

**EVALUATION OF TREATED MATURE PROSOPIS (*Prosopis juliflora*) POD MEAL-
BASED DIET ON GROWTH PERFORMANCE OF PIGS**

DAVID MARI NGUGI

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

EGERTON UNIVERSITY

NOVEMBER 2021

DECLARATION AND RECOMMENDATION

Declaration

This research thesis is my original work and to the best of my knowledge has not been presented for award of a degree at any other university.

Signature  _____


Date 15/11/2021

Ngugi David Marii

KM113/14673/18

Recommendation

This research thesis has been prepared with our supervision and submitted to graduate school with our approval as university supervisors


Signature  _____

Date 15/11/2021

Prof. Anthony King'ori, PhD

Department of Animal Sciences

Egerton University

Signature  _____

Date: 15/11/2021

Dr. Olivier Kashongwe, PhD

Department of Animal Sciences

Egerton University

COPYRIGHT

© Ngugi David Marii

All rights reserved. No part of this research work may be reproduced, stored in retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying or recording without prior written permission of the author or Egerton University on that behalf.

ACKNOWLEDGMENTS

I would like to express my gratitude to the Almighty God who has given me strength during the entire period of my study. I would like to acknowledge Egerton University, Board of Postgraduate Studies and the Department of Animal Sciences for the opportunity to pursue my MSc programme. I would like to extend my gratitude to Network for Eco-farming (NECOFA) and (MANITESE), Centre for Excellence in Sustainable Agriculture and Agribusiness Management (CEESAM) for their financial support in this study. I wish to thank my colleagues and friends for their material inputs and ideas during the project. I am particularly grateful to my supervisors Dr. Olivier Kashongwe and Prof. Anthony King'ori who greatly contributed in the study from proposal development to thesis writing.

ABSTRACT

Smallholder pig production depends heavily on non-conventional feed resources as a means to lower cost of production. Utilization of these feed resources by pigs is however limited by the high fibre content thus leading to poor digestibility. This study evaluated the effect of treatment of ground Prosopis pod meal (GPPM) by natural fermentation, *Lactobacillus Plantarum* and fibrolytic enzyme on digestibility and growth performances in grower pigs. Experiment one consisted of an *in vitro* dry matter digestibility (IVDMD) followed by *in vitro* gas production to assess fermentation kinetics. Samples of GPPM were allocated to four treatments. Treatments included GPPM treated with multi-enzyme (Natuzyne®) (T1); Untreated (GPPM) (T2); GPPM fermented with (*Lactobacillus plantarum* MTD1 Ecosyl ®) (T3) and GPPM treated using natural fermentation (T4). The second and third experiments assessed the performance and cost of feeding pigs based on the best treatment from experiment 1. The second experiment was conducted using thirty Landrace x Large white crosses of 20±3 kg which were allotted to five treatments with six pigs each (replicates). The dietary treatments were; T1- (0% GPPM + 0.035% enzyme), T2- (0% GPPM and 0% enzyme), T3- (10% GPPM and 0.035% enzyme), T4- (20% GPPM and 0.035% enzyme), T5- (30% GPPM and 0.035% enzyme). The hypotheses were tested using mixed model procedures of IBM SPSS Statistics version 22 for the IVDMD while a two-way analysis of variance SAS 9.0 (2002) was used for the feeding trial and the cost of feeding GPPM. Results of the IVDMD indicated a significant difference in IVDMD of T4 (45.60%) compared with T3 (50.30%) and T1 (52.81%). Multi-enzyme pre-treated GPPM (T1) (18.50 ml) had a significantly higher cumulative gas ($p < 0.05$) than naturally fermented GPPM (13.42 ml). This was reflected by the rate of degradation and the time in hours at which rate of degradation was achieved. There were significant differences in the rate of degradation whereby multi-enzyme treated GPPM was higher compared to natural fermented GPPM ($p < 0.05$). Results from the feeding trial indicated that the average daily gain (ADG) of T1, T2 and T3 were not significantly different, however, ADG for T1 (0.634 kg/day) was significantly different from T4 (0.440 kg/day) and T5 (0.421 kg/day). The FCR of T1 (2.707) was the highest while T5 (3.383) was the lowest ($p < 0.05$). The feed cost per weight gain showed that T5 had a relatively lower cost among diets with GPPM. In conclusion, pre-treatment of Prosopis pod with exogenous enzyme improved IVDMD relative to other treatments. The study also demonstrated that GPPM treated with the multi-enzyme complex can be included up to 30% in grower's pig diets without effecting ADG while reducing the cost of feeding.

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATION	ii
COPYRIGHT	iii
ACKNOWLEDGMENTS	iv
ABSTRACT	v
LIST OF FIGURES	x
LIST OF TABLES	x
LIST OF ABBREVIATIONS AND ACRONYMS	xii
CHAPTER ONE	1
INTRODUCTION.....	1
1.1 Background Information	1
1.2 Statement of the problem	2
1.3 Objectives	3
1.3.1 Broad objective	3
1.3.2 Specific objectives	3
1.4 Hypotheses	3
1.5 Justification	3
CHAPTER TWO	5
LITERATURE REVIEW	5
2.1 Introduction.....	5
2.2 Overview of pig production in Kenya	5
2.3 Pig production system in Kenya	6
2.4 Sources of feed ingredients for pigs in Kenya	6
2.5 Pig nutrition	7
2.6 Nutrient Requirements of Pigs.....	7
2.7 Dietary fibre	8
2.7.1 Effects of dietary fibre on pigs.....	9
2.7.2 Approach to improve utilization of fibrous diets by pigs	10
2.7.3 Exogenous enzyme treatment	10
2.7.4 Fermentation treatment	10
2.8 Overview of non-conventional feedstuffs.....	11

2.8.1	<i>Prosopis juliflora</i> tree (<i>Mathenge</i>) in Kenya	12
2.8.2	Chemical composition of mature <i>Prosopis juliflora</i> pods	12
2.8.3	<i>Prosopis juliflora</i> pods as animal feed.....	13
2.9	Research gap	14

CHAPTER THREE15

DETERMINING THE *IN-VITRO* DIGESTIBILITY OF FERMENTED, ENZYME-TREATED AND UNTREATED MATURE *PROSOPIS JULIFLORA* POD-BASED MEAL.....15

3.1	Introduction.....	15
3.2	Materials and methods	16
3.2.1	Study site.....	16
3.2.2	Preparation of experimental materials	17
3.2.3	Preparation of enzyme-treated mature <i>Prosopis</i> pod meal	17
3.2.4	Preparation of naturally fermented mature <i>Prosopis</i> pod meal.....	17
3.2.5	Preparation of mature GPPM fermented with lactic acid bacteria inoculum	17
3.2.6	Proximate analysis	18
3.2.7	Three-way <i>in-vitro</i> digestibility of dry matter determination	18
3.2.8	The <i>in-vitro</i> fermentation phase.....	19
3.2.9	Dry matter digestibility and fermentation kinetics calculations	21
3.4	Results.....	22
3.4.1	The pH of fermented GPPM	22
3.4.2	Nutrient composition of GPPM fermented naturally and with <i>Lactobacillus plantarum</i>	22
3.4.3	The <i>in-vitro</i> digestibility of untreated, enzyme treated, and fermented GPPM..	23
3.4.4	Fermentation kinetics.....	24
3.5	Discussion	25
3.6	Conclusion	28

CHAPTER FOUR.....29

EFFECT OF FEEDING ENZYME TREATED *PROSOPIS JULIFLORA* POD MEAL-BASED DIETS ON THE GROWTH PERFORMANCE OF GROWER PIGS.....29

4.1	Introduction.....	29
4.2	Materials and methods	30

4.2.1	Experimental location	30
4.2.2	Preparation of the <i>Prosopis</i> pod meal and experimental diets	30
4.2.3	Management of experimental animals	32
4.2.4	Experimental design.....	32
4.2.5	Proximate analysis	32
4.2.6	Data collection	33
4.3	Statistical analysis.....	33
4.4	Results.....	33
4.4.1	Chemical composition of the experimental diets and <i>Prosopis juliflora</i> pod meal.....	33
4.4.2	Growth performance of the pigs	34
4.5	Discussion.....	36
4.6	Conclusion	39
CHAPTER FIVE		40
EFFECT OF INCLUSION OF TREATED <i>PROSOPIS JULIFLORA</i> POD MEAL IN THE TOTAL RATION ON COST OF FEEDING GROWER PIGS		40
5.1	Introduction.....	40
5.2	Material and methods.....	41
5.3	Statistical analysis.....	41
5.4	Results.....	42
5.5	Discussion.....	44
5.6	Conclusion	45
CHAPTER SIX		46
GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS.....		46
6.1	General discussion	46
6.1.1	Determining the <i>in-vitro</i> digestibility of fermented, enzyme-treated and untreated mature <i>Prosopis juliflora</i> pod-based meal.....	46
6.1.2	To determine the feed intake, feed conversion ratio and growth rate of grower pigs offered enzyme-treated mature <i>Prosopis juliflora</i> pods meal-based diets	47

6.1.3	Cost of feeding processed <i>Prosopis juliflora</i> and the implications for smallholder farmers	47
6.2	Conclusions.....	47
6.3	Recommendations.....	48
6.4	Areas of further research.....	48
REFERENCES.....		49
APPENDICES.....		57
Appendix A: Ethical clearance		57
Appendix B: National Commission for Science, Technology and Innovation Research Permit		58
Appendix C: Statistical output for the objectives		58
Appendix D: Research pictorial.....		72
Appendix E: Publication.....		73

LIST OF TABLES

Table 2.1 Nutrient requirement of growing-finishing pigs fed <i>ad-libitum</i> amount/kg diet (90% dry matter) based on body weight.	8
Table 2.2 The nutrient content and energy of pods and seeds of <i>Prosopis juliflora</i>	14
Table 3.1 Diet containing <i>Prosopis juliflora</i> pod meal (g/100g).....	20
Table 3.2 The pH of fermented GPPM.....	22
Table 3.3 Nutrient composition of GPPM under untreated, enzyme treated, natural and fermentation with <i>Lactobacillus plantarum</i> -MTD1 (Ecosyl®).....	23
Table 3.4 Fermentation kinetics of fermented untreated and enzyme treated <i>Prosopis juliflora</i> pod meal	24
Table 4.1 Chemical Composition of the experimental diets (g/100g).....	31
Table 4.2 Chemical composition of <i>Prosopis juliflora</i> pod meal.....	34
Table 4.3 Effect of experimental diets on growth performance of pigs	35
Table 5.1 Economics of feeding enzyme treated <i>Prosopis</i> pod meal to grower pigs.....	42
Table 5.2 Feed composition and feed cost computation for experimental diets	43

LIST OF FIGURES

Figure 3.1	The <i>in-vitro</i> digestibility of multi-enzyme, fermented and untreated ground <i>Prosopis juliflora</i> pod meal.....	23
Figure 3.2	Cumulative gas produced in during the 72 hours period Error bar represents mean.....	24
Figure 4.1	Mean weekly weight of pigs fed diets with or without <i>Prosopis juliflora</i> meal and Natuzyme®.	36

LIST OF ABBREVIATIONS AND ACRONYMS

ADF	Acid detergent fibre
ADG	Average daily gain
<i>Ad libitum</i>	free choice feeding
ANOVA	Analysis of variance
ANFs	Anti-nutritive factors
AOAC	Association of official analytical chemists
ASALS	Arid and semi-arid lands
BW	Body weight
Barrows	Castrated males
CP	Crude protein
DM	Dry matter
DE	Digestible energy
EE	Ether extract
FAO	Food and Agricultural Organisation of the United Nations
GE	Gross energy
Gilts	Female pigs under one year that has not farrowed
GM	Gross margin
GDP	Gross domestic product
GMO	Genetically modified organisms
GPPM	Ground Prosopis pod meal
IVDMD	<i>in-vitro</i> dry matter digestibility
KNBS	Kenya Bureau of Statistics
MOLD	Ministry of Livestock Development
NDF	Neutral detergent fibre
NSP	Non starch polysaccharide
NRC	National Research Council
SAS	Statistical analysis system
VAT	Value added tax
VFA	Volatile fatty acids

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Kenya's population is expected to reach 96 million from the current 47 million people, while the urban population is expected to triple from 12 million to 40 million by 2050 (FAO, 2017). This implies that demand for food and especially meat will increase with the main drivers being increasing human population, urbanisation and disposable income (Bett et al., 2012).

Consumption of pork in Kenya surged by 60% from 15,000 to 24,000 tonnes between 2016 and 2017 (Muthui et al., 2019). Such consumption will therefore drive up demand hence incentivising more people to engage in pig farming or its associated value chain thus contributing to employment. Pigs are highly prolific, have short generation interval high feed conversion efficiency and quicker returns in comparison to other livestock (Carter et al., 2013). This coupled with improved pig husbandry techniques results in more farmers adopting pig production (Muthui et al., 2019).

Pig farming in Kenya is mostly practiced by small scale farmers having 10-100 pigs at a given time (FAO, 2012). Small scale farming of pigs is characterised by a diversity of production systems including free-range, semi-intensive and intensive systems. Local and crossbreeds dominates (exotic x indigenous), while exotic are kept by few farmers. Poor disease and parasite control, and inadequate nutrition, however, negatively affect productivity (Carter et al., 2013). Feed cost accounts for 80% of the total cost of production (Manhique et al., 2017). Therefore, any intervention that aims to reduce the cost of production in pig enterprise should focus on feed cost reduction.

The East Africa Community consumes about 6 million metric tonnes of feeds against 1.7 million metric tonnes produced per year (Muthui et al., 2018). This shows that member countries are feed deficient and as a consequence feeds become expensive. Global climate change is also a challenge as most of the feed ingredients used in livestock production are highly prone to droughts and causes fluctuations in the availability and cost (Kang et al., 2009). Therefore more attention has been shifted to non-conventional feed resources that are affordable, locally available and climate-smart, more importantly; leguminous agroforestry fodder trees (Gachui et al., 2017).

Prosopis juliflora is an exotic invasive plant species that was introduced to Kenya in 1980 from Brazil and Hawaii through the fuel wood afforestation project as a means to reclaim desert lands, providing fodder and firewood to local communities (Pasicznik et al.,

2007). The tree produces pods that are sweet, nutritious and highly palatable by animals, but the leaves are unpalatable due to high amounts of antinutritive factors (ANFs) (Odero-Waitituh et al., 2016). The tree is highly resilient as it sprouts quickly upon cutting due to its deep root system (Pasicznik et al., 2007). The utilization of the pods not only provides an affordable and available source of alternative feed ingredient but also reduces its rapid spread.

Levels of inclusion of up to 10% have been used to replace the total diet formulation in improved indigenous layer chicken without affecting weight gain (Manhique et al., 2017). Higher levels of inclusions (> 10%) have been associated with poor growth and production due to the presence of high fibre (11-35%) and the presence of anti-nutritive factors (ANFs) such as, tannins, trypsin inhibitors and saponins (Manhique et al., 2017). Dietary fibre and the associated ANFs adversely affect nutrient utilization by binding or encapsulating to or with amino acids, minerals and fats reducing the accessibility by enzymes hence reduced nutrients absorption. Dietary fibre and antinutritive factors (ANFs) has also been associated with increase of the viscosity and decrease of the enzyme movement through the digesta hence reducing digestibility (De Vries et al., 2012).

Pigs have a higher capacity to handle a fibrous diet than poultry, due to a better fermentative capacity in the cecum and longer digesta retention time (Choct et al., 2010). Moreover, this capacity increases with age. It can therefore be hypothesised that Prosopis pods meal can replace a larger proportion of the pig ration hence reducing the cost of feed considerably. Utilization of treatment methods of fibrous feed material improves fibre digestibility, reduces the levels of ANFs and improves nutrients availability and utilization (De Vries et al., 2012). Hence subjecting the pods to fermentation or enzyme treatment can result in a higher inclusion level in the diet.

1.2 Statement of the problem

The cost of pig feeds is high and almost unaffordable to most farmers. This leads farmers, especially smallholders, to use feeds of low quality and in insufficient quantity to meet pigs' requirements. Importations of feed ingredients to meet market demand further increase the prices without offering a guarantee in quality. In this context, locally available and affordable feed resources represent an opportunity to be explored as alternatives for both farmers and the feed industry. Prosopis pods are among these locally available alternatives but contain high crude fibre, which limits their utilization in monogastric animals. Treatment of Prosopis pods by exogenous enzymes or fermentation has been suggested to improve

digestibility high-crude diets. However, little evidence exists on their effectiveness in this context. This study, evaluated the *in-vitro* digestibility of fermented, enzyme-treated and untreated mature *Prosopis* pod meal and the performance of growing pigs offered treated mature *Prosopis* pod meal-based diets.

1.3 Objectives

1.3.1 Broad objective

The overall objective was to contribute to sustainable production of pigs through the utilization of mature *Prosopis juliflora* pods as a feed resource in Kenya

1.3.2 Specific objectives

- i. To determine the *in-vitro* digestibility of fermented, enzyme-treated and untreated mature *Prosopis juliflora* pod meal.
- ii. To determine the feed intake, feed conversion ratio and growth rate of grower pigs offered treated mature *Prosopis juliflora* pods meal-based diets.
- iii. To determine the effect of inclusion of treated *Prosopis juliflora* pod meal in the total ration on cost of feeding in grower pigs

1.4 Hypotheses

- i. *Ho*: There is no significant difference in *in-vitro* digestibility of fermented, enzyme-treated and untreated mature *Prosopis juliflora* pod meal.
- ii. *Ho*: Inclusion of treated mature *Prosopis juliflora* pods meal in the diet of growing pigs has no significant effect on feed intake, feed conversion ratio and growth rate.
- iii. *Ho*: Inclusion of treated mature *Prosopis juliflora* pods meal in the diet of grower pig has no significant effect on the cost of feeding.

