any anti-nutritive compounds, nutritional deficiencies or excesses in livestock diets could lead to long-term stress.

2.7 *Prosopis juliflora* in Kenya: An overview

The plant *Prosopis juliflora* belongs to the *Fabaceae* family, *Mimosoideae* sub family and the genus *Prosopis*. It is native to south America but was introduced to Africa and Asia as an agroforestry measure for environmental conservation and desert rehabilitation. The plant is thorny having a wide, flat-topped crown with several variaties and genetics (Harris *et al.,* 2003; Prasad & Tewari, 2016). Prosopis (*Prosopis juliflora*) is a small, fast growing, drought-resistant, evergreen plant requiring only small quantiiies of water for its growth due to its rooting system (deep taproot) which can reach 35 metres deep. This makes it suitable for dry areas with an annual rainfall range of 150 and 700 mm (Maundu *et al.,* 2009; Von-Maydell, 1986).

Its invasive nature in various parts of the world has resulted in significant economic, environmental and social losses (Anderson, 2005; Prasad & Tewari, 2016). In Africa alone, Prosopis is believed to have invaded over 4 million hectares, threatening crop and rangeland production, desiccating water resources and displacing native flora and fauna (Witt, 2010).

Figure 4. *Prosopis juliflora* plant infestation in Baringo, Kenya

Source: Shitanda *et al*. (2013)

In Kenya, it was introduced in the early 70s in the coastal town of Bamburi; the planting materials were sourced from Brazil and Hawaii (Choge *et al.,* 2002). Large scale planting of Prosopis plant in the rift valley and coastal provinces was done by Fuelwood afforestation programme and National irrigation board respectively (Maundu *et al.,* 2009). Currently in Kenya, it is widespread in the Rift valley especially Baringo County (Figure 4), north Eastern and coastal regions (Figure 5). Prosopis species are generally fast-growing, drought-resistant, nitrogen-fixing trees or shrubs adapted to poor and saline soils in arid and semi-arid zones (Anderson, 2005). The plant matures in 3 to 4 years producing 2.04 tonnes/acre to 17.65 tonnes/acre depending on plant population, rainfall, management practices and elevation (Kyuma *et al.,* 2018; Sawal *et al.,* 2004). It improves both moisture and organic matter in soils and could be used for soil fertility improvement as it fixes nitrogen in soils (Shitanda *et al.,* 2013).

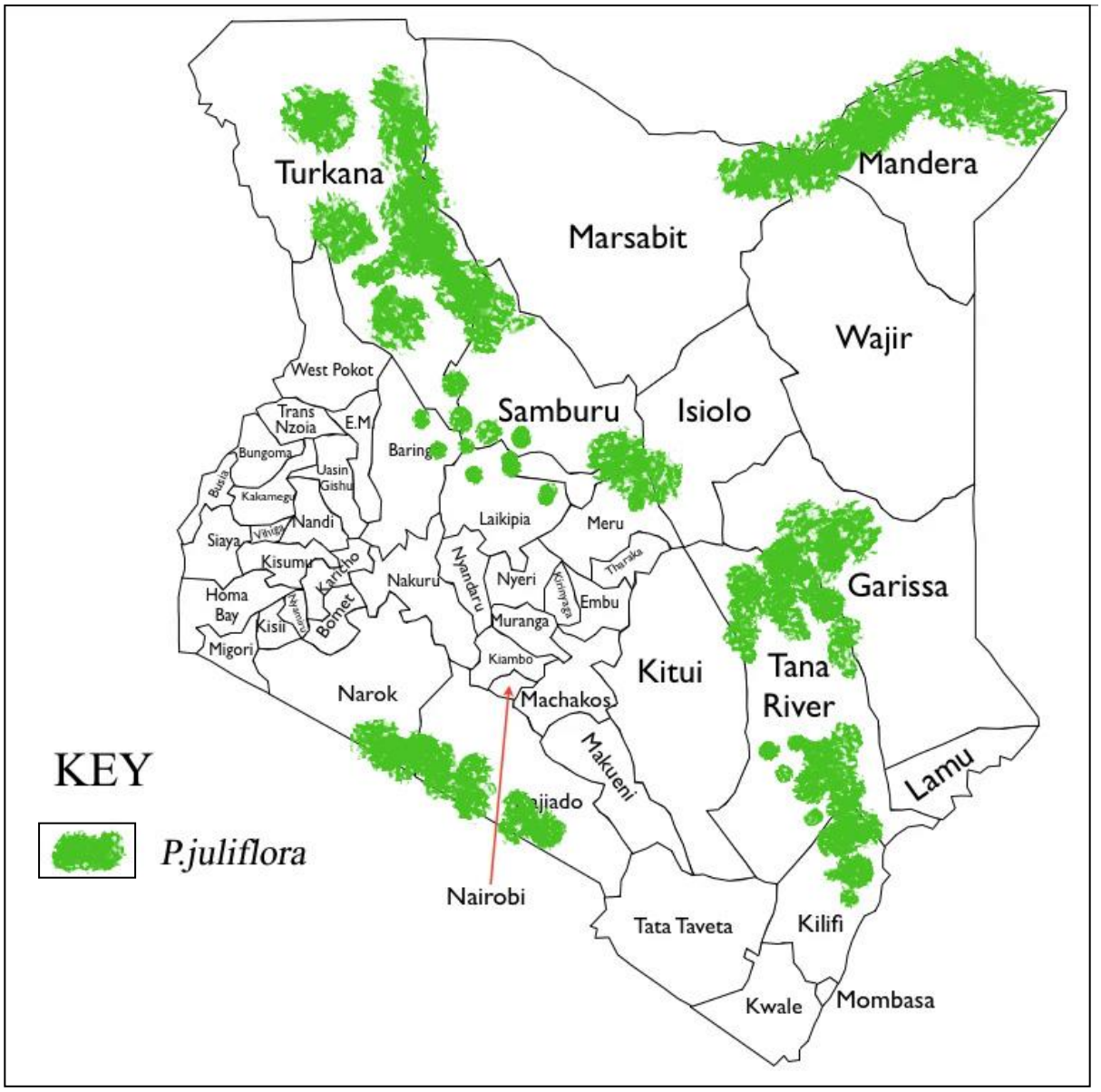

Figure 5. Distribution of *Prosopis juliflora* in Kenya

Source: Sirmah (2009)

2.7.1 Nutritional properties of Prosopis pods

Indigenous knowledge on browse preferences by livestock reported *Prosopis juliflora* as a good livestock feed as it improved performance (Anttila *et al.,* 1993). Several researchers have reported satisfactory nutritional content of Prosopis pods (Choge *et al.*, 2007; Odero-Waitituh *et al.*, 2015; Sawal *et al.*, 2004; Shitanda *et al.*, 2013). Odero-Waitituh (2015) reported that the ME content was 12.8MJ/kg. Shitanda *et al.* (2013), reported that ripe pods contained 46.3±5.18 mg/100g vitamin C; 12% CP of which 7% was digestible (Sawal *et al.*,

2004). Also, Mohammadabadi and Chaji (2018) in experiments to investigate *in vitro* gas production and *in situ* degradation of Mesquite leaves and pods in Arabian camels in Iran, reported potential for use of *Prosopis juliflora* pods and leaves as non-conventional livestock feed resource. However, Odero-Waitituh (2015) reported mineral content of 1.35mg/g and 2.43mg/g for P and Ca, respectively; this was noted to be lower than requirements for most livestock and recommendations made that supplementation is paramount. According to Odero-Waitituh *et al.* (2015) and Shitanda *et al.* (2013) the supplementation is to conform to specific livestock requirements for P and Ca.

2.7.2 Anti-nutritive compounds in Prosopis pods

Mature Prosopis pods are non- conventional feed resources that can be used by all classes of livestock but presence of anti-nutritive compounds hampers their use. The anti-nutritive compounds found in the pods are alkaloids, tannins, phenolics, steroids, terpenes and flavonoids (Odero-Waitituh *et al.,* 2015; Ruiz-Nieto *et al.,* 2020). Odero-Waitituh *et al.* (2015) reported 8 % tannin level and 17% CF in Prosopis pods which interfered with livestock performance with recommendations that treatment could reduce their effects. According to Cruz-Alcedo (1999) mature pods of Prosopis comprise non-starch polysaccharide galactomannans composed of mannose and galactose forming a linear β (1-4)-linked backbone of D-mannose molecules to which single units of D-galactose are attached through α (1-6) linkage that reduces enzymatic digestion, reducing livestock performance (Odero-Waitituh, 2015). According to Cruz-Alcedo (1999) these linkages were completely broken within four hours of *in vitro* microbial fermentation. Treatments methods as enzyme, fermentation, soaking, addition of probiotics have been reported to reduce the anti-nutritive compounds and improved livestock performance (Chovatiya *et al.,* 2018; Mariam *et al.,* 2013; Yusuf *et al.,* 2008).

2.8 Feed additives in livestock nutrition

There are a variety of feed resources available for animal feeding. However, their use is hampered by factors that limit the ability of the animal to ingest, digest the feed resourses, assimilate and metabolize the products from digestion of these feed resources. Various treatments and strategies are available to allow for efficient use of these feed resources such as addition of feed additives, chemical or physical processing of the feed resourses and microbiological processes. Application of these processes significantly reduces the challenges and the dependence of livestock keepers on purchases of sometimes expensive conventional

feed resourses and therefore efficient utilization of locally available unconventional feed resourses (Grigoriev & Volkov, 1985). Processing of the feed resourses allow for the animal to ingest the feed by improving palatability of these feeds, unlocking the bound nutrients making them available for metabolism, efficient utilization and therefore improved livestock performance (Afanasievna *et al.,* 2015).

Feed additives are non-nutritive substances, preparations and/ or micro-organisms that are added to livestock feeds. They are important in livestock performance as their use help in meeting the animal's nutritional needs in terms of energy and maintaining the sugar protein ratio at the required physiological range. (Afanasievna *et al.,* 2015). This results in improved feed intake, growth performance and the efficiency of feed utilization for healthy, economical and eco-friendly livestock production (Sing, 2015). According to Eiben (2008) and Sing (2015) they include but not limited to probiotics, prebiotics, enzymes, organic acids, herbal extracts, antibiotics, and coccidiostats. According to Bimrew (2014), feed additives improve the plane of nutrition by increasing the availability of nutrients from feed and reducing feed wastage by improving feed digestibility. However, resent studies have reported that some feed additives when consumed by livestock, predisposes the animal to ill health and the animal products produced by such animals are unsafe for human consumption and health. For instance, growth promotors and antibiotics are not recommended for use as feed additives as they result in antibiotic resistance in livestock and humans. It is therefore recommended that feed additives that will improve feed utilization and performance but at the same time be safe for animal health and welfare and products produced be safe for human consumption and health should be used. Such feed additives include microbial cultures (probiotics) used in the ruminant to manipulate biochemical processes and therefore ruminal microbial composition and prebiotics (oligosaccharides and lectins) can successfully be used instead of growth promoters and antibiotics in improving livestock production efficiency (Al-Dobaib & Mousa, 2009; Yadav *et al.,* 2016).

Prebiotics are undigestible feed additives that selectively stimulate growth and activity of specific bacteria in the colon resulting in selective fermentation of gut microorganisms (Gibson & Roberfroid, 1995). They therefore induce the growth or activity of beneficial microorganisms (probiotics) like bacteria and fungi. Mode of action of prebiotics is by initiating gut fermentation and production of short chain fatty acids (SCFAs) which provides energy for metabolism and reduce intestinal pH and therefore reduced number of pathogenic

microorganisms in the gut (Van-Loo & Vancraeynest, 2008). They are therefore selectively fermented ingredients that stimulate the growth of certain healthy bacteria called probiotics (Hutkins *et al.,* 2016). They, therefore, cause specific changes, both in composition and /or activity of intestinal microflora conferring health benefits to host (Roberfroid, 2007). Recent studies have demonstrated that combinations of suitable probiotics and prebiotics may provide solutions in elimination of gut diseases and reduce microbial disorders (Gaggìa *et al.,* 2010). Examples of prebiotics are inulin and trans-galactooligosaccharides (Roberfroid, 2007), resistant starch (Zaman *et al.,* 2015), beta glucans (Arena *et al.,* 2014) and pectins (Gomez *et al.,* 2014).

Enzymes are biological catalysts composed of amino acids with vitamins and minerals. They bring about biochemical reactions without undergoing change and are used in the study of the bodys physiology and metabolism. They have been used in many fields for many years such as food processing, leather industries, brewing, baking, detergent industries where proteolytic enzymes are used and lately in the feed industry. This was due to better understanding of their property of enhancing digestion of other undigestible feed resourses (Guenter, 1997). They are useful as feed additives in livestock (especially non-ruminants) that lack endogenous enzymes for the digestion of fibrous feeds. (Khattak *et al.,* 2006). Enzymes improve livestock performance by enhancing nutrient digestibility by breaking down complex structures of anti-nutritive compounds such as non starch polysaccharides (NSP) (arabinoxylans and β-glucans resulting in high feed conversion efficiency and reduced excreta production, therefore, an eco-friendly environment (Choct, 2006; Khattak *et al.,* 2006). This is by cleaving the NSP into smaller polymers with reduced ability to form viscous digesta and enhancing nutrient digestibility (Choct, 1997). For example, supplementating the diets of finishing pigs with phytase enzyme improved digestibility of amino acids and, therefore, the efficiency of feed utilization (Zhang & Komogay, 1999). Also, apparent crude protein digestibility was improved when wheat-based diets were supplemented with beta-glucanase and xylanase in broilers (Wang *et al.,* 2005). Recent studies by Abdullahi *et al.* (2020) reported improved growth of grower rabbits even when inclusion level of tiger nut offal meal was increased to 50% and the diets supplemented with 200ppm of KINGZYME® ENZYME. The enzyme complex is composed of xylanase with the activity of at least 12000 U/g, cellulase - not less than 300 U/g, protease - 1000 U/g, amylase - 350 U/g, β-glucanase with activity at least 3000 U/g and other enzymes - pectinase, lipase.

Growth promoters are feed additives used in livestock to improve growth rates and improve overall efficiency. They include natural hormaones such as testosterones, oestrogen, and progesterone and and syhtetic ones such as melengestrol, trenbolone and antimicrobial agents such as ionophores, penicilins, tetracyclines, sulphonamides (Al-Dobaib & Mousa, 2009). In earlier studies, Coates *et al.* (1963) demonstrated that chicks given prophylactic antibiotic treatment (germ free) had improved growth performance as compared to chicks raised conventionally. This was due to their antagoniostic effect on the foreign gastro intestinal tract microflora resulting in low microbial loads, high efficiency of feed utilization and better chick growth. However, there is evidence in recent studies that subjecting animals to sub optimal levels of antibiotics can lead to future antimicrobial resistance in animals. Also, antibiotic residues in livestock products reduce food safety, predispose consumers to ill health and development of antibiotic resisitance (Al-Dobaib & Mousa, 2009). Other ways of improving feed efficiency, utilization and livestock performance have been explored and documented. For instance, Feighner and Dashkevicz (1987) demonstrated that by use of specific inhibitors, bacterial bile salt hydrolase enzyme activity was modifyied in the gastro-intestinal tract resulting in improved livestock growth. This provides an opportunity for use of the enzyme modification technology in animal growth promotion instead of antibiotics.

Probiotics are defined as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" (FAO, 2001). They are live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance (Fuller, 1989). Probiotics can be classified as bacteria in origin (several species of Lactobacillus, Bacillus etc) and non bacteria in origin (yeast and fungi); spore formers (Lactobacillus) and non spore formers (Bacillus); multi species containing an array of microorganisms and single species containing only a single species of microorganisms; allochthonous probiotics where microorganisms used are usually not habitat to the gastro-intestinal tract (GIT) such as yeast and Autochthonous probiotics where microorganisms used are normal resident of the GIT such as Lactobacillus and Bifidobacterium. Production of probiotics is by fermentation where specific temperature and pH must be maintained depending on the microorganisms involed in the production. Drying is by spray drying or freeze drying (Yadav *et al.,* 2016). In ruminants, the mode of action of probiotics is by modifying rumen fermentation resulting in an increase in population of beneficial bacteria while in non-ruminants is by stimulatory and inhibitory mechanisms; stimulations of non-

specific immunity, brush border disaccharidases and inhibitons of adhesive effect of pathogens; toxins and antagonistic effect on pathogenic micro-organisms (Auclair, 2001).

Probiotics improve the overall health of an organism by improving the microbial balance in the gut thereby improving weight gain and feed efficiency (Yirga, 2015; Shrivastava *et al.*, 2012). It, therefore, provides a potential alternative strategy to the traditional practice of sub therapeutic antibiotic use (Yirga, 2015). Probiotic use reduces the incidence of antibiotic residues in animal products and therefore antibiotic resistance in humans. Zubaidah *et al.* (2012) reported the reduction of *E. coli* and Salmonella microorganisms in faecal samples of rats given probiotics with Lactobacillus species and *Saccharomyces cerevisiae* served as a natural substitute for antibiotics (Mohamed *et al.,* 2015) and anticoccidials (Giannenas *et al.,* 2014). Probiotic microorganisms consist mostly of bacterial strains of Lactobacillus, Bifidobacterium, Lactococcus, Bacillus, Streptococcus, and yeast as Saccharomyces types which have been used for centuries in the food and feed industry (Yirga, 2015). Commonly used probiotic microorganisms are Lactobacillus and Bifidobacterium species (Gram+ bacteria) (Heyman & Ménard, 2002).

Several researchers have reported improved animal performance associated with probiotic use in feeds (Alkhalf *et al.,* 2010; Atella *et al.,* 2015; Mohan *et al.,* 1996; Mohan *et al.,* 1995). According to Nikpiran *et al.* (2013), the use of probiotic SC reduced stress due to low enzyme activity in Japanese quails. This was noted to have led to better feed intake and lower feed conversion ratio and therefore better growth rate. It has been reported that probiotics help reduce the level of cholesterol in livestock. For instance, Alkhalf *et al*. (2010) and Mohan *et al.* (1995) reported reduced serum cholesterol levels in broilers and improved laying performance and low blood cholesterol in white leghorn layers offered diets with probiotics respectively. Also, these animal products produced from probiotics exhibiting low cholesterol could help reduce cardiovascular health risk to consumers as consumption of high cholesterol diets is believed to be a predisposition to cardiovascular diseases.

2.9 Fermentation in enhancement of nutritional value of feeds

Fermentation is the conversion of sugars into organic acids or alcohol, using microorganisms like bacteria, yeast, and fungi (Rose, 1982). It involves oxidative-reactive reactions where organic compounds serve as electron donor and acceptor at the same time (Figure 6). Adenosine triphosphate (ATP) is synthesized by substrate level phosphorylation (Müller, 2001). End products as biomass, enzymes, primary and secondary metabolites and

recombinant products are gotten as a result (Paulová *et al.*, 2013). Fermentation has been used for preserving foods for centuries prior to the invention of pasteurization and sterilization, and every culture has a variety of fermented products as part of its diet (Chaves- López *et al.*, 2014). The primary benefit of fermentation is the conversion of sugars and other carbohydrates to usable end products, production of important nutrients such as peptides (Jyoti *et al.*, 2016), elimination of anti-nutritive compounds and mycotoxins (Biernasiak *et al.*, 2006). The benefits of fermentation include but are not limited to development of aroma, preservation, improvement of nutritional value and reduction of anti-nutritive compounds (Steinkraus, 1995). Fermentation processes can be categorized by the primary metabolites and microorganisms involved: 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).

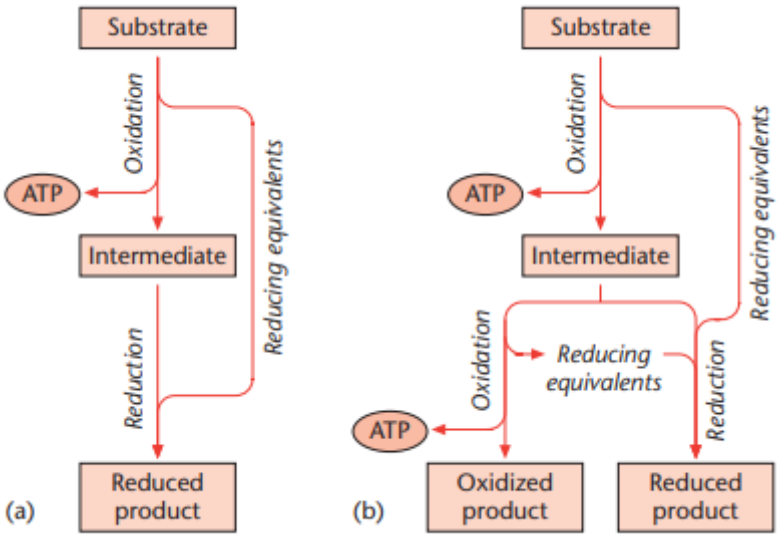

Figure 6. Substrate oxidation and reduction of the generated intermediates (a), oxidation and reduction of the oxidized intermediates (b)

Source: Müller (2001)

2.9.1 Types of Fermentation

Liquid submerged state fermentation (SmF)

Involves inoculation of starter or microbial culture in the liquid substrate or medium to the desired product. There is use of free-flowing substrates where the bioactive compounds are secreted into the liquid medium. The microorganisms involved (bacteria) require high moisture content. However, the parameters that control the fermentation will depend on the type of microorganism being used as the starter culture and the products needed at the end of fermentation (Subramaniyam & Vimala, 2012). Liquid submerged state fermentation has the potential to be used in production of bioherbicides. This is due to the difference in production of bioherbicide where the final product is not a fermentation byproduct but a living biomass of the plant pathogen (Stowell, 1991). Microsclerotia of *Colletotrichum truncatum* with (particle size range = 180 gm to 425 jm) were shown to be stable and effective in controlling hemp sesbania (*Sesbania exaltata*) a weed in soy bean, cotton and rice. Using propagules of *Colletotrichum truncatum* in liquid submerged state fermentation, the bioherbicide produced was weed specific in controlling *Sesbania exaltata* (Jackson *et al.,* 1996; Jackson & Schisler, 1995). Studies by Jackson and Schisler (1995) demonstrated that Microsclerotia *of Colletotrichum truncatum* can be produced in submerged culture, while at the same time be dry and viable. When incorporated in potting media before seeding, they were effective in controlling hemp sesbania seedlings.

Solid state fermentation (SSF)

Solid state fermentation (SSF) is recognized as a biotechnological process in which in the absence or near absence of free water, organisms grow and ferment solid substrates with high water absorption. It is a process that takes place in a solid matrix like a substrate with or without a certain amount of water activity (a_w) or free water for trigger of metabolic processes. This is the moisture needed to support the growth and metabolic activity of microorganisms in the solid substrate (Singhania *et al*. 2010; Pandey *et al.*, 2000; Thomas *et al.* 2013). The lignocellulosic structural characters of plant residues provide a solid support and act as a substrate for the microbial fermentation to produce a certain value-added product through SSF. The solid matrix may be inert or biodegradable with enough moisture to support growth of microorganisms (Pandey *et al.*, 2000). The starter culture is inoculated in the prepared solid substrate with the required moisture content and left to ferment. Solid state

fermentation has applications in several biological fields as production of biologically active secondary metabolites, in feed and food industry, fuel production and production of pharmaucitical products and industrial chemicals. It involves use of bioprocesses as biobeneficiation, bioleaching, bioremediation and biopulping. It is therefore an attractive alternative to liquid submerged fermentation (Pandey, 2003; Singhania *et al.,* 2009).

Unlike most intensive biotechnological processes, solid state fermentation has several advantages such as low production costs, stability of the end products, high proportion of the end products as compared with the initial substrates (Hölker & Lenz, 2005). According to Viniegra-González *et al.* (2003) significantly higher levels of the enzymes invertases, tanases and pectinases were produced by *Aspergillus niger* grown by silid state fermentation as compared with liquid submerged state fermentation. Other advantages of solid-state fermentation over liquid submerged state fermentation include but limitred to lower demand on sterility conditions due to low water requirement and activity, lower catabolic repression, higher end concentration of products, cultivation of microorganisms for use in water-insoluble substrates and use for cultivation of various fungi in mixed and single cultures (Hölker, 2004). It has been used to improve animal performance through its application in animal nutrition in terms of production of enzymes, bioactive components, organic acids, vitamins, and feed additives, by bio-transforming products, biological degradation and detoxification of plant residues/wastes as compared to liquid submerged state fermentation. Therefore, the inclusion of SSF biomass has a great and positive impact on nutritive composition of feed and improvement of animal performance, health, carcass attributes and environmental protection by modulating hemo-biochemical status, gut morphology, gut microbiota, rumen fermentation along with the reduction in enteric methane emission of animals (Díaz-Godínez *et al.,* 2017; Bhargav *et al.,* 2008).

2.9.2 Application of SSF in Animal Nutrition

Solid state fermentation as a technology has been used in the field of animal nutrition, especially in the utilization of highly lignified by-products. It has several applications including; enzyme production, bioactive metabolites, organic acids production, vitamins, biological degradation of anti-nutritional factors from the various by-products and animal feed stuffs. Specific microorganisms produce enzymes capable of biodegrading the lignified substrates into utilizable simple products by animals and human beings (Díaz-Godínez *et al.,* 2017). Enzyme production is higher in solid state fermentation of plant substrates. This is

because plant cell wall has two phases including micro-fibrilar phase which contains micro fibrils of cellulose and matrix phase (non-crystalline phase) which contains polysaccharides (Pectin and hemicelluloses), proteins and phenolic compounds. The enzymes commonly produced by microorganisms include; cellulases, lignases, Xylanases, Laccases, among others. Bacteria and fungi are common microorganisms used as starter cultures; however, fungi are considered the best source of enzyme production through the SSF (Díaz-Godínez *et al.,* 2017; Maleki *et al.* 2016).

Microorganisms use for processing of foods in human diets is as old as mankind (Rose, 1982), representing one of the oldest techniques in food preservation. Lactic acid bacteria are the most widely used microorganism (Rose, 1982), having the following benefits among others, improvement of nutritional value of food, control of intestinal infections, improvement of lactose intolerance, control of some types of cancer, and control of serum cholesterol levels. Some potential benefits may result from growth and action of the bacteria during the manufacture of cultured foods while others from growth and action of certain species of the lactic acid bacteria in the intestinal tract following ingestion of foods containing them (Gilliland, 1990). According to Steinkraus (1995), there are several benefits of fermentation as enhancement of diet through development of flavour, aroma, and texture in food substrates, preservation and shelf-life extension through lactic acid, alcohol, acetic acid and alkaline fermentation, enhancement of food quality with protein, essential amino acids, essential fatty acids and vitamins, improving digestibility and nutrient availability, detoxification of anti-nutrient through food fermentation processes, and a decrease in cooking time and fuel requirement. Also, Chukeatirote (2015), reported proteolytic enzymatic activities during fermentation of soybean contributed to attributes as good texture, appearance, flavour and aroma.

Fermentation, whether spontaneous or microbial culturing in origin improved nutritional value and reduced anti-nutritive compounds in livestock feeds (Aremu *et al.,* 2015; Iyayi & Aderolu, 2004; Sarasvati *et al.,* 2014; Thanh Hang *et al.,* 2019). Spontaneous fermentation of the bean (*Phaseolus vulgaris*), improved protein digestibility, enhanced storage quality of the product and amount of food energy, vitamins, and a partial or complete elimination of anti-nutritional compounds. Enzymes as α amylase produced by *Lactobacillus manihotivorans* during solid state fermentation was able to breakdown starch to provide carbon source to produce lactic acid. The enzymes together with the microorganisms can contribute to protein enhancement of the fermented substrates (Aguilar *et al.,* 2000; Oboh, 2006). Most

phytochemicals in plants such as phytic acid and tannins are reduced during fermentation by the activity of enzymes. The endogenous phytase enzyme produced in legumes and cereals hydrolyses the phytic acid as well as the added yeasts or other microorganisms with phytase activity (Dierick, 1989; Oboh, 2006). Cereal or legume matrices are broken down during fermentation leading to release of bound phytochemicals (Đorđević *et al.,* 2010; Egwim *et al.,* 2013; Esser *et al.,* 1983). Also, β- glucosidase enzymes from *Lactobacillus plantarum* and *Bacillus subtilis* are able to break the glucoside bonds between the sugars and the phytochemicals and therefore inactivating the toxins (Kuo *et al.,* 2006).

Spontaneously fermenting cassava reduced the cyanide with an r^2 value of 98% (Egwim *et al.,* 2013) with fermented products having significant reduction in tannin levels (Esser *et al.,* 1983). Also, the content of crude fibre in fermented cassava was reduced by 83.7% while crude protein increased by 43.8% (Perera *et al.,* 2018). Tannin level decreased (58–71% and 61–70%) after fermentation thereby increasing functionality of *Phaseolus vulgaris* (Granito *et al.,* 2002) and the whole bean fermentation was promising due to the lower cost. According to Iyayi and Aderola (2004), fungal fermentation with *Trichoderma viride* enhanced the nutritive value of agro industry by-products and their use spared the use of up to half the quantity of maize in conventional layers' diet with lower feed costs. In solid state fermentation of cassava pulp-maize, with urea and diammonium phosphate using *Bacillus subtilis* (Thanh Hang *et al*., 2019) reported a conversion of 7.2 g DM of substrate to 1 g of true proteins. Natural fermentation of sorghum, pearl millet and maize improved starch and protein digestibilities (Alka *et al.,* 2012) and pearl millet protein digestibility in vitro (Ali *et al.,* 2003). Also, probiotic fermentation with Lactobacillus spp was reported to improve *in vitro* digestibilities of protein and starch while reducing anti-nutritive compounds, phytic acid, polyphenols and trypsin inhibitor (Sindhu & Khetarpaul, 2001).

Probiotics improve the overall health of an organism by improving the microbial balance in the gut thereby improving weight gain and feed efficiency (Shrivastava *et al.,* 2012; Yirga, 2015). Lactic acid bacteria *Pediococcus pentosasceus, Lactobacillus fermentum, Lactococcus lactis ssp lactis, Lactobacillus pentosus* in spontaneously fermented millet/Sorghum mixture have the ability to survive in the gastrointestinal tract of humans, they can therefore be used as probiotics in food and feed preparations (Oluwajoba *et al.,* 2013). Their use, therefore, provide a potential alternative strategy to the traditional practice of sub therapeutic antibiotic use (Yirga, 2015). Probiotic use therefore reduces the incidence of antibiotic residues in animal products and therefore antibiotic resistance in humans. According to Gorbach (1990),

when fermented or lyophilized milk product with Lactobacillus GG strain was consumed by individuals with diarrhoea, the strain exhibited an antimicrobial substance which had a broad-spectrum activity against a range of bacteria, including *Clostridium difficile.* This specific Lactobacillus is therefore useful in treating recurring diarrhoea caused by toxin produced by *Clostridium difficile* (Silva *et al.,* 1987) and is effective in terminating relapsing colitis due to *C. difficile.* In experiments conducted by Barai *et al.* (2018) to asses' antidiarrheal property of probiotic bacteria in yoghurt using castor oil induced diarrheal mice, they reported an increase in the latent periods, reduced total faecal output, and frequency and fecal water content compared to the negative control group. Similarly, Zubaidah *et al.* (2012) reported the reduction of *E. coli* and Salmonella microorganisms in faecal samples of rats given probiotics. Lactobacillus species and *Saccharomyces cerevisiae* can therefore serve as a natural substitute for antibiotics (Mohamed *et al.,* 2015), anticoccidials (Giannenas *et al.,* 2014) and viricidal (Esser *et al.,* 1983).

There was a systemic augmentation of immune response characterised by an increase in both phagocytic and lymphocytic cell activity when mice were fed fermented milk using *Lactobacillus casei* and *Lactobacillus acidophilus* suggesting their effect on the immuned sytem activation (Perdigon, *et al.,* 1988). In feeding beef calves, Du *et al.* (2018) reported therapeutic characteristics of *Bacillus amyloliqueficiens* and *Bacillus subtillis* incooperated in their diets in terms of growth performance by regulating hormones and improving intestinal and rumen development in growth retareded animals. Also, in feeding broiler chicken using rice bran fermented with *Bacillus amyloliqueficiens* there was improved weight gain and low feed conversion ratio (Supriyati *et al.,* 2015).

In experiments using male Swiss mice, implanted with Ehrlich ascites tumour cells Shahani *et al*. (1983), reported anti-tumour properties of fermented colostrum and milk from use of *Lactobacillus acidophilus* and Lac*tobacillus bulgaricus* due to inhibition of tumour cell proliferation indicated by a 16 to 40% decrease in cell counts and a 13 to 35% decrease in DNA synthesis. Also, Veer (1989), reported that consumption of fermented milks protected women between the ages of 30-60 years from breast cancer with individuals consuming unfermented dairy products especially high fat developing breast cancer (Ronco *et al.,* 2002).

According to St-Onge *et al*. (2000), fermented foods are functional in lowering elevated cholesterol concentrations so long as these bacteria are bile resistant and have the ability to de conjugate bile acids and bind cholesterol. In the large intestine, these bacteria ferment

indigestible carbohydrates and produce short chain fatty acids which alter cholesterol synthesis. Also, in the intestines, they can bind bile acids to cholesterol, resulting in the excretion of bile acid–cholesterol complexes in the faeces. Decreased bile acid recycling through the enterohepatic circulation would result in cholesterol uptake from the circulation into the liver for de novo synthesis of bile acids. Studies done by Li *et al.* (1998) demonstrated that hypercholesterolemia induced by feeding rabbits 25% casein diets for 30 days can be treated by administering *Monascus purpureus* (red yeast rice prepared by fermentation) at 0.4g/ feed/day thereby reducing serum total cholesterol and triglyceride in the rabbits. Also, Sindhu and Khetarpaul (2003) reported reduction in total serum cholesterol when mice were used to study the hypocholesterolemic effect of an indigenously developed and probiotic fermented cereal-pulse food mixture. When using fermented shrimp, Le and Yang (2018) isolated *Lactobacillus plantarum* that reduced cholesterol level in vitro.

Spontaneous fermentation has been reported to lead to feed and food detoxification. Most naturally occurring plant toxins are removed through fermentations. For instance, Egwim *et al.* (2013) reported that naturally fermenting cassava allowed for the growth of *Geotricum candidia* which drastically reduced the substrates pH allowing for the colonization of a pH resistant strain *Corynebacterium lactis*. The bacterium hydrolyses the toxic chemical cyanide in cassava with an r^2 value of 98%, creating food safety. In a series of experiments conducted by Moran (2001), there were reports that fermenting porcine liquid feed using *Lactobacillus plantarum,* potential pathogens were effectively eliminated. Also, studies by Oyewole (1997) reported that Lactic acid bacteria (LAB) fermentation contributed to food safety, improved nutritional value, high shelf life, and acceptability of a wide range of cereal-based foods.

Lucke (2000) reported that LAB adopted to meats improved safety of sausages by means of acid formation. They also reduced the number of pathogens as *Listeria monocytogenes* in meat products. This opens a window for improvement of safety of non-fermented perishable meat and food products without affecting their shelf life. Ojokoh *et al.* (2015) reported dissapearance of contamination microorganisms *Micrococcus luteus, Bacillus subtilis, Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* during fermentation of pearl millet. Also, Oluwajoba *et al.* (2013), when fermenting a combination of sorghum and millet reported suppression of pathogenic microorganisms and survival of microorganisms with probiotic qualities. Lactobacillus spp was found to reduce pathogenic microorganims in fermented milks to safe levels for human consumption (Cunha *et al.*, 2013).

Mould damage of most cereals lead to underutilization of the grain as well as monetary loss. Studies done by Vasanthi *et al.* (2009) reported the possibility of exploiting the use of these cereals when fermented. They reported mould count reduction to 58% at 24 h and to 96% at 36 h of fermentation. Also, Londero *et al.* (2014), in an experiment to analyse the effectiveness of whey fermented with kefir grains as an additive to reduce fungal incidence, demonstrated that fermented whey added to poultry feed acted as a bio preservative, improving its resistance to fungal contamination and increasing its shelf life. Fermentation therefore improved feed and food safety of mouldy cereals and protected feeds from fungal infestation.

Several researchers have reported antioxidant properties of fermented products. During normal cellular and biochemical reactions, free radicals and reactive nitrogen species are generated which are usually counteracted by body's own defence antioxidant system or not, under disease conditions. Fermented milk products prevent the resultant oxidative stress that would otherwise damage the cell macromolecules such as deoxyribonucleic acid, proteins, and lipids and also disturbing signalling transduction inside the cell (Fardet & Rock, 2018; Khan *et al.,* 2019). For instance, fermentation using *Saccharomyces cerevisiae* and *Lactobacillus rhamnosus* improved antioxidant properties of buckwheat, wheat germ, barley and rye (Đorđević *et al.,* 2010). Also, fermenting soybean with Acetobacter sp., Lactobacillus sp., Saccharomyces sp. and Streptomyces sp., conferred on the resulting broth higher antioxidant properties than unfermented soybean broth, with recommendations of the use of fermented soy bean broth as an antioxidant (Yang *et al.,* 2000). These antioxidant properties of fermented products explain the reported dairy product-protective effects against some chronic diseases (Fardet & Rock, 2018).

2.9.3 Spontaneous fermentation

This fermentation is usually spontaneous and uncontrolled involving mixed cultures of yeast, bacteria, and fungi where some microorganisms might work in parallel while others in a sequential manner with a changing dominant micro flora (Blandino *et al.,* 2003; Chaves- López *et al.,* 2014). The products are therefore obtained under local climatic conditions with variable sensory characteristics and quality (Chaves- López *et al.,* 2014; Granito *et al.,* 2002) (Figure 7).

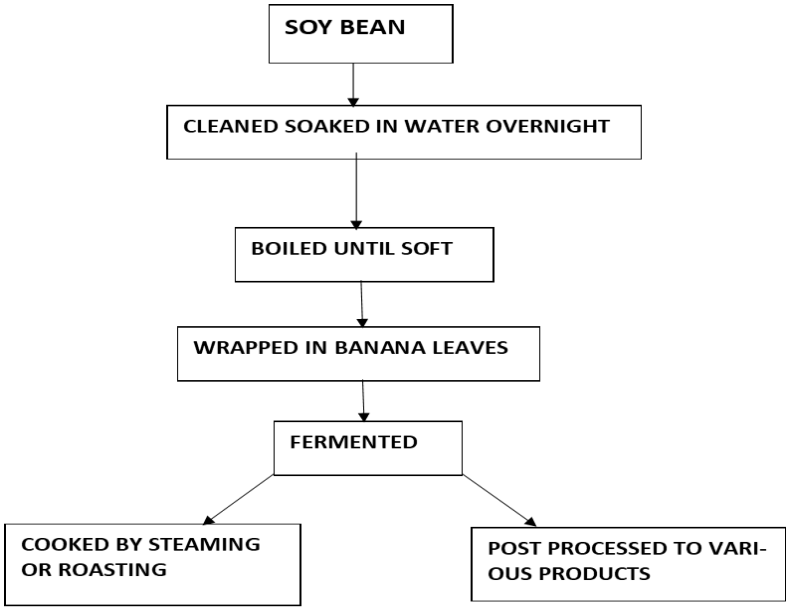

Figure 7. Flow chart of spontaneous fermentation of soybean

Source: Granito *et al.* (2002)

Microbial and biochemical changes during spontaneous fermentation

Microbial population and biochemical changes in spontaneously fermenting substrates depend on type of fermenting substrates and the initial microorganisms that are present, giving an array of microorganisms (Table 1).

Table 1. Probiotics in spontaneously fermenting substrates

Microorganism	Substrate	Source
Bacillus subtilis	Cassava	Perera *et al.* (2018)
Corynebacterium manihot	Cassava	Perera *et al.* (2018)
Aspergillus niger	Cassava	Perera *et al.* (2018)
Lactobacillus acidophillus	Pearl millet	Ojokoh *et al.* (2015)
Lactobacillus brevis	Pearl millet	Ojokoh *et al.* (2015)
Streptococcus thermophillus	Pearl millet	Ojokoh *et al.* (2015)
Pediococcus pentosasceus	Millet + Sorghum	Oluwajoba *et al.* (2013)
Lactobacillus fermentum	Millet + Sorghum	Oluwajoba *et al.* (2013)
Lactococcus lactis ssp lactis	Millet + Sorghum	Oluwajoba *et al.* (2013)
Lactobacillus pentosus	Millet + Sorghum	Oluwajoba *et al.* (2013)

Substrates with high protein encourage growth and activity of proteolytic enzymes leading to production of peptides, amino acids and ammonia (Anggo *et al.,* 2015; Chukeatirote, 2015). This alkaline substrate pH provides selective condition for growth of desired bacteria as Bacillus species and suppression of unfavourable bacteria (Chukeatirote, 2015). However microbial populations during natural uncontrolled fermentations of carbohydrate rich substrates (dehulled maize) had lactic acid bacteria (LAB) and yeast dominating the microbial population up to 48 hours of fermentation after which LAB dominated as fermentation progressed and pH reduced further (Hounhouigan *et al.,* 1994). Hounhouigan *et al.* (1994) reported increase of LAB from 3.2 x 10^6 c.f.u./g (wet wt.) to 2 x 10^9 from 12 to 24 h of fermentation in home-produced dehulled maize. In commercially produced dehulled maize, the yeast count increased from 1.3 x10^5 to 2.5 x 10^7 c.f.u/g after 48 h of fermentation before decreasing. The dominant yeasts were mainly *Candida krusei*, although *C. kefyr, C. glabrata* and *Saccharomyces cerevisiae*, with *S. cerevisae* dominating at the end of fermentation in wine production using grape juice (Fleet *et al.,* 1984). Enterobacteriaceae counts increased slightly during the initial stage of the fermentation, but decreased below the detection level after 24 to 48 hours. *Enterobacter cloacae* was mostly found in commercial dehulled maize and *Escherichia coli* mostly in home-produced dehulled maize (Hounhouigan *et al.,* 1994).

The dominant microorganisms in a fermenting substrate can be due to contamination or natural occurrence in the substrate (Ojokoh *et al.,* 2015). This will determine the substrates pH during fermentation (Anggo *et al.,* 2015). There was a gradual drop in pH during the 72-hour fermentation period of dehulled maize fermentation 41.6%, with pH recording at 72-hour fermentation being the lowest (Hounhouigan *et al.,* 1994). However, in fermentation of soybeans there was an increase in pH up to 8. This was due to Enzymatic degradation of soy proteins into peptides, amino acids, and ammonia (Chukeatirote, 2015). This could be due to differences in substrates in terms of chemical composition. Initial reaction of proteins is enzymatic proteolysis of the proteins while carbohydrates are microbial fermentation.

2.9.4 Fermentation using microbial inoculums

Microbial fermentation involves inoculated or introduction of microorganisms into the fermenting substrate to induce fermentation (Lafon-Lafourcade & Ribereau-Gayon, 1984), the advantage being achievement of a more rapid and even rate of fermentation and product of a more consistent quality (Kunkee & Goswell, 1977). However, the assumption that inoculated microorganism suppress the naturally occurring microorganisms is not strictly correct especially in wine production where different strains of *S. cerevisiae* were present in the produced wine (Heard & Fleet, 1985).

Probiotic fermentation

According to FAO (2001) the meaning of probiotics is “for life” and it is currently used to name bacteria associated with beneficial effects for humans and animals. Fuller (1989) defined a probiotic as a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance. The International Scientific Association on Probiotics and Prebiotics (ISAPP) reinforced the FAO/WHO definition of probiotics, with minor changes: ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’ (Hill *et al.,* 2014).

Probiotic microorganisms consist mostly of bacterial strains of Lactobacillus, Bifidobacterium, Lactococcus, Bacillus, Streptococcus, and yeast such as Saccharomyces types (Rolfe, 2000; Yirga, 2015). They have been used for centuries in the food and feed industry (Yirga, 2015). According to Rolfe (2000), viable and biologically active microorganisms are usually required at the target site in the host, it is therefore essential that the probiotic be able to withstand the host's natural barriers against ingested bacteria by

resisting gastric acid, bile salts and pancreatic enzymes to adhere to intestinal mucosa and readily colonize the intestinal tract and thus exert their beneficial properties (Djezzar *et al.*, 2019). Djezzar *et al.* (2019) reported improvement in improvement in broiler gut health and therefore higher performance when *Saccharomyces cerevisiae* and *Pediococcus acidilactici* was incooperated in broiler diets.

Commonly used probiotic microorganisms are Lactobacillus and Bifidobacterium species (Gram+ bacteria) (Heyman & Ménard, 2002; Rolfe, 2000) and Streptococcus species (Rolfe, 2000). Bacterial probiotics include Lactobacillus species, Bifidobacterium species, *E. coli*, Streptococcus species, *Lactococcus lactis*, some Enterococcus species and non-pathogenic yeast Saccharomyces (Gogineni *et al.*, 2013). Commonly used microorganisms with probiotic effect in the food industry are presented in table 2.

Table 2. Probiotics used in the food industry

Microorganism	**Where used**	**Source**
Lactobacillus bulgaricus	Dairy industry	Gogineni *et al.* (2013)
Lactococcus lactis	Dairy industry	Gogineni *et al.* (2013)
Saccharomyces cerevisiae	Brewing/ food industry	Kunkee and Goswell (1977)
Streptococcus thermophilus	Dairy industry	Heller (2001)
Lactobacillus helveticus	Dairy industry	Heller (2001)
Lactobacillus brevis	Dairy industry	Heller (2001)
Bacillus amyloliquefaciens	Food industry	Du *et al.* (2018)
Bacillus subtilis	Food industry	Du *et al.* (2018); Sarasvati *et al.* (2014)
Leuconostoc mesenteroides	Vegetable industry	Daeschel *et al.* (1987)
Pediococcus acidilactici	Fish industry	Liepe (1983)
Leuconostoc lactis	Dairy industry	Sandine (1988)

Probiotic fermentation relies on the use of defined starter cultures with desirable characteristics to ensure consistency and commercial viability (Hill *et al.*, 2017). Lactic acid bacteria (LAB) are the most commonly used and have an important role in the feed and food industry, fermenting raw vegetables, milk, meat and cereals. The species used belong to the genera Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, and Streptococcus (McKay & Baldwin, 1990). The use of Lactic acid bacteria (LAB) in controlled fermentation where

specific microorganisms are used under specific temperatures in the dairy industry results in production of desirable dairy products with probiotic properties (Parnell-Clunies *et al.,* 1986) Figure 8.

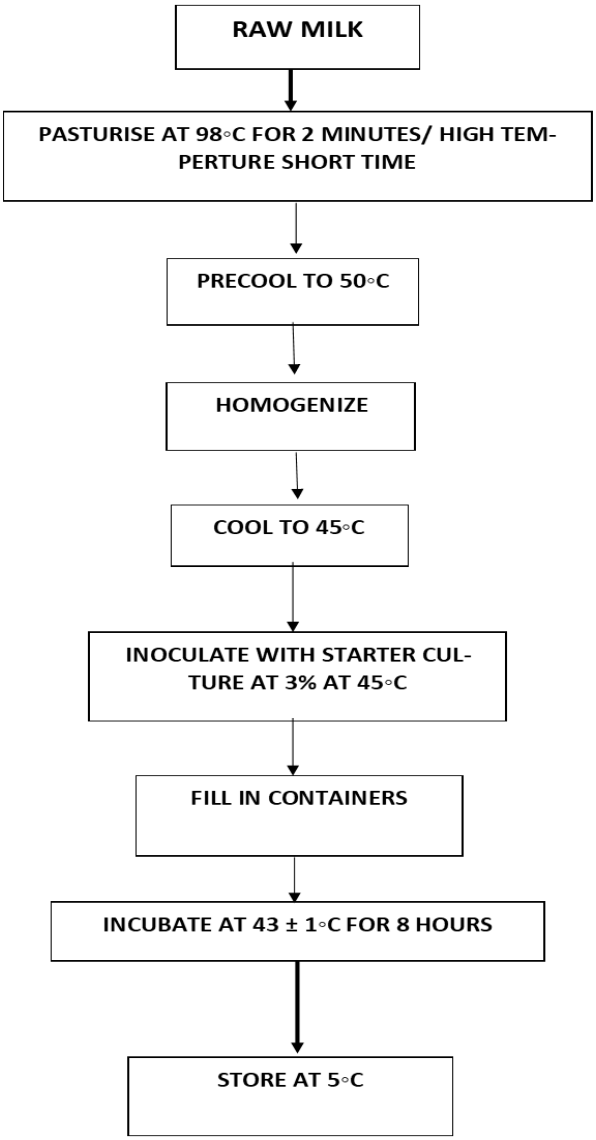

Figure 8. Flow chart of milk fermentation using *S. thermophilus* and *L. bulgaricus*, ratio 1:1

Source: Parnell-Clunies *et al.* (1986).

CHAPTER THREE

PROXIMATE COMPOSITION, AMINO ACID PROFILE, PHYTIC ACID, AFLATOXIN AND TANNIN COMPOSITION OF FERMENTED *P. juliflora* PODS

Abstract

This study evaluated the nutritional and anti-nutritional changes that occur in ground mature Prosopis pods on fermentation. Four fermentation methods (factor A) were used, namely; spontaneous (S) fermentation, microbial/probiotic fermentation using *Saccharomyces cerevisiae* (SC), *Lactobacillus salivarius* (LS) and combined cultures of SC/LS for 24, 48- and 72-hour intervals (factor B) with 3 replicates for each method/treatment in a 4×3 factorial experiment in a completely randomised design (CRD). All the fermentation methods used affected the parameters measured positively. The pods nutritional value was improved $p<0.05$ and anti-nutritive compounds reduced, except for numerical reduction of ether extract (EE), acid detergent fibre (ADF) and neutral detergent fibre (NDF). The crude protein (CP) value increased within the first 24 hours by 10.07%, 11.96%, 11.49% and 8.72% for S, SC, LS and LS/SC respectively. Total essential amino acids (TEAAs) were enriched within the range of 34-50%. Spontaneous fermentation at 72 hours reported the highest value for the limiting amino acids on DM basis (Lysine, 4.81mg/g; methionine, 4.1mg/g and arginine, 6.94mg/g) for growing rabbits. Crude fibre (CF) reduced after 24 hours by 10.43%, 11.31%, 14.01% and 12.18% for S, SC, LS and LS/SC respectively. There was treatment effect on all the anti-nutrient content after 72-hour fermentation in all the methods ($p<0.05$). Phytic acid was reduced from 48.42ppb to 48.02 ppb, 36.11 ppb, and 46.08 ppb for spontaneous, SC, LS and SC/LS respectively. Aflatoxin content was reduced from 6.7 ppb to 6.6 ppb and 4.9 ppb for spontaneous and SC/LS respectively but increased to 12.8 ppb and 6.8 ppb for SC and LS respectively. Tannin content reduced from 74.55ppm to 22.4ppm, 20.46ppm, 34.46ppm and 28.26ppm for spontaneous, SC, LS and SC/LS respectively. Spontaneous fermented substrates reported superior nutritional enrichment especially the limiting amino acids in rabbit nutrition (Lysine, methionine and arginine) and anti-nutrient reduction; it can therefore be used for supplementary rabbit feeding.

Keywords: Anti-nutritive compounds, fermentation, nutritional value, proximate.

3.1 Introduction

The annual population growth in Kenya was estimated at (2-3) % (KNBS, 2009) from an initial population of 40 million in 2009 to 46.7 million in 2019 (KNBS, 2019) with an annual growth rate of 2.2% (KNBS, 2019). Food production should therefore be in tandem with this growth to ensure food security. Three million tonnes of maize produced in 2013 was far below the estimated 4 million tonnes required for human consumption. This situation is worsened further by presence of ASALs in Kenya that comprise 75% of land mass and unfit for crop production (Chisholm, 2014; GoK, 2007). There is need for exploration of non-conventional livestock feed resources that are available all year round, cost effective and with no competition with humans. Prosopis plant has been identified as such a feed resource among others like Acacia, Moringa (Abdulrazak *et al.,* 2000; King'ori *et al.,* 2011; Nouman *et al.,* 2014; Odero-Waitituh *et al.,* 2015). Prosopis species are generally fast-growing, drought-resistant, nitrogen-fixing trees or shrubs adapted to poor and saline soils in arid and semi-arid zones (Anderson, 2005). Chemical analysis of Prosopis pods reported 18% CP (Koech *et al.,* 2010), 13% and 69% sugars and carbohydrates (Choge *et al.,* 2007). Also, Odero-Waitituh *et al.* (2015), reported a metabolizable energy value of 2.8 MJ/kg sample. However, high crude fibre (17 %) in the pods reduced broiler growth (Odero-Waitituh *et al.,* 2016).

Fermentation, both microbial inoculation and spontaneous, has been reported to improve nutritional value of feeds (Aremu *et al.,* 2015; Sarasvati *et al.,* 2014; Yusuf *et al.,* 2008). It makes foods more edible by changing chemical compounds, predigesting feed (Egwim *et al.,* 2013) and breaking down complex protein structures to peptides and free amino acids (Hesseltine & Wang, 1979), thereby allowing for improved digestibility especially so if the materials were fibrous and with anti-nutritive compounds (Hesseltine & Wang, 1979; Sarasvati *et al.,* 2014). Fermentation also reduces starch (Balogun & Oyeyiola, 2011) which could improve on keeping quality when dealing with feed ingredients that are hygroscopic. Also, fermentation detoxifies feeds with aflatoxin and reduces phytic acid and tannins (Assaf *et al.,* 2019; Sarasvati *et al.,* 2014).

Several researchers have reported improved nutritional value and reduced anti-nutritional content when different species and parts of Prosopis pods were fermented. For instance, Yusuf *et al.* (2008) reported that on fermentation of decorticated *Prosopis africana* seed meal, CP% was enhanced from 22.62 to 42.52 resulting in improved broiler growth. Aremu *et al.*

(2015) reported reduction of % CF of Mesquite bean from 11.35±0.44 to 8.22±0.03 and improved % CP from 11.77±0.26 to 15.19±0.02. Also, Sarasvati *et al.* (2014) reported improvement in % CP and reduction in tannin and phytic content when *Prosopis juliflora* pods were fermented with *Bacillus subtilis*, *Bacillus circulans* and *saccharomyces cerevisiae*. This study evaluated the nutritional enrichment (CP and amino acid) and anti-nutritive compounds reduction (CF, tannins, aflatoxin and phytic acid) that occur in GMPP on probiotic (SC and LS) and S fermentations.

3.2 Materials and Methods

3.2.1 Prosopis pods harvesting, drying, storage and milling

Prosopis pods were obtained from Marigat Sub County in Baringo County, about 130 km from the study site. Dry ripened brown mature pods were collected from the ground after shaking the trees. Pods with any blackening or discoloration or evidence of browsing by livestock or attack by insects or moulds were discarded to avoid infection with aflatoxin (Choge *et al.,* 2007). Pods were dried in the sun until a constant weight was achieved. 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 ground pods were kept in airtight containers to prevent moisture attraction.

3.2.2 Study site

Maize and UGMPP sample preparation were done at the Egerton University Animal Nutrition laboratory. They were packed in sample bags and transported to the National Centre for International Research on Animal Gut Nutrition (NCIRAGN), Nanjing Agricultural University, Peoples Republic of China, where fermentation of UGMPP was done. NDF, ADF and amino acid profiling of UGMPP, FGMPP and maize was done at the NCIRAGN, Nanjing Agricultural University, Peoples Republic of China.

Probiotic and spontaneous fermentations of ground mature Prosopis pods and amino acid profile analysis of FGMPP, UGMPP and maize were done at the NCIRAGN, Nanjing Agricultural University, Peoples Republic of China, in November 2018. Nanjing is located at latitude 32°03'42.01" N and longitude 118°46'40.01" E with an average altitude of 150 feet above sea level. https://latitude.to/map/cn/china/cities/nanjing . There are about 115 rainy

days in a year, and the average annual rainfall is 1,100 mm. The annual mean temperature is 3° C in January and 28º C in July. (https://www.climatestotravel.com/climate/china/nanjing)

Proximate, starch, phytic acid, tannins and aflatoxin content analyses was conducted at Animal Science Laboratories, Egerton University. Egerton is located at latitude 0º 23' S and longitude 35º 57' E with an altitude of 2,238 m above sea level. It has a mean daily temperature of 21º C. There is a bimodal rainfall pattern (March to May and June to September) with a mean annual rainfall of 900 - 1,020 mm (Egerton University Meteorological Station, 2019).

3.2.3 Microbial strains and source

Pure cultures of *Lactobacillus salivarius* (LS) and *Saccharomyces cerevisiae* (SC) were used. They were sourced from the intestinal contents of weaned piglets at Nanjing Agricultural University farm. The piglets were slaughtered by intracardiac injection of 1 ml T-61® (Intervet, Germany). Digesta samples were taken from the entire small intestines (ileum, caecum and proximal colon). Homogenized digesta was then serially diluted in 0.9 % NaCl solution and poured onto agar plates containing different selective media for culturing *S. cerevisiae* and *L. salivarius*. Lactobacilli were cultured using De Man, Rogosa and Sharpe (MRS)-agar inoculated with the homogenized digesta for 72 h at 37°C in anaerobic jars using Anaerocult A. *Saccharomyces cerevisiae* were cultured on SABOURAUD-Agar for 5 days at 37° C to prevent bacterial growth. Typical colonies of lactobacilli from MRS-agar plates at the highest dilution rate were picked up and cultivated in MRS-broth overnight. Subsequently they were purified by fractional plating 3-4 times on MRS-agar plates and cultivated. Strain purification was done by spreading of colonies until single identifiable colonies were visible. Cell morphology was verified by microscopic observations as well as Gram-staining. Once the microorganisms were identified, they were cultured in MRS and Yeast peptone media (YPM) broths for *L. salivarius* and *S. cerevisiae* respectively. Microbial dilution and plating were done to get the cfu/g. The microbial counts of LS and SC were 1.0×10^8 cfu/g and 1.0×10^8 cfu/g for LS and SC.