1.5 Justification

The increasing demand for food by human populations has posed a threat to the sustainability of the feed industry (Muthui et al., 2018). Most of the small-scale farmers do not afford to offer their pigs nutritionally adequate diets. Climate change has altered the weather pattern resulting in inadequate crop production for food and feed. As a result, efforts have been focused on the identification and evaluation of alternative feedstuffs that are locally available in situations of climate change. These feedstuffs should also be affordable and can meet the nutrient requirement of pigs with less or no competition with man (Muthui

et al., 2018). Whole mature *Prosopis* pod meal contain crude protein of 7.1-18.5%, and metabolizable energy of 12.791 MJ/Kg (Odero-Waitituh et al., 2016). The energy and protein content compares favourably with maize, rice, and wheat bran; the main feed ingredients for pig feeds used by smallholders in Kenya (Muthui et al., 2018). The pods are locally available and face no competition with man, hence presumably affordable to incorporate in the pigs' feed, with a likelihood of a reduction in feed cost. However, the pod meal is characterized by a high fibre content of 11-35%. Growing pigs can tolerate crude fibre of up to 6%, levels beyond this limit nutrient utilization in the feed (Al-marzooqi et al., 2015). Therefore, there is a need to treat the pod meal using exogenous enzyme or fermentation to enhance digestibility. This study evaluated the utilization of treated *Prosopis* pods as a feed ingredient in growing pigs' diet.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Over the last 30 years, consumption of livestock products has been on the rise with the low and middle-income countries experiencing the highest demand (FAO, 2018). More importantly, meat consumption has tripled with the main drivers being population growth, urbanization, income gain and globalization (FAO, 2018). Global pig production and consumption is mainly dominated by Asia representing 55% of the average pig meat production in comparison to Africa that represents 1.1% (FAO, 2018). In Africa, the livestock revolution has seen meat consumption being projected to increase from 5.5 to 13.3 million metric tonnes between 1997 and 2025 (Bett et al., 2012).

Livestock production in Kenya provides food, income through sale of animals or animal products, employment and as a store of wealth more importantly in rural areas. Livestock production is estimated to contribute 12% of the gross domestic product (GDP), 40% of the total agriculture gross domestic product (GDP) and employment opportunities for 50% of the total agricultural workforce (MOLD, 2012). The animal resource base comprises 17.5 million cattle, 27.7 million goats, 17 million sheep, 3 million camels, 334, 689 pigs and poultry 31 million in total (FAO, 2017). With the expected increase in demand for animal products, the population of the animals is expected to rise with the associated effects of improving income especially in the arid and semi-arid lands (ASALs) of Kenya where almost 60% of the livestock are kept.

2.2 Overview of pig production in Kenya

Pig production plays an important role in social-economic activities among rural and urban communities in Kenya. Pig production is mostly carried out by smallholder farmers that constitute 70% of the total pig production in Kenya with the rest attributed to commercial pork farms (Mbuthia et al., 2014). Pig production is mainly preferred by smallholder farmers due to the minimum inputs and labour requirement, a large number of offspring's per farrowing, short generation intervals and high feed conversion efficiency (Carter et al., 2013). The space required per pig is also small hence more pigs can be kept in a relatively small area especially in the urban slum area in Kenya where a small portion of pig farming is done. These characteristics offer the farmer with an investment that not only requires less initial capital but also give good returns in a relatively short period. Currently, the population of

pigs stands at 334, 689 pigs with a large portion of this being concentrated in Central 27.48% and Western 26.24%, respectively, (FAO, 2012).

2.3 Pig production system in Kenya

Pig production system in Kenya is classified under large-scale intensive farms, small-scale commercial farms and free ranging/backyard systems (FAO, 2012). Large intensive farms are usually organised companies such as Farmers Choice, which keep 5,000 to 30,000 pigs (FAO, 2012). These farms are involved in the entire value chain from breeding of the pigs to slaughtering and finally to meat processing. The commercial small-scale pig production is mainly practiced by farmers in the peri-urban areas surrounding the city and towns. This is characterised by use of exotic breeds under confinement and provision of better nutrition hence higher performance (Levy et al., 2014).

Free ranging or backyard systems are carried out by farmers in western parts of Kenya where pigs are tethered outside. Pigs under this system are left to graze and scavenge for their feeds hence are characterised by low production (Levy et al., 2014; Mbuthia et al., 2014). Pigs under this system have a relatively lower average daily gain (ADG) of 0.182 to 0.480 kg/day in comparison to pigs kept under intensive system with an average daily gain (ADG) of 0.570 to 0.831 kg/day (Carter et al., 2016). Little or no investments are made in regards to shelter, diseases, parasite control and provision of quality feeds. This system though cheap has several disadvantages; low off-take, low reproductive efficiency and high mortality rate (Kagira et al., 2010).

2.4 Sources of feed ingredients for pigs in Kenya

Feed constitutes almost 80% of the total cost of production in pig enterprise making it the single most important component that determines profitability in pig farming (FAO, 2012). Most of the feed ingredients that go into pig feeds are usually by-products of cereal milling and also from oil cakes, most of which are imported from neighbouring countries (Muthui et al., 2019). Feed manufacturers in Kenya are located around urban areas; this, in turn, has a ripple effect on feed cost to most small-scale farmers due to the additional cost involved in transporting the feeds. Moreover, due to inadequate oversight by quality control institutions, the ingredients used to make the feeds have been compromised as a result of adulterations (Kenya Market Trust, 2017). This is further complicated by the moratorium placed by the government that restricts manufactures from importing ingredients that are of genetically modified organisms (GMO) origin to Kenya (Kenya Market Trust, 2017).

Other government regulations that have impacted feed prices are those that restrict the importation of specific feed material like bone meal. Restriction of fishing to allow breeding at specific times of the year has also caused massive fluctuation of fishmeal (Omena) prices in Kenya (Kenya Market Trust, 2017). Therefore, due to high cost, most of the pig farmers have resulted in using surplus farm produce, market and kitchen waste, swills from eateries and institutions and on-farm formulations (Mutua et al., 2020). This though has come at a price as pig productivity is reduced due to nutritional imbalances that characterize these feed materials (Carter et al., 2013).

2.5 Pig nutrition

Pigs are simple stomach animals that have a similar digestive system to man and avian species with minor differences in their digestive organ anatomy and functionalities. Ingested feed undergoes enzyme breakdown in the stomach and in the small intestines and the soluble nutrients absorbed in the small intestines (Martens et al., 2012). The undigested material non-starch polysaccharides (NSP), resistant starch, indigestible protein undergo fermentation in the large intestines and in the cecum (Martens et al., 2012). Through this process fibre undergoes degradation through microbial fermentation to form methane (CH₄), carbon dioxide CO₂ and volatile fatty acids such as acetic acids, butyric acids and propionic acids (Martens et al., 2012).

The digestive system makes the pig inefficient in utilization of vast quantities of grasses, silage and hay, making them highly dependent on grains. For a feed material to be of importance to the pig, digestion and assimilation of nutrients must occur (Jacyno et al., 2016). The digestibility of nutrients is dependent on several factors and can be classified into animal, feed and environmental factors. For instance, young pigs especially newly weaned pigs have limited enzyme secretory and lower absorptive capacity (Torres-Pitarch et al., 2107). This is in contrast to adult pigs that have a more developed and larger gastro-intestinal tract with the residence time of the digesta being comparatively longer (Jacyno et al., 2016).

2.6 Nutrient Requirements of Pigs

Nutrients are chemical compounds present in food required for maintenance of body activities, growth, lactation and gestation by animals. These include fats, protein, carbohydrates, vitamins, minerals and water (Patience, 2012). The provision of dietary energy and nutrients sufficiently to pigs is based on body weight and physiology status (Dourmad et al., 2008).

The nutrient requirement in pigs differs based on the body weight and physiological status, performance potential, and environmental conditions the animal is exposed to (NRC, 2012). The use of modelling, has allowed precise estimation of nutrient requirements in exotic pigs according to housing conditions, body score and performance goals of a typical pig production system (Dourmad et al., 2008). This may not necessarily represent the pigs in the tropics that are either local and crosses whose growth performance is relatively lower than the exotic breeds (Carter et al., 2016).

Table 2.1 Nutrient requirement of growing-finishing pigs fed *ad-libitum* amount/kg diet (90% dry matter) based on body weight.

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

Source: NRC, (2012)

2.7 Dietary fibre

Dietary fibre is a heterogeneous mixture of structural, non-structural polysaccharides and lignin components of plants that cannot be digested by the mammalian endogenous

enzyme (Kerr et al., 2013). This is attributed to the inability of the endogenous enzyme to digest the glycosidic bonds associated with fibre. As a result most of the undigested fibre undergo complete or partial fermentation in the large intestines and caeca to release volatile fatty acids (VFA) and gases Methane (CH₄) carbon dioxide (CO₂) and Hydrogen (H₂) (NRC, 2012).

Cellulose, hemicellulose and pectin are the most abundant while less abundant non-starch polysaccharides (NSP) include mucilage, glucomannans, fructans and gums (Kerr et al., 2013). Digestibility of dietary fibre is highly variable and it is dependent on the physio-chemical characteristics with sugar beet fibre being totally soluble while straws being insoluble in growing pigs (Noblet et al., 2001). Age has been shown to affect the digestibility of fibre in pigs with mature pigs, sows and boars handling relatively higher levels than younger pigs. This is attributed to the high number of cellulolytic bacteria which are 6.7 times higher in sows than growing pigs (Jacyno et al., 2016).

2.7.1 Effects of dietary fibre on pigs

Dietary fibre contributes 25% of the total maintenance energy in growing pigs, boars and sows through volatile fatty acids VFAs (acetate, propionate and butyrate), produced during fermentation (Kerr & Shurson, 2013). Additionally, colonocytes are highly dependent on fermentation products mainly butyrate as a source of energy and regulation of growth and development (Mpendulo et al., 2018). The VFAs produced have also been attributed to promoting gut health through lowering intestinal pH and promoting growth of lactobacillus bacterial species that competitively inhibit the growth of harmful microorganism (Jha & Berrocso, 2016). Increase in dietary fibre has also been shown to improve digestive organ development, hydrochloric acids, bile and enzyme secretion hence improved digestibility of feeds (Mateos et al., 2012). Increasing level of fibre has however, been associated with low nutrient digestibility with digestible energy DE being reduced by 0.01 for every 10g of neutral detergent fibre neutral detergent fibre (NDF) added in the diet (Noblet et al., 2001).

Reduced digestibility in nutrients has been attributed to sequestration of nutrients by fibre where most of the nutrients are encapsulated by limiting the accessibility of enzymes to these nutrients (De Vries et al., 2012). Soluble non-starch polysaccharides (NSP) such as arabinoxylans and glucans has been associated with increased viscosity of the digesta which consequently reduces digestive and absorptive capacity of the gut (Kerr & Shurson, 2013). Moreover, fibre reduces the mean retention time of the food in the gastrointestinal tract and as a result less time is available for efficient digestion (Kerr & Shurson, 2013).

2.7.2 Approach to improve utilization of fibrous diets by pigs

To improve the utilization of fibrous diet by growers' pigs requires disruption of the hemicellulose, cellulose and lignin matrices. Use of chemical, mechanical and biological methods of treatment have proven to be effective in rendering dietary fibre more degradable (Kanengoni et al., 2015).

2.7.3 Exogenous enzyme treatment

Exogenous enzymes, especially cell wall degrading enzymes, are essential in removing side chains, cleaving polymers and removing nutrients tightly bound to the cell wall (De Vries et al., 2012). Carbohydrates, proteases and phytases are the most commonly used enzymes as single or a mixture of multi-enzyme complexes. The efficacy of enzymes in improving nutrient utilization is dependent on the substrate-enzyme match, the practical size of the feed, dosage used, enzyme activities and concentration of non-starch polysaccharides (NSP) (De Vries et al., 2012). Enzyme treatment not only improves digestibility and nutrients but also complements the undeveloped enzyme in young pigs (Torres-Pitarch et al., 2017).

Apparent ileal digestibility and total tract digestibility of gross energy (GE) and neutral detergent fibre NDF was observed to increase when xylanases and beta glucanase was added to poor quality barley and wheat middling in growing pigs (Clarke et al., 2018; Moran et al., 2016). Moran et al. (2016) reported an increase of apparent total tract digestibility (ATTD) of ash, calcium and total phosphorus when a combination of carbohydrase and phytases were supplemented to a diet containing wheat bran. This process is described as the “de-caging effect of enzymes” on nutrients from aleurone layer that contains high amounts of nutrients but also high non-starch polysaccharides (NSP) content. Hooda et al. (2011) reported a weak relationship between digesta viscosity and apparent ileal digestibility of energy and crude protein. Therefore, higher soluble non-starch polysaccharides (NSP) leads to a lower utilization of feeds. The effect of soluble non-starch polysaccharides (NSP) on the digesta viscosity might therefore, not be the most important factor affecting nutrient digestibility in pigs (Hooda et al., 2011).

2.7.4 Fermentation treatment

Fermentation processes have been utilized as a means of feed preservation and feed processing techniques for many years. Fermentation involves the transformational process during which starch and sugars are transformed into lactic acid, volatile fatty acids (VFA), carbon dioxide (CO₂) alcohols and methane (Humer et al., 2016). Fermentation can be

prepared by either using spontaneous fermentation or by use of an inoculum containing specific *Lactobacillus* starter culture with an aim of achieving a pH of 4.5 and below, lactic acid bacteria of $9\log_{10}$ colony forming unit and lactic acid concentration of 150 mmol/l (Missotten et al., 2010). The un-disassociated organic acids produced from the anaerobic fermentation have antibacterial properties mainly attributed to their ability to freely cross the bacterial membranes, reduce the pH and stopping the enzymatic activities of bacterial cells (Plumed-Ferrer et al., 2009).

Fermentation process has been shown to reduce antinutritive factors (ANFs) concentration with Vandenberghe et al. (2021) observing a reduction in total polyphenol and phytic acid 24 hour after natural fermentation. Fermentation was also observed to reduce glucosinolates in rapeseed meal while contributing to improved energy and calcium digestibility for broilers. Further, fermentation has been reported to reduce neutral detergent fibre (NDF) content through production of non-starch polysaccharides (NSP) degrading enzymes by bacteria, while there was an increase in amino acid digestibility in fermented corn-soybean mix (Shi et al., 2017). The decline of pH obtained during fermentation also improves minerals absorption with iron in ferrous form that is poorly absorbed being converted to ferric iron that is highly absorbable (Nkhata et al., 2018).

However, fermentation has been associated with the loss of essential nutrients, vitamins and amino acids through conversion to other substances or utilization by fermentation microflora (Nkhata et al., 2018). Lysine, an essential amino acid can be converted to biogenic amine such as Cadaverine through decarboxylation during fermentation, with a resultant effect being irreversible loss of amino acids (Missotten et al., 2010). Moreover, presence of biogenic amine has been attributed to decrease in palatability of the feed. Therefore, it is advisable to ferment the ingredient of interest but not the complete diet (Missotten et al., 2010).

2.8 Overview of non-conventional feedstuffs

Pig production in the tropics is only tolerated if the pigs do not compete with humans for feed resources (Kambashi et al., 2014) but with increasing human population the competition is becoming inevitable and as a result more non-conventional feed resources are being utilized. Non-conventional feed resources include crop residues, slaughter waste, agro-industrial, pods from leguminous trees and household kitchen waste. These are highly abundant, relatively affordable, rich in fibre, low in energy and carbohydrates and contain a relatively high amounts of anti-nutritive factors (Kambashi et al., 2014). These non-

conventional feedstuffs have shown potential as feed materials for animals more specifically leguminous trees such as *Prosopis juliflora* that produced edible pods (Manhique et al., 2017; Odero-Waitituh et al., 2016). The pods and leaves from these trees are rich in proteins and can be a potential source of protein for livestock during most times of the year.

2.8.1 *Prosopis juliflora* tree (Mathenge) in Kenya

Prosopis juliflora is a leguminous tree native to most arid and semi-arid regions in North America and parts of Asia. However, the tree is considered an exotic invasive plant species that was introduced from Brazil and Hawaii to Kenya as a mean to reclaim desert lands, providing fodder and firewood to local communities in the 1980s through the fuelwood afforestation project (Pasicznik et al., 2007). *Prosopis juliflora* tree is utilized as a source of firewood, timber, charcoal and its flower produces adequate amount of nectar and pollen needed for honey production (Pasicznik et al., 2007). The tree is wide spreads in the dry and hot lowland areas making it a potential threat to most parts of Kenya that consist 80% arid and semi-arid areas (Maundu et al., 2009).

The tree is distributed in parts of Garissa, Baringo, Tana River and Mandera County in Kenya. Due to the invasive nature of the tree, prosopis has been attributed to transformation of agro and silvo-pastoral grazing areas into a thick thorny bush inaccessible to people and animals causing economic damages to local communities (Maundu et al., 2009). Most of the eradication programs put in place to control the plant have however failed because of its hardiness and as a result, attention has shifted to utilization as a tool to control its spread (Mahgoub et al., 2004).

Prosopis juliflora has a tap root that is 6-9 meters long that enables it to exploit the deep aquifers while the bark is rough and dull red in colour (Schachtschneider et al., 2013). The tree produces pods that are curved with 4mm thickness, 1cm wide and 15 cm in length with a light yellow hard exocarp, fleshy mesocarp and woody endocarp when dry but it's green when unripe (Schachtschneider et al., 2013). The seeds enclosed in an endocarp are very small, almost 5mm in diameter and weigh about 40 mg with 25 seeds per pod.

2.8.2 Chemical composition of mature *Prosopis* pods

The pod in this case implies the pericarp (mesocarp, exocarp) and the seeds while the pulp is the mesocarp and exocarp. Several authors have reported variations in the nutrient content of the pods (Table 2.2). Manhique et al. (2017) observed a diverse variation in Baringo, Garissa and Kajiado County in Kenya, a variation attributed to location and age at

which the pods are harvested. The pods are rich in saccharose (20-25% of dry matter) with 70% of the sugar being sucrose making the pods to have a sweet taste. Mature dry Prosopis pod contains a DE of 2880 kcal/kg, 2682 kcal/kg ME and 4340 kcal/kg GE while the pericarp contains 2642 kcal/kg DE, 2466 kcal/kg ME and 4291 kcal/kg GE (Sawal et al., 2004). Prosopis pods contain 0.24% calcium and 0.13% total phosphorus (Odero-Waitituh et al., 2016). These levels are below the requirement of growing pigs 0.66% Calcium and 0.56% total phosphorus respectively. Therefore, supplementation of diet with Phosphorus and Calcium is needed. The pod however contains several ANFs such as tannins, trypsin inhibitors that depress nutrient utilization and feed intake. Al-Harhi et al. (2019) reported a lower protein quality of Prosopis pods in comparison to soybean meal as a results to the ANFs.