3.2.4 Preparation of microbial inoculums

Yeast peptone media and MRS agar media were prepared in quadruples under sterile conditions. Sterile 50 ml media bottles and 50 ml YPM in beakers were prepared and

inoculated with 0.5 ml of LS and SC. They were incubated at 37^{o} C for 24 h on a rotary shaker (120 rpm) after which turbidity was observed indicating microbial growth. The inoculums cell density was adjusted to 10^{8} cfu ml-1 by appropriate dilution using sterile distilled water.

3.2.5 Inoculation of substrate in solid state fermentation

Under sterile conditions, 66 g of GMPP was weighed and put in sample fermentation bags. A mixture of 2 ml of inoculum and 32 ml of buffered saline solution were added. They were then hand mixed thoroughly and incubated at 37^{o} C for 24, 48 and 72 hours. At every 24 hours' interval, pH was measured and microbial counting was done.

3.2.6 Solid media and sample preparation for plating and microbial counting

In a 1000 ml calibrated beaker, 49 g of Yeast Peptone agar was weighed, added into the beaker and distilled water added up to the 1000 ml mark and mixed thoroughly using an American Scientific Vortex mixer. It was then transferred to a universal bottle and sterilized in the autoclave for 1 hour at 105^{o} C. After cooling, it was removed and poured onto sterile Petri dishes in the sterile cabinet. They were then left to solidify in the cabinet and kept in sterile cabinets for plating. In a 1000 ml calibrated beaker, 63 g of MRS agar was weighed, added into the beaker and distilled water added up to the 1000 ml mark and mixed thoroughly using an American Scientific Vortex mixer. It was then transferred to a universal bottle and sterilized in the autoclave for 1 hour at 105^{o} C. After cooling, it was removed and poured onto sterile Petri dishes in the sterile cabinet and spread plate method used. They were then left to solidify in the cabinet and kept in sterile cabinets for plating

Using serial dilution method in isolation of microorganisms, 0.5 g of fermented substrate and 4.5 ml of buffered saline solution were added into a bijou bottle and mixed thoroughly using an electric shaker for 5 minutes to allow for uniform distribution of the microbes in the mixture; Vortex Genie 2 model, https://www.scientificindustries.com/vortex-genie-2.html. This bijou bottle was labelled 10^{1}. Seven bijou bottles were labelled from 10^{2} up to 10^{8} for dilution and 0.9 ml buffered saline solution was added to each of the 7 bottles. One ml was then added to the seven bottles in dilution where 1 ml of 10^{1} was added to 10^{2} then 10^{3} up to 10^{8} after shaking and mixing well. From dilution of 10^{6}, 10^{7} and 10^{8}, 0.1 ml was plated in duplicate for every experimental unit. Plating for control and SC/L were for both YPA and MRS. Plating for each experimental unit was done in duplicate. The plated petri dishes were

incubated for 48 hours before microbial colony counting. Plating of the experimental units was done every 24 hours. At the end of the fermentations, pH readings were done for every experimental unit using Sartorius pb10 model, https://www.sartorius.com/shop/us/en/usd/ph-meters/c/M_pH-Meters?page=1&.

3.2.7 Experimental units and design

A factorial arrangement in a completely randomized design was used in the study. There were four treatments (factor A) and three fermentation time (factor B). Each treatment was at 24, 48 and 72 hours, making four experimental units at each level with three replicates each. The total number of experimental units were 12 replicated 3 times giving a total of 36 experimental units. Using PQRS software, the four treatments were randomly allocated to the fermentation containers such that there was one container for each experimental unit with three replicates per treatment for every 24-hour interval (3 containers/every 24 h/treatment) for the three intervals (12 experimental units/ treatment) (Table 3). Fermentation was done in airtight containers.

Table 3. Experimental layout

Fermentation duration/time	**Treatment**			
24 hours	SC	LS	S	SC/LS
	LS	SC	S	SC/LS
	SC/LS	SC	LS	S
48 hours	LS	SC/LS	SC	S
	S	SC	SC/LS	LS
	SC	LS	SC/LS	S
72 hours	SC/LS	S	SC	LS
	S	SC/LS	SC	LS
	LS	SC	S	SC/LS

Key: SC = *Saccharomyces cerevisiae*; LS = *Lactobacillus salivarius*; S = Spontaneous fermentation.

3.2.8 Proximate analysis, detergent fibre and anti-nutritive compounds content

Dried samples of FGMPP, UGMPP, and feed ingredients were analysed for proximate composition and detergent fibres using the standard procedures of the Association of Official Analytical Chemists (AOAC, 1995) and Goering and Van-Soest (1975) respectively. Moisture was determined using the AOAC oven drying method 950.46 (AOAC, 1995). Crude protein was determined using combustion of AOAC crude protein analysis method 992.15 (AOAC, 1995). Crude fibre was determined using the AOAC method 962.09 (AOAC, 1995). Ether extract was determined according to Soxhlet AOAC method 923.03 (AOAC, 1995). Ash was determined using the AOAC method 920.153 (AOAC, 1995).

Determination of dry matter

Equation 1. Calculate of dry matter

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

Determination of crude protein

Equation 2. Calculation of % CP

% 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 DM basis

Determination of NDF

Equation 4. Calculation of % NDF

$$\text{NDF (\%)} = \frac{(m2 - m1 * C1)}{m} \times 100\ \%$$

Where:
m1: empty bag weight g
m: sample and bag weight g
m2: sample and bag weight after drying g
C1: empty bag weight after drying/m1 (correction factor)

Determination of ADF

Equation 5. Calculation of % ADF

$$\text{ADF (\%)} = \frac{(m2 - m1 * C1)}{m} \times 100\ \%$$

Where:

m1: empty bag weight g

m: sample and bag weight

m2: sample and bag weight after drying g

C1: empty bag weight after drying/m1 (correction factor)

Determination of ether extract

Equation 6. Calculate of % EE

(Wt of lipids /wt of sample DM basis) * 100

Determination of ash

Equation 7. Calculation of % organic matter.

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

Determination of gross energy, total tannins, total aflatoxins and phytic acid

Gross energy was determined using an atomic bomb calorimeter (e2K bomb calorimeter, www.cal2k.com, South Africa (Appendix D). Total tannins were determined as described by Abdulrazak and Fujihara (1999) (Appendix D). Total aflatoxin determination was done according to the procedure by Vicam AflaTest® for corn, grains, and feeds (VST, 2000) (Appendix D). Phytic acid determination was done according to the procedure by Raboy *et al*. (2000) (Appendix D).

3.2.8 Statistical analysis and model

Data from proximate, amino acid and ANC analysis were subjected to normality and homogeneity of variance tests using SPSS Statistics 25.0.0 software (2017). They were then subjected to analysis of variance using general linear model (GLM) of Statistical Analysis Systems (SAS, 9.1.3) computer package (2005). Mean separation were determined using the Tukey's range test. Where two means were compared a two-paired t- test was used. Probability values of ($p<0.05$) were considered significant. The following model was used;

$Y_{ijk} = \mu + A_i + Bj + (AB)ij + \varepsilon ijk$

Where:

Y_{ijk} = observation of k^{th} experimental unit.

μ = overall mean,

A_i = effect of level of factor A

Bj = effect of level of factor B

(AB)ij = effect of interaction of factors A and B.

εijk = random error associated with Y_{ijk}

3.3 Results

3.3.1 Microbial count and pH of the fermenting substrates

Microorganisms were present in the first 24 hours for the spontaneously fermenting substrate and for the SC/LS fermenting substrates only. After 48 and 72 hours, there was no microbial count recorded for all the fermentation methods. For single culture of LS and SC, there were no microorganisms in all the experimental units. The pH dropped in the first 24 hours after which it went up in all the fermenting substrates. However, the pH drop for spontaneous fermentation was significantly slower ($p<0.05$) as compared to all the treatments (Table 4).

Table 4. Mean microbial count and pH of UGMPP during solid state fermentation

Fermentation time		0 hrs	24 hrs	48 hrs	72 hrs
Fermentation type					
L. salivarious	pH	4.46	3.98[a]	3.96 [a]	3.99 [a]
S cerevisiae	pH	4.46	3.95 [b]	3.98 [a]	3.99 [a]
S cerevisiae/*L salivarius*	pH	4.46	3.99 [a]	3.95[b]	3.99 [a]
Spontanous	pH	4.46	3.99 [a]	3.97 [a]	3.95 [b]
SEM	-	-	0.006	0.006	0.005
P value	-	-	0.004	0.03	0.001
Fermentation type					
			Cfu/g	Cfu/g	Cfu/g
L. salivarious	MC/MRS	-	0	0	0
S cerevisiae	MC/YPA	-	0	0	0
S cerevisiae/*L salivarius*	MC/MRS	-	4	1	0
	MC/YPA	-	170	0	0
Spontanous	MC/MRS	-	Countless	0	0
	MC/YPA	-	6	2	0

MC = microbial count; MRS = Ma, Rogosa and Sharpe; UGMPP = unfermented ground mature Prosopis pods; YPA = yeast peptone agar; SEM = standard error of the means; [a,b] means within the same column with different superscripts are significantly different at ($p<0.05$).

3.3.2 Chemical composition of FGMPP

Proximate, starch, NDF and ADF fractions of FGMPP using different methods of fermentation are shown in Table 5. All fermentation methods used affected the proximate, starch, NDF and ADF fractions positively. Moisture reduced leading to an increase in DM, Ash content improved indicating mineral enrichment during fermentation. Spontaneous and probiotic fermentations improved crude protein content of ground Prosopis pods. The crude protein value increased within the first 24 hours by 10.07%, 11.96%, 11.49% and 8.72% for spontaneous, SC, LS and LS/SC respectively. There was no significant increase in CP in the 48 and 72hr fermentations.

Table 5. Proximate, NDF and ADF composition of UGMPP and FGMPP using different fermentation method and time (%DM)

		Moisture	%CP	%CF	%EE	% Starch	%Ash	%NDF	%ADF
					UGMPP				
		9.06^{a}	14.79^{a}	22.67^{a}	4.35^{NS}	10.52^{a}	4.94^{b}	34.44^{NS}	22.91^{NS}
Method	**Time**				**FGMPP**				
S	**24 hrs**	7.62^{b}	16.28^{b}	20.33^{b}	4.31^{NS}	7.53^{b}	5.06^{b}	34.45^{NS}	22.12^{NS}
	48 hrs	7.61^{b}	16.3^{b}	20.32^{c}	4.35^{NS}	7.55^{b}	5.04^{b}	35.78^{NS}	22.68^{NS}
	72 hrs	7.63^{b}	16.28^{b}	21.48^{c}	4.26^{NS}	7.55^{b}	5.03^{b}	34.47^{NS}	21.80^{NS}
SC	**24 hrs**	7.3^{c}	16.56^{c}	20.13^{d}	4.1^{NS}	6.5^{c}	5.02^{b}	34.06^{NS}	22.39^{NS}
	48 hrs	7.33^{c}	16.44^{c}	20.11^{d}	4.01^{NS}	6.55^{c}	5.05^{b}	35.06^{NS}	22.35^{NS}
	72 hrs	7.34^{c}	16.59^{c}	20.14^{d}	4.15^{NS}	6.65^{c}	5.01^{b}	32.61^{NS}	20.53^{NS}
LS	**24 hrs**	7.85^{d}	16.49^{c}	19.49^{e}	4.28^{NS}	7.25^{c}	5.5^{a}	33.62^{NS}	21.83^{NS}
	48 hrs	7.8^{e}	16.44^{b}	19.3^{e}	4.15^{NS}	7.16^{c}	5.06^{b}	36.68^{NS}	23.94^{NS}
	72 hrs	7.81^{e}	16.57^{c}	19.47^{e}	4.28^{NS}	7.25^{c}	5.09^{b}	35.20^{NS}	23.28^{NS}
LS/SC	**24 hrs**	8.52^{f}	16.08^{d}	19.91^{b}	4.14^{NS}	9.31^{d}	5.05^{b}	31.76^{NS}	19.60^{NS}

	48 hrs	8.58^{f}	16.02^{d}	19.79^{b}	4.11^{NS}	9.27^{d}	5.06^{b}	32.61^{NS}	20.06^{NS}
	72 hrs	8.53^{f}	16.08^{d}	20.05^{b}	4.19^{NS}	9.35^{d}	5.02^{b}	34.38^{NS}	22.92^{NS}
SEM		0.03	0.03	0.01	0.07	0.03	0.06	1.19	0.99

SEM = standard error of mean; different superscripts within the same column denotes statistical difference at ($p<0.05$); NS = not significant; UGMPP = unfermented ground mature Prosopis pods; FGMPP = fermented ground mature Prosopis pods; S = spontaneous fermentation; SC = *Saccharomyces cerevisiae*; LS = *Lactobacillus salivarius*; NDF = neutral detergent fibre; ADF = acid detergent fibre; CP = crude protein; CF = crude fibre.

Crude fibre content decreased after 24 hours by 10.43%, 11.31%, 14.01% and 12.18% for spontaneous, SC, LS and LS/SC respectively after which increasing fermentation time did not affect the CF content significantly. However, spontaneous fermentation resulted in higher CF values (5.66%) at 72 hrs when compared to fermentation at 24 hours.

3.3.3 Amino acid profile of FGMPP

Amino acid profiles is presented in Table 6. There was general increase of all the amino acids analysed. There was 34-50%. enrichment of essential amino acid (EAAs) in the fermented products especially the ones that are limiting in grain-based diets and alfalfa-based diets for rabbits Lysine and arginine increased on fermentation (Table 6).

Table 6. Essential Amino acid profile of maize, unfermented and fermented GMPP (mg/g DM) during 72-hour fermentation

Amino Acid	Arginine*	Histidine	Leucine	Lysine*	Methionine*	Phenylanine	Threonine	Tryptophan	Valine
Maize	3.94^{bc}	1.66^{f}	2.83^{e}	2.98^{b}	4.56^{g}	2.11^{e}	3.38^{cd}	4.27^{c}	4.27^{c}
UGMPP	4.95^{c}	1.2^{c}	0.36^{c}	3.65^{c}	4.57^{g}	1.38^{a}	0.05^{a}	0.05^{a}	4.44^{cd}
24hr S	7.13^{e}	1.62^{f}	0.27^{b}	4.52^{f}	4.3^{f}	2.01^{cd}	3.65^{d}	3.94^{b}	4.97^{e}
48hr S	6.11^{d}	1.34^{d}	0.26^{b}	3.77^{cd}	4.14^{e}	1.91^{c}	3.08^{c}	4.02^{b}	3.98^{b}
72hr S	6.94^{e}	1.57^{e}	0.3^{b}	4.81^{g}	$4.1d^{e}$	1.89^{c}	0.01^{a}	4.42^{d}	5.12^{f}
24hr SC	9.1^{f}	2.02^{b}	0.01^{a}	0.73^{a}	4.06^{d}	1.87^{c}	4.21^{e}	4.8^{e}	5.25^{g}
48hr SC	5.69^{d}	1.21^{c}	0.1^{a}	0.67^{a}	3.94^{d}	2.02^{d}	2.86^{b}	4.73^{e}	3.9^{b}
72hr SC	0.004^{a}	0.01^{a}	5.6^{h}	4.59^{f}	1.45^{b}	2.06^{d}	6.51^{f}	7.7^{f}	5.09^{f}
24hr LS	0.34^{b}	0.01^{a}	6^{j}	5.16^{g}	1.39^{b}	1.62^{b}	9.74^{j}	13.35^{g}	5.38^{h}
48hr LS	0.33^{b}	0.01^{a}	5.85^{i}	5.08^{g}	1.42^{b}	3.78^{h}	10.62^{k}	13.87^{h}	5.94^{i}
72hr LS	0.31^{b}	0.01^{a}	0.57^{d}	4.15^{e}	1.36^{ab}	4.2^{i}	9.1^{i}	13.4^{g}	4.82^{d}
24hr SC/LS	0.36^{b}	0.01^{a}	4.7^{f}	3.65^{c}	1.29^{a}	3.76^{h}	7.29^{g}	13.65^{gh}	4.43^{cd}
48hr SC/LS	0.35^{b}	0.01^{a}	5^{f}	3.8^{d}	1.34^{a}	3.34^{g}	7.71^{h}	13.94^{i}	0.02^{a}

72hr SC/LS	0.01^{a}	0.01^{a}	5.43^{gh}	4.35^{ef}	1.62^{c}	2.67^{f}	9.74^{j}	14.21^{j}	5.15^{f}
SEM	0.03	0.01	0.03	0.03	0.03	0.02	0.2	0.18	0.11

* Limiting amino acid in rabbit nutrition; SEM = standard error of mean; different ^{a,b,c} superscript on the same column denotes significant difference at ($p<0.05$); GMPP = ground mature Prosopis pods; S = Spontaneous fermentation; SC = *Saccharomyces cerevisiae* fermentation; LS = *Lactobacillus salivarius* fermentation; SC/LS = *Saccharomyces cerevisiae* and *Lactobacillus salivarius* fermentation

3.3.4 Anti-nutritive compounds in FGMPP after 72hrs of fermentation

Anti-nutrient content of UGMPP and FGMPP using different fermentation methods are presented in Table 7. All fermentation methods significantly ($p<0.05$) reduced phytic acid, tannins and aflatoxin contents of GMPP.

Table 7. Changes in anti-nutrient compounds content on fermenting GMPP for 72 hours using different methods

	Treatments						
Parameter	**Trt 1**	**Trt 2**	**Trt 3**	**Trt 4**	**Trt 5**	**SEM**	**p-value**
Phytic acid ppb	48.42^{a}	48.02^{a}	36.11^{b}	46.08^{c}	42.95^{d}	0.09	<0.0001
Aflatoxin ppb	6.7^{b}	6.6^{b}	6.4^{ab}	6.5^{b}	4.9^{a}	0.34	0.02
Tannins ppm	74.55^{a}	22.4^{b}	20.46^{c}	34.52^{d}	28.26^{e}	0.23	<0.0001

SEM = standard error of means; ^{a,b,c,d} different superscript are different at ($p<0.05$); Trt 1 = ground mature Prosopis pods (GMPP); Trt 2 = spontaneous fermentation; Trt 3 = *Saccharomyces cerevisae* (SC); Trt 4 = *Lactobacillus salivariuos* (LS); Trt 5 = SC/LS fermentation.

3.4 Discussion

Microbial count and pH of substrates

Presence of microbial colonies in the first 24 hours for S and SC/LS cultures resulted in microbial growth within the same period while absence of microbial colonies for SC and LS plates within the same period could have been due to initial growth and death of microbial cells in the first 24 hours (Table 4). There could have been fast growth of the microorganisms supported by presence of starch and non-starch polysaccharides that provided the carbon skeleton needed for microbial growth. The carbon sources could have been utilized within the first 24 hours of fermentation resulting in the drop in pH and depletion of carbon sources in the substrate. Studies by Cruz-Alcedo (1999), reported fast bacterial growth in the first four hours during microbial fermentation in vitro. This could explain the reason for zero recordings of microbial population on further plating, beyond 24 hours. Also, Ahlawat *et al.* (2009), reported fast bacterial growth in fermenting substrates, between 10 to 18 hours after which the population began to decline and sustaining growth of microorganisms was only

possible by provision of extra carbon sources for growth. In this study, the initial fermenting substrate was not enriched and there was no addition of substrate as fermentation progressed. The finding on microbial count in this study is contrary to reports by Balogun and Oyeyiola (2011) that there was continuous microbial growth when *Prosopis africana* seeds were naturally fermented. The variation in microbial growth reported in this study could be due to different fermenting substrate (*Prosopis juliflora* pods) have pericarp and seeds as compared to seeds and different processing methods used by Balogun and Oyeyiola (2011) before fermenting the substrates. The carbohydrate content of Prosopis pericarp constitutes about 80% of total carbohydrate in the whole pod (Harden & Zolfaghari, 1988). In this study, whole pods were ground which could have resulted in higher carbohydrate content of the substrates. This could have caused rapid fermentation and fast depletion of carbon sources for fermentation and therefore microbial death within the first 24 hours of fermentation.

There was a decrease in pH of the fermenting substrates in all fermentation methods used. Starch content in ground mature *Prosopis juliflora* pods decreased significantly; this could have provided the needed carbon skeleton for fermentation and production of metabolites as alcohol and lactic acid; therefore, reduction in pH. This is in agreement with studies by Sarasvati *et al.* (2014) when investigating the effects of fermentation on nutritional quality of *Prosopis juliflora* pods as alternative fish feed where decreased pH of fermenting substrates was reported. Results of natural fermentation using sorghum as a substrate reported 28% reduction in pH after 36 hours fermentation period (Ibrahim *et al.,* 2005). Also, Song *et al.* (2008) reported a drop in pH when soybean meal was fermented using microbial inoculums and spontaneous methods. In this study, the increase in pH at different fermentation methods and duration could have been due to the assimilation of organic acids and urea hydrolysis (Raimbault, 1998).

Moisture

Moisture content decrease and increase in DM in the fermented products in this study is probably due to reduction in starch content which could have led to reduction in hygroscopic tendency of ground Prosopis pods and therefore low moisture content. In this study, the moisture content of the ground pods was 9.4 % which is comparable to 9.7 % reported by (Odero-Waitituh *et al.,* 2015). Also, Choge *et al.* (2007) and Preeti *et al.* (2015) reported that Prosopis pods had a carbohydrate content of 69% and a 30% sugar content respectively, conferring in them hygroscopic property which increased the moisture content (Odero-

Waitituh, 2015). In this study, decrease in moisture content of the fermented substrates could be due to a decrease in starch content and therefore reduction in the hygroscopic tendencies. Also, the increase in DM content of the fermented substrates in this study could be due to the microorganisms' biomass. (Haltrich *et al.,* 1996; Igwe *et al.,* 2012). Fermentation therefore reduced the moisture content and improved the keeping quality. This can protect the fermented pods from mycotoxins attack, improve the keeping quality of fermented pods and ensure longevity in case of use for large-scale feed manufacturing.

Crude protein and amino acids

Improvement of CP was observed in all the fermentation methods (Table 5), which is in agreement with (Aremu *et al.,* 2015; Yusuf *et al.,* 2008) for spontaneous fermentations and (Sarasvati *et al.,* 2014; Thi Huyen *et al.,* 2019) for microbial fermentations. Fermentations with LS and SC gave the highest CP value probably due to immediate microbial activity from the inoculation leading to fast microbial action, microbial protein synthesis, microbial growth and production of microbial enzymes for substrates breakdown (Sarasvati *et al.,* 2014). Fermentation with a combination of LS/SC exhibited the lowest CP values probably due to competition by the microorganisms for the substrates or antagonistic mode of action between the microorganisms which could have reduced the total microorganisms present in the final fermented substrate (Blandino *et al.,* 2003).

All the fermented substrates exhibited higher protein and amino acid content. These microorganisms and the microbial enzymes are proteins which are composed of amino acids as the building blocks. In this study, these microorganisms and enzymes could be responsible for the higher CP and amino acid values in fermented substrates Several researchers have reported high enzyme activity in fermenting substrates. Bairagi *et al.* (2004) reported production of α amylase and cellulases in fermenting substrates of Leucena leaves inoculated with intestinal contents of fish. Also, various enzymes were produced during spontaneous fermentation of *Prosopis africana* seeds. For example, α amylase is produced by Bacillus species (Achi, 1992; Souza 2010); Xylanases are produced by *Saccharomyces cerevisiae* (Haltrich *et al.,* 1996); Pectinases are produced by *Bacillus subtilis* (Ahlawat *et al.,* 2009). These microorganisms and the microbial enzymes are proteins (Haltrich *et al.,* 1996) which are composed of amino acids as the building blocks. In this study, these microorganisms and enzymes could have been responsible for the higher CP and amino acid values in fermented substrates (Haltrich *et al.,* 1996; Igwe *et al.,* 2012).

Crude fibre

Reduction in CF content of Prosopis pods was observed in all the fermentation methods used. Various enzymes are produced during fermentation, pectinases are produced by *Bacillus subtilis* during spontaneous fermentation (Ahlawat *et al.*, 2009); xylanases are produced by *Saccharomyces cerevisiae* (Haltrich *et al.*, 1996) which act together with cellulases in a synergy relationship exhibiting an additive effect on cellulose breakdown (Hu *et al.*, 2011). Crude fibre content of the fermenting substrate with microbial inoculation SC, LS and SC/LS was lower compared to spontaneously fermented substrate. This could have been due to microbial inoculations of the substrates allowing for immediate microbial growth and enzymatic production, therefore immediate and fast breakdown of CF (Hu *et al.*, 2011).

Starch

Starch content reduced with fermentation in all the fermentation methods used (Table 5). This is in agreement with results reported by Okine *et al.* (2005) where starch decreased when potatoe pulp was ensiled with or without bacterial inoculants. Achi (1992) isolated *Bacillus subtilis, B. licheniformis, B. megaterium, Staphylococcus epidermidis* and Micrococcus spp in spontaneously fermenting *Prosopis africana* seeds. Further, Souza (2010) reported that bacteria of the genus Bacillus produced the enzyme α amylase which is responsible for breakdown of starch. In the present study, α amylase could have been produced by the fermenting Prosopis pod substrate that could have hydrolysed the starch to oligosaccharides during fermentation therefore reducing the starch content.

Phytic acid

The reduction in phytic acid content during fermentation could have been due to microbial breakdown of glucosidic bonds between the sugars and the phytochemical in the structure of Prosopis pods. The results of this study are in agreement with Vig and Walia (2001) who reported a decrease in phytic acid of rapeseed with increasing fermentation time when using *Rhizopus oligosporus* as an inoculum. Also, Sarasvati *et al.* (2014), reported reduction in phytic acid content of *Prosopis juliflora* pods on solid state fermentation using *Bacillus circulans, Bacillus subtilis* and *Saccharomyces cerevisiae.* According to Achi (1992), *Bacillus subtilis* was present in naturally fermenting *Prosopis africana* seeds. The general reduction in phytic acid in fermenting substrates could be due to the action of phytase enzyme that is produced when pH of substrates drop. This is responsible for the breakdown of phytic acid (El Hag *et al.*, 2002). In this study, it is probable that, phytase enzyme was produced in the fermenting substrates due to the sudden pH drop and was responsible for the breakdown of the bonds and therefore reduction in the phytic acid content of the fermented substrates.

Aflatoxins

All the samples analyzed for aflatoxins had concentrations below the 20ppb recommendations by Allen (2003) for livestock feeds. However, aflatoxin content of UGMPP was higher than values reported by Choge *et al.* (2007) of 5.8 ppb. All fermented substrates had lower level of aflatoxin regardless of the fermentation method used. The reduction in aflatoxin level could have been due to microbial action in all the fermenting substrates. SC/LS fermentation had the lowest aflatoxin level possibly due to a synergistic action of SC and LS. According to Boudergue *et al.* (2009), live cells of *Saccharomyces cerevisiae* plus their cell wall components were effective in detoxifying aflatoxin B_1 in feeds. Megharaj *et al.* (1997) reported that mixed bacterial cultures of Bacillus detoxified aflatoxin contaminated feeds. It is probable that in this study, mixed cultures of Bacillus produced during spontaneous fermentation of Prosopis pods (Achi, 1992) and the inoculated *Saccharomyces cerevisiae* could have detoxified the fermented substrates and reduced the aflatoxin content. According to De Oliveira and Corassin (2014), combined cultures of *Saccharomyces cerevisiae* and lactic acid bacteria were effective in detoxifying aflatoxin by physical binding of the toxin to the microbial cell walls. In this study, the SC/LS mixture had the lowest level

of aflatoxin. This could have been due to the combined action of *Saccharomyces cerevisiae* and lactic acid bacteria in detoxification.

Tannins

All fermentation methods used reduced the total tannin content. This is in agreement with studies by Sarasvati *et al.* (2014) who investigated the effects of microbial fermentation on nutritional quality of *Prosopis juliflora* pods as alternative fish feed and reported decreased tannins on fermentation. Also, Bairagi *et al.* (2004) reported that intestinal content of fish had *Bacillus subtilis* and *Bacillus circulans* which when used as an inoculant in fish diets, reduced tannins. In this study, spontaneous fermentation of mature *Prosopis juliflora* pods exhibited the highest total tannin reduction values (69.96%) compared to all the microbial fermentations investigated. This is in accordance with studies by Olagunju *et al.* (2018) where there were reports of 75% tannin reduction when tamarind seeds (*Tamarindus indica L.*) were naturally fermented. *Saccharomyces cerevisiae* and Bacillus spp were used in production of the enzyme tannase which is responsible in breaking down tannins (Belmares *et al.*, 2004). In this study, the microorganisms were either inoculated or present in all the fermenting substrates. It is possible that the reduction of tannins observed could have been due to production of the enzyme tannase during fermentation of all the substrates regardless of the method of fermentation, leading to reduction of tannins in the fermented substrates.

3.5 Conclusions and Recommendations

3.5.1 Conclusions

i. Fermentation; spontaneous or with SC, LS or LS/SC resulted in improved CP and essential amino acids while CF, phytic acid and tannins decreased in fermented substrates.

ii. Spontaneous fermentation was more effective in reducing tannin content and amino acid enrichment especially the limiting amino acids for rabbits; Lysine, methionine and arginine.

3.5.2 Recommendations

i. Use of fermented Prosopis pods is recommended in rabbit diets, so long as the dietary requirements are met as per the feeding standards and nutrient requirements of individual class of livestock.

ii. Since spontaneous fermentation reported the highest amino acid profile especially the limiting ones for rabbits, it is recommended that this method of fermentation would be the ideal treatment method of GMPP intended for rabbit diets.

iii. Further research should be conducted to establish the efficacy of SC and a combination of SC and Lactobacillus species in reducing aflatoxin in Prosopis pods.

iv. Further research should be done on the efficacy of SC in reduction of phytic acid in Prosopis pods.

CHAPTER FOUR

FAECAL NUTRIENT DIGESTIBILITIES OF GROUND MATURE *Prosopis juliflora* PODS IN RABBITS: EFFECT OF FERMENTATION AND ADDITION OF CORNSTARCH

Abstract

The study was conducted to determine the digestibilities of unfermented ground mature *Prosopis juliflora* pods (UGMPP) and fermented ground mature *Prosopis juliflora* pods (FGMPP). In a completely randomized design (CRD) experiment, 15 bucks with a mean weight of 2.96±0.25 (mean ± SE) were each housed in cages (75×55×40) cm^3. They were offered the five experimental diets consisting of FGMPP, UGMPP, FGMPP plus cornstarch, UGMPP plus cornstarch and non protein diet. The study investigated apparent digestibility coefficients of UGMPP and FGMPP for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and ash and if these digestibility coefficients were affected by addition of cornstarch. Also, true and apparent digestibilities of CP were investigated. Results showed that DM and nutrient digestibilities for UGMPP were higher ($p<0.05$) than all other treatments. DM digestibilities were; 0.68 for UGMPP; 0.64 for FGMPP plus cornstarch; 0.58 for UGMPP plus cornstarch, and 0.54 for FGMPP. Crude protein digestibilities were; 0.76 for UGMPP; 0.73 for FGMPP plus cornstarch; 0.6 for UGMPP plus cornstarch, and 0.72 for FGMPP. Use of non-protein diets (NPD) increased true digestibility coefficients of UGMPP and FGMPP from 0.76 and 0.72 to 0.84 and 0.77 respectively. The study concluded that although UGMPP reported the highest digestibility coefficients, FGMPP was superior in terms of total nutrients intake due to high dry matter intake (DMI). The study recommended the use of FGMPP in rabbit diets and also, further studies to be conducted to investigate the effect of addition of cornstarch when FGMPP is fed to rabbits.

Keywords: Cornstarch, digestibility, fermentation, *Prosopis juliflora*, soluble fibre

4.1 Introduction

Nutrient analysis is the first step in estimating the feeding value of feedstuffs (Guevara *et al.,* 2008). Nutritive value of feeds is determined by a number of factors, including composition, odour, texture and taste (Schneider & Flat, 1975). These factors are generally measurable in the case of the animal as digestibility and intake (Khan *et al.,* 2003). Digestibility is a measure of the availability of nutrients (Khan *et al.,* 2003). Nutrient digestibility in livestock

is a crucial determinant of nutrient bioavailability and therefore related to performance (Guevara *et al.,* 2008).

The common way to perform digestibility trials is to collect all feed consumed and all faeces excreted during a certain number of days (Guevara *et al.,* 2008). The nutrient differences of the feaces and the feed on analysis gives what was retained by the animal and therefore digestibility, expressed as a percentage and is reffered to as the digestibility coefficient. This is the most reliable method of measuring a feed's digestibility (Guevara *et al.,* 2008). The feaces is composed of endogenous (cell disquamation, bacterial and enzyme debris) materials of animal origin that was not part of the feed and the undigested component of the feed. This gives a lower digestibility coefficient as more nutrients will appear in feaces in apparent digestibility trials (De Lange *et al.,* 1990). Use of non protein diet to correct for the values of apparent digestibility gives the true coefficient of digestibility especially protein and amino acid components. This is the difference in feacal protein and amino acid values in apparent digestibility and non protein diets. There are other indirect methods as use of internal markers to determine digestibility (Lippke, 2002). These markers must be totally indigestible and un-absorbable (Jagger *et al.,* 1992). Also, *in vitro* digestibility techniques can be used. They provide a quick, inexpensive, and precise prediction of *in vivo* digestibility (Khan *et al.,* 2003).

There are plenty of non conventional fibrous and non fibrous feedstuffs that can be included in livestock diets in Kenya. Some of these feed ingredients have the ability to improve production efficiencies of livestock (Engle, 2017; King'ori *et al.,* 2011; Muthui *et al.,* 2018). However, their level of inclusion is often limited due to the little information available on their nutritive value, total mean retention time, and the nutrient imbalance that they might present (Safwat *et al.,* 2015). Mature pods of *Prosopis juliflora* is one such feed resource in Kenya (King'ori *et al.,* 2011). Several researchers have reported its attributes and availability (King'ori *et al.,* 2011; Odero-Waitituh, 2015) in arid and semi-arid lands (ASALs) in countries where it was introduced. However, ANC (high tannin-8% and CF-17.7%) in Prosopis pods reduced animal performance, with recommendations that feeding value could be enhanced by treating the pods (Mariam *et al.,* 2013; Odero-Waitituh, 2015). Addition of cornstarch to feeds was reported to improve digestibility coefficients of fibrous feed and livestock performance (Guo *et al.,* 2006; Shi & Noblet 1994). Digestibility studies of the pods have not been done to determine the digestibility coefficients of FGMPP and UGMPP in rabbits. This would inform their inclusion in rabbit feeds. The objective of this study was to

determine if apparent and true feacal nutrient digestibilities of ground mature pods of *Prosopis juliflora* in rabbits are affected by fermentation. Also, the study was to asses if inclusion of cornstarch to the FGMPP and UGMPP affected nutrient digestibilities in rabbits. The results of the study will provide guidelines on the inclusion of UGMPP and FGMPP in rabbit diets.

4.2 Materials and Methods

4.2.1 Study site

Prosopis juliflora pod collection, drying, milling, storage and fermentation is as described in 3.2.1. Fermented ground mature *Prosopis juliflora* pods, unfermented ground mature *Prosopis juliflora* pods and faecal sample preparation was done at the Egerton University, Animal Nutrition laboratories. Proximate and gross energy analysis was conducted at Animal Nutrition laboratories, Egerton University. Egerton University's location is as described in 3.2.2.

4.2.2 Experimental animals and management

The study was conducted using 15 New Zealand White adult bucks from Tatton Agricultural Park (TAP), Rabbit Unit, Egerton University. They were housed individually in cages (75×55×40) cm^3 fitted with half inch wire mesh for comfort and to allow for faeces and urine passage. Below the floor of each cage, there was another quarter inch wire mesh fitted to allow for urine passage but not faeces as described by Schneider and Flat (1975). This allowed for collection of all faeces. The cages were disinfected with kupacide® before introduction of the rabbits. The rabbits were dewormed with ascarex® one week before the commencement of the experiment.

Tatton Agriculture Park observes measures for maintenance of biosecurity and prevention of disease transmission by preventing unnecessary entry into the unit and provision of a foot bath with Kerol® if individuals who enter the unit. Authority to use live animals was obtained from the Institute of Primate Research on compliance with international code of animal ethics in research (Appendix B). Authority to conduct the research was obtained from National Commission for Science Technology and Innovation in compliance with international code for permission and clearance to conduct research (Appendix A). Continuous observation of the rabbits was done to ensure that they were free from any stress due to excessive

environmental disturbances. All welfare aspects of the rabbits were well taken care of. Feed and water were supplied *ad-libitum* using metalic feeders and drinkers throughout the experimental period. The rabbits were given a 7-day acclimatization period to the diets and cages, followed by a 5-day faecal collection period.

4.2.3 Dietary treatments

The diets were formulated to meet the nutrient requirements for their category as per the standard (Deblas & Mateos, 2010). Feed preparation, milling and mixing was done at the Tatton Agriculture Park feed mill. Sodium selenite was added to the non-protein diet to prevent the diet from going rancid. Spontaneously fermented GMPP (FGMPP) and UGMPP were used in dietary formulations (Table 8). Dietary treatments 1 to 5 were offered *ad-libitum*. The diets were constituted as follows;

Diet 1 as treatment 1/Trt 1 non protein diet;

Diet 2 as treatment 2/Trt 2 FGMPP;

Diet 3 as treatment 3/Trt 3 UGMPP;

Diet 4 as treatment 4/Trt 4 FGMPP+30% cornstarch;

Diet 5 as treatment 5/Trt 5 UGMPP+30% cornstarch;

Table 8. Composition of experimental diets

Ingredient	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
Cornstarch	74.3	-	-	30	30
Sucrose	25.0	-	-	-	-
Sunflower oil	0.09	-	-	-	-
Vitamin /mineral premix*	0.5	0.5	0.5	0.5	0.5
Sodium selenite	0.11	-	-	-	-
FGMPP	-	99.5	-	69.5	-
UGMPP	-	-	99.5	-	69.5
Total	100	100	100	100	100

*To provide per diet: vitamin A(10,000 iu), vitamin D (20,000 iu), vitamin E (1355 iu.), vitamin K (4500 mg), choline (350 mg), folic acid (1 mg), copper sulphate 7500 manganese sulphate (12,250 mg), zinc sulphate (12,250 mg), sodium chloride (210,000 mg), potassium chloride (50,000 mg), Di-calcium phosphate (10,000 mg)

4.2.4 Experimental design

Fifteen adult bucks with similar body weights were used. Each cage had one rabbit representing an experimental unit. Using PQRS software, each rabbit was randomly allocated to one of the 15 cages/experimental units. There were three rabbits for each treatment (3 rabbits/treatment) in a completely randomized design (CRD) (Table 9).

Table 9. Experimental layout

R3T5	R3T1	R3T3	R2T2	R2T4
R2T3	R1T1	R1T5	R1T3	R1T2
R1T4	R3T1	R3T4	R3T2	R2T5

Key: T1-treatment 1, T2-treatment 2, T3-treatment 3, T4-treatment 4, T5- treatment 5, R-replicate

4.2.5 Data collection

Feed intake was determined daily as the difference between the feed offered and refusals. Feed refusals collection was done every morning 09.00 hrs before the next feeding. Faeces were collected daily at 0900 hours, weighed and 50% kept in the refrigerator at -20^{o} C in plastic sample bags, according to the procedure by McDonald (2010). At the end of the 5-day collection period, the deep-frozen faeces were removed and oven dried at 60^{o} C for 24 hours. They were then put in the desiccator for 2 hrs. The 5 days collected faeces from each cage (experimental unit) were pooled and ground to pass through a 5 mm sieve and kept airtight in sample bags for analysis.

4.2.6 Digestible nutrients and energy determination

The difference approach and total collection method was used to calculate digestible energy (DE) and nutrients in UGMPP, FGMPP, cornstarch + FGMPP, cornstarch + UGMPP and non-protein treatment (McDonald, 2010; Adeola, 2001). Energy and nutrient in diet minus energy and nutrient in faeces = energy and nutrients retained in the body

Calculations

Digestible nutrients

Equation 8. Calculation of digestible energy

DE = GES – GEF

Source: McDonald (2010)

Where DE is digestible energy

GES is Gross energy in treatment

GEF is Gross energy in faeces

Apparent digestible nutrients

The collected excreta and nutrient intake in the adult buck was used to determine apparent and true nutrient digestibility using the following formula:

Equation 9. Calculation of apparent nutrient digestibility.

Apparent nutrient digestibility (AND) % = 100× (NI-NE) /NI)

Source: McDonald (2010)

Where:

NI represents the nutrient intake (g)

NE represents the nutrient excreted (g)

True digestible nutrients

Equation 10. Calculation true nutrient digestibility

True nutrient digestibility (TND) % = 100× ((NI-NE)-NENPD)/NI)

Source: Takagi *et al.* (2002)

Where:

NI represents the nutrient intake (g)

NE represents the nutrient excreted (g).

NENPD represents the nutrient excreted in the non-protein diet (g).

Digestibility coefficient

Equation 11. Calculation of digestibility coefficient

Digestibility coefficient (DC) = percentage digestibility/100

4.2.7 Statistical analysis

Digestible energy was computed using Equation 8; apparent nutrient digestibility was computed using Equation 9; true nutrient digestibility was computed using Equation 10 and Digestibility coefficient was computed using Equation 11. Data were first subjected to normality and homogeneity of variance tests using SPSS Statistics 25.0.0 software (2017). They were then subjected to analysis of variance using general linear model (GLM) of Statistical Analysis Systems (SAS, 9.1.3) computer package (2005). The differences among treatment means were determined using the Tukey's range test. Analysis for apparent digestibilities of gross energy of FGMPP and UGMPP and true and apparent digestibilities of CP were done using a two-sample t test. Probability values of ($p<0.05$) were considered significant. The following model was used;

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

Where:

Y_{ij} = observation of i^{th} dietary treatment.

μ= overall mean,

α_i= effect of i^{th} dietary treatment, (i=1…..5)

$\varepsilon_{ij=}$ random error associated with Y_{ij}

4.3 Results

Proximate fractions and gross energy of UGMPP and FGMPP are presented in Table 10.

Table 10. Proximate fractions and gross energy (GE) of UGMPP and FGMPP (on DM basis)

Parameter	UGMPP	FGMPP
% DM	91.7[a]	95[b]
% CF	20.5[a]	22.3[b]
% CP	14.2[a]	16.5[b]
% EE	4.3	4.35
% Ash	3.4a	5.4[b]
GE in MJ/kg sample	14.05[a]	15.56[b]

UGMPP = unfermented ground mature *Prosopis juliflora* pods; FGMPP = fermented ground mature Prosopis pods; DM = dry matter; CF = crude fibre; CP = crude protein; EE = ether extract; [a,b,c,d] superscript within the same raw are different at ($p<0.05$).

Dry matter intake (DMI), faecal output (FO), apparent digestibilities of UGMPP, FGMPP, UGMPP plus corn starch and FGMPP plus cornstarch are presented in Table 11. The DMI was highest ($p<0.05$) in FGMPP with addition of corn starch compared to all other treatments

Table 11. DMI, faecal output and apparent digestibility coefficients of UGMPP and FGMPP with and without cornstarch

Parameter	UGMPP	FGMPP	UGMPP+CS*	FGMPP+CS*	R^2	p-value
DMI (g)	248[a]	381[b]	190[c]	576[d]	0.99	<0.0001
FO (g)	84[a]	177[b]	75[a]	199[c]	0.99	<0.0001
GE MJ/	0.65[a]	0.53[b]	-	-	0.92	<0.0001
DM	0.67[a]	0.54[b]	0.58[c]	0.64[d]	0.99	<0.0001
CP	0.65[a]	0.63[b]	0.6[c]	0.73[b]	0.99	<0.0001
CF	0.6[a]	0.24[b]	0.32[c]	0.26[b]	0.97	<0.0001
Ash	0.62[a]	0.69[b]	0.64[c]	0.78[d]	0.99	<0.0001
EE	0.72[a]	0.55[ac]	0.09[b]	0.48[c]	0.98	<0.0001

[a,b,c,d] different superscripts within the same raw are significantly different at $p<0.05$; UGMPP = unfermented ground mature Prosopis pods; FGMPP = fermented ground mature Prosopis pods; DM = dry matter; CF = crude fibre; CP = crude protein; EE = ether extract; DMI = dry matter intake; FO = faecal output; GE = gross energy (mega joules/kg sample); CS* = addition of 30% cornstarch.

The results of true and apparent CP digestibility coefficients are presented in Table 12.

Table 12. True and apparent CP digestibility coefficient of UGMPP and FGMPP

	Coefficient of apparent digestibility	Coefficient of true digestibility
Sample		
UGMPP	0.64	0.76[b]
FGMPP	0.63	0.69[a]
SEM	0.59	0.58
p-value	0.29	0.001

SEM = standard error of means; [a, b] different superscripts within the same column are significantly different at $p<0.05$; UGMPP = unfermented ground mature *Prosopis juliflora* pods; FGMPP = fermented ground mature *Prosopis juliflora* pods.

4.4 Discussion

Results from the current study show that fermentation of GMPP and addition of corn starch improved palatability and therefore DMI of both FGMPP and FGMPP with corn starch. Similar results of increased intake of fermented products due to improved aroma and palatability were reported in experiments by Chukeatirote (2015); Egwim *et al.* (2013) and Israilides *et al.* (1981) when soybean was spontanously fermented; indigenous Nigerian foods were naturally fermented and wheat straw was fermented with yeast (*Aureobasidium pullulans)* respectively. The implication is that fermented products have good sensory attributes regardless of the fermentation method (Uyoh *et al.*, 2009). When investigating effect of dietary cornstarch levels on growth performance, digestibility and microscopic structure in the white shrimp (*Litopenaeus vannamei*) reared in brackish water, dry matter intake improved on addition of cornstarch up to 20% of diet after which it started to decrease (Guo *et al.*, 2006). In this study, DMI of FGMPP significantly ($p<0.05$) increased with addition of corn starch.

In this study, the DMI for UGMPP diet was 248 g as compared to 381 g by FGMPP. Fermentation of the pods decreased DM digestibility while addition of corn starch improved the digestibility of FGMPP but decreased the digestibility of UGMPP. In studies to characterize *Prosopis juliflora* seed galactomannan, Cruz-Alcedo (1999) reported presence of soluble fibre in *Prosopis juliflora* seeds with suggestions by Trocino *et al.* (2013) that these soluble fibres improved intakes and therefore digestibility and performance of growing rabbits. Other studies on the effect of fermentation on soluble fibre content of fermented substrates demonstrated reduction in soluble fibre. Cruz-Alcedo (1999) reported destruction of the galactose-mannose linkages that make the carbohydrates in *Prosopis juliflora* seeds within 4 hours of microbial fermentation *in-vitro*. Also, Egwim *et al*. (2013) while reviewing fermented foods in Nigeria, reported predigestive processes involvement in improvement of digestibility of fermented products and therefore animal performance. In this study, it is possible that FGMPP was composed of the nutrients such as amino acids and peptides which were predigested and therefore no more digestion was possible because they are the smallest building blocks and therefore absorbed as they are. This might have caused a lower digestibility coefficient of DM of the FGMPP as compared to UGMPP. The digestibility of CP, CF and ash followed the same pattern. Crude fibre digestibility was higher for UGMPP which could have been due to the low overall DMI and therefore lower CF intake. In this

study, CP digestibility coefficient of 0.76 for UGMPP was significantly higher ($p<0.05$) than all other treatments. This was similar to CP digestibility recorded by Batista *et al.* (2002) who reported total tract digestibility coefficients of 0.76 to 0.82 of mesquite (*P. juliflora*) pods depending on the moisture content of the samples. The lower DMI recorded in this study for UGMPP as compared to FGMPP can explain the higher digestibility coefficient of CP due to lower rate of passage, longer exposure to digestive enzymes and juices and therefore higher digestibility coefficient. However, it is important to note that as much as digestibility coefficient of CP was higher in UGMPP, due to the higher DMI recorded for FGMPP, the total CP retention was higher. In this study, addition of cornstarch decreased UGMPP but increased FGMPP digestibility coefficients (Table 11). The soluble fibres in UGMPP could have formed substrate for digestion and provided readily available energy for enzymatic reactions for higher digestibility coefficients in UGMPP (Cruz-Alcedo, 1999; Trocino *et al.*, 2013). Fermentation of UGMPP reduced soluble fibre content of UGMPP and therefore decreased readily available energy content. In this study, it is probable that addition of cornstarch to the FGMPP improved digestion coefficients of nutrients by providing energy for enzymatic digestive processes and microbial activity. Cruz-Alcedo (1999) and Trocino *et al.* (2013) reported that presence of soluble fibre in *Prosopis juliflora* pods improved feed digestibility and therefore improvement of livestock performance were recorded.

4.5 Conclusions and Recommendations

4.5.1 Conclusions

i. Results from the current study suggest that negatively affected true digestibility coefficient of CP and apparent digestibility coefficients of nutrients (CP, CF, EE and GE). However, it had a positive effect on ash apparent digestibility.

ii. It's important to note that the palatability for UGMPP was low due to the low DMI. The total nutrients that were assimilated for the treatment with UGMPP was therefore lower when compared with FGMPP.

4.5.2 Recommendations

i. This study recommends the use of FGMPP in rabbit diets as it had higher palatability. It is expected that higher nutrient intake would translate to improved performance.

ii. Studies to be conducted to investigate the effect of addition of cornstarch to FGMPP-based diets fed to rabbits.

iii. Studies to be conducted to investigate the effect of different levels of FGMPP intake on digestibility coefficients.

CHAPTER FIVE

GROWTH RATE, BLOOD HAEMATOLOGICAL AND METABOLITE INDICES, OF GROWER RABBITS FED FERMENTED GROUND MATURE PROSOPIS PODS

Abstract

This study was conducted to determine the effect of feeding fermented ground mature Prosopis pods (FGMPP) on growth rate, blood haematological and metabolite indices. In a randomized complete block design (RCBD), sixty (60), 42-day old New Zealand White grower rabbits, weighing 0.5 ± 0.04 (mean ± SE) were housed in cages (75×55×40) cm^3. The study investigated growth rate and changes in blood haematological and metabolite indices during 6 weeks' growth period from 42 to 84 days. The results showed that inclusion of FGMPP in the rabbit grower diets reduced mortality and resulted in better ADG ($p<0.05$). There was treatment effect ($p<0.05$) on average daily feed intake (ADFI) while feed conversion ratio (FCR) and final weights were similar ($p>0.05$) across all treatments. Lymphocyte %, red blood cell count (RBC), haemoglobin content (HB), packed cell volume (PCV) and platelet counts were positively influenced by inclusion of FGMPP in the diets of growing rabbits. Blood glucose, cholesterol, cortisol and calcium contents were significantly influenced ($p<0.05$) by the dietary treatments. The study concluded that 30% maize in grower rabbits' diets can be replaced by FGMPP.

Keywords: Blood, fermentation, growth, haematology, metabolite, Prosopis pods.

5.1 Introduction

Growth and development are the integration of all facets of animal science including nutrition, and meat science (Trenkle & Marple, 1983) and genetics or animal breeding and physiology (Owens *et al.,* 1993; Trenkle & Marple, 1983). It encompasses biochemistry, endocrinology, and animal management (Owens *et al.,* 1993). It can be measured by observing the change in body weight per unit of time or by plotting body weight against age (Trenkle & Marple, 1983). Growth is defined as an increase in tissue mass. Mass increases by increased reproduction or cell multiplication (hyperplasia) early in life and increase in cell size due to growth in size of the cell components (hypertrophy) later in life. The growth curve, being mass or cumulative weight plotted against age, is sigmoid, consisting of a pre-pubertal accelerating phase plus a post pubertal decelerating phase (Owens *et al.,* 1993).

Growth of an animal is the most important indicator of nutritional status of feeds, although maximum growth rate is set genetically (Owens *et al.,* 1993). It is therefore one of the parameters that is used to evaluate the nutritive value of feeds. Growth experiments are conducted during pre-pubertal accelerating phase to determine if the nutrient being tested is able to sustain the high nutritional demands for the growth phase.

Other important parameters to consider when testing a feed is quality and safety and therefore safety and quality of animal products to the consumers bearing in mind animal welfare issues that compromise quality (FAO, 2014). Nutritional deficiencies or toxicities can cause animal welfare issues which can be manifested as long-term stress. There are several blood parameters that help in indication of feed toxicity to livestock (Isaac *et al*., 2013). For instance, blood haematological parameters, metabolite indices (Aro *et al.,* 2013) and antibody titres (Khobondo *et al.,* 2019) are important in health and physiological status screening of animals. Blood haematology and chemistry can therefore be a reliable media that can be used to monitor nutritional staus, physiological effects of diets and therefore health status of animals (Shousha *et al.,* 2017). Franck *et al.* (2016) used blood haematological and chemistry indices to investigate the effects of dietary treatment on nutritional and physiological status of rabbits successfully where it was demonstrated that different diets have significant differeces in blood chemistry and haematology. Inclusion of *Prosopis juliflora* pods in the diets of laying indigenous chicken did not affect, laying percentage, growth and antibody titer and the products were therefore considered safe for human consumption (Khobondo *et al.,* 2019). In toxicological studies to investigate the effects of *P. juliflora* pods on internal organ physiology and anatomy using rats, it was reported that the pods exhibited LD_{50} greater than 5000 mg/kg and recommendations made that it can be used by animals and humans with a degree of safety and tolerance (Kimani *et al*., 2014; Wamburu *et al.,* 2015).

In clinical studies conducted by Colfer *et al.* (1950) on mice, rats and rabbits, there was a significant relationship between temporary lymphopaenia (drop in blood lymphocytes) and injection of adrenocorticotropic hormone (ACTH). When animals are exposed to conditions that are not favourable, ACTH is produced from the anterior pituitary gland under natural conditions (Medugu *et al*., 2010), therefore it can be used as an indicator of stress (Cufer *et al.,* 1998). Also, Tanchev *et al.* (2014), reported that transportation induced stress in rabbits significantly ($p<0.05$). This was manifested by elevated levels of cortisol and ACTH. The two are blood metabolite indices scientifically important in detecting level of stress in

animals. Stress originating from disease can be demonstrated by use of changes in haematological parameters (Hinton *et al.,* 1982) which are useful tools in disease diagnosis in rabbits and other livestock. Chineke *et al.* (2006) and Etim *et al.* (2014b) demonstrated that apart from genotype, age sex, breed and animal management system, differences in levels of haematological and metabolite indices may be influenced by nutritional factors. Red blood cell count (RBC), packed cell volume (PCV) and lymphocytes percentage, were significantly lower ($p<0.05$) in high tannin diets. However, mean corpuscular haemoglobin concentration (MCHC), neutrophil and basophil counts were superior ($p <0.05$) in high tannins diets in broilers (Medugu *et al.,* 2010). Etim *et al.* (2014a) reported that dietary contents influence the effectiveness of body metabolic processes and physiology of rabbits and therefore blood profile. According to Etim *et al.* (2014b) haematological studies are a useful tool in assessing the physiological status of rabbits as influenced by diets.

Rabbits unlike poultry, beef and swine can be produced on a wide range of feedstuff materials like forages, cereal by-products, soybeans waste (Iyeghe-Erakpotobor *et al.,* 2006) and unconventional feed resources as mature *Prosopis juliflora* pods which is in less competition with man (Iyeghe-Erakpotobor *et al.,* 2006; Odero-Waitituh, 2015). *Prosopis juliflora* is readily available in ASALs areas of Kenya (King'ori *et al.,* 2011). Several researchers have reported positive results when *Prosopis juliflora* pods were included in livestock diets. Wanjohi *et al.* (2017a) reported 20% inclusion of Prosopis pods in indigenous chicken diets supported growth. Also, Khobondo *et al.* (2019), reported an inclusion level of 10% to have supported growth and egg production with no significant statistical effect on antibody titres of indigenous chicken, showing that there are no immunological reactions when Prosopis pods are fed to livestock. However, in broiler feeding Odero-Waitituh *et al.* (2016) reported reduced broiler performance as level of Prosopis pods replacing maize increased beyond 20%. Various treatment methods resulted in reduction of anti-nutritive compounds and improvement of livestock performance (Mariam *et al.,* 2013; Sarasvati *et al.,* 2014; Yusuf *et al.,* 2008). Also, Oluwajoba *et al.* (2013) reported the ability of Lactic acid bacteria *Pediococcus pentosasceus, Lactobacillus fermentum, Lactococcus lactis ssp lactis, Lactobacillus pentosus* produced during spontaneous fermentation of millet/Sorghum mixtures to survive in the gastrointestinal tract of humans thereby confering probiotic effects and improving gut health. Suggestions were made that these fermentation products could be used as probiotics in food and feed preparations. No studies have been done to investigate the effect of Prosopis pods on haematological parameters and if, fermenting the Prosopis pods

could improve rabbit performance and therefore allow for higher inclusion rates. The objective of this study was to evaluate growth performance, and blood haematological and metabolite changes that occur when fermented ground mature *Prosopis juliflora* pods are fed to grower rabbits.

5.2 Materials and Methods

5.2.1 Study site

Prosopis juliflora pods collection, drying, milling, storage and fermentation is as described in 3.2.1. Study site's location is as described in 3.2.2. Blood samples for haematology and blood metabolites analysis were collected at Egerton University and transported to Medhills Laboratories, Nakuru for, haemoglobin, red blood cell count, basophil, eosinophils, neutrophils, lymphocytes, glucose, calcium, cholesterol and cortisol determination.