2.8.3 Prosopis pods as animal feed

Prosopis pods are consumed by a wide range of animals in their native environment upon getting dry and falling on the ground. High sugar content makes the pods highly palatable to animals an adaptive mechanism that helps in seed dispersal (Felker et al., 2001). The hard capsule surrounding the seed makes the whole seed indigestible hence grinding will render the pods and seeds digestible and reduce seed dispersal (Peña-Avelino et al., 2013). The pods are consumed by goats, sheep, cattle, camels, pigs and poultry either as whole pods or in milled processed form. Further the seeds which contains a relatively higher amount of crude protein than the pods are usually extracted and fed to animals. The pods have been included in the diets of poultry; Al-marzooqi et al. (2015) observed that replacement of maize with ground mature Prosopis pod meal at 10% in the diet lowered the cost of production while having no negative impact on growth parameters such as feed intake, feed conversion ratio and body weight gain.

Partial replacement of barley with *Prosopis juliflora* pod meal at 20% provided to fattening lambs and goats did not affect meat characteristics, nutrients digestibility and growth performances (Mahgoub et al., 2004). Obeidat and Shdaifat (2013) further observed an increase in milk production in ewes fed 25% Prosopis pod meal. Additionally, the weaning weight of lambs from ewes fed Prosopis pod meal-based diet also increased. However, detrimental effects on growth rates were observed when Prosopis pods meal were fed to cows at levels above 60% (Peña-Avelino et al., 2013). Cows particularly observed to be affected by jaw paralysis which ultimately resulted to low feed intake (Peña-Avelino et al., 2013).

Table 2.2 The nutrient content (% DM) and energy (MJ/kg DM) of pods and seeds of *Prosopis juliflora*

Plant part	DM	CP	CF	EE	Ash	NDF	ADF	GE	References
Pods	94.4	12.5	19.2	1.89	4.37	45.9	29.7	17.3	Manhique et al. (2017)
Pods	90.3	11.4	17.7	2.80	2.7	25.3	15.4	17.5	Odero-Waitituh et al. (2016)
Pods	90.2	12.8	18.4	1.1	5.1	N.R	N.R	18.7	Al-Marzooqi et al. (2015)
Pods	91.4	15.2	18.6	2.61	6.04	28.1	20.0	N.R	Al-Harhi et al. (2019)
Seeds	96.1	22.6	6.90	6.46	4.04	N.R	N.R	N.R	Yusuf et al. (2008)

N.R not reported

2.9 Research gap

The current literature suggest that competition of food by humans and livestock and climate change pose a major threat to the sustainability of the animal feed industry. The literature also shows that most of the small-scale farmers are unable to afford to offer their pigs nutritionally adequate diets. As a result, efforts have been focused on the identification and evaluation of alternative feedstuffs that are locally available and adaptable in the current situations of climate change. Whole *Prosopis* pods meal contains crude protein and metabolizable energy that compares favourably with maize, rice, and wheat bran; the main feed ingredients for pig feeds used by smallholders in Kenya. Additionally, the pods are locally available and face no competition with man, hence presumably affordable to incorporate in the pigs' ration, with a likelihood of a reduction in feed cost. The utilization of the pods is relatively low due to the higher fibre that potentially reduce digestibility. Several intervention to overcome high fibre content in fibrous diet such as fermentation and use of fibrolytic enzymes have been used with success. This study addresses how the utilization of *Prosopis* pods meal as a feed ingredient in growing pigs diet can be improved using interventions such as fermentation and enzyme treatment.

CHAPTER THREE

DETERMINING THE *IN-VITRO* DIGESTIBILITY OF FERMENTED, ENZYME-TREATED AND UNTREATED MATURE *PROSOPIS JULIFLORA* POD-BASED MEAL

Abstract

The objective of this study was to determine the effects of enzyme supplementation and fermentation on *in-vitro* dry matter digestibility (IVDMD) and the fermentation kinetics of Prosopis pods meal for pig feeding. There were four treatment groups; T₁ Ground Prosopis pods meal (GPPM) treated with multi-enzyme (Natuzyme®); T₂ Untreated (GPPM); T₃ GPPM fermented with (*Lactobacillus plantarum* MTD1 Ecosyl®) inoculum and T₄ GPPM treated using natural fermentation. An IVDMD trial was conducted using pepsin-pancreatin hydrolysis method. The pepsin-pancreatin hydrolysed materials were mixed with faeces from barrows for *in-vitro* gas measurement. Fermentation kinetics parameters were computed from cumulative gas collected from *in-vitro* gas production. The IVDMD and fermentation kinetics were analysed using the mixed model procedures in SPSS Statistics version 22. Results indicated a significant difference in IVDMD of T₄ (45.6%) compared with T₃ (50.3%) and T₁ (52.81%). T₁ was significantly different from T₂ (49.1%) ($p < 0.05$). Cumulative gas produced from multi-enzyme pre-treated GPPM (18.502 ml) was significantly higher ($p < 0.05$) than naturally fermented GPPM (13.429 ml). There was significant differences in the rate of degradation (R_{max}) ml/hr when untreated (0.677) and multi-enzyme treated GPPM (0.786) compared to *Lactobacillus plantarum* (0.493) and natural fermented GPPM (0.425). The time at which R_{max} was reached for multi-enzyme pre-treatment (5.140) was significantly shorter ($p > 0.05$) compared to natural fermentation (19.069). Pre-treatment of Prosopis pod meal with multi-enzyme treatment compared to the untreated Prosopis pod meal improved the *in-vitro* dry matter digestibility. The rate and the extent of gas produced in natural and inoculated fermentation was significantly reduced as a result of fermentation.

3.1 Introduction

The animal feed industry has been confronted with increased costs of cereals and oilseeds mainly because of increased demands by emerging markets for human consumption and increased use of these products for energy production (De Vries et al., 2012). In Kenya like most developing countries, pig farming is practised mainly by smallholders farmers characterized by low input and limited resources (Kagira et al., 2010; Kambashi et al., 2014). Furthermore, the high cost of conventional feed has prompted the use of locally available

feed resources. A survey in Kenya confirmed that only 30% of pig farmers use commercial feeds while the rest included some form of alternative feed material in their feeding regime (Mbuthia et al., 2014). These locally available materials are often of low digestibility due to their high cell wall contents, making them unusable by pigs which lack the endogenous enzyme needed to break the lignocellulose bonds (Muthui et al., 2019).

Physical, chemical and biological processing have the potential to increase utilization of highly fibrous alternative feed materials (De Vries et al., 2012). Processing methods, including the use of particle size reduction, exogenous enzymes and fermentation improves digestibility and fermentation kinetics of poor-quality feed materials (Lee et al., 2018; Zangaro et al., 2019). The use of lactic acid bacteria fermentation has not only been utilized as a means of feed preservation over the years but has been associated with a reduction of the total fibre content through hydrolysis of the neutral detergent fibre while improving the crude protein levels (Odero-Waitituh et al., 2020; Wang et al., 2018). Lyberg et al. (2006) reported an improvement of organic matter digestibility and crude protein content after lactic acid fermentation of wheat and barley-based diets. This is supported by a recent study that concluded that use of lactic acid fermentation improved the *in vitro* dry matter digestibility and the fermentation kinetic of high fibrous rapeseed meal (Jang et al., 2021).

The *in-vitro* method for simulating pig digestive system provides a rapid ethical and less expensive technique unlike the use of *in-vivo* method to evaluate digestibility of feed ingredients. The *in-vitro* digestibility methods that simulate the gastrointestinal tract of pigs have been used for the prediction of *in-vivo* digestibility values (Zangaro et al., 2019). The *in-vitro* dry matter digestibility and gas production provide a better view on the effect of processing technologies on the degradability and volatile fatty acid production of feeds in the hindgut of pigs (Zangaro et al., 2019). This study was conducted to determine the effects of pre-treatment of Ground Prosopis pod meal (GPPM) meal interventions with multi-enzyme complex Natuzyme®, fermentation with *Lactobacillus plantarum* and natural fermentation on *in-vitro* dry matter digestibility and the fermentation kinetics.

3.2 Materials and methods

3.2.1 Study site

An *in-vitro* experiment was conducted at Egerton University, Animal Nutrition laboratory. The University is situated at 0° 23' S, 35° 55' N within Njoro Sub-County, Nakuru County. The altitude is 1800 meters above sea level with an average annual rainfall of 900-

1,200 mm. The area has average daily temperatures ranging from 17°C- 22°C (Egerton University Department of Agriculture Engineering, Metrological station 2018).

3.2.2 Preparation of experimental materials

Mature Prosopis pods were collected from Marigat Sub-County located 0° 20'N and 35° 37'E (Mwangi et al., 2005) by hand-picking from the ground underneath the prosopis trees after vigorous shaking the trees. The pods were allowed to dry, sorted then milled (Choge et al., 2007) and used in the making of the treatments.

There were four treatments with 3 replicates each;

T₁: GPPM treated with an enzyme (Natuzyme®)

T₂: Untreated GPPM

T₃: GPPM fermented with lactic acid bacteria inoculum

T₄: GPPM treated using natural fermentation

3.2.3 Preparation of enzyme-treated mature Prosopis pod meal

Natuzyme® (Bioproton PTY Ltd, Australia) is a multi-enzyme complex distributed in Kenya by Cooper limited Kenya. The enzyme contained (12,000 units/g of xylanase, 6,000 units/g of cellulase, 1,500 units/g of phytase, 700 units/g of beta-glucanase, 700 unit/g protease and 400 unit/g of alpha-amylases) presented in powder form. The enzyme was added to the samples of GPPM in triplicates and mixed thoroughly. The enzyme was included at the rate of 350mg/kg of feedstuff in the dry form as per the manufacturer's instructions and recommendations.

3.2.4 Preparation of naturally fermented mature Prosopis pod meal

A mixture of 1 kg mature GPPM with distilled water at a ratio of 1:2.75 (wt. /vol) in triplicate was incubated at room temperatures (22°C) for 7 days using 2 kg plastic bottles (Jørgensen et al., 2010). The plastic containers were sealed tightly to create anaerobic conditions. After 7 days, pH of individual samples was recorded using portable pH meter (pH/ORP/ Temperature Combo Tester - HI98121 HANNA instruments) and a sample was obtained for proximate analysis.

3.2.5 Preparation of mature GPPM fermented with lactic acid bacteria inoculum

An Inoculant containing a single strain of commercial *Lactobacillus Plantarum*-MTD1 (Ecosyl® Products, Ltd., Stokesley, England) was used as the starter culture. Three

individual samples of 1kg of mature GPPM were mixed with distilled water at a ratio 1:2.75 (wt. /vol) and the culture added to the mixture (Jorgensen et al., 2010). The inoculated mature GPPM was then incubated at room temperatures in tightly sealed 2 kg plastic bottles for 7 days in the laboratory. A sample was also collected for proximate analysis. The pH of individual samples was measured and recorded using a digital hand-held pH meter (pH/ORP/Temperature Combo Tester - HI98121 HANNA instruments).

3.2.6 Proximate analysis

The proximate analysis conducted on the samples included dry matter determination by drying in a hot air oven at 105°C for 24 h (AOAC, 2006), ash determination by burning samples in a muffle furnace at 550°C for 8h (AOAC, 2006), ether extract soxhlet method (using ether) (AOAC, 2006). Total nitrogen for crude protein (N x 6.25) determination was obtained using micro-Kjeldahl method (AOAC, 2006). Constituents of the cell wall, neutral detergent fibre (NDF) and acid detergent fibre (ADF), were determined using the Van Soest method (Van Soest et al., 1991). Hemicellulose was determined as a difference between the neutral detergent fibre (NDF) and the acid detergent fibre (ADF).

3.2.7 Three-way *in-vitro* digestibility of dry matter determination

To simulate the digestive process in the pigs' stomach and intestines an *in-vitro* digestibility trial was conducted according to Boisen and Fernhdez (1997). There were four treatments with 3 replicates each. Treatment 1: GPPM (control), Treatment 2: enzyme-treated mature *Prosopis* pod meal. Treatment 3: Mature GPPM naturally fermented and Treatment 4: Mature GPPM fermented using a commercial *Lactobacillus plantarum*-MTD1 (Ecosyl® Products, Ltd., StoKesley, England).

Step one (Porcine Stomach Simulation Phase)

Ground feed sample of 0.4 g was weighed and placed in a 100 ml conical flask. Sodium phosphate buffer solution, 200 ml (0.1 M, pH 6.0) was added to the flask and carefully mixed with the sample by stirring. To simulate the stomach digestive process, 80ml of 0.2M HCl was added and the pH adjusted to 2.0 with 1M HCl or 1M NaOH solutions. This was followed by addition of prepared 5 mL pepsin porcine grade enzyme with 4x USP activity (Pepsin from porcine gastric mucosa powder, ≥ 250 units/mg solid Sigma-Aldrich Corp., St. Louis, MO, USA) containing 1 mg pepsin per ml 0.02 M HCl. To each conical flask, 2ml Chloramphenicol C-0378; Sigma-Aldrich Corp., St. Louis, MO, USA (0.5g/100ml

ethanol) was added to inhibit bacterial growth. The flasks were closed and incubated in a water bath at 39° C and stirred continuously for 2 hours.

Step two (Porcine Intestines Simulation)

This step simulated the intestinal digestion of the pig. The mixture from step one was mixed with 80 ml of phosphate buffer (0.2M, pH 6.8) and 20 ml of 0.6M NaOH. The pH was adjusted to 6.8 using 1M HCl or 1M NaOH to provide a stable environment for intestinal enzymes to thrive. To the mixture, 10.6 ml of artificial pancreatin P-1750 Sigma-Aldrich Corp., St. Louis, MO, USA (porcine grade enzyme with 3 x USP activities) containing 100 mg/1 litre buffer was added and incubated at 39°C with continuous stirring for 4 hours. The residues were filtered through a nylon bag (pores size of (42 µm) washed with distilled water, followed by washing two times using 20 ml, 95% ethanol, and 20 ml, 99.5% acetone. The residues were dried on an oven at 70°C for 12 hours and weighed.

3.2.8 The *in-vitro* fermentation phase

The rate of fermentation of the hydrolysed substrate was assessed *in-vitro* by cumulative gas production technique adapted to the pig by Bindelle et al. (2007). A 200 mg sample was used for the third step of this procedure involving microbial fermentation.

i. Donor Animals

The donor animals were 3 Large White barrows with an average weight of 25±3 kg. Pigs were fed a diet containing Prosopis pods meal for 7 days to allow for the adaptation to the diet (Table 3.1). The pig's diet contained no antibiotics. Faecal samples were collected directly from the rectum of the pig and immediately placed in a flask flushed with CO₂ to avoid exposure to aerobic condition and kept in a water-bath at 39°C until time of use. This was achieved by flushed with CO₂. The faeces of the three pigs were pooled together in equal amounts to reduce within animal variation.

Table 3.1 Diet containing *Prosopis juliflora* pod meal (g/100g)

Ingredients	Quantity in g/100g
Maize	60
Soybean meal	21.4
Fishmeal (Omena)	2
<i>Prosopis juliflora</i> pod meal	12
Vegetable oil	2
Di-calcium phosphate	2
Lysine	0.2
Methionine	0.2
Vitamin and mineral premix	0.1
Iodized salt	0.1
Calculated composition	
ME (MJ/Kg)	13.2
Crude protein	17.25

*Vitamin premix: Vitamin and mineral premix: vitamin A 8,000 IU; vitamin D3 2,000; vitamin E 37.5 mg; vitamin K-3 0.925 mg; vitamin B2 8.43 mg; vitamin B12 0.04 mg; nicotinic acid 34.5 mg; pantothenic acid 26 mg; 450 mg Fe; 400 mg Cu; 250 mg Zn; 150 mg Mn; 0.5 mg I; 0.25 mg Se: Omena (*Rastrineobola argentea*)

i. Inoculum Preparation and Incubation

The faecal mixture was blended to homogenise and then filtered through double-layered cheesecloth. The filtrate was mixed at a ratio of 1 part filtrate to 20 part buffer solution on a (volume/volume basis (Bauer et al., 2001).The buffer solution composed of micro and macro minerals, reducing solution and carbonate buffer (Menke et al., 1979). The inoculum prepared was then poured into calibrated 100 ml Poulten and Graf GmbH FORTUNA™ Precision Gas Syringe containing 200 mg substrate in a thermostatically controlled water bath at 39°C. The syringe pistons were lubricated using oil to allow ease of movement during gas measurement throughout the entire process. The whole process was carried out in anaerobic conditions by continuously bubbling of CO₂.

Two syringes containing the inoculum only were used as blanks. Each treatment had 3 replicates and the amount of gas produced during fermentation was measured at 0, 2, 5, 8, 12, 16, 20, 24, 30, 36, 48 and 72 hours. The total gas produced at a particular period of time was

computed as the total increase in volume minus the volume of gas recorded in the blank. The experimental scheme was as follows: 2 runs x 4 treatment groups x 3 replicate + 2 blanks (contained inoculum only).

3.2.9 Dry matter digestibility and fermentation kinetics calculations

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

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

where:

DM_{In} and DM_{RS} are the initial (DM) and residual (DM) respectively

Gas accumulation curves recorded during the 72 hours of fermentation was modified according to monophasic model (Groot et al., 1996).

$$G = \frac{A}{\left[1 + \frac{B^e}{t^e} \right]}$$

where:

G (mL/g DM) = the amount of gas produced per gram of Dry matter (DM) incubated, at time T after incubation.

A (mL/g DM) = the asymptotic gas production.

B (h) = the time after incubation at which half of the asymptotic amount of gas has been formed

C = constant determining the sharpness of the switching characteristic of the profile.

$$R_{max} = \left[A \times (C^B) \times B \times (T_{max}^{(-B-1)}) \right] / \left[1 + (C^B) \times (T_{max}^{(-B)}) \right]^2$$

$$T_{max} = C \times \left[\left(\frac{B-1}{B+1} \right)^{\left(\frac{1}{B} \right)} \right]$$

R_{max} the maximum rate of gas production (ml g⁻¹ x h) and T_{max} , the time at which R_{max} is accomplished was computed using the formula by Bauer et al. (2001).

3.3 Statistical analysis

Data was analysed IBM SPSS Statistics version 22. Assumption for normality and homogeneity of variance of the data was checked using Shapiro-Wilk and Levenes test statistics respectively with data assumed to be normal when ($p > 0.05$). Data on pH was analysed using an independent T test. The IVDMD during hydrolysis, total gas production and fermentation kinetics were analysed using the mixed model procedures. Mean separation

was conducted using Tukey's HSD (Honest Significant Difference) test at 0.05 level of significance. Application of Excel solver in Microsoft excel was used in curve fitting and in the computation of fermentation kinetics.

$$Y_{ijk} = \mu + T_i + G_k + \varepsilon_{ijk}$$

where;

Y_{ijk} = Observation on the dependent variables

μ = overall mean

T_i = Fixed effect due to the i_{th} treatment

G_k = Random effect due to trial

ε_{ijk} = the random error

3.4 Results

3.4.1 The pH of fermented GPPM

The method of fermentation had no effect on the pH of the GPPM (Table 3.2).

Table 3.2 The pH of fermented GPPM

Treatments	pH	<i>p</i> value
Natural	4.6 ±0.08	0.1705
Inoculated	4.4 ±0.07	

Means in the same column with no superscript letters are similar (at 5% level of significance). n=5, ± standard deviation.

3.4.2 Nutrient composition of GPPM fermented naturally and with *Lactobacillus plantarum*

The proximate composition of the fermented is presented on Table 3.3.

Table 3.3 Nutrient composition of GPPM under untreated, enzyme treated, natural and fermentation with *Lactobacillus plantarum*-MTD1 (Ecosyl®)

Nutrient components (g/100g) in DM basis	Natuzyne© multi-enzyme GPPM (T ₁)	Unfermented GPPM (T ₂)	Inoculated fermentation (T ₃)	Natural fermentation (T ₄)
Organic Matter	94.35	94.35	94.15	93.96
Ether Extract	1.34	1.34	1.26	1.17
Crude protein	14.48	14.48	15.50	16.40
NDF	47.41	47.41	41.50	43.41
ADF	27.13	27.13	23.35	26.5
Carbohydrates	78.52	78.52	77.37	76.39
Crude fibre (CF)	31.92	31.92	26.08	26.31
Hemicellulose	20.28	20.28	18.15	16.91

NDF: Neutral Detergent fibre, ADF: Acid detergent fibre, CF: Crude fibre

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

T₁ resulted in the highest digestibility (52.81 ± 1.17) relative to the rest while natural fermentation T₄ had the least (45.6 ± 1.66). T₄ significantly differed from T₁ ($p < 0.05$). Inoculation using *Lactobacillus Plantarum* (T₃) resulted to a higher IVDMD compared to natural fermentation (T₄). There was a 3.68% increase in IVDMD (Fig. 1) after treating the pods with the multi-enzyme (T₁) (52.81 ± 1.17), relative to the untreated (T₂) (49.13 ± 2.14).

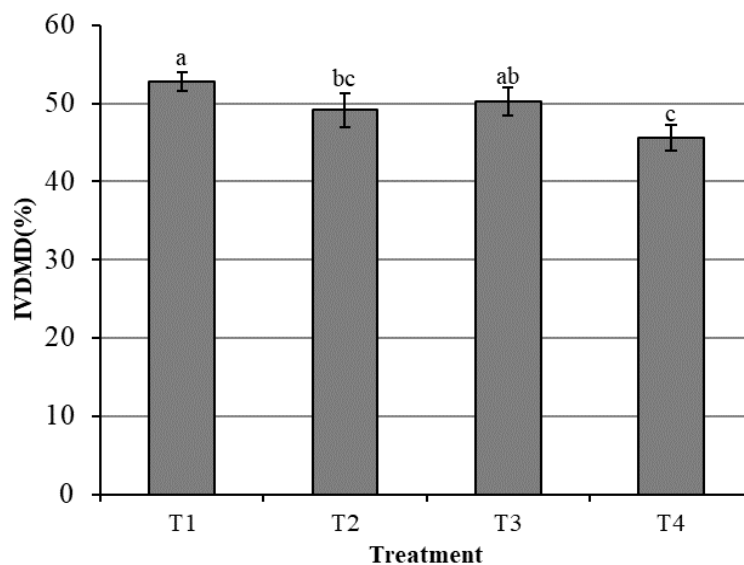


Figure 3.1 The *in-vitro* digestibility of multi-enzyme, fermented and untreated ground Prosopis pod meal. Error bars represent Mean (\pm SE) IVDMD of GPPM.

3.4.4 Fermentation kinetics

Cumulative gas production showed a curvilinear shape (Figure3.2).

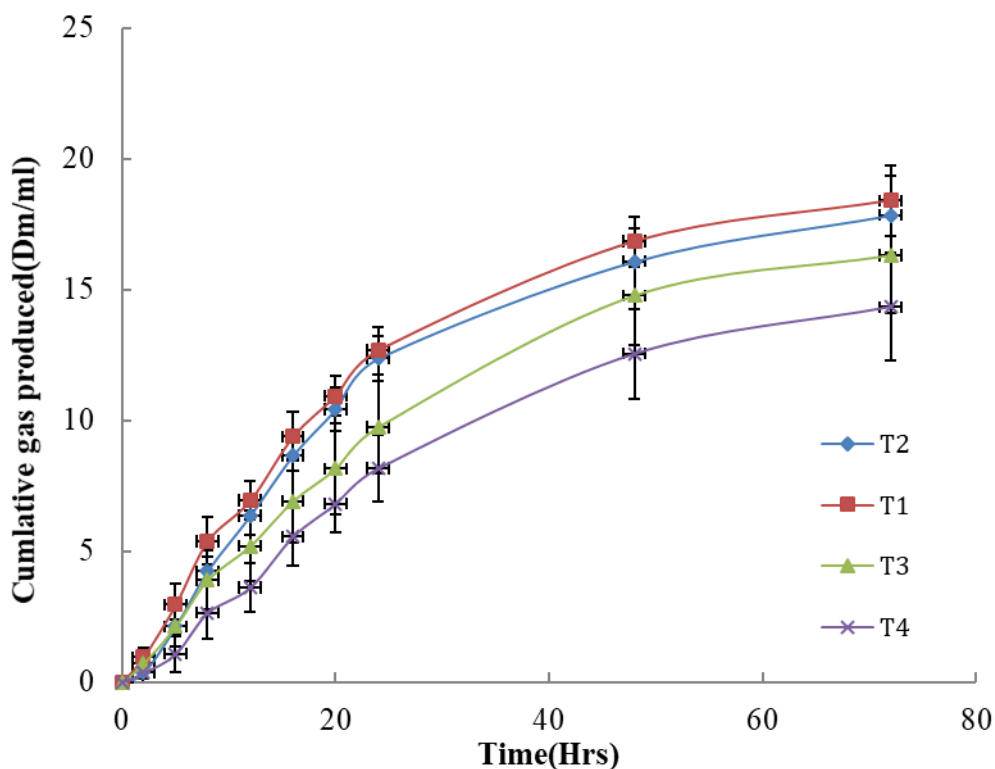


Figure 3.2 Cumulative gas produced in (DM/ml) during the 72 hours period Error bar represents mean (\pm SE)

Treatment 4 (13.42 ± 1.63) had the overall lowest G (ml/g DM) and IVDMD in contrast to T1 (18.50 ± 1.27) which had the highest G (ml/g DM) and IVDMD. However, T1, T2 and T3 were not significantly different $p > 0.05$ (Table 3.4).

Table 3.4 Fermentation kinetics of fermented untreated and enzyme treated Prosopis pod meal

Treatments				
Parameters	T1	T2	T3	T4
G(ml/g DM)	$18.50^a \pm 1.27$	$17.74^{ab} \pm 1.48$	$16.53^{ab} \pm 2.19$	$13.42^b \pm 1.63$
A	$22.19^{ab} \pm 2.26$	$19.72^{ab} \pm 1.84$	$23.22^a \pm 4.63$	$15.28^b \pm 2.30$
C	1.488 ± 0.15	1.734 ± 0.22	1.94 ± 0.48	2.091 ± 0.23
B	$20.34^b \pm 1.53$	$18.36^b \pm 1.01$	$33.98^a \pm 6.76$	$22.01^{ab} \pm 1.74$
T _{MAX} (hrs.)	$5.14^b \pm 1.84$	$7.91^{ab} \pm 1.01$	$13.01^{ab} \pm 5.68$	$19.06^a \pm 1.22$
R _{MAX}	$0.78^a \pm 0.09$	$0.67^a \pm 0.04$	$0.49^b \pm 0.08$	$0.42^b \pm 0.04$
pH	6.89 ± 0.03	6.91 ± 0.04	6.89 ± 0.09	6.95 ± 0.02

Means within a row with the different superscript letters across rows are statistically different $p < 0.05$. T1= enzyme treated Prosopis pod meal, T2= untreated Prosopis pod meal, T3= *Lactobacillus plantarum* inoculated Prosopis pod meal, T4= naturally inoculated Prosopis pod meal. \pm represent standard error of mean .G (ml/g DM): total gas produced corrected in dry matter basis; A: the asymptotic gas production, B: the time after incubation at which half of the asymptotic amount of gas has been formed, C: constant determining the sharpness of the switching characteristic of the profile, T_{MAX} : the time at which R_{max} is accomplished, R_{MAX} : the maximum rate of gas production ($ml\ g^{-1}\ x\ h$).

Enzyme treated GPPM (T1) had the highest rate of gas production R_{max} (0.78 ± 0.09), while naturally fermented pod meal T4 had the lowest rate (0.42 ± 0.04). The rate of gas production was similar between enzyme treated (T1) and control group (T2). T_{MAX} representing the time at which the maximum rate of gas production was achieved was significantly different ($p < 0.05$). Enzyme treated Prosopis pod meal took the shortest time 5.14 ± 1.84 hrs while naturally fermented and inoculated took a longer time. The pH was not significantly different across the different treatment $p > 0.05$.

3.5 Discussion

Alternative feed resources are the predominant feed materials available for pigs in most parts of Kenya (Muthui et al., 2019). The quality of these alternative feeds depends on their nutrient content and the efficiency by which these feed resources are digested and utilized by pigs. The utilization of *in vitro* digestibility fermentation kinetics provides an approach to investigate various alternative feed resources and the various technologies that can be used to improve their utilization and their digestibility. However, various effects cannot be evaluated such as feed intake caused by anti-nutritive factors and the interaction between the digestive system and feed (Kambashi et al., 2014).

Fermentation of feed material can be influenced by several factors: fermentation temperatures/environmental temperatures, feed: water ratio and inoculant used for the fermentation process (Missoten et al., 2010). The results for pH recorded on the 7th day showed that natural fermentation and *Lactobacillus plantarum* fermentation were not different $p > 0.05$. The use of *Lactobacillus plantarum* however had a lower pH (4.4) that lies in the range of 3.5-4.5, considered to be appropriate for a good fermented feed while allowing for effective acid hydrolysis of structural carbohydrates (Plumed-Ferrer et al., 2009).

Fermentation resulted in an improvement in nutritional contents of the pods and a reduction in fibre constituents. Unfermented Prosopis pod in this study had a CF of 31.92%,

26.315% and 26.08% when fermented naturally and with *Lactobacillus plantarum* inoculant respectively. Fermentation has been reported to reduce fibre constituents in fermented feeds (Koo et al., 2018). Odero-waitituh et al. (2020) reported values similar to this study with a crude fibre of naturally fermented (26.6%) and unfermented Prosopis pod meal (32.9%). Wang et al. (2018) reported a decrease in the concentration of hemicellulose and NDF because of the acid hydrolysis and the ability of microbial enzymes released by microbes during ensiling to degrade the fibre. This could explain the decrease in NDF after fermentation.

Consequently, fermentation both *Lactobacillus Plantarum* and natural fermentation lead to loss of organic matter and total carbohydrates. Similarly, Jørgensen et al. (2010) reported a decrease in total starch, sucrose, fructose and total NSP when barley and wheat were subjected to fermentation. The crude protein of the fermented pods increased in comparison to unfermented. The slight increase in protein content may also be due to a decrease of carbohydrate ratio in the total mass resulting in redistribution of nutrient percentages (Olagunju et al., 2018). The improvement in the crude protein content of Prosopis pod meal after fermentation observed in this study was also reported by Odero-waitituh et al. (2020).

Addition of fibre degrading enzyme improved the *in-vitro* digestibility of dry matter of the GPPM by 3.68% compared to the control and by 2.51% and 7.21% respectively compared to *Lactobacillus plantarum* inoculated and naturally fermented GPPM. The cumulative gas production of multi-enzyme treated GPPM was also higher than the control. Enzymes have been postulated to cause disruption and hydrolysis of the plant cell wall integrity and consequently led to release of nutrients encapsulated by the cell wall constituents (De Vries et al., 2012). This could explain the increase in digestibility and cumulative gas production on enzyme treated GPPM. Similarly Lee et al. (2018) reported an improved in IVDMD when solvent and cold extracted canola cake rich in crude fibre was treated with multi-enzyme complex. The multi-enzyme treated GPPM was similar to *Lactobacillus plantarum* inoculated GPPM but significantly different to natural fermentation in terms of cumulative gas produced and IVDMD.

Fermentation using natural fermentation (T4) decreased IVDMD by 3.5%, while *Lactobacillus plantarum* (T3) improved by 1.2 % compared to the untreated GPPM (T2) ($p>0.05$). The lower IVDMD of natural fermentation compared to *Lactobacillus plantarum* could be partly explained by lower fibre content associated with fermentation with *Lactobacillus plantarum*. Additionally natural fermentation is associated with higher nutrient

losses due to the longer time it takes to achieve a lower pH that inhibit growth of organism such as yeast associated with loss of organic matter (Huyen et al., 2020). Contrary, to our study Jørgensen et al. (2010) reported that the ileal digestibility of dry matter increased by 6 and 3 percentage units by fermentation of barley and wheat, respectively. Barley and wheat contain a higher amount of starch composition 64.8% and 69.5% respectively. Gelatinization and starch swelling might happen during the fermentation process making the starch granules more accessible for α -amylase in the small intestine (Zentek & Boroojeni, 2020). The higher ileal digestibility of barley and wheat could be as a result of increased starch digestibility. This is unlike Prosopis pods which contain a lower proportion of starch but more of soluble sugars (Choge et al., 2007), that are usually lost during the fermentation process.

The *in-vitro* gas production methods can provide a better mechanism for feed evaluation as they are ethical, superior, faster and less expensive than *in-vivo* method (Coles et al., 2005). Fermentation (T4) and (T3) did not result in an improvement in cumulative gas production compared to the control. Additionally, both the rate of gas produced (R_{max}) in natural and inoculated fermentation was significantly lower compared to the control despite the lower amount of crude fibre associated with fermentation. It has been reported that Prosopis pod meal is a rich source of saccharose (20-25% DM) and reducing sugars (10-20% DM) (Chaturvedi & Sahoo, 2013).

Pre-treatment using fermentation could have led to losses of some rapidly fermentable carbohydrates as evident by the reduction of hemicellulose and total carbohydrates. These rapidly fermentable components could have been hydrolysed or used up during microbial fermentation (Koo et al., 2018). This therefore, meant that the residues comprised of slowly fermentable and non-fermentable components whose rate of degradation was lower. This is in agreement with previous findings of (Bindelle et al., 2007) who reported a relatively slower fermentation rate in feed ingredients especially those rich in insoluble and less in soluble fibre. The lower half time asymptotic associated with multi-enzyme treated and untreated compared to the GPPM treated with *Lactobacillus plantarum* (T₃) implied that fermentable organic matter depleted within a shorter period of incubation time.

R_{max} can be obtained rapidly following incubation in case of fermentation of soluble feedstuff components which do not require colonization under high microbial density (Groot et al., 1995). R_{max} is negatively correlated to T_{max} , therefore substrate with higher R_{max} results to a lower T_{max} . Inoculated, multi-enzyme treatment and control did not significantly differ in T_{max} . However, multi-enzyme treatment and control resulted to a lower numeric value than both natural and inoculated fermentation. This could be explained by the low

amount of fermentable carbohydrates associated with fermented GPPM. From the study pre-treatment technologies affected the digestibility of Prosopis pod meal. This conclusion was similar to that of Groot et al. (1996) who concluded that pre-treatment technologies applied to the substrate affect digestibility and degradability of substrate in the hindgut of pigs.

3.6 Conclusion

Based on this study, pre-treatment of Prosopis pod with exogenous enzyme improved *in-vitro* digestibility compared to untreated, natural and *Lactobacillus plantarum* induced-fermentation. Cumulative gas produced improved slightly but was not significant. Naturally fermented Prosopis pod meal had a low digestibility and fermentability, therefore, it is not a suitable pre-treatment method for improving the digestibility of the pods for pigs.

CHAPTER FOUR

EFFECT OF FEEDING ENZYME TREATED *PROSOPIS JULIFLORA* POD MEAL-BASED DIETS ON THE GROWTH PERFORMANCE OF GROWER PIGS

Abstract

A feeding trial was conducted to investigate the effect of inclusion of enzyme treated ground *Prosopis* pod meal (GPPM) on the performances of growing pigs. Thirty Landrace x Large White crosses of 20 ± 3 kg with an equal number of barrows and gilts were allotted to five treatment diets in a randomized complete block design with each treatment having six pigs. The dietary treatments were: T1 - containing 0% GPPM and 0.035% enzyme per kg of diet, T2 - containing 0% GPPM and 0% enzyme, T3 - containing 10% GPPM and 0.035% enzyme, and T4 - containing 20% GPPM and 0.035% enzyme, T5; diet containing 30% GPPM and 0.035% enzyme per kg of diet, respectively. The enzyme was added to the dietary treatment according to manufacturer's instructions at a rate of 350mg/kg feed material. Results from proximate analysis showed that the level of ADF and NDF increased as the amount with the level of inclusion of GPPM in the diet. The average daily gain (ADG) of T1, T2 and T3 were not significantly different. However, ADG for T1 (0.63 ± 0.07 kg/day) was significantly different from T4 (0.44 ± 0.04 kg/day) and T5 (0.42 ± 0.05 kg/day). Pigs fed T5 showed the lowest daily feed intake (DFI) relative to T1. The DFI of pigs fed T1 was not different from those fed T2, T3 and T4 ($p>0.05$) but T1 significantly differed ($p<0.05$) with T5. Feed conversion ratio (FCR) increased as the levels of GPPM increased with T1 (2.70 ± 0.24) and T5 (3.38 ± 0.340) having the lowest and highest FCR respectively. Therefore, GPPM with fibrolytic enzyme can replace 30% of growers pig diet without affecting average daily gain. However average daily feed intake and feed conversion ratio significantly reduced at 30% inclusion.