5.2.2 Experimental animals and management

The study was conducted at Tatton Agriculture Park using 60 (42 days old) New Zealand White grower rabbits (30 bucks and 30 does). They were housed in cages (75×55×40) cm^3. The rabbits were reared for 3 days on standard grower diet for adaptation (Deblas & Mateo, 2010). During this period, disease control measures were carried out. The rabbits were dewormed with ascarex® and dusted with Sevin® for control of internal and external parasites respectively. Before introducing the rabbits to the experimental cages, watering and feeding troughs were thoroughly cleaned, disinfected with kupacide® and dusted with Sevin® against external parasites. Management of Tatton Agriculture Park rabbit unit was as outlined in section 3.2.1.

5.2.2 Dietary treatments

Ingredients for formulation of experimental diets were milled and mixed at the Tatton Agriculture Park feed mill, Egerton University. Proximate analysis was done on the feed ingredients used before experimental diets formulation. Five dietary treatments were formulated consisting of control/standard diet for the class of rabbit, 15% UGMPP, 30% UGMPP, 15% FGMPP and 30% FGMPP based diets (Table 13).

Table 13. Composition of experimental diets

	Diets				
Ingredient	**30%UGMPP**	**15%UGMPP**	**30%FGMPP**	**15%FGMPP**	**Control**
Maize	-	15	-	15	30
Wheat bran	22.3	15	21.5	15.2	20
Maize germ	13.5	20.5	14	20	15
Rice germ	13	14	15	15	13
UGMPP	30	15	-	-	-
FGMPP	-	-	30	15	-
SFC	18.2	17.5	16.5	16.8	19
Bone meal	2.0	2.0	2.0	2.0	2.0
Iodized salt	0.5	0.5	0.5	0.5	0.5
Vit premix*	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Calculated analysis					
CP (%)	16.2	15.9	16.2	15.9	15.8
ME (MJ/)	9.78	9.82	9.78	9.82	9.84
CF (%)	15.9	15.8	15.9	15.8	15.5

UGMPP = unfermented ground mature *Prosopis juliflora* pods; FGMPP = fermented ground mature *Prosopis juliflora* pods; SFC = sunflower cake; CP = crude protein, ME = metabolizable energy (mega joules/kg sample); CF = crude fibre; *To provide per diet: vitamin A(10,000 i. u), vitamin D (20,000 i. u), vitamin E (5 i.u.), vitamin K (2.5 mg), choline (350 mg), folic acid (1 mg), manganese (56 mg), iodine (1 mg), iron (20 mg), copper (10 mg), zinc (50 mg), cobalt (1.25 mg).

The rabbits were reared on formulated standard diets for each class for the control/reference diet and the diets with inclusion of UGMPP and FGMPP were formulated to requirements for each class of rabbits (Deblas & Mateo, 2010).

5.2.3 Experimental design

Sixty 42-day old New Zealand White grower rabbits with similar body weights were used. Each cage had 3 rabbits representing an experimental unit. Using the PQRS software, they were randomly allocated to twenty experimental units consisting of two groups with equal bucks and does. Using the same software, the five dietary treatments were then randomly allocated to the experimental units in four replicates per treatment, each consisting of six grower bucks and six grower does (12 rabbits/treatment) in a randomized complete block design (RCBD) based on sex (Table 14). Each rabbit per cage was ear numbered and parameter taken on the same rabbit throughout the experimental period, 42 days.

Table 14. Experimental layout

Males	R1T2	R1T1	R2T2	R2T5	R2T3
	R2T1	R1T3	R1T5	R2T4	R1T4
Females	R3T1	R4T1	R3T3	R3T4	R3T5
	R3T2	R4T2	R4T4	R4T3	R4T5

Key: T1-treatment 1, T2-treatment 2, T3-treatment 3, T4-treatment 4, T5- treatment 5, R-replication

5.2.4 Data collection

Feed intake, weekly weights and blood parameters

Feed intake (FI) was calculated as the difference of feed offered and feed left by the end of each day for each of the experimental unit. The average daily intake from each cage represented one experimental unit. Average daily live weight gain for each experimental unit was represented by the average cage weight gain divided by seven. Weights were taken weekly throughout the experimental period and final weight taken at the end of 6 weeks feeding period.

Blood samples were taken at the beginning of the experiment and at the end of the sixth week feeding period. Before commencement of the experiment, one rabbit from each experimental unit was randomly selected, 5 ml of blood sample (2 ml and 3 ml) drawn from the ear vein into 2 different vials according to Suckow and Douglas (1997) for haematology and metabolites analysis respectively. The sera from the 3 ml collected samples were separated by centrifuging at 3000 r.p.m for 30 minutes then frozen at -20^{o} C until analysis for cortisol, cholesterol and Ca was carried out. Glucose and full haemogram analyses were done on the other 2 ml sample vials with the anticoagulant.

5.2.5 Statistical analysis

Data from average daily feed intake (ADFI), final weights, average daily gains (ADG), feed conversion ratio (FCR), blood haematology and metabolite indices were subjected to normality and homogeneity of variance test using SPSS Statistics 25.0.0 software (2017). They were then subjected to analysis of variance using general linear model (GLM) of Statistical Analysis Systems (SAS, 9.1.3) computer package (2005). Where significant differences were observed in the initial weights, they were used as covariates. The differences among treatment means were determined using the Tukey's range test. Probability values of ($p<0.05$) were considered significant. Statistical model used was as follows;

$Y_{ijk}= \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$

Where:

Y_{ij} = observation of i^{th} dietary treatment in the j^{th} sex.

μ= overall mean,

α_i= effect of i^{th} dietary treatment, (i=1,…..5)

β_j= effect of j^{th} sex, (j=1,2)

$\varepsilon_{ijk=}$ random error associated with Y_{ijk}

5.3 Results

Proximate composition of feed ingredients used to formulate experimental diets is presented in Table 15.

Table 15. Proximate composition and energy content of feed ingredients used in formulation of experimental treatments (on DM basis)

Ingredient	%DM	%CF	%CP	%EE	%Ash	ME	%CH_2O
MG	90.8	8.7	9.76	12.15	2.7	14.89	62.75
Maize	88.6	2.8	8.21	7.12	0.7	14.31	71.63
RH	93.7	29.2	7	5.51	17.5	7.91	33.59
WB	89.2	11.6	16.65	7.81	4.7	12.28	47.95
SFC	94.5	29.42	22.8	15.36	5.8	11.96	22.26
FGMPP	84.2	26.58	13.96	1.77	4.89	8.29	37.57
UGMPP	88.25	32.9	10.23	2.62	3.81	8.03	37.68

SFC = sunflower cake; MG = maize germ; RH = rice husks; WB = wheat bran; UGMPP = unfermented ground mature *Prosopis juliflora* pods; FGMPP = fermented ground *Prosopis juliflora* pods; CP = crude protein, ME = metabolizable energy (mega joules/kg); CF = crude fibre; EE = ether extract; CH_2O = carbohydrates.

Results for ADFI, ADG, final weights, FCR and % mortality of the growing rabbits are presented in Table 16.

Table 16. Performance of grower rabbits fed UGMPP and FGMPP-based diets

Treatments	Initial wt(g)	Final wt(g)	ADG (g)	ADFI (g)	FCR	% mortality
30%UGMPP	440.75	1242.5	19.75^{b}	81.25	4.32	16.7
15%UGMPP	481.00	1304.25	19.5^{b}	78.5	3.86	25
30%FGMPP	458.50	1486.5	22.25^{c}	75.75	3.64	8.33
15%FGMPP	539.00	1242.25	19.00^{b}	76.75	4.25	16.7
Control	588.00	1181.75	15.75^{a}	66.75	3.92	25
SEM	41.94	79.01	1.25	3.58	0.27	-
P value	0.13	0.12	0.04	0.14	0.49	-

SEM = standard error of means; $^{a, b, c}$ = means in the same column with different superscripts are significantly different ($P<0.05$); UGMPP = unfermented ground mature *Prosopis juliflora* pods; FGMPP = fermented ground *Prosopis juliflora* pods.

There was treatment effect ($p<0.05$) for ADG. Growth parameters were similar ($p>0.05$) for all treatments except for rabbits fed 30% FGMPP inclusion that had a higher ($p<0.05$) ADG. Weekly ADG for the grower rabbits is presented in Figure 9. The diet with 30% FGMPP inclusion showed a higher ($p<0.05$) weekly ADG during the experimental period.

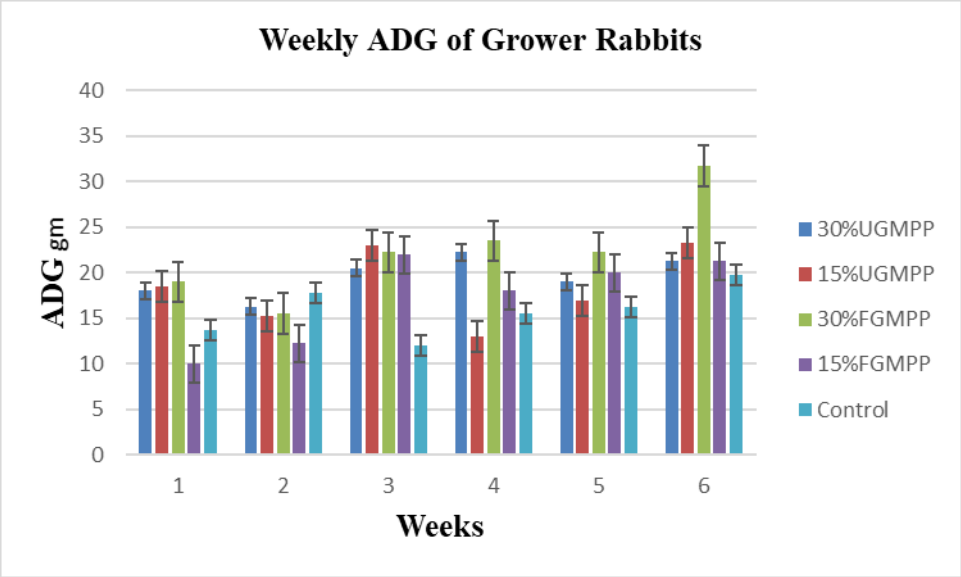

Figure 9. Weekly ADG of 42-day old grower rabbits fed FGMPP and UGMPP based diets.

Haematological components and blood metabolite indices are presented in Tables 17 and 18. Treatments affected blood haematological indices ($p<0.05$) except for eosinophils. All blood metabolite indices were positively ($p<0.05$) affected by the treatment. When the rabbits were fed diets with 30% FGMPP inclusion, red blood cell (RBCs) and platelet count were higher ($p<0.05$) compared to all other treatments, while the same treatment reported results that were similar to the control ($p>0.05$) for lymphocyte %, haemoglobin values, haematocrite/PCV % and monocytes %. Neutrophil % was higher ($p<0.05$) in the diet with 15% FGMPP compared to all the other treatments, while Basophil count for the treatments with 30% and 15% FGMPP inclusion compared with the control.

Table 17. Haematological indices of grower rabbits fed UGMPP and FGMPP- based diets

Indices	Treatments							
	Initial	**Trt 1**	**Trt 2**	**Trt 3**	**Trt 4**	**Trt 5**	**SEM**	**NPR**
WBC 10^9 /L	3.18	6.25^{a}	5.05^{b}	6.48^{a}	5.21^{b}	8.45^{c}	0.07	5.2-12.5*
Lymphocyte %	67.67	62.08^{a}	72.85^{b}	76.98^{c}	64.5^{d}	78.75^{c}	0.43	30-85*
Monocytes %	3.82	3.5^{a}	5.93^{b}	$5.0b^{c}$	4.83^{dc}	4.85^{dc}	0.21	0-4*
H/N %	24.08	13.85^{a}	13.85^{a}	17.43^{a}	22.55^{b}	15.8^{a}	0.83	20-75*
PCV %	31.87	33.65^{a}	40.7^{b}	43.35^{c}	40.6^{b}	44.25^{c}	0.32	33-50*
RBC 10^{12}/L	4.25	4.85^{a}	6.13^{b}	7.18^{c}	6.2^{b}	6.35^{b}	0.1	5.1-7.9*
HGB g/dl	9.44	10.8^{a}	12.23^{b}	13.65^{c}	12.6^{b}	13.98^{c}	0.19	10-17.4*
MCV/ FL	71.72	68.2^{a}	65.45^{b}	62.63^{c}	64.83^{bc}	67.7^{ab}	0.52	60-69*
Platelets 10^9/L	213.15	238^{a}	259.75^{a}	367.5^{b}	255.75^{a}	254^{a}	9.37	250-650*
Eosinophils 10^9 /L	0.06	0.01	0.02	0.01	0.02	0.02	0.007	0-4*
Basophils 10^9 /L	0.47	1.13^{a}	0.47^{b}	0.22^{c}	0.22^{c}	0.33^{bc}	0.05	0-1*

SEM = standard error of means; $^{a, b, c}$ = means in the same row with different superscripts are significantly different (P<0.05); Trt 1 = 30% UGMPP (unfermented ground mature Prosopis pods); Trt 2 = 15% UGMPP; Trt 3 = 30% FGMPP (fermented ground mature Prosopis pods); Trt 4 = 15% FGMPP; Trt 5 = Control; WBC = white blood cells; RBC = red blood cells; HGB = haemoglobin; MCV = mean corpuscular volume/mean cell volume; PCV = packed cell volume/haematocrite. H/N = heterophils/neutrophils; NPR = normal physiological range

*Source: Normal Biochemistry values for *Oryctolagus cuniculus* – European rabbit. http://wildpro.twycrosszoo.org/S/00Ref/Haematology/Haem_13_O_cuniculus.htm

Table 18. Blood metabolite indices of grower rabbits fed FGMPP and UGMPP-based diets

	Treatments							
Indices	**Initial**	**Trt1**	**Trt 2**	**Trt 3**	**Trt 4**	**Trt 5**	**SEM**	**NPR**
Glucose mmol/l	4.87	6.65^{a}	5.68^{b}	7.95^{c}	5.15^{d}	8.29^{c}	0.08	5.5-8.2*
Cortisol nmol/l	127.09	28.75^{a}	37.25^{b}	25.25^{a}	31.68^{ab}	49.75^{c}	1.53	-
Cholesterol mmol/l	3.03	2.6^{a}	3.03^{b}	2.13^{c}	2.33^{c}	2.54^{a}	0.05	0.1-2.0*
Calcium mmol/l	8.85	2.95^{a}	2.05^{b}	3.05^{d}	3.13^{cd}	2.95^{ac}	0.12	2.2-4.0*

SEM = standard error of means; $^{a, b, c, d}$ = means in the same row with different superscripts are significantly different (P<0.05); Trt 1 = 30% UGMPP (unfermented mature Prosopis pods); Trt 2 = 15% UGMPP; Trt 3 = 30% FGMPP (fermented ground mature Prosopis pods); Trt 4 = 15% FGMPP; Trt 5 = control; NPR = normal physiological range;

*Source: Normal Biochemistry values for *Oryctolagus cuniculus* – European rabbit. http://wildpro.twycrosszoo.org/S/00Ref/Biochemistry/Bioch_13_O-cuniculus.htm

5.4 Discussion

In this study, use of ground mature *Prosopis juliflora* pods had no influence on final weight, average daily feed intake (ADFI) and feed conversion ratio (FCR). Similar results were reported by Adamu *et al.* (2011) when they fed *Prosopis africana* pulp to grower rabbits. However, inclusion of 30% FGMPP in the diets of grower rabbits resulted in higher ($p<0.05$) ADG in this study. Chovatiya *et al.* (2018), reported improved weight gain when *Labeo rohita* fingerlings were fed treated *Prosopis juliflora* pods using fermentation and on addition of probiotics. Improvement of performance parameters of animals fed fermented products was due to efficiency of feed utilization from improved nutritional value and availability rather than feed intake and better intestinal integrity and pathogen control in the intestines (Mourão *et al.*, 2006; Xu *et al.*, 2020). In this study, there was an improvement in ADG while ADFI was not significantly different among treatments. This could be an indication of efficiency of feed utilization due to improved nutritional value on fermentation. Feed conversion ratio for the control treatment was within the range reported by Attia *et al.* (2014) for growing rabbits. Trocino *et al.* (2013), reported the positive effects of soluble fibre to the gut stabilization and reduction of gastrointestinal disorders of growing rabbits. Cruz-Alcedo (1999) reported presence of soluble fibre in the galactomannans of Prosopis seeds. According to Chaucheyras-Durand and Durand (2010) and Trocino *et al*. (2013), dietary

fermented products and soluble fibre conferred probiotic effect on the gut of livestock. In this study, probably the 30 % FGMPP inclusion allowed for a more efficient feed utilization due to a better gut environment created by probiotic and soluble fibre effect of the fermented mature *Prosopis juliflora* pods, therefore a higher ADG.

In this study, mortality of the grower rabbits decreased as inclusion of fermented ground mature Prosopis pods increased (Table 17), the lowest mortality reported in the rabbits offered 30% FGMPP inclusion in the diet. Trocino *et al.* (2013), reported that use of soluble fibre that consisted of gums, fructans and pectins in the diet of growing rabbits reduced mortality significantly. Cruz-Alcedo (1999) reported that Prosopis seed galactomannans consisted of soluble fibre that can be used as a dietary soluble fibre source. Gómez-Conde *et al.* (2007) and Margüenda *et al.* (2012) reported high mortality when growing rabbits were fed insoluble fibre regardless of the sources, caused by deterioration of mucosal morphology due to an increase in *C. perfringens* in ileal and caecal contents. In this study, it is possible that the soluble fibre in both fermented and unfermented ground mature Prosopis pods could have improved gut environment by promoting desired caecal fermentation in the diets (Cruz-Alcedo, 1999).

Results in this study indicated that feeding grower rabbits fermented mature ground Prosopis pods improved the physiological status of the rabbits by positively changing blood haematological parameters such as lymphocyte %, white blood cell count, neutrophils %, basophils count and haematocrite %. This is in agreement with Adedirea *et al.* (2013), who demonstrated that, fermenting the cowpea husks with a combined microbial strain of *Trichoderma reesi* + *Rhizopus oligosporus* improved nutritional value as demonstrated by improved feed conversion ratio and superior ($p<0.05$) or comparable to control ($p>0.05$) blood haematological parameters (haematocrite, red blood cell count and haemoglobin) during growth phase. Fermentation of highly fibrous feeds improved the nutritional value and therefore the utilization these feed materials as rabbit feeds were enhanced. Sarasvati *et al.* (2014) on *in vitro* studies and Yusuf *et al.* (2008) on feeding broilers reported improved nutritional qualities of *Prosopis juliflora* on fermentation.

In the present study, 30% UGMPP inclusion had lower blood haematological indices (packed cell volume, haemoglobin and red blood cell count) indicating that this inclusion in the diets interfered with synthesis of some blood haematological components. However, Adamu *et al.* (2011) was of a contrary opinion when investigating the effects of *Prosopis africana* pulp on

nutrient digestibility, carcass components and blood composition of growing rabbits. Cruz-Alcedo (1999) reported little or no variation in proximate and anti-nutrient composition of different species of Prosopis pods. The total tannin composition of *Prosopis pallida* pulp was 4.23 % (Felker *et al.,* 2003). This was lower than 7.4% of total tannins reported in this study. Supplementation of pigs with dietary tannins significantly decreased red blood cell count, haemoglobin content and packed cell volume (Lee *et al.,* 2010). In this study, the challenge reported by feeding 30% UGMPP seem to have been rectified by fermentation as treatment with FGMPP had higher ($p<0.05$) values for the same parameters. This could have been due to decrease in total tannin content reported in this study, from 7.4% to 2.2% on fermentation.

This is an indication that treatment for 30% FGMPP inclusion possibly conferred on the rabbit's higher feed utilization efficiency and therefore a higher physiological function. Blood haematological parameters are important in ensuring the body is well nourished and waste products are removed (Ofsthun, 1993). Red blood cell count, haemoglobin and packed cell volume are important in the functioning of cardiovascular system. Red blood cells and haemoglobin are used in transport of oxygen and carbon dioxide, thus ensuring that the body is free from hypoxia (Black, 1940) and body's acid-base balance or pH (Albers, 1970) is maintained within the narrow limits that allow for optimum physiological functioning of the body. Red blood cells transport carbon dioxide to the lungs for expiration and therefore excretion and oxygen from the lungs to the body tissues for cellular respiration and metabolism. According to Cass and Dalmark (1973) and Swietach *et al.* (2010), this physiological function occurs due to presence of haemoglobin and the anion exchange isoforms (AE1), isoform of the anion-exchange transporter/ Band 3 which are the two key molecular elements in the Red Blood Cells. Rubana and Aulik (1989) reported that accumulation of lactic acid in blood was low in individuals with higher levels of haemoglobin. High level of haemoglobin is important for effective transport of oxygen and prevention of acidaemia loads when individual values of the anaerobic threshold are surpassed (Rubana & Aulik, 1989). Adequate supply of oxygen by haemoglobin therefore prevents a shift by the working muscle to anaerobic respiration (Garbus *et al.,* 1967). Blood packed cell volume is an important indicator of hydration status of an animal and therefore the ability of blood to perform its physiological function of transporting oxygen, carbon-dioxide and protecting the body from invading foreign bodies (Nakyinsige *et al.,* 2013). Medugu *et al.* (2010), reported that stressed broilers portrayed an inferior/low blood packed cell volume when high tannin sorghum was fed. This indicated that nutritional factors

interfered with level of blood constituents. In this study, higher RBC count and higher haemoglobin level was an indication of a normal physiological function and therefore higher ($p<0.05$) ADG (Tables 14 and 15).

Blood haematological components useful in the body's defence (lymphocyte %, monocyte % and neutrophil %) were higher ($p<0.05$) or comparable ($p>0.05$) with the control in rabbits fed 30% and 15% FGMPP inclusion in diet. In studies to investigate the relationship between ACTH and lymphopaenia, Colfer *et al.* (1950), found evidence that stress triggered the production of ACTH which in turn caused reduction in the level of lymphocyte % in an individual's body by shifting neutrophil/heterophil: lymphocyte ratio in favour of an increase in neutrophils (Jenkins, 2008). Higher levels of lymphocyte in the treatments with FGMPP in this study indicated that anti-nutritive compounds present in UGMPP (7.4% tannins; 22% crude fibre) could have been reduced by fermentation (Aremu *et al.*, 2015; Sarasvati *et al.*, 2014) and therefore lowering their concentrations and effects on animal performance. In this study UGMPP based diets produced the lowest lymphocyte and monocyte %. This is in agreement with the findings of Medugu *et al.* (2010) when they fed broilers high tannin sorghum diets. Odero-Waitituh (2015), fed broilers graded levels of GMPP and reported low final weights as GMPP inclusion was increased in the diets. This could have been caused by high tannin content (8%) in the Prosopis pods. Basophils and eosinophils did not show any trend in their counts, and the eosinophils were not significantly ($p>0.05$) different across treatments. This is in agreement with reports by Medugu *et al.* (2010) that there was no effect of treatment of high tannin sorghum for broiler diet on these haematological parameters.

Blood metabolites were significantly influenced ($p<0.05$) by all treatments. Diets with 30% FGMPP inclusion were lower ($p<0.05$) in blood cholesterol and cortisol content compared to all other treatments. Blood glucose level for all treatments was within the range (5.15-8.29) mmol/L; though significant ($p<0.05$) it was within the level postulated by (Harcourt-Brown, F. M., & Harcourt-Brown, S, 2012) of (7.6 ± 2.6) mmol/L for normal rabbits. However, the initial kits' blood glucose level was lower than the normal range (4.87 mmol/L). This could have been due to the inability of young rabbits to regulate their blood glucose levels due to insufficient glycogen reserves (Jenkins, 2008). Idahor *et al.* (2018) reported significant changes on blood glucose of rabbits as affected by dietary inclusion of sheabutter nut meal and blood glucose levels can be used to determine if rabbits are stressed. Elevated blood glucose levels is an indication of stress, liver damage and impaired physiological function (Lepitzki & Woolf, 1991). Reports by Njidda and Isidahomen (2011) indicate that blood

glucose level should fall within the physiologically acceptable range for the rabbit to be considered healthy. However, high levels are desired as they indicate presence of enough energy in form of gluc ose for cellular metabolism. In this study, glucose level was significantly higher ($p<0.05$) in rabbits offered control and 30% FGMPP inclusion diet compared to all the other treatments. This could be an indication of the ability of the diets to provide enough glucose for cellular metabolism.

Feeding grower rabbits FGMPP based diets regardless of the inclusion level lowered ($p<0.05$) cholesterol level. St-Onge (2000) reported that fermentation products contained bacteria which became resident in the gastro-intestinal tract of animals fermenting food-derived indigestible carbohydrates causing increased production of short-chain fatty acids, which decrease circulatory cholesterol concentrations either by inhibiting hepatic cholesterol synthesis or by redistributing cholesterol from plasma to the liver. However, Omidi *et al.* (2013) reported an increase in high density lipoprotein (HDL) cholesterol and decrease in low density lipoprotein (LDL) cholesterol in ostriches offered *Prosopis farcta* beans. Blood calcium level for all the treatments fell within the normal physiological range for rabbits. Though blood calcium content was ($p<0.05$) significant, it showed no consistent trend that could be attributed to treatment effect. High calcium level immediately post weaning could possibly be an indication that all the rabbits were physiologically stressed during weaning. According to Benson and Paul-Murphy (1999), blood calcium levels in rabbits are majorly regulated by dietary sources, presence or absence of vitamin D doesn't affect absorption. Although within the normal physiological range, treatment with 15 % FGMPP inclusion resulted in the highest ($p< 0.05$) blood calcium 3.13 mmol/l. This could indicate better calcium absorption. Initial blood cortisol level was high (127.09 nmol/L). It is possible that weaning caused stress to the rabbits which were manifested by high cortisol level. This decreased with age of the rabbits regardless of the treatment offered (Table 18). Feeding the rabbits with FGMPP significantly ($p<0.05$) lowered the blood cortisol level compared to the other treatments. Using the cortisol level to measure presence of stress, there is evidence in this study that feeding the rabbits 30% FGMPP reduced the stress levels from weaning significantly when compared to all other treatments. Possibly due to FGMPP conferring probiotic effect on the intestines, efficiency of feed utilization and physiological function.

5.5 Conclusions and Recommendations

5.5.1 Conclusion

Inclusion of 30% FGMPP in the diet resulted in higher ADG. However, it had no effect on ADFI. Red blood cell count, PCV, HB were significantly higher, while cholesterol and cortisol were significantly lower in the rabbits fed 30% FGMPP.

5.5.2 Recommendation

It is therefore recommended that 30% of maize grain in grower rabbits' diet can be replaced by FGMPP.

CHAPTER SIX

CARCASS EVALUATION AND ECONOMICS OF FEEDING FERMENTED GROUND MATURE PROSOPIS PODS TO GROWER RABBITS

Abstract

This study was done to investigate the effect of inclusion of graded levels of fermented ground mature Prosopis pods (FGMPP) replacing maize in the diets of growing rabbits on carcass characteristics, sensory characteristics and economic benefit. Sixty (60), 42-day old rabbits weighing 0.5 ± 0.04 (mean ± SE) were housed in cages measuring (75×55×40) cm^3; three rabbits of the same sex per cage. In a randomized complete block design (RCBD) 5 diets; control (formulated standard grower diet), 15% unfermented ground mature *Prosopis juliflora* pods (UGMPP), 30% UGMPP, 15% fermented ground mature Prosopis pods (FGMPP) and 30% FGMPP replacing maize in formulated standard grower diets were offered in four replicates per treatment (six males and six females). For carcass characteristics parameters, analysis of data was done using the general linear model (GLM) of Statistical Analysis Systems (SAS). Tukey's range procedure at ($p<0.05$) significance was used to separate means. Descriptive sensory evaluation was done using SPSS Statistics 25.0.0 software (2017) and cost benefit analysis was done using excel spreadsheets 2010. The results showed that 30% FGMPP inclusion resulted in economic benefit ($p<0.05$). There was treatment effect on caecal pH, dressing %, salty taste and grittiness of meat. The study concluded that replacing 30% maize in diets of grower rabbits with FGMPP did not affect consumer preference of the meat and made economic sense. This will ensure sustainability in rabbit production, improvement of farmer livelihoods, food and nutritional security to the Kenyan human population.