4.1 Introduction

Pigs play crucial social economic roles to smallholders farmers in Kenya while providing a cushion in times of financial crisis (Kagira et al., 2010). Pig farming is becoming highly relevant to farmers shifting from ruminants to non-ruminants due to the short breeding cycle, high fecundity and high feed conversion ratio (Chia et al., 2019). However, pig production has recently been experiencing increased cost of feed ingredients mainly due to the increased competition for food cereals and oilseeds with man (De Vries et al., 2012).

Smallholder farmers have begun to focus and rely more on alternative feed resources such as leguminous tree pods (acacia and *Prosopis juliflora* pods) and leaves (*Calliandra*

calothyrsus and *Sesbania sesban* leaves). *Prosopis juliflora* tree is an exotic invasive plant widely distributed in arid and semi-arid regions due to its ability to withstand harsh and dry climatic conditions of Kenya (Odero-Waitituh et al., 2016). *Prosopis juliflora* pods from these trees have been evaluated by several authors and were found to be of good nutritional quality for both ruminants and non-ruminants (Manhique et al., 2017; Odero-Waitituh et al., 2016). However, the pods contain a high level of plant cell wall components that lower the efficiency of utilization of the nutrients by non-ruminants which lack endogenous fibre digestive enzymes (De Vries et al., 2012). Al-Marzooqi et al. (2015) reported that *Prosopis* pods meal contain 69.5% cellulose, 53.5% hemicellulose and 97% higher lignin content relative to maize.

Dietary fibre including pectins, cellulose, hemicellulose, β -glucans, fructans, oligosaccharides, lignin, and resistant starch affects the voluntary feed intake of pigs which ultimately affect growth performance (Muthui et al., 2019). Furthermore, the apparent digestibility of fibre is (40-50%) in growing pigs, this inevitably reduces the energy value of feed (Noblet et al., 2001). One way to counteract the effect of low digestibility of the pod and improve the nutritive values of the pod has been the use of exogenous enzymes (Al-Marzooqi et al., 2015). Use of exogenous enzymes has been associated with reduction of viscosity of digesta and improvement in energy, protein and fats digestibility. This in turn increases the surface area between food, absorptive surface of the gut and endogenous enzymes (Muthui et al., 2019). The objective of this study was to determine the effect of feeding enzyme treated *Prosopis* pod meal based diets on the growth performance of grower pigs.

4.2 Materials and methods

4.2.1 Experimental location

The feeding trial was conducted in a commercial pig breeding farm in Molo Sub-County, Nakuru County. The area is 0° 35 9' South and 0° 35 15' East. The area is 2400 – 3100 mm above sea level and receives an average annual rainfall of 1200-1500 mm. The average temperature is 12°C in the coolest seasons, 23°C during the wet seasons and 27°C during the hot dry periods (Nyakeya et al., 2018).

4.2.2 Preparation of the *Prosopis* pod meal and experimental diets

The diets were formulated using maize germ, wheat bran, sunflower seed meal, omena (*Rastrineobola argentea*) and GPPM (Table 4.1).

Table 4.1 Chemical Composition of the experimental diets (g/100g)

Ingredients	Treatments				
	T1	T2	T3	T4	T5
Wheat bran	15.0	15.0	8.50	4.00	2.80
Maize germ	53.2	53.2	48.6	42.7	32.8
Vegetable oil	5.0	5.0	5.0	5.0	5.0
GPPM	0.0	0.0	10.0	20.0	30.0
Omena	6.0	6.0	5.50	5.0	5.0
Sunflower seed cake	17.5	17.5	19.0	20.0	21.0
Lysine	0.90	0.90	0.90	0.90	0.90
Methionine	0.50	0.50	0.50	0.50	0.50
DCP (granular 24%)	0.50	0.50	0.50	0.50	0.50
Limestone	0.85	0.85	0.85	0.85	0.90
Iodized salt	0.25	0.25	0.25	0.25	0.25
Vitamin premix*	0.250	0.25	0.25	0.25	0.25
Mycotoxins binder	0.10	0.10	0.10	0.10	0.10
Natuzyme® enzyme	0.035	0.000	0.035	0.035	0.035
Chemical analysis					
Dry matter	88.35	88.35	88.75	88.52	90.47
Crude Protein (CP)	17.01	17.01	17.02	16.81	17.01
Ether Extracts	7.90	7.90	6.93	5.715	6.51
Ash	8.27	8.32	8.33	12.33	13.11
NDF	32.63	32.63	34.38	35.63	37.13
ADF	11.10	11.10	12.60	13.65	16.94
GE (MJ/kg DM)	17.10	17.10	16.00	16.00	16.10

T1 = 0% GPPM and 0.035% enzyme per kg of diet; T2 = 0% GPPM and 0% enzyme per kg of diet; T3 = 10% GPPM and 0.035% enzyme per kg of diet, T4 = 20% GPPM and 0.035% enzyme per kg of diet, T5= 30% GPPM and 0.035% enzyme per kg of diet; GPPM ground Prosopis pod meal; *Vitamin and mineral premix: vitamin A 8,000 IU; vitamin D3 2,000; vitamin E 37.5 mg; vitamin K-3 0.925 mg; vitamin B2 8.43 mg; vitamin B12 0.04 mg; nicotinic acid 34.5 mg; pantothenic acid 26 mg; 450 mg Fe; 400 mg Cu; 250 mg Zn; 150 mg Mn; 0.5 mg I; 0.25 mg Se CP =Crude Protein; NDF= neutral detergent fibre; ADF= acid detergent fibre; GE= Gross energy.

Collection and preparation of GPPM is similar to that explained in chapter 3. Natuzyme® was included at the rate of 350 mg/kg of feed in dry form as per the manufacturer's instructions and recommendations.

The compositions (per kg of diet) of the diets were as follows:

1. T1 = diet containing 0% GPPM and 0.035% enzyme
2. T2 = diet containing 0% GPPM and 0% enzyme
3. T3 = diet containing 10% GPPM and 0.035% enzyme
4. T4 = diet containing 20% GPPM and 0.035% enzyme
5. T5 = diet containing 30% GPPM and 0.035% enzyme

4.2.3 Management of experimental animals

The experimental animals were identified using ear tags. They were placed in pens with concrete floors (3m x 3m) that had dry wood shavings as beddings. Experimental animals were dewormed using subcutaneous injectable Ivermectin® to control external parasite and provided with injectable multivitamin before the start of the experiment. The pigs used for the experiment were fed on the experimental diets for 7 days of adaptation before the beginning of the data collection. The experimental animals were fed from concrete troughs. Water was provided *ad libitum* using drinking-nipples throughout the feeding trial (35 days) period. Biosecurity measures were put in place to prevent diseases while the well-being of the pigs was monitored to identify sick animals.

4.2.4 Experimental design

Experimental animals used were 15 barrows and 15 gilts with an average weight of 20±3kg, which were crosses of Landrace and Large white. They were randomly allotted to the five treatment diets in a randomized complete block design with sex as a blocking factor. There were two pens per treatments with each pen having 3 pigs each. Barrows and gilts were kept in separate pens during the entire experimental period.

4.2.5 Proximate analysis

The feed samples from each experimental diet were collected and taken to Egerton University, Animal Nutrition Laboratory for proximate analysis. The proximate analyses and constituents of the cell wall, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were conducted using methods described in Chapter 3.

4.2.6 Data collection

Individual pig body weight from each pen was recorded on a weekly basis using a digital weighing scale generic weighing scale 50kg portable hanging balance with 10 grams accuracy. The weekly weights recorded were used to compute the body weight gains. Feed was offered at 0800 am while refusals were collected during the next day prior to feeding. The refusals were weighed using a digital weight balance then used to compute daily feed intake. Body weight gains and daily feed intake were used to compute the feed conversion ratio. The formulae (1, 2 and 3) show the equations used to calculate the production performances:

$$1. \text{Average daily gain (ADG) in kg/day} = \frac{(\text{Final Weight} - \text{Initial Weight})}{7 \text{ days}}$$

$$2. \text{Daily feed intake} = \text{Feed offered (kg)} - \text{Feed refusal (kg)}$$

$$3. \text{FCR} = \text{Feed intake} / \text{Weight gain.}$$

4.3 Statistical analysis

Data were analysed using SAS 9.0 (2002) using a two-way analysis of variance. Mean separation was conducted using Tukey's HSD (Honest Significant Difference) test at 0.05 level of significance. Means were considered different if $p < 0.05$. Initial weight of the pigs was fitted as a covariate while sex was used as a blocking factor.

The model used was:

$$Y_{ijk} = \mu + T_i + \beta_k + S_f + \varepsilon_{ijk}$$

where;

Y_{ijk} = response variable of interest (ADG, FCR)

μ = population mean

T_i = fixed i^{th} treatment effect (T1, T2, T3, T4 and T5)

S_f = fixed effect of sex (gilts and barrows)

β_k = fixed effect of initial weight used as a covariate

ε_{ijk} = random error

4.4 Results

4.4.1 Chemical composition of the experimental diets and *Prosopis juliflora* pod meal

The chemical composition of the diets and the *Prosopis* pod meal was done in duplicates. The results of DM, CP NDF, and ADF of the diets and *Prosopis* pod meal are presented in Table 4.1 and Table 4.2.

Table 4.2 Chemical composition of Prosopis pod meal

Nutrient components (g/100g)	Ground <i>Prosopis juliflora</i> pod meal
Dry matter	92.94
Ash	6.35
Ether Extracts	1.34
Crude protein	14.48
NDF	47.41
ADF	27.13

Results of proximate analysis (Table 4.2) showed an increase of NDF and ADF as the level of inclusion of GPPM increased in the diet. Diets with 30% Prosopis pod meal with enzyme (T5) had the highest NDF and ADF, 37.13 and 16.94% respectively. The ash content also increased as the level of inclusion of Prosopis pod meal increased. The diets were isonitrogenous and isocaloric.

4.4.2 Growth performance of the pigs

Pigs fed the diets readily accepted the diet though in the third week they experienced diarrhoea which was treated (bolus containing S/DIME[®] 16% Solution Sulphadimidine). The diets had a significant effect on FCR ($p<0.05$) with T1 and T5 being significantly different (Table 4.3).

Table 4.3 Effect of experimental diets on growth performance of pigs

Item	Dietary treatments					P value	
	T1	T2	T3	T4	T5	Diet	Sex
ADG	0.63±0.05 ^a	0.517 ^{ab} ±0.07	0.516 ^{ab} ±0.06	0.440 ^b ±0.04	0.420 ^b ±0.05	<.0001	0.55
FCR	2.707 ^a ±0.19	2.833 ^a ±0.14	3.058 ^a ±0.32	3.275 ^a ±0.32	3.383 ^b ±0.33	0.037	0.77
DFI	1.467 ^a ±0.05	1.439 ^a ±0.05	1.477 ^a ±0.06	1.330 ^{ab} ±0.08	1.271 ^b ±0.04	<.0001	0.01
IW	22.720±0.58	21.593±0.89	22.295±0.07	22.405±0.80	21.688±0.25	0.525	0.12
FW	40.950 ^a ±0.95	37.370 ^b ±0.23	37.580 ^b ±0.42	35.650 ^c ±0.05	34.470 ^c ±0.68	0.002	0.02

Means within a row with the different superscript letters are statistically different ($p < 0.05$). SEM: standard error of mean. T1 = 0% GPPM and 0.035% enzyme per kg of diet; T2 = 0% GPPM and 0% enzyme per kg of diet; T3 = 10% GPPM and 0.035% enzyme per kg of diet, T4 = 20% GPPM and 0.035% enzyme per kg of diet, T5 = 30% GPPM and 0.035% enzyme per kg of diet; ADG = Average daily gain (kg/day); DFI daily feed intake (kg); FCR feed conversion ratio, IW = initial weight (kg), FW = final weight (kg).

Higher inclusion levels of GPPM in the diets led to an increase in the FCR. Pigs fed on T1 (2.707) and T5 (3.383) had the lowest and the highest FCR respectively. Diet significantly affected ADG ($p < 0.05$) while sex did not affect the ADG ($p = 0.5554$). The mean body weight gain of T1, T2 and T3 were not significantly different. However, as the levels of inclusion of GPPM increased, the ADG of the pigs decreased with T1 (0.634 kg/day) being significantly different from T4 (0.440 kg/day) and T5 (0.420 kg/day). Diet and sex had an effect ($p < 0.05$) on the DFI with barrows having a relatively higher weight compared to gilts. Diet with 30% GPPM and enzyme had the lowest DFI that significantly differed from T1, T2 and T3 at ($p < 0.05$).

Initial weight was not significantly different across all the experimental diets conversely, sex ($p = 0.122$) did not affect initial weight however, diet had a significant effect ($p < 0.05$) on the final weight of pigs (Table 4.3). Mean final weights for pigs fed on diet with 30% Prosopis pod meal and enzyme was significantly lower compared to T2. However, T5 and T4 had similar final weights. Weekly body weights were significantly different from the dietary treatment ($p < 0.05$) while the body weights increased from week 1 to week 5 ($p < 0.05$) for both male and female pigs. The model was highly significant with 96.8% variation being explained. Pigs fed on diet T5 had a lower weekly weight relative to other pigs fed on the other diets for the whole feeding period (Figure 4.1).

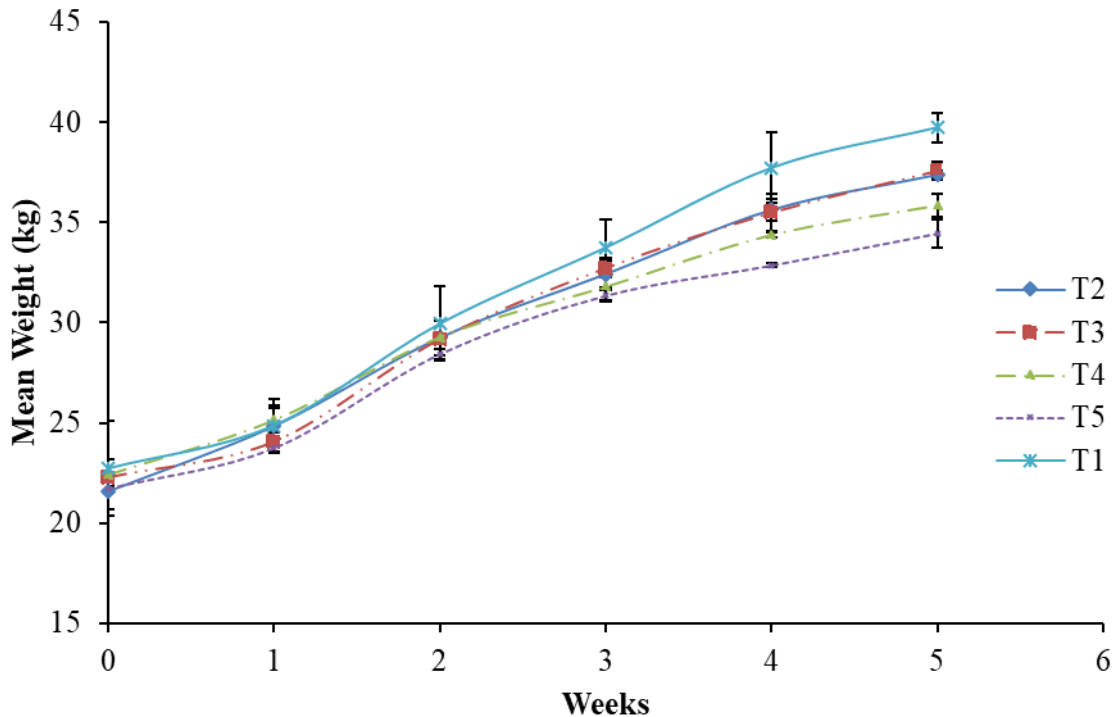


Figure 4.1 Mean weekly weight of pigs fed diets with or without *Prosopis juliflora* meal and Natuzyme®.

T1 = 0% GPPM and 0.035% enzyme per kg of diet; T2 = 0% GPPM and 0% enzyme per kg of diet; T3 = 10% GPPM and 0.035% enzyme per kg of diet, T4 = 20% GPPM and 0.035% enzyme per kg of diet, T5= 30% GPPM and 0.035% enzyme per kg of diet. Error bars indicate \pm Standard error

4.5 Discussion

The DM, ash, crude protein, NDF and ADF composition of GPPM in this study were within the range cited by several authors (Sawal et al., 2004).Manhique et al. (2019) reported that the proximate composition of *Prosopis juliflora* pods from Baringo was 94.4% DM, 12.56% CP, 4.37% ash, 45.87% NDF and 29.71% ADF which were close to those in this study. However, the DM content was higher while both ash and crude protein were lower than those reported in study. Samples for this study were collected from communal grazing lands. The presence of livestock in the silvo-pastoral systems contributes to a higher total carbon and nitrogen contents on leguminous shrubs which in turn causes change the nutritional contents of the various parts of the plant (Lira et al., 2020).This could explain the higher crude protein associated with the pods from this study.

The nutrient content of the GPPM in this study contained a higher crude protein of 14.48% in comparison to maize bran 11%, maize grain 8.25%, and industrial and kitchen

swills which are the most common used feedstuffs for pig production in Kenya (Muthui et al., 2019). This, therefore, indicates that GPPM contains a crude protein level that can be valuable for pig nutrition.

4.5.1 Production performance of grower pigs

Feed intake is influenced by many factors including animal characteristics such as body weight, genetic adaptations, and feed factors such as nutrients density, digestibility, and the presence of anti-nutritive factors (Choct et al., 2010). Similarly, factors that affect feed intake also influence feed conversion ratio. In this study, there was a significant decrease in feed intake and a reduction in feed efficiency as measured by increasing FCR when GPPM was included at 30% + enzyme (T5) a diet with 371.3g/kg DM NDF and 169.40 g/kg DM ADF.

Several reasons could have contributed to the reduced feed intake and feed efficiency. Firstly, the physical characteristics of Prosopis pod meal is usually highly fibrous with high amount of ADF and NDF. Diets with a high amount of fibre are associated with an increased bulkiness in the gut and thus reduces feed intake (Ndou et al., 2014). Further, the high amount of insoluble fibre could have led to increased water holding capacity (as most of these insoluble fibres such as cellulose behave like sponges within the gastrointestinal tract) that eventually lead to gut fill, thus depressing feed intake (Jang et al., 2019; Ndou et al., 2014). Our results corresponds to Ndou et al. (2014) who reported that the gut capacity was attained when weaners pigs were offered a diet containing an NDF of 367 g/kg DM and ADF of 138 g/kg DM above which feed intake starts to reduce. It is interesting to note that values for NDF and ADF of the diet with 30% GPPM had surpassed the values by (Ndou et al. 2014) where feed intake starts decreasing. However, this could not in entirety explain the decrease in feed conversion ratio and feed intake as the effect of fibre on gut fill diminishes as the pig ages (Ndou et al., 2014). Therefore, other factors such as the level of tannins could have resulted in decreased feed intake.

Although effect of tannins in Prosopis pod meal on performance were not investigated, tannins are usually bitter and astringent, and thus affects feed intake. In fact, tannins have been attributed to reduced dietary protein digestibility (Annor et al., 2017) as a result of their ability to form insoluble complexes with both digestive enzymes and dietary protein thus reducing feed conversion ratio. The low feed intake and feed efficiency from this study are consistent with (Pinheiroer et al., 1993) who reported a significant reduction in feed intake and feed conversion when Prosopis pod meal replaced the 30% maize soybean mixture in the

diets of pigs. Other authors have also reported that nutrient intake were depressed in animals that consumed diets containing more than 200 g/kg of Prosopis pod meal due to the large amounts of fibre and tannins that suppressed feed intake (Obeidat et al., 2008; Odero-Waitituh et al., 2016).

Results from the average daily gain (ADG) of T2 compared to T3, T4, and T5 were not significantly different though there was a decrease in the feed intake as the level of GPPM increased across the diets. This is in contrast to (Pinheiroer et al., 1993) who reported a reducing ADG as prosopis gradually replace a maize-soybean mixture in the diet of finishing pigs, at 30% level of inclusion. A number of reasons could explain this, first as evident from the *in-vitro* trial multi-enzyme complex increases IVDMD of Prosopis pod meal. This is due to the fact that multi-enzyme mixtures have been known to improve the digestibility of protein, energy and fats by reducing the viscosity of the digesta and hydrolysing the non-starch polysaccharides (Torres-Pitarch et al., 2017). This increases the contact between the feed and digestive enzymes as well as the digestive surfaces. Secondly, the addition of multi-enzyme has been reported to improve fibre digestibility as a result of fermentation of the fibre in the hindgut with the volatile fatty acids produced contributing to the net energy requirements of the pigs needed for growth (Nkosi et al., 2020). This could therefore explain why the inclusion of Prosopis pod meal up to 30% of the diet had no negative effect on growth rate as the enzyme probably counteracted the effects of increasing fibre in the diets. Similarly, Kwon et al. (2020) reported higher inclusion levels of palm kernel meal (PKM) for growing-finishing pigs of 12% compared to a conservative recommended inclusion level of 10% due to supplementation of β -mannanase enzyme to diets containing PKM.

The addition of enzyme to the diet without GPPM (T1) resulted in a 22.63% increase in the ADG of pigs compared to T2 though the differences were not significant. This could be explained by the fact that T1 and T2 had met the nutrients requirements of pigs whilst having minimal fibre content The growth of animals fed these diets reached the commercial expectation, and thus, significant improvement by multi-enzyme over that which had already been achieved would have been unlikely. This finding is consistent with other studies e.g. (l'Anson et al., 2014; Nkosi et al., 2020) who reported that the extent to which enzyme supplementation improves nutrient digestibility tends to be low when using highly digestible ingredients thus improvement in growth rates are not observed.

4.6 Conclusion

Inclusion of GPPM with Natuzyme® multi-enzyme complex at 30% resulted to a decline in the feed intake. Inclusion of GPPM with Natuzyme® multi-enzyme complex at 30% resulted to a decline in the feed conversion ratio.

CHAPTER FIVE

EFFECT OF INCLUSION OF TREATED *PROSOPIS JULIFLORA* POD MEAL IN THE TOTAL RATION ON COST OF FEEDING GROWER PIGS

Abstract

Feed cost account 70% of the total cost of production in pig production enterprises. The use of non-convectional feed material that are cheaper and can be a viable option to counteract the high cost of feed. The objective of this study was to determine cost of pig production of inclusion of treated mature *Prosopis* pod meal in growing pig's diets. Data collected from daily feed intake and average daily weight gain during the feeding trial in chapter 4 was used in the computation of the feed cost per weight gain. The feed cost was then calculated as the product of feed intake per pig and the cost per kg of each treatment diet. Total feed cost per kilogram of gain of each treatment was then calculated as the total feed cost divided by the body weight gain of each pig per treatment diet. Data was analysed using SAS 9.0 (2002) using a two way analysis of variance with sex as a blocking factor. Mean separation was conducted using Tukey's HSD. From the results T5 (124.54 Kes/kg) resulted to the lowest feed cost per kilogram gain compared to T2, T3 and T4 (132.20 Kes/kg). Enzyme inclusion resulted to a lower feed cost per kilogram gain when T1 (111.13 Kes/kg) compared to T2 (140.80 Kes/kg) ($p < 0.05$). Inclusion of GPPM with Natuzyme® multi-enzyme complex at 30% resulted to the lowest cost of production compared to other diets containing *Prosopis juliflora* pods. The inclusion of enzyme on the diets lowered the cost per kg weight gain.

5.1 Introduction

Feed cost is a major factor constraining increased pork production thus impacting pork producer's profitability (Choi et al., 2015). The high cost of conventional feed has meant more livestock producers use agro-industrial by-products and/or unusual feeds such as *Prosopis* pods meal (GPPM). These alternative feeds may be economically advantageous in reducing feeding costs and can play an important role in feeding livestock under various management systems. Carter et al. (2013) observed that for farmers in western Kenya to earn profits from pig enterprises, pig rearing must include some zero or low-cost feedstuffs.

Bio-economic value analysis is important in ensuring that animal production makes economic sense especially when a feed is being investigated to replace the conventional feed. Several researchers have reported increased feed costs per unit muscle deposition when livestock feed ingredients were treated. For instance, Odero-Waitituh et al. (2020) reported a reduction in cost when fermented *Prosopis* pod meal and seeds were included in the animal's

diets. Further, combining exogenous enzyme with non-convectional ingredient was observed to be a valid practice to reduce the cost of feeding and to allow better utilization of non-convectional feed material. These feed materials are regarded to be of low digestibility due to their fibre content that is difficult to be utilized by the pig endogenous enzymes (Al-Khalaifah et al., 2020). However, there is scanty information available on the economics implication of feeding grower pigs on enzyme treated Prosopis pod meal. The study aim was to evaluate the economic implication of feeding enzyme treated prosopis pod meal on the cost of feeding. The objective of this experiment was therefore to determine the cost of production inclusion of enzyme-treated mature Prosopis pod meal in growing pig diets.

5.2 Material and methods

Data collected from daily feed intake and average daily weight gain during the feeding trial in Chapter 4 was used in the computation of the feed cost per weight gain. The feed cost per diet was computed by multiplying the price per kilogram of each ingredient by the proportion of each ingredient including the enzyme where appropriate in the five diets presented at Table 5.2. Recent prices of ingredients at the time of conducting the experiments were used (2nd January 2020). The cost of per kg of each ingredient was calculated based on the collection fee paid for the case of Prosopis pod meal, transport cost and the cost for milling and mixing.

Total feed cost was then calculated as the product of total feed consumed during the experimental period and the cost per kg of each diet. Thus, the total feed cost per kilogram of gain (Kes/kg) equals total feed cost divided by total body weight gain. This methodology compares the feed cost for 1 kg weight gain (Choi et al., 2015).

$$\text{Feed cost per weight gain} = \frac{\text{Feed cost (Kes/kg)} \times \text{Feed intake per head/kg}}{\text{Weight gain per head /kg}}$$

5.3 Statistical analysis

Data collected from feed cost per weight gain (Kes/kg) were subjected to the analysis of variance using the general linear model procedure of statistical analysis system version 9.0. Mean separation was conducted using Tukey's HSD (Honest Significant Difference) test at 0.05 level of significance. Means were considered different if $p < 0.05$. Initial weight of the pigs was fitted as a covariate while sex was used as a blocking factor.

The model used was:

$$Y_{ijk} = \mu + T_i + \beta_k + S_f + \varepsilon_{ijk}$$

where;

Y_{ijk} = response variable of interest

μ = population mean

T_i = fixed i_{th} treatment effect (T1, T2, T3, T4 and T5)

S_f = fixed effect of sex (gilts and barrows)

β_k = fixed effect of initial weight used as a covariate

ε_{ijk} = random error

5.4 Results

Table 5.1 Economics of feeding enzyme treated Prosopis pod meal to grower pigs

Item	Dietary treatments					P value	
	T1	T2	T3	T4	T5	Diet	Sex
FC:ADG	111.1 ^a ±0.02	140.80 ^b ±2.0	137.98 ^b ±7.8	132.20 ^b ±3.6	124.54 ^c ±0.8	0.03	0.52
ADG	0.63±0.05 ^a	0.517 ^{ab} ±0.07	0.516 ^{ab} ±0.06	0.440 ^b ±0.04	0.420 ^b ±0.05	<.0001	0.55
Cost/kg (Kes)	43.7	43.4	41.9	39.9	38.0		

Means within a row with the different superscript letters are statistically different ($p < 0.05$).

T1 = 0% GPPM and 0.035% enzyme per kg of diet; T2 = 0% GPPM and 0% enzyme per kg of diet; T3 = 10% GPPM and 0.035% enzyme per kg of diet, T4 = 20% GPPM and 0.035% enzyme per kg of diet, T5 = 30% GPPM and 0.035% enzyme per kg of diet; ADG = Average daily gain (kg/day); FC:ADG = Feed cost per kilogram gain of the diets (Kes/kg), Kes: Kenya shilling, ± represent standard error of mean

Table 5.2 Feed composition and feed cost computation for experimental diets

Ingredients	Price Kes/kg	Proportion of the diets					Cost of fraction per 100kg				
		T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Wheat bran	18	15	15	8.5	4	2.8	270	270	153	72	50.4
Maize germ	28	53.2	53.2	48.6	42.7	32.8	1489.6	1489.6	1360.8	1195.6	918.4
Vegetable oil	120	5	5	5	5	5	600	600	600	600	600
GPPM	8	0	0	10	20	30	0	0	80	160	240
Omena	120	6	6	5.5	5	5	720	720	660	600	600
Sunflower seed cake	30	17.5	17.5	19	20	21	525	525	570	600	630
Lysine	250	0.9	0.9	0.9	0.9	0.9	225	225	225	225	225
Methionine	720	0.5	0.5	0.5	0.5	0.5	360	360	360	360	360
DCP (granular 24%)	90	0.5	0.5	0.5	0.5	0.5	45	45	45	45	45
Limestone	6	0.85	0.85	0.85	0.85	0.9	5.1	5.1	5.1	5.1	5.4
Iodized salt	7	0.25	0.25	0.25	0.25	0.25	1.75	1.75	1.75	1.75	1.75
Vitamin premix*	250	0.25	0.25	0.25	0.25	0.25	62.5	62.5	62.5	62.5	62.5
Mycotoxins binder	320	0.1	0.1	0.1	0.1	0.1	32	32	32	32	32
Natuzyne® enzyme	870	0.035	0	0.035	0.035	0.035	30.45	0	30.45	30.45	30.45
		100	100	100	100	100	4366.4	4335.95	4185.6	3989.4	380.9
		Cost of feed in Kes/kg					43.7	43.4	41.9	39.9	38

From Table 5.1, T5 had the lowest feed cost per kilogram gain when compared with T2, T3 and T4 ($p < 0.05$). T1 resulted to a lower feed cost per kilogram gain compared to the negative control that had a significantly higher feed cost per kilogram gain. Inclusion of Prosopis pod meal at 30% + enzyme resulted to a 16.23 KES in savings in the feed cost per kg when compared to the T2. Effects of sex on feed cost per kilogram gain was not significant at ($p < 0.05$).

5.5 Discussion

The high cost of feed is a result of the competition between man and livestock for these feed ingredients (Madubuike et al., 2006). The smallholders farmers rearing pigs in the tropics are often capital constrained therefore they are unable to access commercial feeds, hence the use of cheaper alternative feed ingredients seems to be a more attractive option (Asindu et al., 2020). The cost of feeding the pigs was lower in T5 compared to T2, T3 and T4. Diets with 30% Prosopis pod meal with enzyme T5 had the highest inclusion of Prosopis pod meal; as a consequence, it replaced a considerable amount of wheat bran and maize bran which were considerably expensive. Prosopis pod meal for the study were obtained at a lower cost relative to other energy or protein ingredients used in this study as only the cost of labour for collection; transport and milling cost were factored in. These results were consistent with the findings of Obeidat et al. (2013) in a feeding trial with lambs using Prosopis pod meal-based diets.

The use of enzyme resulted in a higher growth rate as observed in Table 4.3 this could explain the low cost per kg when T1 was compared to T2. Although the inclusion of Prosopis pod meal up to 30% led to a decline in growth rates, the lower market price of GPPM compensated for the reduced weight gain on 30% GPPM so that the cost per kg carcass was not affected by diet. However, it should be noted that as the distance to areas where Prosopis pods are obtained increases, the cost of the pods tends to increase. As such, the use of Prosopis pods might be less economical in peri-urban areas than in remote countryside farms which are closer to Prosopis pod tree vegetation. Kambashi et al. (2014) observed that utilization of forages for pig's nutrition was making good economic returns to farmers in rural areas where forages were abundant and closer to the production areas than in peri-urban areas where transport cost made it an uneconomical solution.

5.6 Conclusion

Inclusion of GPPM with Natuzyme® multi-enzyme complex at 30% of the total ration resulted to a lower cost of production compared to other *Prosopis juliflora* -based diets. The inclusion of enzyme in the diets lowered the cost per kg weight gain of the grower pigs relative to those that were not provided the enzymes.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

The majority of smallholder farmers in Kenya heavily rely on non-conventional feed materials such as swills and kitchen leftovers (Carter et al., 2013). These materials have been the major cause of poor performances of pigs in Kenya as they are considered to be bulky due to high fibre and contain anti-nutritive factors that reduce intake (Muthui et al., 2019). Though *Prosopis juliflora* pods are reported to be of nutritional value to animals, inclusion in non-ruminant feed has been limited by a high fibre content (Al-Harathi et al., 2019). There is limited information that treatment with exogenous enzyme or fermentation could improve the digestibility of GPPM and therefore improve growth performance of pigs. This study was therefore conducted to determine:

- i. The *in-vitro* digestibility of fermented, enzyme-treated and untreated mature *Prosopis juliflora* pod meal.
- ii. Feed intake, feed conversion ratio and growth rate of grower pigs offered treated mature *Prosopis juliflora* pods meal-based diets.
- iii. Effect of inclusion of treated *Prosopis juliflora* pod meal in the total ration on cost of feeding in grower pigs.

These objectives were achieved using *in-vitro* and *in-vivo* experiments and the hypotheses tested by analysis of variance using SPSS and SAS linear and non-linear models. From the findings presented in Chapters 3 and 4, processing techniques was found to improve the nutritive value of mature *Prosopis* pods meal and their utilization by growing pigs. In the following paragraphs, implications of these findings are explained:

6.1.1 Determining the *in-vitro* digestibility of fermented, enzyme-treated and untreated mature *Prosopis* pod-based meal

Digestibility value is the best indicator of the nutritive value of a feed (NRC, 2012). Therefore, overcoming the limitation of poor digestibility associated with high fibre through biotechnological interventions can be a viable option. This objective evaluated the effects of fermentation and exogenous enzymes of GPPM on digestibility and fermentation kinetics.

Both fermentation (natural and *Lactobacillus plantarum* inoculated GPPM) interventions resulted in an improvement in crude protein while the total fibre constituents decreased. Pre-treatment of *Prosopis* pod meal with both multi-enzyme treatment compared to the untreated *Prosopis* pod meal improve the *in-vitro* dry matter by 3.68%. Natural

fermentation resulted in the lowest IVDMD and cumulative gas produced due to the low fermentable carbohydrates. The total gas from treatments remained unaffected. However, natural fermentation and *Lactobacillus Plantarum* led to a reduced rate and the extent of gas produced implying lower fermentability of fermented GPPM.

6.1.2 To determine the feed intake, feed conversion ratio and growth rate of grower pigs offered enzyme-treated mature *Prosopis juliflora* pods meal-based diets

The inclusion of GPPM with Natuzyme® multi-enzyme complex up to 30% resulted in a decline in the feed intake. This was probably associated with the increased bulkiness of high fibrous diets and possibly some tannin. There was also a general increase in the feed conversion ratio as the level of GPPM increased in the diet, with 30% resulting in the highest FCR. However, the growth rate remained unaffected as the level of *Prosopis* pod meal increased although the feed intake reduced. This was indicative of the celluloses, xylanases and proteases present in the multi-enzyme complex that degraded the fibre in the *Prosopis* pod meal.

6.1.3 Cost of feeding processed *Prosopis juliflora* and the implications for smallholder farmers

The cost per kg weight gain also decreased as more of the *Prosopis* pod meal replaced the total diet, with 30% inclusion showing the lowest cost of feed per kg weight gain of grower pigs. Therefore, the inclusion of enzyme-treated GPPM can help reduce the cost of production.

6.2 Conclusions

- i. Pre-treatment of *Prosopis* pod meal with exogenous enzyme significantly increased *in-vitro* digestibility compared to untreated, natural and *Lactobacillus plantarum* induced-fermentation. Natural fermentation resulted to a significantly lower IVDMD and cumulative gas.
- ii. Inclusion of GPPM with Natuzyme® multi-enzyme complex in grower pig diet at 30% had similar growth rates compared to the control but resulted to a significant decline in the feed intake and feed conversion ratio.
- iii. Inclusion of GPPM with Natuzyme® multi-enzyme complex in grower pig diet at 30% of the total ration resulted to a significantly lower cost of production compared to other *Prosopis* pod-based diets.

6.3 Recommendations

These are general recommendations to farmers and researchers:

- i. Use fibrolytic enzymes to treat mature Prosopis pod meal to improve digestibility.
- ii. Use enzyme treated mature Prosopis pod meal to replace 30% of the total diet of grower pigs.
- iii. Inclusion of GPPM with Natuzyme® multi-enzyme complex at 30% of the total ration reducing the cost of feeding grower pigs.

6.4 Areas of further research

- i. Evaluate the effects of Prosopis pod meal treated with an exogenous enzyme on the volatile fatty acid profile and methane production in pigs through an *in-vitro* study.
- ii. Evaluate the effects of feeding Prosopis pod meal in grower pigs on carcass quality and sensory attributes.