Keywords: Carcass, **c**ost benefit, fermentation, *Prosopis juliflora*, sensory characteristics

6.1 Introduction

The Kenyan human population was estimated at 47.6 million (KNBS, 2019) in the 2019 census up from 37.7 million people in the 2009 census (KNBS, 2009). The estimated annual growth rate was 2.2% (KNBS, 2019). According to Djurfeldt and Wambugu (2011) maize is Africa's largest contemporary staple crop and food with an estimated 87% of Kenyans consuming it as a dietary staple food at an average intake of 400 gm per person per day

(Shephard, 2008). In 2013, Kenya produced 3 million tonnes of maize which was far below the estimated national consumption of 4 million tonnes (Chisholm, 2014). The effect of global warming has worsened the situation with unpredictable weather patterns experienced interfering with crop and maize production (Ochieng *et al.,* 2016). Tongruksawattana and Wainaina (2019) reported drought, pests and excessive rainfall as the outcome of climate change as demonstrated by the study over a period of 10 years (2000-2010). In Kenya, maize is used in formulating commercial feeds for non-ruminant livestock. However, production of maize is majorly rain fed (Lewis *et al.,* 1998), with a reduction in production in the recent years due to climate change (Mati, 2000; Ochieng *et al.,* 2016). This has led to fluctuation in availability and price during periods of scarcity, creating the need for use of livestock feed ingredients that are available throughout the year and with less competition with man.

Prosopis is an invasive multipurpose dry land tree or shrub native to South America, Central America and the Caribbean. It was introduced to Eastern Africa in the 1970s through collaborative projects involving local governments and outside agencies. It is readily available in ASALs areas of Kenya (King'ori *et al.,* 2011). Wanjohi *et al.* (2017a) reported that indigenous chicken offered diets with 20% Prosopis pods inclusion exhibited growth rate similar to the reference diet/ control. However, studies done by Odero-Waitituh (2015) to investigate the possibility of using mature ground Prosopis pods in broilers, reported reduction in feed intake and growth rate as Prosopis pods inclusion levels. It was suggested that, it could have been due to high crude fibre and tannins and recommended that treating the pods could improve the utilization of the pods. Aremu *et al.* (2015) and Sarasvati *et al.* (2014) reported that fermentation significantly reduced the anti-nutritive compounds in Prosopis pods with animals exhibiting positive performance indices (Chovatiya *et al.,* 2018).

Food quality and safety is important as it safeguards consumers against diseases and poor health. Poor animal nutrition management not only affects livestock health, welfare and productivity but also animal products quality and safety. It is therefore important to observe and practice all animal management aspects and practices that ensure that product quality and safety is not compromised (FAO, 2014). Evaluation of sensory characteristics is therefore done to ensure the animal product produced is acceptable to consumers (Akinboye *et al.,* 2018). According to Odero-Waitituh (2015), mature pods of *Prosopis juliflora* pods was suitable for broiler feeding but feed intake and growth rate were impaired by high crude fibre (17%) content. Wanjohi *et al.* (2017b) reported comparable carcass sensory attributes with the control when indigenous chicken were fed diets with 20% mature Prosopis pod inclusion.

Mature Prosopis pods have successfully been tested and used as human food (Choge *et al.,* 2007), chicken feed ingredient; (Wanjohi *et al.,* 2017a), without any harmful immunological effects (Khobondo *et al.,* 2019). Toxicological tests done on rats by Kimani *et al.* (2014) and Wamburu *et al.* (2015) reported LD_{50} that exceeded 5000 mg/kg which was safe for use by humans and animals with a degree of safety and tolerance.

Cost-benefit analysis is done to ensure that producing the animal product will be cheaper per unit gain with the test ingredient being used to replace the conventional feed. Several researchers have reported increased feed costs per unit muscle accreditation when livestock feed ingredients were treated. Maidala *et al.* (2011) reported increased feed costs when weaner rabbits were fed diets containing soybean (*Glycine max* (L) Merrill) product processed using different methods. Similarly, Akintunde *et al.* (2015) reported increased feeding costs when Japanese Quails were fed Processed Pigeon Pea (*Cajanus cajan*) Seeds. However, Yusuf *et al.* (2008) reported reduced feed costs when broiler chickens were fed decorticated fermented *Prosopis africana* seed meal. Maidala (2015) reported reduction in feeding costs when broiler chickens were fed diets with processed african locust bean. Bio-economic implications, product safety and quality are important parameters to be considered to ensure product quality when fermented Prosopis pods are used in livestock diets. This study evaluated rabbit carcass, meat sensory characteristics, caecal contents pH changes, and economic implications of feeding fermented ground mature Prosopis pods to grower rabbits.

6.2 Materials and Methods

6.2.1 Study site

Prosopis juliflora pods collection, drying, milling, storage and fermentation protocol is as described is as described in 3.2.1. Study site location is as described in 3.2.2.

6.2.2 Experimental animals and management

This was carried out as outlined in 5.2.1

6.2.3 Dietary treatments

This was carried out as outlined in 5.2.2

6.2.4 Experimental design

This was carried out as outlined in 5.2.3

6.2.5 Data collection for evaluation of carcass quality

On the 42nd day, one rabbit from each experimental unit was selected randomly, identified, and fasted overnight with *ad-libitum* provision of drinking water before slaughter for carcass characteristics and caecum contents pH determination respectively. Slaughtering was done according to the welfare law (Lafuente & López, 2014). The rabbits were slaughtered following the cervical dislocation method, then skinned and eviscerated (Martin *et al.*, 2016). Thigh meat was used for sensory evaluation.

The following weights were recorded; live, dressed carcass, heart, front limbs, hind limbs, anterior portion with all ribs minus the front limbs, posterior portion minus the limbs, front and hind claws and head. Caecal contents were collected for pH determination according to the procedure by Guedes *et al.* (2009). Carcass characteristics and organ weights (head, heart, liver, lung, and kidney) were weighed and expressed as a percentage of the live weight before slaughter.

Sensory characteristic determination

Evaluation of descriptive sensory characteristics was conducted at Guildford Dairy Institutes sensory room, Dairy and Food Science and Technology Department, Egerton University. Description of Egerton University's location is as described in 3.2.2. *Sample collection*

Meat samples of the rabbits slaughtered at 12 weeks of age from each diet were obtained from the thigh parts. Good manufacturing practices were observed at all times. The meat not required for immediate analysis was frozen (-18^{o}C) or stored as appropriate.

Selection of panellists

Selection of panellists was done by administering of pre-screening questionnaires to 20 candidates. The pre-screening questionnaires included questions about availability, food habits, flavour, texture and aroma of different products and questions about allergenicity to meat-based products. This was done to select candidates who were verbal with respect to sensory properties and able to participate in the rabbit meat sensory evaluation. During the orientation sessions, the panel agreed on the attributes to use for evaluation, evaluated several meat samples from the rabbits offered (control/reference, 30% FGMPP and 30% UGMPP diets), and rated their intensities (agreed upon by consensus by the panellists). From the pre-screening questionnaires, 12 of the candidates were selected according to procedure by Meilgaard *et al.* (2000) as verbal with respect to sensory properties. They were then trained on both qualitative and quantitative meat discrimination. During training a sensory lexicon was developed with 15 descriptors (Appendix G)

Sample preparation

Frozen meats, in different containers, were thawed using running tap water for 6h. This was followed by sample preparation by boiling the meats for 40 minutes in different aluminium pots labelled with random three-digit numbers. The boiled meats were then cut into small pieces of about $2cm^3$ using a kitchen knife. Ceramic plates divided into five (according to the assigned codes) were used to present the cut meat samples to the panellists. Stainless-steel fork and knife were also availed to each one of the panellists. Water was provided for cleansing and rinsing the palate between samples. They evaluated the meat samples for appearance, aroma and flavour using the sensory descriptors developed during training.

6.2.6 Economics of using FGMPP and UGMPP

Costs were estimated by identifying the input items that varied across the treatments, quantifying their level input in each treatment and estimating the unit price of each input. The inputs considered were FGMPP, UGMPP, Rice husks, Wheat Bran, Maize germ, maize, sunflower cake, bone meal, iodized salt and vitamin premix. The output item was the grower meat got as dressed carcass weight and its price estimated.

The following expression; Equation 12 was used in estimating the benefits and costs:

$$BC = \frac{(KP \times P_1)}{\sum (\%\, I_{(1, 2, 3 \ldots r)} \times P_{0\,(1, 2, 3 \ldots r)})\, F}.$$

Equation 12

Source: Author

Where: -

BC is benefit-cost.

KP is no. of kilograms of final product.

P_1 is price of final product.

I is ingredient.

P_0 is price of corresponding ingredient.

% is percentage ingredient in 1 diet

F is no. of kilograms feed fed

6.2.7 Statistical analysis

Data from carcass characteristics and caecum contents pH were subjected to normality and homogeneity of variance test using SPSS Statistics 25.0.0 software (2017). They were then subjected to analysis of variance using general linear model (GLM) of Statistical Analysis Systems (SAS, 9.1.3) computer package (2005). The differences among treatment means were determined using the Tukey's range test. Probability values of ($p<0.05$) were considered significant.

Using SPSS Statistics 25.0.0 software (2017), data from sensory evaluation were subjected to normality and homogeneity of variance test. Outliers were identified and removed from the data. Principal component analysis (PCA) was then done to identify the significant factors that caused the greatest variabilitie in the sensory attributes. Significant factors and non-significant factors were then subjected to analysis of variance using general linear model (GLM) of Statistical Analysis Systems (SAS, 9.1.3) computer package (2005). The

differences among treatment means were determined using the Tukey's range test. Probability values of ($p< 0.05$) were considered significant.

Data from price of feed ingredients and price of meat were fitted into the cost benefit analysis equation; equation 12. This was done in Excel program in Microsoft Office 2010 and the cost benefit of feeding the various diets computed. They were then subjected to analysis of variance using general linear model (GLM) of Statistical Analysis Systems (SAS, 9.1.3) computer package (2005). The differences among treatment means were determined using the Tukey's range test. Probability values of ($p< 0.05$) were considered significant. The treatment with highest cost benefit value was considered cost effective and therefore the best one. Statistical model used is as outlined in 5.2.5.

6.3 Results

Carcass characteristics, caecal contents pH, organ weights are presented in Table 19.

Table 19. Carcass characteristics, pH of caecal contents and organ weights of grower rabbits fed UGMPP and FGMPP- based diets

	Treatments					
Parameters	**30% UGMPP**	**15% UGMPP**	**30% FGMPP**	**15% FGMPP**	**Control**	**SEM**
Rabbits slaughtered	4	4	4	4	4	
Live wt (g)	1340.25[a]	1253.00[a]	1359.25[a]	1230.25[a]	1211.75[a]	72.55
Dressing %	48.23[a]	46.47[b]	48.95[a]	46.98[b]	45.36[b]	0.68
Caecal pH	6.56[a]	6.52[a]	6.55[a]	6.60[a]	6.17[b]	0.05
Body components						
Head	9.95[a]	9,91[a]	9.71[a]	11.02 [a]	11.12[a]	0.47
Claws	2.94[a]	2.89[a]	2.67[a]	2.9[a]	2.79[a]	0.14
Anterior part	12.50[a]	11.82[a]	12.56[a]	13.27[a]	10.20[a]	0.77
Posterior part	15.12[a]	13.81[a]	15.20[a]	14.52[a]	12.75[a]	0.78
Heart	0.30[a]	0.33[a]	0.28[a]	0.32[a]	0.25[a]	0.03
Liver	2.80[a]	3.17[a]	3.44[a]	3.54[a]	2.93[a]	0.27
Lungs	0.65[a]	0.50[a]	0.57[a]	0.59[a]	0.43[a]	0.06
Kidneys	0.74[a]	0.76[a]	0.68[a]	0.66 [a]	0.69[a]	0.05
Front limbs	7.3[a]	7.21[a]	7.36[a]	8.14[a]	6,88[a]	0.32
Hind limbs	12.96[a]	12.02[a]	13.70[a]	13.27[a]	11.60[a]	0.49

SEM = standard error of means; [a, b, c, d] = means in the same row with different superscripts are significantly different ($P<0.05$); Trt 1 = 30% UGMPP (unfermented mature Prosopis pods); Trt 2 = 15% UGMPP; Trt 3 = 30% FGMPP (fermented ground mature Prosopis pods); UGMPP; Trt 4 = 15% FGMPP; Trt 5 = control; DCW = Dressed carcass weight; Body components % live weight.

There was treatment effect ($p<0.05$) on caecal pH contents and dressing percentage. Sensory and meat quality are presented in Tables 20 and 21.

Table 20. Effect of feeding grower rabbits FGMPP and UGMPP-based diets on sensory attributes of meat

Attribute	30% UGMPP	15% UGMPP	30% FGMPP	15% FGMPP	Control	SEM	p-value
Overall rating							
Overall	4.42	5.5	5.5	4.91	5.08	0.35	0.18
Appearance	5.42	5.25	5.67	4.42	5.33	0.41	0.27
Flavour	5.67	5.58	5.25	4.02	4.83	0.39	0.45
Texture	4.67	5.67	5.58	5.5	4.75	0.37	0.17
Appearance							
Colour	4.33	3.83	4.0	4.58	4.5	0.44	0.71
Oiliness	4.83	3.0	4.08	4.58	4.25	0.47	0.08
Denseness	4.17	3.92	4.25	4.33	4.67	0.51	0.88
Flavour							
Salty taste	2.67^{a}	2.42^{a}	2.58^{a}	4.83^{b}	3.92^{ab}	0.45	0.0008
Oily	4.0	3.08	2.5	3.3	3.58	0.47	0.23
Chicken	4.5	4.17	4.58	4.33	4.17	0.43	0.94
Beefy	3.33^{a}	1.83^{ac}	2.58^{a}	3.9^{ab}	1.75^{ac}	0.44	0.003
Texture							
Particles	3.08	3.17	4.5	3.75	3.91	0.46	0.19
Grittiness	3.67^{a}	1.83^{b}	4.67^{ad}	2.58^{ab}	4.17^{a}	0.45	0.0002
Tenderness	5.58^{a}	4.58^{ab}	3.67^{b}	4.33^{ab}	5.41^{a}	0.47	0.04
Juiciness	4.25	3.5	3.5	4.17	5.0	0.5	0.19
Rubbery	3.17	3.75	4.58	3.67	4.0	0.51	0.4
Aroma							
Chicken	4.58	5.5	4.83	4.58	4.42	0.42	0.4
Residual							
Teeth adhesion	3.42	3.5	4.0	3.67	3.58	0.47	0.91

Metallic aftertaste	3.0	2.83	4.5	3.25	3.0	0.44	0.07

SEM = standard error of means; [a, b, c, d] = means in the same row with different superscripts are significantly different (P<0.05); Trt 1 = 30% UGMPP (unfermented mature Prosopis pods); Trt 2 = 15% UGMPP; Trt 3 = 30% FGMPP (fermented ground mature Prosopis pods); UGMPP; Trt 4 = 15% FGMPP; Trt 5 = control.

Table 21. Principal components with factor loadings of descriptive sensory properties of meat from grower rabbits fed FGMPP-based diets

	Principal Components					
Attribute	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Colour	-.008	.360	-.277	**.599**	.315	-.221
Denseness	.297	-.268	-.423	.255	.291	.320
Oiliness	.024	**.649**	.051	.212	.538	-.277
Chicken aroma	-.673	-.343	.298	.120	-.123	-.325
Salty taste	-.029	.365	.144	.469	**-.610**	-.191
Chicken	-.637	-.225	.431	.264	.067	-.015
Beefy	.446	.382	-.025	.313	-.349	.188
Tenderness	-.379	.479	-.112	-.249	.195	.347
Juiciness	-.117	.535	.312	-.019	-.199	.315
Rubbery	.299	-.458	-.047	.574	-.071	.359
Grittiness	.537	.035	**.597**	.057	.282	-.170
oily	-.520	.333	.326	.071	.056	**.432**
Particles	**.793**	.007	.381	-.015	-.030	-.044
Teeth adhesion	.515	.010	.487	-.292	.054	.089
Metallic	-.172	-.338	.483	.310	.308	.239
Eigenvalues	2.875	2.022	1.745	1.451	1.251	1.037
Variance explained (%)	19.168	13.477	11.633	9.675	8.34	6.913

Extraction Method: Principal Component Analysis

Rotation Method: Varimax with Kaiser Normalization

KMO 0.503

Bartlett's Test: Chi square value 196.229; $p<0.0001$

There was no treatment effect ($p>0.05$) on overall rating of the meat.

Economics of feeding FGMPP to grower rabbits is presented in Table 22. There was economic benefit ($p<0.05$) of feeding FGMPP to grower rabbits.

Table 22. Economics of feeding grower rabbits FGMPP and UGMPP-based diets

	Treatments						
Parameter	**30% UGMPP**	**15% UGMPP**	**30% FGMPP**	**15% FGMPP**	**Control**	**SEM**	**p-value**
Initial wt (kg)	0.45	0.48	0.49	0.5	0.53	0.04	0.13
Final wt (kg)	1.34	1.25	1.36	1.23	1.29	0.07	0.14
Wt gain (kg)	0.89^{a}	0.77^{b}	0.87^{a}	0.73^{c}	0.76^{b}	0.008	<0.0001
Total FI (kg)	3.41^{a}	3.23^{b}	3.02^{c}	3.15^{d}	2.95^{e}	0.014	<0.0001
DC wt (kg)	0.65^{ab}	0.58^{b}	0.67^{a}	0.60^{ab}	0.55^{bc}	0.013	0.0004
BC value	3.56^{a}	2.78^{b}	3.76^{c}	2.92^{d}	3.03^{e}	0.01	<0.0001

SEM = standard error of means; $^{a, b, c}$ = means in the same raw with different superscripts are significantly different ($P<0.05$); Trt 1 = 30% UGMPP (unfermented mature Prosopis pods); Trt 2 = 15% UGMPP; Trt 3 = 30% FGMPP (fermented ground mature Prosopis pods); Trt 4 = 15% FGMPP; Trt 5 = control; BC = cost benefit; FI = feed intake; DC = Dressed carcass; Wt = weight; The feed with the least cost per unit weight gain and highest BC is desired; * Calculations were done based on the prevailing price of ingredients at the time of study.

6.4 Discussion

All the carcass parameters measured were not significantly ($p>0.05$) different except for dressing %, and caecal content pH. The dressing % was within the range of 43.36 and 49.95. Similar results were reported by Adamu *et al.* (2011) and Igwebuike, *et al.* (1995) who fed grower rabbits on *Prosopis africana* pulp and sorghum waste respectively. However, García *et al.* (1993) recorded a higher dressing % when rabbits weighing above 2 kg live weights were slaughtered.

The dressing % was significantly higher ($p<0.05$) in all the treatments with Prosopis pod inclusion as compared to the control. García *et al.* (1993) and Templeton (1968) reported that dressing % was positively correlated with the slaughter weight. It is probable that the significant difference ($p<0.05$) in dressing % reported in this study is due to lower live weights recorded in the rabbits slaughtered (Table 19). Organ weights were similar ($p>0.05$) across the treatments. Similar results were reported by Adamu *et al.* (2011) for grower rabbits fed *Prosopis africana* pulp. This could be an indication that the inclusion levels of UGMPP and FGMPP was within safe and tolerable limits.

Caecal pH was significantly lower ($p<0.05$) in the control (6.17±0.05) as compared to all the other treatments (Table 19). The pH recorded in the control treatment in this study are in accordance with the caecal content's pH of 6.1 ± 0.09 at 12 weeks of age, when rabbits were fed control diet (Abdel-Khalek *et al.,* 2011) and in the range of 6.01 and 6.17 when growing rabbits were fed corn and barley-based diets (Belenguer *et al.,* 2000). When low fibre diets were fed to growing rabbits, it was reported that the caecal contents pH were significantly lower ($p<0.05$) when compared with rabbits fed high fibre diets. This was due to fast caecal fermentation and production of VFAs due to readily fermentable carbohydrates in low fibre diets (Gidenne, 1995). For this study, the control diet was corn based. This suggests that there was a higher rate of caecal fermentation in the control diet than the other treatments with mature ground Prosopis pod inclusion. Contrary opinion has been reported by several researchers with suggestions that lower caecal pH, is an indication of higher caecal fermentation when growing rabbits were fed fibrous feeds and therefore higher production of short chain volatile fatty acids (SCFAs). Alvarez *et al.* (2007) reported caecal content pH within the range of 5.66 to 5.71 when investigating the effects of type and level of fibre on digestive physiology and performance in reproducing and growing rabbits.

The caecal content pH of the diets with ground mature Prosopis pods were significantly higher ($p<0.05$) than the control treatment, which was corn based (Table 19). This is in accordance with (pH range of 6.3 to 6.5) of Montiel *et al*. (2013) who investigated the possibility of using inulin as a growth promoter in diets for rabbits. Mišta *et al.* (2018) reported caecal content pH of 6.54 in a comparative *in vitro* study of caecal microbial activity in brown hares and domestic rabbits which were offered the same diet.

According to Gidenne *et al.* (1998), volatile fatty acids are the main products of microbial hindgut fibre fermentation with high SCFAs production recorded when feeding alfalfa, a soluble fibre source. Soluble fibre sources are favourable in promoting gut health, improved growth and reduced mortality (Alvarez *et al.,* 2007; Trocino *et al.,* 2013). Vernay (1987) reported that colonic fermentation produced 64.4 umol/g butyric acid (SCFA) as compared to 73.0 umol/g in the caecum. This provided additional energy to colonocytes in the colon of non-ruminant livestock (Xiao *et al.,* 2015). Caecal and microbial fermentation of indigestible sugars in the hindgut decreased luminal pH and extended gut wall tissue. This facilitated absorption of key dietary minerals across hindgut and enhanced excessive blood nitrogen (N) flow into the cecum for use as N source for bacterial growth, enhanced N retention in caecotrophic animals (De Blas *et al.,* 1999; Xiao *et al.,* 2015). In this study, there could have been a faster rate of passage of the treatments with ground mature Prosopis pods and a higher colonic fermentation rate and therefore higher production of SCFAs which were absorbed in the colon walls providing energy. Also, the fermentation in the caecum and colon could have improved nitrogen retention by increasing the volume of recycled microbial proteins in the ceacotrophs and therefore higher ADG values in the treatment with 30% FGMPP inclusion.

Sensory evaluation results for the attributes that were significant according to the PCA were similar ($p>0.05$) except for salty taste and grittiness ($p<0.05$). The treatment with 30% FGMPP had lower (desirable to the consumers) ($p<0.05$) salt taste while grittiness for the same treatment was similar ($p>0.05$) to the control. According to Tasić *et al.* (2017) grittiness is an indicator of poor-quality meat. The similarity in the control and 30% FGMPP inclusion treatment in grittiness is an indication of similar meat quality. Daily salt intake in humans is high due to the diets consisting of processed foods with high salt content. This predisposes people to cardiovascular diseases such as high blood pressure. Low salt diets are therefore recommended. In this study, low salt content of the meat from rabbits fed on 30% FGMPP based diets would be ideal for preventing cardiovascular diseases in humans (Jiménez-Colmenero *et al.,* 2001).

The overall rating of the descriptive sensory attributes evaluated such as, appearance, texture and flavour were similar across all treatments (Table 20). Ausol and Mukhtar (2011) reported that sensory attributes from meat of broilers fed treated or soaked Prosopis seeds were similar with the reference diet. Similar results were reported by Ashayerizadeh *et al.* (2018) when broiler

chicken were fed fermented rapeseed meal; Wanjohi *et al.* (2017b) when Kenyan indigenous chicken were fed Prosopis pods; Al-Marzooqi *et al.* (2015) when chicken were fed enzymatic treated and untreated Prosopis pods and Park *et al.* (2005) when pigs were fed fermented food waste. According to Guerrero *et al.* (2018) meat quality is affected by several factors as diet, production system, age with meat from goats reared with milk replacers as opposed to dams' milk exhibiting differences in sensory attributes (Argüello *et al.,* 2005). For this study, age and production system were the same across all treatments. The only effect on descriptive sensory attributes was therefore the diets. Guerrero *et al.* (2013) reported that some of the dietary factors that determine differences in quantitative and qualitative properties of meat are physical properties, chemical properties, addition of additives and composition of the diets. According to Farmer (1994), many of the nutrients in meat are also involved in flavour formation therefore there is a linear relationship between nutritional aspect of meat and its flavour. In this study, similar sensory attributes exhibited by meat in all the treatments is an indication that all the diets provided adequate nutrients to allow for normal metabolism in muscular tissues.

The cost benefit (BC) values increased as inclusion of UGMPP and FGMPP increased (Table 22). The highest BC was recorded in meat from rabbits offered treatments with 30% FGMPP. This was in accordance with reports by Yusuf *et al.* (2008) who reported that there was economic benefit of feeding fermented decorticated *Prosopis africana* seed meal as compared to all other diets. This is contrary to reports by Akintunde *et al.* (2015) and Maidala *et al.* (2011) that cooking, roasting and salting of feed ingredient offered to rabbits increased the cost of feeding. Fermenting ground mature Prosopis pods increased the cost benefit values and therefore lower costs per unit gain in this study. Weight gain and dressed carcass weights for the rabbits offered 30% UGMPP and 30% FGMPP were similar (Table 22). However, the rabbits offered 30% UGMPP exhibited the highest total feed intake as compared to all the other treatments. It may seem that the benefit of cost of fermentation in this study could have been from efficiency of feed utilization, improved gut health, increased feed intake resulting in increased deposition of muscles and therefore weight gain (Chovatiya *et al.,* 2018; Montiel *et al*., 2013). This is in accordance with studies conducted by Maidala (2015) on effects of different treatment methods of African locust bean on broiler meat cost benefit analysis. Fermentation in this study was

spontaneous, which could have resulted in lower costs as compared to when microbial fermentation was used. Also, muscle tissue deposition depends on efficiency of feed utilization.

6.5 Conclusions and recommendations

6.5.1 Conclusions

Based on the results of this study, it is concluded as follows

i Inclusion of 30% FGMPP did not affect carcass and sensory characteristics. However, it had effect on pH of caecum contents

ii Inclusion of 30% FGMPP had the highest cost benefit value.

6.5.2 Recommendations

i Fermented ground mature Prosopis pods can substitute 30 % maize in grower rabbit diets.

ii It is important to always check for the price of maize as compared to the price of fermented ground mature Prosopis pods as these prices are subject to seasonal fluctuations. This will inform the suitability of 30% FGMPP inclusion in diets of grower rabbits.

CHAPTER SEVEN

EFFECTS OF FEEDING FERMENTED GROUND MATURE PROSOPIS PODS ON LACTATION PERFORMANCE IN RABBITS

Abstract

The aim of this study was to investigate the effect of including fermented ground mature Prosopis pods (FGMPP) in lactating does diets on lactation performance and does weights. The study investigated kits weight gain and doe weight changes during the four-week lactation period. Fifteen primiparous does weighing 3.05 ± 0.47 (mean ± SE) with a litter of six kits each, weighing 0.61 ± 0.045 (mean ± SE) were individually housed in cages measuring (75×55×40) cm^3. In a completely randomized design (CRD), 5 diets; control (formulated standard breeder diet), 15% unfermented ground mature Prosopis pods (UGMPP), 30% UGMPP, 15% fermented ground mature Prosopis pods (FGMPP) and 30% FGMPP replacing maize in formulated lactating breeder diets were offered in three replicates per treatment. There was no treatment effect on does weight changes and kits weights. The study concluded that 30% maize in lactating doe diets can be replaced by FGMPP.

Keywords: Energy balance, fermentation, kits, growth, maize, *Prosopis juliflora.*

7.1 Introduction

Energy deficit caused by milk production demands in lactating does may lower receptivity, conception and ovulation rates, embryonic and foetal survival as well as foetal growth in primiparous rabbit does (Olotunmogun *et al.,* 2017). This results in poor reproductive and lactation performance (Fortun-Lamothe & Prunier, 1999). According to Fortun-Lamothe (1998), this can be prevented by increased energy intake with a positive effect on conception rate, reproductive and lactation performance. Breeding does; both lactating and pregnant have high nutrient requirements due to extra physiological demands that production and reproduction put on their bodies (Latu *et al.,* 2017). Also, physiological state (pregnancy verses non-pregnancy) concurrent with lactation significantly influences the weight and performance of does (Xiccato *et al.,* 2004; Xiccato *et al*., 1995). Energy in the lactating does' diet must be adequate to support lactation in terms of quantity and quality, with a desired balance of protein and energy

(Olotunmogun *et al*., 2017) and therefore support fast kits growth. Energy balance is calculated from the difference between the energy supply from the feed and the various energy requirements of the animal (Fortun-Lamothe, 2006). There is high energy output associated with milk production in lactating does which is not entirely compensated for by feed intake. Therefore, does meet this energy deficit by increasing the mobilization of body reserves leading to loss of body energy and therefore, loss of condition and weight (Xiccato *et al*., 1999). This is observed in the first 3 weeks of lactation when litter weight gain is highly correlated with milk production (r = 0.91) (Fernández-Carmonan *et al.,* 2005).

Lactating does offered cereal starch-enriched diets, exhibited a higher feed intake to balance for energy. This energy supplement is mainly utilized to increase their milk energy production (higher milk yield with higher energy content) for the kits have high energy requirements (Maertens *et al.,* 2006). Intake of energy that supports milk production without compromising body reserves is possible by use of rearing technologies that stimulate feed intake (Rommers *et al.,* 1999) and provision of diets with high energy content that results in better litter performance with lower body reserve depletion (Pascual *et al*., 2003; Xiccato *et al.,* 1995). High milk yield of these does is usually related to a greater negative energy balance and lower fertility values affecting subsequent litter size (Pascual *et al*., 2003). Pascual *et al.* (2000) studied the effect of high-energy diets on improvement of milk production and body condition of does and reported that a reduction in depletion of body reserves can be realized when dietary energy sources are from starch rather than fat sources. This resulted in an increase of lipid body reserves and therefore improvement of subsequent reproductive and productive efficiency of breeding does (Fortun-Lamothe & Lebas, 1996).