REFERENCES

- Annor, G. A., Tyl, C., Marcone, M., Ragae, S., & Marti, A. (2017). Why do millets have slower starch and protein digestibility than other cereals?. *Trends in Food Science & Technology*, *66*, 73-83.
- Al-Harhi, M. A., Attia, Y. A., Al-Sagan, A. A., & Elgandy, M. F. (2019). Nutrients profile, protein quality and energy value of whole *Prosopis* pods meal as a feedstuff for poultry feeding. *Italian Journal of Animal Science*, *18*(1), 30–38.
- Al-Khalaifah, H., Shahin, S. E., Omar, A. E., Mohammed, H. A., Mahmoud, H. I., & Ibrahim, D. (2020). Effects of graded levels of microbial fermented or enzymatically treated dried brewer's grains on growth, digestive and nutrient transporter genes expression and cost effectiveness in broiler chickens. *BMC Veterinary Research*, *16*(1), 1-15.
- Al-Marzooqi, W., Al-Kharousi, K., Kadim, I. T., Mahgoub, O., Zekri, S., Al-Maqbaly, R., & Al-Busaidi, M. (2015). Effects of feeding *Prosopis juliflora* pods with and without exogenous enzyme on performance, meat quality and health of broiler chickens. *International Journal of Poultry Science*, *14*(2), 76–88.
- AOAC. (2006). *Official Methods of Analysis*. Association of Official Analytical Chemists. (18th ed.).
- Asindu, M., Ouma, E., Elepu, G., & Naziri, D. (2020). Farmer demand and willingness-to-pay for sweet potato silage-based diet as pig feed in Uganda. *Sustainability*, *12*(16), 6452.
- Bauer, E., Williams, B. A., Voigt, C., Mosenthin, R., & Verstegen, M. W. A. (2001). Microbial activities of faeces from un-weaned and adult pigs, concerning selected fermentable carbohydrates. *Animal Science*, *73*(2), 313–322.
- Bett, H., Musyoka, M., Peters, K., & Bokelmann, W. (2012). Demand for meat in the rural and urban areas of Kenya: A focus on the indigenous chicken. *Economics Research International*, *2012*(2), 1–10.
- Bindelle, J., Buldgen, A., Boudry, C., & Leterme, P. (2007). Effect of inoculum and pepsin-pancreatin hydrolysis on fibre fermentation measured by the gas production technique in pigs. *Animal Feed Science and Technology*, *132*(1–2), 111–122.
- Boisen, S., & Fernández, J. A. (1997). Prediction of the total tract digestibility of energy in feedstuffs and pig diets by *in-vitro* analyses. *Animal Feed Science and Technology*, *68*(3), 277–286.
- Carter, N. A., Dewey, C. E., Thomas, L. F., Lukuyu, B., Grace, D., & de Lange, C. (2016).

- Nutrient requirements and low-cost balanced diets, based on seasonally available local feedstuffs, for local pigs on smallholder farms in Western Kenya. *Tropical Animal Health and Production*, 48(2), 337–347.
- Carter, N., Dewey, C., Mutua, F., de Lange, C., & Grace, D. (2013). Average daily gain of local pigs on rural and peri-urban smallholder farms in two districts of Western Kenya. *Tropical Animal Health and Production*, 45(7), 1533–1538.
- Chaturvedi, O. H., & Sahoo, A. (2013). Nutrient utilization and rumen metabolism in sheep fed *Prosopis juliflora* pods and Cenchrus grass. *SpringerPlus*, 2(1), 1-7.
- Chia, S. Y., Tanga, C. M., Osuga, I. M., Alaru, A. O., Mwangi, D. M., Githinji, M., & Dicke, M. (2019). Meal on Growth Performance, Blood Profiles and Economics of Growing Pigs in Kenya. *Animals*, 9(705), 1–19.
- Choct, M., Dersjant, L., McLeish, J., & Peisker, M. (2010). Soy oligosaccharides and soluble non-starch polysaccharides: A review of digestion, nutritive and anti-nutritive effects in pigs and poultry. *Asian-Australasian Journal of Animal Sciences*, 23(10), 1386–1398.
- Choge, S. K., Pasiecznik, N. M., Harvey, M., Wright, J., Awan, S. Z., & Harris, P. J. (2007). *Prosopis juliflora* pods as human food, with special reference to Kenya. *Water SA*, 33(3), 419–424.
- Choi, H., Jeong, J., Kim, D., Lee, Y., Kwon, H., & Kim, Y. (2015). Influence of rapeseed meal on growth performance, blood profiles, nutrient digestibility and economic benefit of growing-finishing pigs. *Asian-Australasian journal of animal sciences*, 28(9), 1345.
- Clarke, L., Sweeney, T., Curley, E., Gath, V., Duffy, S., Vigers, S., & O'Doherty, J. (2018). Effect of β -glucanase and β -xylanase enzyme supplemented barley diets on nutrient digestibility, growth performance and expression of intestinal nutrient transporter genes in finisher pigs. *Animal Feed Science and Technology*, 238, 98–110.
- Coles, L., Moughan, P. J., & Darragh, A. J. (2005). *In-vitro* digestion and fermentation methods, including gas production techniques, as applied to the nutritive evaluation of foods in the hindgut of humans and other simple-stomached animals. *Animal Feed Science and Technology*, 123, 421-444.
- De Vries, S., Pustjens, A. M., Schols, H. A., Hendriks, W. H., & Gerrits, W. J. J. (2012). Improving digestive utilization of fibre-rich feedstuffs in pigs and poultry by processing and enzyme technologies: A review. *Animal Feed Science and Technology*, 178(3–4), 123–138.
- Dourmad, J. Y., Étienne, M., Valancogne, A., Dubois, S., van Milgen, J., & Noblet, J. (2008). A model and decision support tool for the nutrition of sows. *Animal Feed Science and*

- Technology*, 143(1), 372–386.
- FAO. (2012). *Pig sector in Kenya; FAO Animal production and livestock country review No. 3*. FAO, Rome. <https://www.fao.org/3/i2566e/i2566e.pdf>.
- FAO. (2017). *Africa sustainable livestock (ASL) 2050 Country brief: Kenya*. FAO, Rome. <https://www.fao.org/documents/card/en/c/I8978EN/>.
- FAO. (2018). *Shaping the future of livestock: sustainably, responsibly, and efficiently*. FAO, Rome. <https://www.fao.org/publications/card/en/c/I8384EN/>.
- Felker, P., Pasiecznik, N., Harris, P., Harsh, L., Cruz, G., Tewari, J & Maldonado, L. (2002). The *Prosopis juliflora*-'*Prosopis pallida*' complex: a monograph. *Forests Trees and Livelihoods*, 172, 1-162.
- Gachuri, A. N., Carsan, S., Karanja, E., Makui, P., & Kuyah, S. (2017). Diversity and importance of local fodder tree and shrub resources in mixed farming systems of central Kenya. *Forests Trees and Livelihoods*, 26(3), 143–155.
- Groot, J., Cone, J., Williams, B. A., Debersaques, F., & Lantinga, E. A. (1996). Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. *Animal Feed Science and Technology*, 64(1), 77–89.
- Hooda, S., Metzler-Zebeli, B. U., Vasanthan, T., & Zijlstra, R. T. (2011). Effects of viscosity and fermentability of dietary fibre on nutrient digestibility and digesta characteristics in ileal-cannulated grower pigs. *British Journal of Nutrition*, 106(5), 664–674.
- Humer, E & Schedle, K. (2016). Fermentation of food and feed: A technology for efficient utilization of macro and trace elements in monogastrics. *Journal of Trace Elements in Medicine and Biology*, 37(20), 69-77.
- Huyen, N. T., Martinez, I., & Pellikaan, W. (2020). Using lactic acid bacteria as silage inoculants or direct-fed microbials to improve *in-vitro* degradability and reduce methane emissions in dairy cows. *Agronomy*, 10(10), 1482.
- Jacyno, E., Pietruszka, A., Biel, W., Kołodziej-Skalska, A., Matysiak, B., Kawecka, M., & Sosnowska, A. (2016). Effect of sow age on the apparent total tract digestibility of nutrients in the diet. *South African Journal of Animal Sciences*, 46(3), 230–236.
- Jha, R., & Berrocoso, J. (2016). Dietary fibre and protein fermentation in the intestine of swine and their interactive effects on gut health and on the environment: A review. *Animal Feed Science and Technology*. 212(1), 18-26.
- Jørgensen, H., Sholly, D., Pedersen, A. Ø., Canibe, N., & Knudsen, K. (2010). Fermentation of cereals. Influence on digestibility of nutrients in growing pigs. *Livestock Science*, 134(3), 56–58.

- Kagira, J M, Maingi, N., Kanyari, P. W. N., Githigia, S. M., Ng'ang'a, J. C., & Gachohi, J. M. (2010). Characteristics of pig trade in low income settings in Busia District, Kenya. *Tanzania Veterinary Journal*, 27(1), 27–35.
- Kambashi, B., Picron, P., Boudry, C., Théwis, A., Kiatoko, H., & Bindelle, J. (2014). Nutritive value of tropical forage plants fed to pigs in the Western provinces of the Democratic Republic of the Congo. *Animal Feed Science and Technology*, 191, 47–56.
- Kanengoni, A. T., Chimonyo, M., Ndimba, B. K., & Dzama, K. (2015). Potential of using maize cobs in pig diets - A review. *Asian-Australasian Journal of Animal Sciences*, 28(12), 1669–1679.
- Kerr, B., & Shurson, G. C. (2013). Strategies to improve fibre utilization in swine. *Journal of Animal Science and Biotechnology*, 4(1), 1.
- Kilimo Trust. (2017). *Characteristics of markets for animal feeds raw materials in the East African Community: Focus on maize bran and sunflower oil seed cake*. A Kilimo Trust and IFAD report.
- Koo, B., Kim, J., & Nyachoti, C. M. (2018). Nutrient and energy digestibility, and microbial metabolites in weaned pigs fed diets containing Lactobacillus–fermented wheat. *Animal Feed Science and Technology*, 241, 27-37.
- Kwon, W. B., & Kim, B. G. (2015). Effects of supplemental beta-mannanase on digestible energy and metabolizable energy contents of copra expellers and palm kernel expellers fed to pigs. *Asian-Australasian journal of animal sciences*, 28(7), 1014.
- l'Anson, K. A., Choct, M., & Brooks, P. H. (2014). Effects of dietary xylanase and liquid feeding on growth performance and nutrient digestibility of weaner pigs fed diets high in millrun (wheat by-product). *Animal Production Science*, 54(7), 972-980.
- Lee, J. W., Patterson, R., & Woyengo, T. A. (2018). Porcine in vitro degradation and fermentation characteristics of canola co-products without or with fibre-degrading enzymes. *Animal Feed Science and Technology*, 241, 133–140.
- Levy, M., Dewey, C., Weersink, A., Mutua, F., Carter, N., & Poljak, Z. (2014). Evaluating critical factors to the economic feasibility of semi-intensive pig rearing in western Kenya. *Tropical Animal Health and Production*, 46(5), 797–808.
- Lira Junior, M. A., Fracetto, F. J. C., Ferreira, J. da S., Silva, M. B., & Fracetto, G. G. M. (2020). Legume silvopastoral systems enhance soil organic matter quality in a sub-humid tropical environment. *Soil Science Society of America Journal*, 84(4), 1209–1218.
- Lyberg, K., Lundh, T., Pedersen, C., & Lindberg, J. E. (2006). Influence of soaking,

- fermentation and phytase supplementation on nutrient digestibility in pigs offered a grower diet. *Animal Science*, 82, 853-858.
- Madubuike, F. N., Ekenyem, B. U., & Obih, T. K. O. (2006). Performance and cost evaluation of substituting rubber seed cake for groundnut cake in diets of growing pigs. *Pakistan Journal of Nutrition*, 5(1), 59-61.
- Mahgoub, O., Kadim, I. T., Al-Ajmi, D. S., Al-Saqry, N. M., Al-Abri, A. S., Richie, A. R., & Forsberg, N. E. (2004). Use of local range trees (*Prosopis* spp.) pods in feeding sheep and goats in the Sultanate of Oman. *Zaragoza : CIHEAM Options Méditerranéennes : Série A. Séminaires Méditerranéens*, 59, 191–195.
- Manhique, A O., Kingori, A. M., & Khobondo, J. (2019). Effect of incorporation of ground *Prosopis juliflora* pods in layer diet on weight gain, egg production, and natural antibody titer in KALRO genetically improved indigenous chicken. *Tropical Animal Health and Production*, 51(8), 2213–2218.
- Martens, S. D., Tiemann, T. T., Bindelle, J., Peters, M., & Lascano, C. E. (2012). Alternative plant protein sources for pigs and chickens in the tropics - Nutritional value and constraints: A review. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*. 113(2), 101-123.
- Mateos, G. G., Jiménez-Moreno, E., Serrano, M. P., & Lázaro, R. P. (2012). Poultry response to high levels of dietary fibre sources varying in physical and chemical characteristics. *Journal of Applied Poultry Research*, 21(1), 156–174.
- Maundu, P., Kibet, S., Morimoto, Y., Imbumi, M., & Adeka, R. (2009). Impact of *Prosopis juliflora* on Kenya's semi-arid and arid ecosystems and local livelihoods. *Biodiversity*, 10(3), 33–50.
- Mbuthia, J. M., Rewe, T. O., & Kahi, A. K. (2014). Breeding objectives for pigs in Kenya. II: economic values incorporating risks in different smallholder production systems. *Tropical Animal Health and Production*, 47(2), 361–367.
- Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor in vitro. *The Journal of Agricultural Science*, 93(1), 217–222.
- Missotten, J. A., Michiels, J., Owyn, A., De Smet, S., & Dierick, N. A. (2010). Fermented liquid feed for pigs. *Archives of animal nutrition*, 64(6), 437-466.
- MOLD. (2012). *Republic of Kenya ministry of livestock development strategic plan 2008-2012*. MOLD

- Moran, K., de Lange, C. F. M., Ferket, P., Fellner, V., Wilcock, P., & van Heugten, E. (2016). Enzyme supplementation to improve the nutritional value of fibrous feed ingredients in swine diets fed in dry or liquid form. *Journal of Animal Science*, *94*(3), 1031–1040.
- Mpendulo, C. T., Chimonyo, M., Ndou, S. P., & Bakare, A. G. (2018). Fibre source and inclusion level affects characteristics of excreta from growing pigs. *Asian-Australasian Journal of Animal Sciences*, *31*(5), 755–762.
- Muthui, N. J. (2019). *Influence of nutrition and value chain governance on enterprise performance in smallholder pig production in Kenya*. Doctoral dissertation, Egerton University.
- Muthui, N. J., Matofari, J. W., King'ori, A. M., & Hülsebusch, C. G. (2019). Estimation of daily nutrient allowances for pigs fed with alternative feed resources in smallholder enterprises in Kenya. *Tropical Animal Health and Production*, *51*(4), 799–808.
- Mutua, F., Lindahl, J., & Randolph, D. (2020). Possibilities of establishing a smallholder pig identification and traceability system in Kenya. *Tropical Animal Health and Production*, *52*(2), 859-870.
- Mwangi, E., & Swallow, B. (2005). Invasion of *Prosopis juliflora* and local livelihoods: Case study from the lake Baringo area of Kenya. *Nairobi, Kenya: World Agroforestry Centre*, *22*, 1-68.
- Ndou, S., Gous, R., & Chimonyo, M. (2013). Prediction of scaled feed intake in weaner pigs using physico-chemical properties of fibrous feeds. *British Journal of Nutrition*, *110*(4), 774-780.
- Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science and Nutrition*. *6*(8), 2446-2458.
- Nkosi, B. D., Seshoka, M. L., Fourie, P. J., Kanengoni, A. T., Malebana, I., & Thomas, R.S. (2020). Dietary enzyme addition on the growth performance and carcass characteristics of pigs fed diets containing avocado oil cake silage. *Tropical Animal Health and Production*, *52*, 2945-2953.
- Noblet, J., & Le Goff, G. (2001). Effect of dietary fibre on the energy value of feeds for pigs. *Animal Feed Science and Technology*, Vol. 90, 35–52.
- NRC (2012). *Nutrient Requirements of Swine*, (11th Ed.). National Academy Press, Washington DC, United states of America.
- Nyakeya, K., Jembe T., Miruka, J. B., & Mugo, J. (2018). Assessment of the fisheries status

- in river Molo to guide the management on its fisheries Technical. *African Journal of Education, Science and Technology*, 4(4), 10–20.
- Obeidat, B. S., & Shdaifat, M. (2013). Partial substitution of barley grain with *Prosopis juliflora* pods in lactating Awassi ewes' diets: Effect on intake, digestibility, and nursing performance. *Small Ruminant Research*, 111(1–3), 50–55.
- Obeidat, B. S., Abdullah, A. Y., & Al-Lataifeh, F. A. (2008). The effect of partial replacement of barley grains by *Prosopis juliflora* pods on growth performance, nutrient intake, digestibility, and carcass characteristics of Awassi lambs fed finishing diets. *Animal Feed Science and Technology*, 146(1–2), 42–54.
- Odero-Waitituh, J. A., King'ori, A. M., & Guliye, A. Y. (2016). Effect of replacing maize with milled mature pods of *Prosopis juliflora* on performance of finishing broiler chicken. *Livestock Research for Rural Development*, 28(2).
- Odero-Waitituh, J. A., Kingori, A. M., & Ambula, M. K. (2020). Bio-economic implications of feeding fermented ground mature *Prosopis Juliflora* pods to grower rabbits. *Journal of Economic Impact*, 2(2), 43-49.
- Olagunju, O. F., Ezekiel, O. O., Ogunshe, A. O., Oyeyinka, S. A., & Ijabadeniyi, O. A. (2018). Effects of fermentation on proximate composition, mineral profile and antinutrients of tamarind (*Tamarindus indica L.*) seed in the production of daddawa-type condiment. *Food Science and Technology*, 90 (2018), 455–459.
- Pasiecznik, N. M., Choge, S. K., Rosenfeld, A. B., & Harris, P. J. C. (2007, September). Underutilised crops for famine and poverty alleviation: a case study on the potential of the multipurpose *Prosopis* tree. In *5th International Symposium on New Crops and Uses: Their Role in a Rapidly Changing World* (pp. 326-46).
- Patience, J. F. (2012). *Feed Efficiency in Swine*. Wageningen Academic Publishers Netherlands.
- Peña-Avelino, L., Pinos-Rodríguez, J., Yáñez-Estrada, L., Juárez-Flores, B., Mejia, R., & Andrade-Zaldivar, H. (2014). Chemical composition and *in vitro* degradation of red and white mesquite (*Prosopis laevigata*) pods. *South African Journal of Animal Science*, 44(3), 298.
- Pinheiro, M., de Sousa, R., & Espíndola, G. (1993). Effects of adding mesquite pod meal to pigs in the finishing phase. *Pesquisa Agropecuária Brasileira*, 28(12), 1443-1449.
- Plumed-Ferrer, C., & Von Wright, A. (2009). Fermented pig liquid feed: Nutritional, safety and regulatory aspects. *Journal of Applied Microbiology*, 106(2), 351-368.
- Sawal, R., Ratan, R., & Yadav, S. B. S. (2004). Mesquite (*Prosopis juliflora*) pods as a feed