Al-Marzooqi *et al*. (2015) and Odero-Waitituh *et al*. (2016) reported the metabolizable energy content of mature Prosopis pods to be 18.7 MJ/kg DM sample and 12.8MJ/kg DM sample respectively. This was comparable to maize but it was noted that when fed to livestock, anti-nutritive compounds such as tannins- 8%, and crude fibre- 17% interfered with livestock performance (Odero-Waitituh *et al*., 2016). It was reported that treatment of the pods could result in reduction of these anti-nutritive compounds and their effects on livestock performance. Aremu *et al*. (2015) reported that fermentation significantly reduced the anti-nutritive compounds in Prosopis pods. This therefore creates an opportunity of using fermented mature *Prosopis*

juliflora pods as an energy source in livestock diets. This study therefore investigated the effect of replacing maize with fermented ground mature *Prosopis juliflora* pods in breeder doe diets on weight changes of lactating does and weight gain of their kits.

7.2 Materials and Methods

Study site

Mature *Prosopis juliflora* pods collection, drying, milling, storage and fermentation is as described in 3.2.1. Study site's location is as described in 3.2.2.

7.2.1 Experimental animals and management

The study was conducted at Tatton Agriculture Park, Egerton University. Twenty New Zealand primiparous does, full or half siblings were individually housed in individual cages (75×55×40) cm^3 and reared on standard breeder diet (Deblas & Mateo, 2010) for one week. During this period, the rabbits were dewormed with ascarex® and dusted with Sevin® for control of internal and external parasites respectively. Before moving the rabbits into the experimental cages, watering and feeding troughs were thoroughly cleaned, disinfected with kupacide® and dusted with Sevin® against external parasites. After one week, the rabbits were mated around the same time, 2±1 day.

The study was conducted using 15 lactating does with similar body weights (3.05 ± 0.47) kg, with a litter size of (0.61 ± 0.05) kg each. They were selected from the 20 lactating primiparous does with a kindling interval of 2±1 days. Their litter sizes were adjusted to six kits on the day of kindling by cross-fostering. Using PQRS software, 15 does of similar body weight plus their six kits were randomly assigned to experimental cages individually and reared on formulated standard breeder diet (Deblas & Mateo, 2010) for 2 days. On the third day, using PQRS software, the dietary treatments were randomly assigned to the cages such that there were 3 lactating does per treatment. There was a 2-day adaptation period. Management of Tatton Agriculture Park rabbit unit was as outlined in section 4.2.2.

7.2.2 Dietary treatments

Proximate analysis was done on the feed ingredients used before formulation of experimental diets. The dietary treatments offered to the rabbits were control/standard diet, 15% FGMPP, 15% UGMPP, 30% FGMPP and 30% UGMPP based diets, formulated to a nutrient content of 10.8 MJ/kg sample feed ME and 18% CP (Deblas & Mateos, 2010) (Ta

ble 23).

Table 23. Composition of dietary treatments

	Treatments				
Ingredient	**30%UGMPP**	**15%UGMPP**	**30%FGMPP**	**15%FGMPP**	**Control**
Maize	5	20	5	20	35
Wheat bran	10	9.5	10	10	10.0
Maize germ	25	24	24	25	22.0
Rice germ	10	10.5	10.2	9.3	11.5
UGMPP	30	15	-	-	-
FGMPP	-	-	30	15	-
SFC	18.0	18.0	17.8	17.7	18.5
Bone meal	2.0	2.0	2.0	2.0	2.0
Iodized salt	0.5	0.5	0.5	0.5	0.5
Vit premix*	0.5	0.5	0.5	0.5	0.5
Totals	100	100	100	100	100
Calculated analysis					
CP (%)	18	18.2	18.2	18.4	18.4
ME (MJ/)	10.9	10.8	10.8	10.7	10.7
CF (%)	15	15.3	15.3	15.5	15.5

UGMPP = unfermented ground mature pods of Prosopis juliflora; FGMPP = fermented ground mature pods of Prosopis juliflora; SFC = Sunflower cake; CP = Crude protein, ME = Metabolizable Energy MJ/kg sample; CF = Crude Fibre; * To provide per diet: vitamin

A(10,000 i. u), vitamin D (20,000 i. u), vitamin E (5 i.u.), vitamin K (2.5 mg), choline (350 mg), folic acid (1 mg), manganese (56 mg), iodine (1 mg), iron (20 mg), copper (10 mg), zinc (50 mg), cobalt (1.25 mg).

7.2.3 Experimental design

Fifteen New Zealand White lactating does with similar body weights were used. Each cage had one doe plus 6 kits representing an experimental unit. They were randomly allocated to 15 experimental units. The five dietary treatments were then randomly allocated to the experimental units such that there was one lactating doe plus 6 kits in each experimental unit. Each dietary treatment was replicated three times (3 does plus 18 kits for each treatment) in a completely randomized design (CRD) (Table 24).

Table 24. Experimental layout

R1T5	R1T2	R1T1	R1T4	R3T2
R2T1	R3T3	R2T3	R2T4	R2T5
R3T1	R1T3	R2T2	R3T4	R3T5

Key: T1-treatment 1, T2-treatment 2, T3-treatment 3, T4-treatment 4, T5- treatment 5, R-replication

7.2.4 Data collection

Feed intake (FI) was calculated as the difference of feed offered and feed left over by the end of each day for each experimental unit. The average intake from each cage represented one experimental unit. Average live weight gain or loss for each experimental unit was represented by the average cage weight gain or loss. Weights for does were taken independently from the kits. Cage doe weight and average kit weight represented one experimental unit. Weight measurements were taken weekly throughout the experimental period and final weight taken at the end of 4 weeks feeding period.

7.2.5 Statistical analysis

Data from feed intake and weekly weights were subjected to normality test using SPSS Statistics 25.0.0 software (2017). They were then subjected to analysis of variance using general linear

model (GLM) of Statistical Analysis Systems (SAS, 9.1.3) computer package (2005). Where significant differences were observed in the initial weights, they were used as covariates. The differences among treatment means were determined using the Tukey's range test. Probability values of ($p< 0.05$) were considered significant. The following statistical model was used;

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

Where:

Y_{ij} = observation of i^{th} dietary treatment.

μ= overall mean,

α_i= effect of i^{th} dietary treatment, (i=1…..5)

$\varepsilon_{ij=}$ random error associated with Y_{ij}

7.3 Results

Performance characteristics of does together with their 6 kits per cage representing an experimental unit are presented in Table 25. Does' final weights and Kits growth were similar ($p >0.05$) across all treatments.

Table 25. Performance characteristics of kits and lactating does fed UGMPP and FGMPP - based diets

	Treatments						
Parameter	**Trt 1**	**Trt 2**	**Trt 3**	**Trt 4**	**Trt 5**	**SEM**	**P value**
Kits wts							
Initial wt	0.62	0.62	0.59	0.6	0.62	0.03	0.06
Final wt	2.02	1.79	2.23	2.1	1.85	0.17	0.39
Wt gain	1.4	1.3	1.7	1.46	1.23	0.16	0.34
Does wt							
Initial wt	3.09	2.88	2.83	3.14	3.28	0.24	0.001
Final wt	2.98	2.83	2.98	2.88	2.9	0.05	0.17
Cage FI	7.1	6.43	7.19	7.0	7.0	0.47	0.81

SEM = standard error of means; Trt 1 = 30% UGMPP; Trt 2 = 15% UGMPP; Trt 3 = 30% FGMPP; Trt 4 = 15% FGMPP; Trt 5 = Control; FGMPP = Fermented ground mature Prosopis pods; UGMPP = Unfermented mature Prosopis pods.

All the treatments reported a negative weight gain during the four-week lactation period indicating negative energy balances of the does during lactation except for 30%FGMPP inclusion where higher final weights (2.98±0.05) kg were recorded as compared with the weight recorded at the initial weight (2.83±0.24) kg, indicating a positive energy balance.

7.4 Discussion

The similarity ($p>0.05$) in kits' weight gains is an indicator of similar milk production and quality across all treatments. Litter sizes were equalized to six kits per doe in each cage so that only treatment effect in the diets offered was observed. Ajayi *et al.* (2018) reported that litter size affected kits' growth with smaller litter sizes growing faster than larger litter sizes due to availability of more milk for the kits. Maertens *et al.* (2006), in a review on quantity, quality and non-dietary factors affecting rabbit milk, reported that this anomaly can be corrected by the practice of equalizing litter size at parturition. In commercial rabbit strains, the does are capable of expressing their maximal yield aptitude and therefore similar weight gains in kits. Equalization of kits in this study allowed for only treatment effect to be observed and therefore similar weight gains.

Results for doe weights showed similarity ($p>0.05$) across all the treatments, although does offered 30% FGMPP were heavier than at parturition. According to Fortun-Lamothe and Lebas (1996), high starch and energy diets in lactating does increased lipid reserves and protected the does from weight loss. It is probable that during fermentation the nutrient enrichment and therefore higher nutrient values of the fermented mature Prosopis pods could have increased the energy intake and resulted in the lactating does exhibiting a positive energy balance. According to Maertens *et al.* (2006), kits' energy requirement during the first three weeks of lactation is highest due to the fast growth of kits that solely depends on milk. Does therefore have to produce high quality milk in terms of energy content to sustain the fast growth rate of kits. According to Partridge *et al.* (1983) energy requirements for lactation especially during peak lactation are higher than can be realized with normal energy provisions in commercial diets. Energy dense feeds should therefore be provided. Otherwise, the does mobilizes its body reserves to produce milk for the kits resulting in negative energy balance (Xiccato *et al.*, 1999).

7.5 Conclusion and Recommendation

7.5.1 Conclusion

Results have demonstrated that there was no treatment effect of feeding FGMPP on kits growth and doe weight changes during the four-week lactation period.

7.5.2 Recommendation

It is therefore recommended that up to 30% maize in breeder does diets can be replaced by FGMPP.

CHAPTER EIGHT

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General discussions

Cereal based feed ingredients are used in compounding commercial rabbit feeds. They are also food for man and are produced under natural rainfall. As a result of global warming, frequent drought, sometimes floods and crop pests have been experienced with some regions recording complete crop failure (Tongruksawattana & Wainaina, 2019). Recently, locust invasion in the ASALs of Kenya resulted in destruction of thousands of hactares of crop and pastureland worsening the food insecurity situation (Lacave, 2020). In 2013, Kenya produced 3 million tonnes of maize against an estimated consumption of 4 million tonnes. This deficit scenario has been consistent over the years necessitating maize importation (Chisholm, 2014; Kariuki *et al.*, 2020). This has caused competition between man and livestock especially during periods of scarcity, leading to low rabbit growth and reproductive performance associated with fluctuation in quality, quantity and prices of commercial rabbit feeds. Therefore, there is need to evaluate available non-conventional feed resources to ease the burden on cereals. This study determined whether fermentation improved the nutritional value and reduced anti-nutritive compounds in ground mature Prosopis pods for rabbit feeding. The hypotheses tested were:

i Fermentation has no significant influence on the nutrient; dry matter (DM), crude proteins (CP), ether extract (EE), ash and anti-nutrient content; total tannins, phytic acid, aflatoxin and crude fibre (CF) of ground mature Prosopis pods.

ii Fermentation and addition of corn starch has no influence on gross energy (GE) values, apparent (CF, CP, DM and GE) digestibilities and true CP digestibilities of ground mature Prosopis pods.

iii Inclusion of fermented ground mature Prosopis pods (FGMPP) in grower rabbits' diet does not affect average daily feed intake (ADFI), average daily weight gain (ADWG), feed conversion ratio (FCR), and % mortality, level of blood metabolites and haematological parameters.

iv Inclusion of fermented ground mature Prosopis pods (FGMPP) in grower rabbits' diet does not affect carcass characteristics, pH of the caecum contents, descriptive sensory characteristics and economic benefit.

v Inclusion of FMGPP in lactating does diet does not affect lactation performance (doe weight changes and kits growth rate).

In a completely randomized design (CRD) with 3 replicates, fermentation of the ground mature Prosopis pods was done naturally and by use of microorganisms *Saccharomyces cerevisiae* (SC), *Lactobacillus salivarius* (LS) and a combination of SC and LS. The fermented substrates were then oven dried for 24 hours at 60^{o} C before proximate, acid detergent fibre (ADF), neutral detergent fibre (NDF), and amino acid analysis done. Fermentation method that exhibited the best amino acid profile (spontaneous fermented substrate) in reference to rabbit nutrition was used in subsequent experiments (digestibility, growth and lactation). Digestibility experiment was conducted at Tatton Agriculture park (TAP) using 15 adult New Zealand White bucks in a CRD. Feeding trial was conducted at Tatton Agriculture Park Egerton University where completely randomized design (CRD) and randomized complete block designs (RCBD) were used. Proximate analysis of feed ingredients used was conducted at Animal Nutrition laboratories, Egerton University.

Mixed statistical analyses software's (SAS, PQRS and SPSS) were used. Statistical package for the social sciences (SPSS) was used for normality and variance tests and principal component analysis (PCA) for identification of significant factors in descriptive sensory attribute evaluation. A Probabilities, quantiles and random samples software (PQRS) was used for descriptive statistics and randomization of the experimental treatments and animals. Statistical analysis systems (SAS) software was used in quantitative data analysis using ANOVA in GLM procedures. Means were separated using Tukey's range procedure and p value of ($p<0.05$) was considered significant.

8.2 Implications of the findings

8.2.1 Feeding UGMPP and FGMPP- based diets to grower New Zealand White Rabbits

Results in this study reported lower percentage mortality when the rabbits were offered diets with 15% FGMPP and 30% FGMPP inclusion. Similarly, analysis of blood samples of rabbits offered these diets reported superior ($p<0.05$) haematological and metabolite indices. Rabbits offered diets with 30% FGMPP reported the lowest ($p<0.05$) cortisol level. Cortisol is a hormone produced by animals that are stressed. These stresses can be nutritional (Medugu *et al.,* 2010) or environmental (Tanchev *et al.,* 2014). Perdigon *et al.* (1988), reported improved functioning of the immune system in mice fed *Lactobacillus casei* and *Lactobacillus acidophilus* fermented milk. In this study, low levels of cortisol indicated lower level of stress. Fermented ground mature Prosopis pods (FGMPP) possibly conferred on the rabbits a favourable gastro-intestinal functioning by fermentation microorganisms present colonizing the caecum and intestines therefore providing a probiotic effect leading to improved health and low mortality.

8.2.2 Effect of feeding FGMPP- based diet on growth rate, blood haematological and metabolite indices of grower New Zealand White Rabbits

Several researchers have reported conflicting results from studies on use of blood haematology and metabolite indices in rabbits to investigate effect of treatments of dietary origin. For example, Adedirea *et al.* (2013) reported significant changes in blood haematology and chemistry when rabbits were fed fermented cowpea husks using cultures of *Asperillus niger, Rhizopus oligosporus, Trichoderma reesei, A. niger + R. oligosporus, A. niger + T. reesei* and *R. oligosporus + T. reesei*. Ogbuewu *et al.* (2010), reported significant ($p<0.05$) effect of dietary treatments on blood chemistry, weight gain and linear body measurements of pre-pubertal buck rabbits fed different levels of neem (*Azadirachta indica a. juss*) leaf. Also, investigations by Bamikole *et al.* (2010), reported better ($p<0.05$) performance and superior haematological indices when rabbits were fed un threshed grain amaranth seed head. However, other researchers were of a contrary opinion. For instance, Harcourt-Brown, F. M and Harcourt-Brown, S (2012); Kozma *et al.* (1974) and Melillo (2007) in clinical studies investigating changes in blood haematological and metabolite indices as affected by disease and nutrition, indicated that disease

effect was significant as opposed to nutrition and suggested that these blood haematological and metabolite indices are more useful when investigating disease conditions.

Pathak (2009) reported that sick animals exhibit reduced feed intake and that good plane of nutrition reduces the predisposition of animals to diseases. Amino acids are important in haemoglobin synthesis, especially the ones that cannot be synthesized by the body, essential and or are needed in large quantities and are therefore limiting in rabbit nutrition (L-arginine, Lysine and methionine) (Adamson & Fisher, 1973; Borsook, 1958; Daniel Jr & Krishnan, 1967). In this study, after 72-hour fermentation, there was significant ($p<0.05$) enhancement of L-arginine and Lysine from (4.95 to 6.94) mg/g DM and (3.65 to 4.81) mg/g DM respectively. This could have resulted in rabbits fed 30% FGMPP inclusion exhibiting higher haemoglobin, RBCs and PCV. In this study, treatment with 30% FGMPP inclusion and control had significantly higher ($p<0.05$) blood glucose than all the other treatments. However, these levels were still within the normal physiological range for healthy rabbits (Harcourt-Brown, F. M, & Harcourt-Brown, S, 2012). Similar findings were reported by Adedirea *et al.* (2013). However, Harcourt-Brown, F. M and Harcourt-Brown, S (2012); Kozma *et al.* (1974) and Melillo (2007) were of contrary opinion; they indicated that rabbit blood glucose levels were not affected by nutrition. Blood glucose provides ready energy for cellular metabolism. High blood glucose is therefore beneficial so long as it is within the normal physiological range that allows for normal body functions. In this study, significantly higher ($p<0.05$) blood glucose levels in the rabbits offered diets with 30% FGMPP inclusion could have resulted in better cellular metabolism and therefore higher ADG.

Rabbits offered diets with 30% FGMPP inclusion had the lowest ($p<0.05$) cholesterol levels (2.13 mmol/L) and the highest ($p<0.05$) platelet count ($367.5 * 10^9$/L). *In vitro* fermentation of GMPP improved arginine content from (4.95 to 6.94) mg/g DM. Le1 and Yang (2018) in studies investigating cholesterol lowering ability of lactic acid bacteria in naturally fermented salted shrimp, reported that *Lactobacillus plantarum* FB003 had the best ability to lower cholesterol *in vitro.* Li *et al.* (1998*)* reported similar results in *in vivo* animal models when they investigated the efficacy of *Monascus purpureus*-fermented rice (red yeast rice) in lowering blood cholesterol in animal models of hypercholesterolaemic patients. Wang and Tall (2016) reported that hypercholesterolemia is a risk factor for intravenous blood clotting due to its impact on atherosclerotic lesional cells such as macrophages. Giroux *et al.* (1999) reported that dietary

supplementation with L-arginine has anti-hypercholesterolemic effects in the blood of rabbits. Also, Bode-Böger *et al.* (1998) when studying the effect of L-arginine in hypercholesterolaemic rabbits *in vivo,* reported that dietary supplementation inhibited platelet aggregation and thromboxane A synthesis and therefore prevented intravascular blood clotting. This was by improvement in production and activity of nitric oxide synthase in production of nitric oxide and reduction of vascular oxidative stress and therefore maintenance of integrity of the walls of cardiovascular system (Boger *et al.,* 1997; Dobutovi *et al.,* 2011). The effect of platelet count on platelet aggregation measured with impedance aggregometry (MultiplateTM analyzer) and with light transmission aggregometry, showed that platelet count was significantly inversely affected by platelet aggregation (Femia *et al.,* 2013). In this study, low cholesterol in rabbits offered 30% FGMPP inclusion diet could have resulted from higher levels of arginine after GMPP fermentation resulting in a significantly higher ($p<0.05$) platelet count due to low degree of platelet aggregation. This could have conferred to the rabbits offered 30% FGMPP inclusion diets a physiologically efficient cardiovascular function, superior feed utilization efficiency and therefore a higher average daily gain (ADG).

8.2.3 Evaluation of carcass and sensory attributes, pH of caecal contents and economic benefit associated with fermenting ground mature Prosopis pods in rabbit diets

Contrary to expectations, caecal contents pH values of 12-week-old grower rabbits fed ground mature Prosopis pods were significantly ($p<0.05$) higher than the control. Lower caecal contents' pH is an indication of high fermentation rate and therefore higher production of short chain fatty acids (SCFAs) (Alvarez *et al.,* 2007; Trocino *et al.,* 2013). However, the lower pH did not translate to higher ADG. There is a possibility that fermentation progressed in the colon, which allowed for production of SCFAs and absorption of the SCFAs in the colon for energy. Also, fermentation of the ground mature Prosopis pods could have conferred a probiotic and prebiotic effect on the rabbits offered 30% FGMPP based diet (Montiel *et al.,* 2013) resulting in significantly higher ADG.

8.2.4 Effect of feeding FGMPP- based diets on lactation performance of lactating rabbits

Results in this study, show that all the treatments had similar effect on the lactation performance of does and growth rate of kits. This is an indication that the energy provided by all the diets were similar to provide for similar quality and quantity of milk. However, the positive energy balance in 30% FGMPP inclusion diet indicated sufficient energy provision to the lactating does and therefore positive energy balance. Rolls *et al.* (1984) in studies to investigate the effects of diet and obesity on body weight regulation during pregnancy and lactation in the rat, reported a positive relationship between pups' growth performance and mother's maternal energy intake with a correlation coefficient of r=0.84. This could have resulted in heavier does after four weeks of lactation

8.3 Conclusions

Objective 1

All the fermentation methods used reported a significant effect ($p<0.05$) on nutritional value improvement and anti-nutritive factors reduction; spontaneous or with SC, LS or LS/SC improved the nutritional value and reduced the anti-nutrient content of UGMPP. However, spontaneous fermentation was more effective in reducing tannin content and enrichment of the amino acids that are limiting amino acids for rabbits; Lysine, methionine and arginine.

Objective 2

Results from this study reported significantly highest ($p<0.05$) digestibility coefficient of UGMPP for DM and CP compared to all other treatments. However, a lower DMI of UGMPP is an indication of lower palatability. The lower palatability resulted in a slower rate of feed passage in the gastro intestinal tract and therefore better digestibility. As much as FGMPP reported a lower digestibility coefficient, the higher DMI resulted in higher total nutrient retention.

Objective 3

Treatment with 30% FGMPP dietary inclusion resulted in the highest ($p<0.05$) ADG and lowest percentage mortality. Also, the treatment with 30% FGMPP reported superior ($p<0.05$) blood haematology and chemistry. Based on the results of this study, it is concluded that up to 30% maize grain in rabbit diets can be replaced by fermented ground mature Prosopis pods. Also, when investigating whether dietary factors have an effect on rabbit physiology, blood haematology and metabolite indices are useful tools in such experiments. These parameters give an indication on the health status of the rabbits and therefore availability of the nutrients and the efficiency of feed utilization.

Objective 4

Treatment with 30% FGMPP dietary inclusion resulted in the highest ($p<0.05$) cost benefit. There was no significant treatment effect ($p<0.05$) on carcass and sensory characteristics. Based on the results of this study, it is concluded that up to 30% maize grain in rabbit diets can be replaced by fermented ground mature Prosopis pods.

Objective 5

In this study there was no significant difference ($p>0.05$) among all the treatments. However, fermenting the mature Prosopis pods reduced tannin content from 7.4% to 2.4% and increased gross energy from (14.05 to 15.56) MJ/kg sample.

8.4 Recommendations

Objective 1

Fermented Prosopis pods is recommended in rabbit diets, so long as the feeding standards and nutrient requirements and allowances of individual class of livestock are considered. Sevety two hours spontanously fermented substrates reported the highest content of limiting amino acids in rabbit nutrition, it is best method of fermentation to be used. Further research should be conducted to establish the efficacy of SC and a combination of SC and LABs in reducing aflatoxin in Prosopis pods and SC in reduction of phytic acid in mature Prosopis pods.

Objective 2

The study recommends the use of FGMPP in rabbit diets. Although UGMPP recorded higher nutrient digestibility coefficients, the palatability was lower. Therefore, higher palatability for FGMPP translates to higher nutrient retention. Studies to be conducted to investigate the effect of addition of cornstarch to FGMPP-based diets fed to rabbits and the effect of different levels of FGMPP intake on digestibility coefficients.

Objective 3

It is therefore recommended that 30% of maize in grower rabbits' diet can be replaced by FGMPP

Objective 4

Fermented ground mature Prosopis pods can substitute 30 % maize in grower rabbit diets. However, it is important to always check for the price of maize as compared to the price of fermented ground mature Prosopis pods as these prices are subject to seasonal fluctuations. This will inform the suitability of 30% FGMPP inclusion in diets of grower rabbits.

Objective 5

It is therefore recommended that up to 30% maize in breeder does diets can be replaced by FGMPP.

8.5 Areas for further research

i Further research to establish the effect of feed deprivation and antinutritional factors on blood haematological and metabolite indices.

ii Further research to identify and characterize the microorganisms present in spontaneously fermented ground mature *Prosopis juliflora* pods and investigate the possibility of using these preparations as commercial probiotic preparations in feed and food industry.

iii Determine the effect of FGMPP and other fermented feed products on digestive disorders (natural and due to treatment with antibiotics) in weaned rabbits and their effects on gastro-intestinal tract morphology and function.

iv Identification of the SCFAs (propionic acid, acetic acid, and butyrate) produced in the caecum of growing rabbits and therefore the SCFA that causes the changes in caecal pH.

v Investigate the extent of fermentation and absorption of VFAs in the colon. This would explain the higher ADG in the diet with inclusion of 30% FGMPP while pH was high in the caecal contents' contrary to expectations.

8.6 Recommendations for stakeholders and policy makers

This study has demonstrated that up to 30% maize in rabbit diets at grower and breeder stages can be replaced FGMPP. This level reported a higher ADG and lower mortality. It also reported superior blood haematology and chemistry and was cost effective when included in rabbit diets when compared to maize. It is therefore concluded that FGMPP can be utilized as a livestock feed ingredient. Therefore, there is need to:

i Explore logistics of setting up collection centres, pod milling and Prosopis plant products processing firms in areas where Prosopis plant grows. This will allow for harvesting and selling of the mature pods and other products to these centres which will mitigate the rapid encroachment of grazing land by spread of Prosopis plant and ultimately improve the livelihoods of the rural population in areas where Prosopis plant grows.

ii Explore the possibility of incorporating Prosopis pods in livestock feeds in untreated or treated forms depending on the class of livestock for which the feed is being manufactured and their efficiency of utilizing Prosopis pods. This should be done as per results and recommendations of various studies on inclusion levels of mature Prosopis pods.

i ii Set up demonstration units for professional management of Prosopis bushes to maximize the benefits; pod production, wood fuel, apiculture and soil conservation.

iv Capacity building of farmers and stakeholders on the importance of use of fermentation technology in improving food safety and quality and reduction of health problems related to consumption of unsafe foods.

v Promote use of non-conventional feed resources and capacity build the relevant stakeholders on exploration and use of other locally available non-conventional livestock feed resources to improve livestock performance, nutritional security, income and farmer livelihoods.

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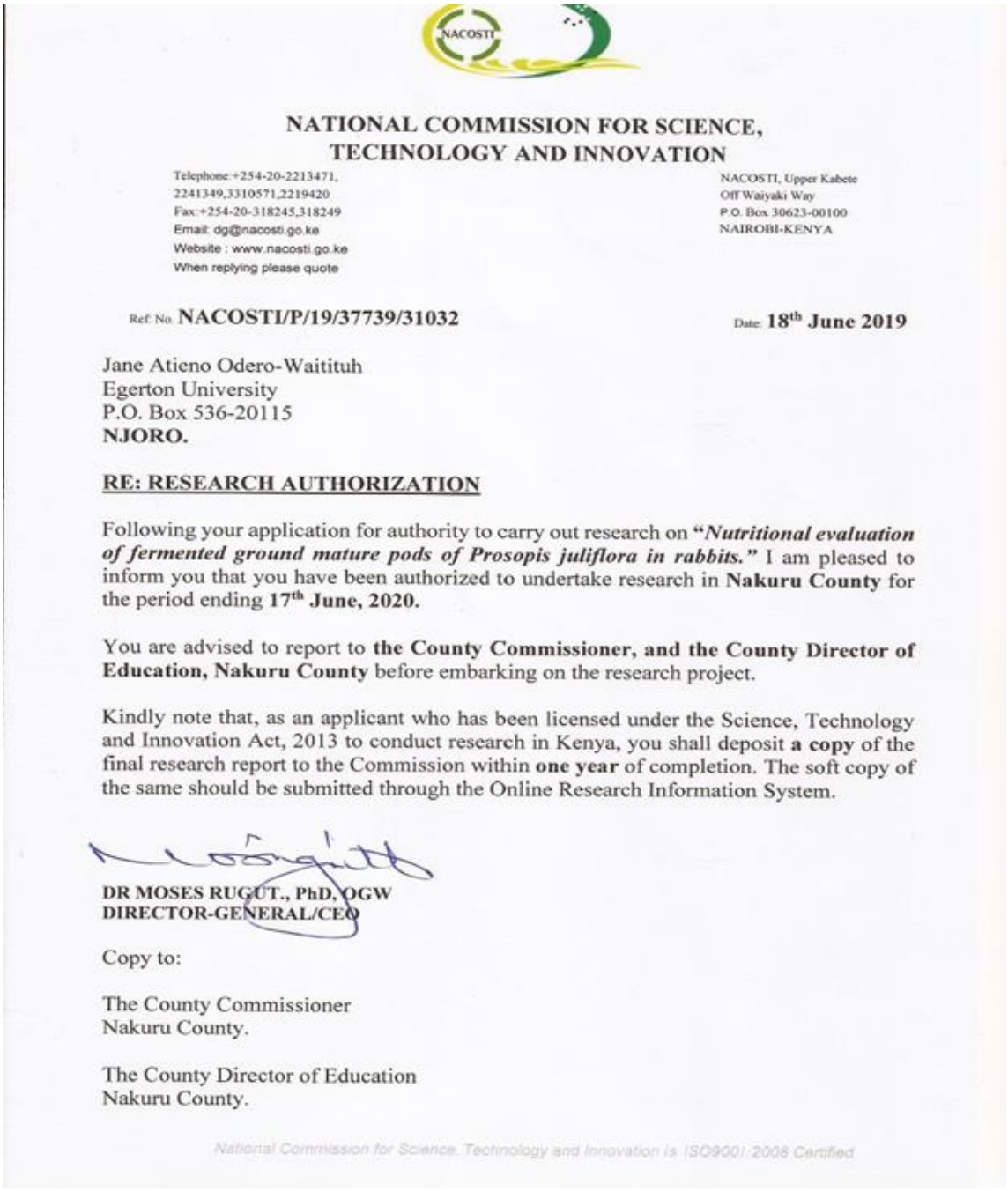
APPENDICES

Appendix A. (NACOSTI permit) National Commission for Science, Technology and Innovation clearance certificate



NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION

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Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/19/37739/31032**

Date: **18th June 2019**

Jane Atieno Odero-Waitituh
Egerton University
P.O. Box 536-20115
NJORO.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Nutritional evaluation of fermented ground mature pods of Prosopis juliflora in rabbits."*** I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for the period ending **17th June, 2020.**

You are advised to report to **the County Commissioner, and the County Director of Education, Nakuru County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

DR MOSES RUGUT., PhD, OGW
DIRECTOR-GENERAL/CEO

Copy to:

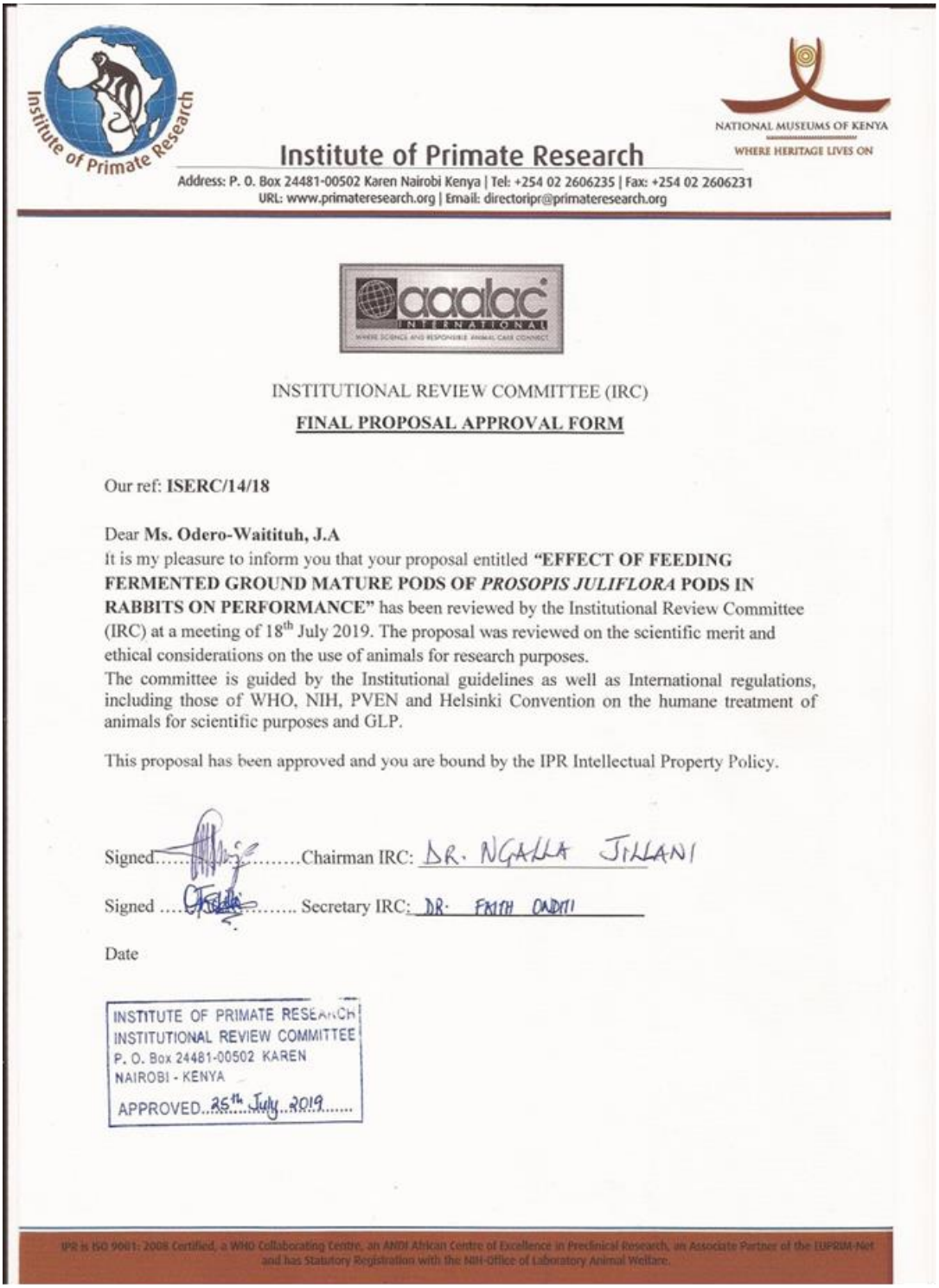
The County Commissioner
Nakuru County.

The County Director of Education
Nakuru County.

National Commission for Science, Technology and Innovation is ISO9001: 2008 Certified

Authority to conduct the study was given by (NACOSTI), the research clearance permit number NAOSTI/P/19/37739/31032 (Appendix A).

Appendix B. Research ethical clearance (Institute of Primate Research)



Institute of Primate Research

Address: P. O. Box 24481-00502 Karen Nairobi Kenya | Tel: +254 02 2606235 | Fax: +254 02 2606231
URL: www.primateresearch.org | Email: directoripr@primateresearch.org

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INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: **ISERC/14/18**

Dear **Ms. Odero-Waitituh, J.A**

It is my pleasure to inform you that your proposal entitled **"EFFECT OF FEEDING FERMENTED GROUND MATURE PODS OF *PROSOPIS JULIFLORA* PODS IN RABBITS ON PERFORMANCE"** has been reviewed by the Institutional Review Committee (IRC) at a meeting of 18th July 2019. The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes.

The committee is guided by the Institutional guidelines as well as International regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR Intellectual Property Policy.

Signed.....Chairman IRC: DR. NGALLA JILLANI

Signed .....Secretary IRC: DR. FAITH OMITI

Date

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED..25th July 2019.....

IPR is ISO 9001: 2008 Certified, a WHO Collaborating Centre, an ANDI African Centre of Excellence in Preclinical Research, an Associate Partner of the EUPRIM-Net and has Statutory Registration with the NIH-Office of Laboratory Animal Welfare.

Authority to use live animals in the experiment was given by the Institute of Primate Research (IPR) research proposal approval reference number ISERC/14/2018 (Appendix B).

Appendix C. SAS input procedure and output for analysis of Caecum pH

```
DATA Ceacum;
INPUT trt block pH @@;
DATALINES;
1 1 6.51 1 1 6.57 1 2 6.74 1 2 6.42
2 1 6.48 2 1 6.52 2 2 6.67 2 2 6.41
3 1 6.54 3 1 6.53 3 2 6.57 3 2 6.56
4 1 6.56 4 1 6.73 4 2 6.57 4 2 6.55
5 1 6.3 5 1 6.03 5 2 6.2 5 2 6.14
;
PROC GLM;
CLASS block trt;
MODEL ph=block trt;
LSMEANS trt / PDIFF TDIFF STDERR  ADJUST=TUKEY;
RUN;
```

The SAS System 13:03 Thursday, January 6, 2000 1

The GLM Procedure

Class Level Information

Class	Levels	Values
block	2	1 2
trt	5	1 2 3 4 5

Number of observations 20

The GLM Procedure

Dependent Variable: pH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.50243000	0.10048600	9.27	0.0005
Error	14	0.15177000	0.01084071		
Corrected Total	19	0.65420000			

R-Square	Coeff Var	Root MSE	ph Mean
0.768007	1.606771	0.104119	6.480000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
block	1	0.00018000	0.00018000	0.02	0.8993
trt	4	0.50225000	0.12556250	11.58	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	1	0.00018000	0.00018000	0.02	0.8993

trt	4	0.50225000	0.12556250	11.58	0.0002

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

trt	pH LSMEAN	Standard Error	LSMEAN Pr > |t|	Number
1	6.56000000	0.05205938	<.0001	1
2	6.52000000	0.05205938	<.0001	2
3	6.55000000	0.05205938	<.0001	3
4	6.60250000	0.05205938	<.0001	4
5	6.16750000	0.05205938	<.0001	5

Least Squares Means for Effect trt

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

Dependent Variable: pH

i/j	1	2	3	4	5
1		0.543308	0.135827	-0.57726	5.331209
		0.9810	0.9999	0.9763	0.0008
2	-0.54331		-0.40748	-1.12057	4.787901
	0.9810		0.9935	0.7934	0.0023
3	-0.13583	0.407481		-0.71309	5.195382
	0.9999	0.9935		0.9500	0.0011

4	0.577265	1.120573	0.713092	5.908474
	0.9763	0.7934	0.9500	0.0003
5	-5.33121	-4.7879	-5.19538	-5.90847
	0.0008	0.0023	0.0011	0.0003

Appendix D. Steps in determination of content of NDF, ADF, gross energy, tannins, aflatoxins and phytic acid

To determine NDF, a pencil was used to put a mark on a drying filter bag, which was then weighed. A weight of 0.5g±0.05g of sample was put in the filter bag and sealed. One blank filter bag and the weighed samples were put in the fibre analyzer for digestion. Two thousand (2000) ml of previously prepared NDF solutions, 20g sodium sulphite (Na_2SO_3), 4ml α amylase were added and the lid closed. The heat and agitate buttons were put on for processing for 90 minutes. After which valve was opened and the liquid drained. Two thousand (2000) ml of hot water and 4.0 ml α-amylase were added and heat and agitate buttons switched on for 5 minutes. This was repeated twice. The sample bags were then removed and immersed in 250 ml beakers with acetone for 5 min. The samples were then put into fuming cupboard, to allow for the acetone to evaporate and then put into the oven at 105^0C for 8 hours. Filter bags were then put in a desiccator for 30minutes and weighed.

To determine ADF, a pencil was used to put a mark on a drying filter bag, which was then weighed. A weight of 0.5g±0.05 g of sample was put in the filter bag and sealed. One blank filter bag and the weighed samples were put in the fibre analyzer for digestion. Two thousand (2000) ml of previously prepared ADF solution, 20g Na_2SO3, 4ml α amylase were added and the lid closed. The heat and agitate buttons were put on for processing for 90 minutes. After which valve was opened and the liquid drained. Two thousand (2000) ml of hot water and 4.0 ml α-amylase were added and heat and agitate buttons switched on for 5 minutes. This was repeated twice. The sample bags were then removed and immersed in 250 ml beaker with acetone for 5 min. The samples were then put into fuming cupboard, to allow for the acetone to evaporate and then put into the oven at 105^0C for 8 hours. Filter bags were then put in a desiccator for 30minutes and weighed.

Gross energy was measured using an atomic bomb calorimeter (e2K bomb calorimeter, www.cal2k.com, South Africa), where 0.5g of the sample was compressed into a pellet and placed in the combustion vile and placed in the bomb container of the calorimeter and lid closed. Thirty (30) psi pressure was injected into the bomb container which was then transferred into bomb calorimeter and lid closed. The bomb was then ignited. The gross energy (GE) value was displayed on the bomb calorimeter screen in MJ/kg sample and the reading taken.

To determine tannins, 0.2 g of the sample was weighed and put into tubes. 10ml of acetone was added to each tube and the contents shaken for 10 minutes. The contents were then centrifuged at 4000 rotations per minute for 5 minutes at $4^{o}C$. Zero point zero five (0.05) ml of tannic acid extract was be pipetted into 100ml volumetric flasks. Tannic acid was then added to the other 100 ml volumetric flasks at 0 ml tannic acid (1 flask), 0.2 ml (2 flasks) and 0.5 ml (2 flasks). Zero-point five (0.5) ml of follin-denis reagent was added to all the volumetric flasks containing the extract from the samples and the tannic acid. Distilled water was then added to three-quarter volume of the volumetric flask. Two-point five (2.5) ml of sodium carbonate (Na_2CO_3) was then added to all the volumetric flasks at intervals of 3 minutes and filled with distilled water to the mark. Spectrophotometer readings were then done after 30 minutes to get the absorbance.

To determine aflatoxin, where 50 gms of ground sample were extracted by using 80% methanol and filtered through a fluted filter paper (Whatman #2). A 10 ml portion of the filtrate was diluted 1:5 with distilled water. The diluted extract was filtered through a glass microfibre filter paper (Vicam 31955). Two ml were added to an aflatest P immunoaffinity column and were allowed to drain at approximately 1–2 drops/second. The column was washed twice with 5 ml portions of distilled water. Aflatoxins were eluted from the column (approximately 1–2 drops/second) with 1 ml of methanol into a glass cuvette. One ml of aflatest developer (Vicam LP) was added to the cuvette, and the contents were mixed for 1 minute. The cuvette was placed in a fluorometer, and the aflatoxin content was read immediately in ppb (equivalent to µg/).

To determine phytic acid, one (1) g of the sample was weighed and transferred into a 1.5 ml microfuge tubes to which 1 ml of 0.4M hydrochloric acid (HCl) was added and incubated for 12 hours at $4^{o}c$ to extract the phytic acid. The sample was then vortexed briefly and 20µl aliquot transferred into microtitre plates and supplemented with 180 µl of Chen's reagent (1 volume 6N H_2SO_4, 1 volume 2.5% ammonium molybdate; 1 volume 10% ascorbic acid, 2 volumes H_20). After mixing the sample containing phytic acid with Chen's reagent, the reaction resulted in the formation of phospho-molybdate compound which had a blue coloration and the colour intensified depending on phytic acid concentration. The assay was then allowed to develop for 2h at room temperature and optical density at 490 nm was measured. Spectrophotometric reading was converted to phytic acid by multiplying with the conversion factor 3.5484 and expressed as mg $^{-1.}$ The standards were prepared by dissolving 0.174 grams of di-Potassium hydrogen

phosphate (K_2HPO_4) in 1-liter distilled water to give a concentration of 1Mm K_2HPO_4. Eight standards were prepared in order of increasing concentration by pipetting 15, 30, 45, 60, 75, 105 and 120µL and supplementing it with 100.

Appendix E. Steps in blood haematological and metabolite analyses

Blood Glucose testing using Glucometer (URIT)

Sample: Coagulant blood.

Materials: Glucometer, Test strips.

Procedure

Blood strips were inserted into the glucometer, a drop of blood sample was put on the edge of the strip and results appear on the strips calibration read in mmol/L.

Blood Cortisol testing using i-chroma™

Materials

i-chroma™ reader, i-chroma™ cartridge, i-chroma™ chip

Sample

The sample type for i-chroma™ cortisol is whole blood /blood serum or plasma

Procedure

30µl of blood serum was pippeted and transferred to a tube containing the detection buffer. The detection buffer tube was tightly closed and buffer plus sample in the buffer tube were then mixed thoroughly by shaking the tube about 10 times. This mixture was used in analysis immediately. 75 µl of sample mixture was pippeted onto the sample well in the cartridge. The laoded sample cartridge was inserted in to the i-chroma slot/incubator at $25^{O}C$ for 10 minutes. The sample loaded cartridge was then inserted into the cartridge and readings done immediately. The instrument for the i-chroma™ test calculated the results automatically and displayed cortisol concentration of the tet sample in nmol/L.

Blood cholesterol testing using biochemistry analyzer

Materials

MonoReagent, standard, biochemistry analyzer, incubator, pipettes

Sample

Serum or plasma

Procedure

Samples and reagents were brought to room temperature and pipeted into leballed tubes as tabulated below

	Blank	Sample	Standard
MonoReagent	1.0 ml	1.0 ml	1.0ml
Sample	-	10 µl	-
Standard	-	-	10 µl

Tube contents were mixed and incubated for 5 minutes at 37^{O}C. Absorbance of the sample and the standard was read at 500nm against the reagent blank.

Blood calcium testing using biochemistry analyzer

Materials

Reagents A and B, standard, biochemistry analyzer, pippettes.

Sample and serum

Procedure

A working reagent was made by mixing reagent A and Reagent B.

Sample, calcium standard and working was pippetted into labelled test tubes as displayed in the table below.

	Blank	Standard	Sample
Calcuim standard	-	10 µl	-
Sample	-	-	10 µl
Working reagent	1.0ml	1.0ml	1.0ml

The mixture in the test tubes were mixed thoroughly and let to stand at room temperature for 2 minutes. Absorbance of the standard and sample was read at 610nm against the blank within the first one hour.

Appendix F. Pre-screening questionnaire and contract for pre-screening of panelists

1. Name: _____
2. Phone (home and business): _____
3. Are there any weekdays (M–F) that you will not be available on a regular basis?

   _____
4. Do you have any of the following? Dentures _____ Diabetes

   _____ Oral or gum disease _____ Hypoglycemia _____

   Food allergies _____ Hypertension _____
5. Do you take any medications which affect your senses, especially taste and smell?

   _____
6. Are you currently on a restricted diet? If yes, explain.

   _____
7. How often do you eat fast foods out in a month? _____
8. What is (are) your favourite food(s)? _____

   _____
9. What is (are) your least favourite food(s)? _____

   _____
10. What foods can you not eat? _____

    _____
11. What foods do you not like to eat? _____

    _____
12. Is your ability to distinguish smell and tastes

Better than average _____ Average _____ Worse than average _____

13. If a recipe calls for vinegar and there is none available, what would you substitute?

    _____
14. What are some other foods that taste like yoghurt? _____

    _____
15. How would you describe the difference between flavour and aroma? _____

    _____
16. How would you describe the difference between flavour and texture? _____

_____

17. Describe some of the noticeable flavours in sausages. _____

_____

18. Describe some of the noticeable flavours in pizza. _____

_____

19. Describe some of the noticeable flavours in boiled beef. _____

_____

20. Is your sensitivity to textural characteristics in foods

Better than average, _____ Average _____ Worse than average _____

21. Describe some of the textural properties of foods in general. _____

_____

22. Describe some of the particles one finds in foods. _____

_____

23. Describe some of the properties which are apparent when one chews on a food.

_____

24. Describe the differences between spongy and rubbery. _____

_____

25. What are some textural properties of bread? _____

_____

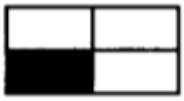
26. For what type of products is texture important? _____

_____

27. Instructions: mark on the line at the right to indicate the proportion of the area that is shaded.

EXAMPLES.

None ⊢——/——⊣ All



None ⊢—/—⊣ All

1.

None ⊢——————⊣ All

2.

None ⊢——————⊣ All

Contract

I _____ agree to be a panellist for **DESCRIPTIVE QUALITY Sensory evaluation of meat of rabbits fed standard diet and diets with inclusions of 15% Prosopis pods (UGMPP), 30 % UGMPP, 15% fermented Prosopis pods (FGMPP) and 30% FGMPP** for a period of one week.

I agree to comply with terms and conditions.

Signature ……

Date ……

Appendix G. Sensory lexicon developed during training

Term	Definition	Rating scale
Colour	Actual colour of the sample	1 = White 7 = Brown
Denseness	Compactness of the cross-section	1= Less compact 7 = Very compact
Oiliness	Presence of visible oil	1 = None 7 = High
Chicken aroma	Aromatic associated with cooked chicken	1 = None 7 = High
Salty taste	Taste associated with iodized salt	1 = None 7 = High
Chicken flavour	Flavour associated with cooked chicken	1 = None 7 = High
Beefy flavour	Flavour associated with cooked beef	1 = None 7 = High
Tenderness	Ease of chewing	1 = Tough 7 = Tender
Juiciness	Moisture released by the product in the mouth as a result of chewing	1 = None 7 = High
Rubbery	Degree to which sample returns to original shape after some deformation	1 = None 7 = High
Grittiness	Amount of small, hard particles between teeth during chew	1 = None 7 = High
Oily residual	Degree to which mouth feels oily after swallowing	1 = None 7 = High
Particle residuals	The amount of particles left in mouth after swallowing	1 = None 7 = Many
Teeth adhesion	Mouth residues that remain stuck on teeth	1 = None 7= High

Metallic after-taste	Metallic flavour similar to the one produced by iron (II) sulphate	1 = None 7 = High

Appendix H. Prosopis tree in a homestead in Baringo, Kenya.

Source: Author

Appendix I. Flowering Prosopis plant

.Source: https://www.google.com/search?q=Prosopis+tree+picture+at+flowering&client=firefox-b&tbm

Appendix J. Mature pods on a Prosopis plant

Source: https://www.google.com/search?q=Prosopis+tree+picture+with+mature+pods&client=firefox-b&tbm

Appendix K. Mature Prosopis pods

Source: Author

Appendix L. Ground mature Prosopis pods

Source: Author

Appendix M. New Zealand White rabbit

Source: https://www.google.com/search?q=Newzealand+white+rabbit+picture&ie=utf-8&oe=utf-8&client=firefox-b

Appendix N. Internal organs, head and limbs

Source: Author

Appendix O. Carcass with internal organs

Source: Author

Appendix P. Publications and presentations

Publications

Animal Research International (2020) 17(2): 3736 – 3746 3736

LACTATION PERFORMANCE OF NEW ZEALAND WHITE RABBITS FED FERMENTED GROUND MATURE *PROSOPIS JULIFLORA* PODS REPLACING MAIZE

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ABSTRACT

Rabbit production is one of the enterprises expected to ensure food and nutrition security in developing countries. However the availability of feed ingredients especially energy sources is a challenge. There is therefore, the need to evaluate non-conventional feed resources such as mature Prosopis juliflora pods that are available throughout the year. Fluctuation in the weight of does during lactation is an indication of energy changes in the body of the doe and therefore energy content of the feeds offered. A study was conducted at Egerton University to investigate the effect of replacing maize with fermented ground mature P. juliflora pods (FGMPP) in lactating doe diets on lactation performance and energy balance. The study investigated kits weight gain and doe weight changes during the four week lactation period. Fifteen primiparous does weighing 3.05 ± 0.47 kg with a litter of six kits each, weighing 0.61 ± 0.05 kg were individually housed in cages measuring 75 x 55 x 40 cm^3. In a completely randomized design (CRD) of 5 diets; control (formulated standard breeder diet), 15 % unfermented ground mature pods of P. juliflora (UGMPP), 30 % UGMPP, 15 % FGMPP and 30 % FGMPP replacing maize in standard breeder diets were offered in three replicates per treatment. The nutritional value of mature Prosopis pods improved ($p<0.05$) on fermentation. There was no treatment ($p>0.05$) effect in weight of kits and does. Up to 30 % maize in lactating doe diets can be replaced by FGMPP.

Keywords: Anti-nutrients, Fermented *Prosopis juliflora* pods, Non-conventional feed resource, Rabbit

Available Online
Journal of Economic Impact
http://www.scienceimpactpub.com/jei

BIO-ECONOMIC IMPLICATIONS OF FEEDING FERMENTED GROUND MATURE *Prosopis juliflora* PODS TO GROWER RABBITS

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HIGHLIGHTS

- *Prosopis juliflora* plant is readily available in arid and semi-arid lands (ASALs) of Kenya to produce mature pods throughout the year.
- Its inclusion in livestock diets compromises performance due to anti-nutrients which can be reduced using fermentation.
- The study investigated the effect of inclusion of graded levels of fermented ground mature *Prosopis juliflora* pods (FGMPP) replacing maize in the diets of growing rabbits on growth and economic benefit.
- The study shows that 30% FGMPP inclusion resulted in a superior average daily gain and economic benefit.
- Findings of the study will ensure sustainability in rabbit production, improvement of farmer livelihoods, and improvement of food and nutritional security to the Kenyan human population.

ABSTRACT

Prosopis juliflora plant is readily available in arid and semi-arid lands (ASALs) of Kenya, producing mature pods throughout the year. However, its inclusion in livestock diets compromises performance due to anti-nutrients which can be reduced using fermentation. At Tatton Agriculture Park, Egerton University, a study was done to investigate the effect of inclusion of graded levels of fermented ground mature *Prosopis juliflora* pods (FGMPP) replacing maize in the diets of growing rabbits on growth and economic benefit. Sixty (60), 42-day old rabbits weighing 0.5 ± 0.04 kg (mean ± SD) were housed in cages measuring (75 * 55 * 40) cm; three rabbits of the same sex per cage. In a randomized complete block design (RCBD) 5 diets; control (formulated standard grower diet), 15% unfermented ground mature pods of *Prosopis juliflora* (UGMPP), 30% UGMPP, 15% FGMPP and 30% FGMPP replacing maize in formulated standard grower diets were offered in four replicates per treatment (six males and six females). Analysis of data was done using the general linear model (GLM) of Statistical Analysis Systems (SAS). Tukey's range procedure at ($p<0.05$) significance was used to separate means. The results show that 30% FGMPP inclusion resulted in a superior ($p<0.05$) average daily gain (ADG) and economic benefit ($p<0.05$). The study concluded that replacing 30% maize in diets of grower rabbits with FGMPP will make economic sense. This will ensure sustainability in rabbit production, improvement of farmer livelihoods, and improvement of food and nutritional security to the Kenyan human population.

Keywords: Economic benefit; fermentation; growth; non-conventional feed resource.
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Chapter 4

SMALLHOLDER FARMING SYSTEMS: CHALLENGES AND OPPORTUNITIES

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ABSTRACT

Smallholder farming systems comprise keeping of livestock and crops. It involves nutritional inter-relationships of integration and interdependency between livestock and crop systems and crop and crop systems. There is nutrient circulation between crops and livestock when there is the feeding of crops and crop residues to livestock and livestock manure use as fertilizer on crops. When there are no nutritional leaks, an equilibrium is created between the various systems. However, normal nutritional leaks are expected to occur when livestock manure is used to fertilize crops for human consumption. To avoid reduction of nutrients in one component of the system, there must be nutritional replenishments. There are an estimated 500 billion smallholder farms worldwide supporting livelihoods of about 2 billion people, most of who are in Sub-Sahara Africa and Asia. Smallholders make more than 60% of

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Descriptive Sensory Characteristics of Meat from Grower Rabbits Fed on Fermented Ground Mature *Prosopis juliflora* Pods Based-diets

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Abstract: The effect of inclusion of graded levels of fermented ground mature *Prosopis juliflora* pods (FGMPP) replacing maize grain in grower rabbits' diets on sensory attributes was investigated. Thigh muscles were obtained from 12-week-old rabbits fed on five diets comprising: control (formulated standard grower diet), 15% UGMPP, 30% UGMPP, 15% FGMPP and 30% FGMPP replacing maize in standard grower diets. Deep-frozen meats from the rabbits were thawed and boiled in different aluminium pots, cut into small pieces of about 2 cm^3 placed in ceramic plates and presented to 12 panellists. Questionnaires were used for sensory attribute profiling. Data was analysed using SPSS Statistics 25.0.0 and the general linear model (GLM) of Statistical Analysis Systems (SAS) softwares for Principal Component Analysis (PCA) and analysis of variance (ANOVA) respectively. Tukey's range procedure was used to separate means at ($p<0.05$) significance. The PCA indicated that grittiness, particles, oiliness, colour, salty taste and oily taste contributed greatest to the observed variability. According to ANOVA, there was no treatment effect ($p>0.05$) in overall rating, appearance, flavour and colour of the meat. However, there was treatment effect ($p<0.05$) on beefy taste, tenderness, salty taste and grittiness. The study concluded that 30% maize grain in diets of grower rabbits' diet can be replaced with FGMPP as it did not affect consumer preference of the meat.

Keywords: Carcass, Fermentation, Mature *Prosopis juliflora* Pods, Grower Rabbits, Sensory Evaluation