- resource for livestock. *Asian-Australasian Journal of Animal Sciences*, 17(5), 719-725.
- Schachtschneider, K., & February, E. C. (2013). Impact of *Prosopis* invasion on a keystone tree species in the Kalahari Desert. *Plant Ecology*, 214(4), 597–605.
- Shi, C., Zhang, Y., Yin, Y., Wang, C., Lu, Z., Wang, F., & Wang, Y. (2017). Amino acid and phosphorus digestibility of fermented corn-soybean meal mixed feed with and fed to pigs. *Journal of Animal Science*, 95(9), 3996.
- Torres-Pitarch, A., Manzanilla, E. G., Gardiner, G. E., O’Doherty, J. V., & Lawlor, P. G. (2019). Systematic review and meta-analysis of the effect of feed enzymes on growth and nutrient digestibility in grow-finisher pigs: Effect of enzyme type and cereal source. *Animal Feed Science and Technology*, 251, 153-165.
- Van Soest, P. J., Robertson, J., & Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74(10), 3583–3597.
- Wang, S., Guo, G., Li, J., Chen, L., Dong, Z., & Shao, T. (2018). Improvement of fermentation profile and structural carbohydrate compositions in mixed silages ensiled with fibrolytic enzymes, molasses and *Lactobacillus plantarum* MTD-1. *Italian Journal of Animal Science*, 18(1), 328-335.
- Vandenbergh, L. P., Pandey, A., Carvalho, J. C., Letti, L. A., Woiciechowski, A. L., Karp, S. G., & Soccol, C. R. (2021). Solid-state fermentation technology and innovation for the production of agricultural and animal feed bio-products. *Systems Microbiology and Bio-manufacturing*, 1(2), 142-165.
- Yusuf, N.D., Ogah, D., & Hassan, M. (2008). Effect of decorticated fermented seed meal (*Prosopis Africana*) on growth performances of broiler chickens. *International Journal of Poultry Science*, 7(11), 1054–1057.
- Zangaro, C. A., Patterson, & Woyengo, T. A. (2019). Porcine *in-vitro* digestion and fermentation characteristics of corn wet distillers’ grains and dried distiller’s grains with solubles without or with multi-enzyme. *Animal Feed Science and Technology*, 254, 114205.
- Zentek, J., & Boroojeni, F. G. (2020). Bio-technological processing of poultry and pig feed: Impact on the composition, digestibility, anti-nutritional factors and hygiene. *Animal Feed Science and Technology*, 268, 114576.

APPENDICES

Appendix A: Ethical clearance

EGERTON

TEL: (051) 2217808
FAX: 051-2217942



UNIVERSITY

P. O. BOX 536
EGERTON

EGERTON UNIVERSITY RESEARCH ETHICS COMMITTEE

EU/RE/DVC/009

Approval No. EUREC/APP/109/2021

15th January, 2021

David Marii Ngugi
Department of Animal Science
Egerton University
P.O.Box 536- 20115
EGERTON
Telephone: +254703709346
E-mail: Davidmarii013@gmail.com

Dear Mr. Ngugi,

RE: ETHICAL CLEARANCE APPROVAL: EVALUATION OF INCORPORATION OF TREATED MATURE PROSOPIS (*Prosopis juliflora*) POD MEAL IN THE DIET ON GROWTH PERFORMANCE OF PIGS

This is to inform you that *Egerton University Research Ethics Committee* has reviewed and approved your above research proposal. Your application approval number is *EUREC/APP/109/2021*. The approval period is *15th January, 2021 –16th January, 2022*.

This approval is subject to compliance with the following requirements:

- i. Only approved documents including (informed consents, study instruments, MTA) will be used
- ii. You are required to adhere Institutional Experimental Animals use and Care policy.
- iii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Egerton University Research Ethics Committee*.
- iv. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Egerton University Research Ethics Committee* within 72 hours of notification
- v. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *Egerton University Research Ethics Committee* within 72 hours
- vi. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.
- vii. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

- viii. Submission of an executive summary report within 90 days upon completion of the study to *Egerton University Research Ethics Committee*.

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

Yours sincerely,

A handwritten signature in black ink, appearing to be 'R. Ngiure'.


Prof. R. Ngiure,


CHAIRMAN, EGERTON UNIVERSITY RESEARCH ETHICS CTTEE
RN/BK/BK



Appendix B: National Commission for Science, Technology and Innovation Research


Permit


REPUBLIC OF KENYA


NATIONAL COMMISSION FOR
SCIENCE, TECHNOLOGY & INNOVATION.

Ref No: **989352** Date of Issue: **29/September/2020**


RESEARCH LICENSE




This is to Certify that Mr., David Ngugi Mariri of Egerton University, has been licensed to conduct research in Nakuru on the topic: EVALUATION OF INCORPORATION OF TREATED MATURE PROSOPIS (Prosopis juliflora) POD MEAL IN THE DIET ON GROWTH PERFORMANCE OF PIGS for the period ending : 29/September/2021.

License No: **NACOSTI/P/20/6926**

989352
Applicant Identification Number


Director General
NATIONAL COMMISSION FOR
SCIENCE, TECHNOLOGY &
INNOVATION

Verification QR Code



NOTE: This is a computer generated License. To verify the authenticity of this document,
Scan the QR Code using QR scanner application.

Appendix C: Statistical output for the objectives

Experiment one

Analysis of Variance

Fermentation kinetics

Cumulative gas produced (DM/ml)

Analysis of variance

Tests of Between-Subjects Effects

Dependent Variable: Cumulative gas produced (DM/ml)

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.	
Intercept	Hypothesis	6575.805	1	6575.805	34.296	.108
	Error	191.734	1	191.734 ^a		
Treatment	Hypothesis	89.825	3	29.942	3.855	.026
	Error	147.577	19	7.767 ^b		
Trial	Hypothesis	191.734	1	191.734	24.685	.000
	Error	147.577	19	7.767 ^b		

a. MS(trial)

b. MS(Error)

Separation of means using Tukey's

Cumulative gas produced (DM/ml)

Tukey's HSD^{a,b}

Treatment	N	Subset	
		1	2
T ₄	6	13.42987	
T ₃	6	16.53492	16.53492
T ₂	6	17.74434	17.74434
T ₁	6		18.50167
Sig.		.065	.621

Means for groups in homogeneous subsets are displayed.

Asymptote point of the curve (A)

Analysis of variance

Tests of Between-Subjects Effects

Dependent Variable: **Asymptote point of the curve(A)**

Source		Type III Sum of Squares	DF	Mean Square	F	Sig.
Intercept	Hypothesis	9702.096	1	9702.096	15.594	.158
	Error	622.176	1	622.176 ^a		
Treatment	Hypothesis	224.589	3	74.863	3.241	.045
	Error	438.866	19	23.098 ^b		
Trial	Hypothesis	622.176	1	622.176	26.936	.000
	Error	438.866	19	23.098 ^b		

a. MS(trial)

b. MS(Error)

Mean separation

Asymptote point of the curve(A)

Tukey's HSD^{a,b}

Treatment	N	Subset	
		1	2
T ₄	6	15.28704	
T ₂	6	19.72216	19.72216
T ₁	6	22.19480	22.19480
T ₃	6		23.22028
Sig.		.094	.598

Constant determining the sharpness of the switching characteristic of the profile: C

Analysis of variance

Tests of Between-Subjects Effects

Dependent Variable: Constant determining the sharpness of the switching characteristic of the profile:C

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Intercept	13455.218	1	13455.218	62.875	.080
Error	213.998	1	213.998 ^a		
Treatment	890.674	3	296.891	4.443	.016
Error	1269.693	19	66.826 ^b		
Trial	213.998	1	213.998	3.202	.089
Error	1269.693	19	66.826 ^b		

Means separation

C

Tukey HSD^{a,b}

Treatment	N	Subset	
		1	2
T ₂	6	18.36114	
T ₁	6	20.34258	
T ₄	6	22.01898	22.01898
T ₃	6		33.98815
Sig.		.865	.086

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 66.826.

**Time after incubation at which half of the asymptotic amount of gas has been formed
(B hrs)**

Analysis of variance

Tests of Between-Subjects Effects

Dependent Variable: Time after incubation at which half of the asymptotic amount of gas has been formed (B hrs)

Source		Type III Sum of Squares	DF	Mean Square	F	Sig.
Intercept	Hypothesis	79.046	1	79.046	60.462	.081
	Error	1.307	1	1.307 ^a		
Treatment	Hypothesis	1.237	3	.412	2.315	.108
	Error	3.384	19	.178 ^b		
Trial	Hypothesis	1.307	1	1.307	7.340	.014
	Error	3.384	19	.178 ^b		

a MS(Trial)

b MS(Error)

Means separation

Time after incubation at which half of the asymptotic amount of gas has been formed (B hrs)

Tukey HSD^{a,b}

Treatment	N	Subset
		1
T ₁	6	1.48873
T ₂	6	1.73397
T ₃	6	1.94600
T ₄	6	2.09058
Sig.		.097

Means for groups in homogeneous subsets are displayed. Based on observed means.

The error term is Mean Square(Error) = .178.

Tmax

Analysis of variance

Tests of Between-Subjects Effects

Dependent Variable: Tmax

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Intercept Hypothesis	3056.840	1	3056.840	1787.210	.015
Error	1.710	1	1.710 ^a		
Treatment Hypothesis	675.686	3	225.229	3.635	.032
Error	1177.276	19	61.962 ^b		
Trial Hypothesis	1.710	1	1.710	.028	.870
Error	1177.276	19	61.962 ^b		

a. MS(trial)

b. MS(Error)

Means separation

Tmax

Tukey HSD^{a,b}

Treatment	N	Subset	
		1	2
T ₁	6	5.150	
T ₂	6	7.911	7.911
T ₄	6	19.069	19.069
T ₃	6		13.013
Sig.		.336	.100

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 61.962.

Rmax

Analysis of variance

Tests of Between-Subjects Effects

Dependent Variable: Rmax

Source		Type III Sum of Squares	Df	Mean Square	F	Sig.
Intercept	Hypothesis	8.501	1	8.501	64.686	.079
	Error	.131	1	.131 ^a		
Treatment	Hypothesis	.494	3	.165	5.945	.005
	Error	.526	19	.028 ^b		
Trial	Hypothesis	.131	1	.131	4.747	.042
	Error	.526	19	.028 ^b		

a. MS(trial)

b. MS(Error)

Means separation

Rmax

Tukey's HSD^{a,b}

Treatment	N	Subset	
		1	2
T ₄	6	.42505	
T ₃	6	.49308	
T ₂	6	.67681	.67681
T ₁	6		.78563
Sig.		.073	.674

Means for groups in homogeneous subsets are displayed.

Based on observed means. The error term is Mean Square (Error) = .028.

The pH of the fermentation broth

Analysis of variance

Tests of Between-Subjects Effects

Dependent Variable: pH

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Corrected Model	.007 ^a	3	.002	.655	.605
Intercept	509.067	1	509.067	143881.771	.000
Treat	.007	3	.002	.655	.605
Error	.025	7	.004		
Total	525.330	11			
Corrected Total	.032	10			

a. R Squared = .219 (Adjusted R Squared = -.115)

Mean comparison

Ph

Tukey'sHSD^{a,b,c}

Treat	N	Subset
		1
T ₃	2	6.8850
T ₁	3	6.8933
T ₂	3	6.9050
T ₄	3	6.9500
Sig.		.612

Data for the second objective

Experiment two

Daily feed intake (DFI)

The ANOVA Procedure

Dependent Variable: Daily feed intake (DFI)

Source	DF	Sum of			
		Squares	Mean Square	F Value	Pr > F
Model	9	0.91166201	0.10129578	8.35	<.0001
Error	40	0.48498899	0.01212472		
Corrected Total	49	1.39665100			

R-Square	Coeff Var	Root MSE	DFI Mean
0.652749	7.871571	0.110112	1.398861

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treat	4	0.32142394	0.08035599	6.63	0.0003
Sex	1	0.08730986	0.08730986	7.20	0.0105
Weeks	4	0.50292821	0.12573205	10.37	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for DFI

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

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	0.012125
Critical Value of Studentized Range	4.03913
Minimum Significant Difference	0.1406

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treat
A	1.47722	10	10%+ Enzyme
A			
A	1.46688	10	Positive Control
A			
A	1.43945	10	Ctrl-Negative
A			
B A	1.33891	10	20%+ Enzyme
B			
B	1.27184	10	30%+ Enzyme

Daily weight gain

The ANOVA Procedure

Class Level Information

Class	Levels	Values
Treat	5	Ctrl-Neg D10 D20 D30 positive
Sex	2	F M
Weeks	6	0 1 2 3 4 5

Number of observations 60

The ANOVA Procedure

Dependent Variable: Daily Weight Gain

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.81051605	0.09005734	10.07	<.0001
Error	40	0.35776002	0.00894400		
Corrected Total	49	1.16827607			

	R-Square	Coeff Var	Root MSE	ADG Mean	
	0.693771	18.60223	0.094573	0.508395	
Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treat	4	0.27579896	0.06894974	7.71	0.0001
Sex	1	0.00316328	0.00316328	0.35	0.5554
Weeks	4	0.53155381	0.13288845	14.86	<.0001

Tukey's Studentized Range (HSD) Test for ADG

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

Alpha	0.05
Error Degrees of Freedom	40
Error Mean Square	0.008944
Critical Value of Studentized Range	4.03913
Minimum Significant Difference	0.1208

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treat
A	0.63424	10	Positive
A			
B A	0.5164	10	10%+ Enzyme
B A			
B A	0.51721	10	CtrlNeg
B			
B	0.44021	10	20%+ Enzyme
B			
B	0.42000	10	30%+ Enzyme

Feed conversion ratio

The GLM Procedure

Class Level Information

Class	Levels	Values
Treat	5	Ctrl-Neg D10 D20 D30 Positive
Sex	2	F M
Weeks	6	0 1 2 3 4 5

Number of observations 60

Dependent Variable: Feed Conversion Ratio

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	9	20.03754557	2.22639395	4.60	0.0003
Error	40	19.35485587	0.48387140		
Corrected Total	49	39.39240144			
	R-Square	Coeff Var	Root MSE	Feed conversion ratio Mean	
	0.508665	23.28103	0.695609	2.987878	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	4	5.61571446	1.40392861	2.90	0.0337
Sex	1	0.03931181	0.03931181	0.08	0.7771
Weeks	4	14.38251930	3.59562982	7.43	0.0001

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Treat	Standard		LSMEAN	
	FCR LSMEAN	Error	Pr > t	Number
CtrlNeg	2.83337539	0.21997077	<.0001	1
D10	3.05838267	0.21997077	<.0001	2
D20	3.27507481	0.21997077	<.0001	3
D30	3.28323501	0.21997077	<.0001	4
positive	2.70712049	0.21997077	<.0001	5

Least Squares Means for Effect treat

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

Dependent Variable: FCR

i/j	1	2	3	4	5
-----	---	---	---	---	---

1		-1.00361	-1.41986	-1.4461	1.38629
	0.8522	0.6188	0.6024	0.6398	
2	1.003606		-0.41626	-0.44249	2.389895
	0.8522		0.9935	0.9917	0.1391
3	1.419864	0.416259		-0.02623	2.806154
	0.6188	0.9935		1.0000	0.0562
4	1.446096	0.44249	0.026231		2.832386
	0.6024	0.9917	1.0000		0.0529
5	-1.38629	-2.3899	-2.80615	-2.83239	
	0.6398	0.1391	0.0562	0.0529	

Overall weekly weights

The ANOVA Procedure

Dependent Variable: Weekly Weights

Sum of						
Source	DF	Squares	Mean Square	F Value	Pr > F	
Model	10	1798.891654	179.889165	148.54	<.0001	
Error	49	59.341762	1.211056			
Corrected Total	59	1858.233416				
	R-Square	Coeff Var	Root MSE	weights Mean		
	0.968065	3.657832	1.100480	30.08558		
Source	DF	Anova SS	Mean Square	F Value	Pr > F	
Treat	4	45.186419	11.296605	9.33	<.0001	
Weeks	5	1742.039609	348.407922	287.69	<.0001	
Sex	1	11.665626	11.665626	9.63	0.0032	

Tukey's Studentized Range (HSD) Test for weights

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

Alpha	0.05
Error Degrees of Freedom	49
Error Mean Square	1.211056
Critical Value of Studentized Range	4.00497
Minimum Significant Difference	1.2723

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treat
A	31.4556	12	positive
A			
B A	30.2319	12	10%+ Enzyme
B			
B	30.1831	12	CtrlNeg
B			
B C	29.8058	12	20%+ Enzyme
C			
C	28.7516	12	30%+ Enzyme

Effect of the Treatment on the Feed cost per Kg gain

The GLM Procedure

Dependent Variable: FC_ADG FC:ADG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1136.577316	284.144329	6.23	0.0352
Error	5	228.189622	45.637924		
Corrected Total	9	1364.766938			
R-Square		Coeff Var	Root MSE	FC_ADG Mean	
0.832800		5.223013	6.755585	129.342	

Appendix D: Research pictorial



a) Drying of the *Prosopis juliflora* pods



b) Landrace x Large White pigs used for the experiment



c) Weighing of feeds

Appendix E: Publication

International Journal of Veterinary Sciences and Animal Husbandry 2021; 6(5): 11-16



ISSN: 2456-2912
VET 2021; 6(5): 11-16
© 2021 VET
www.veterinarypaper.com
Received: 07-07-2021
Accepted: 09-08-2021

Ngugi DM
Egerton University, Faculty of
Agriculture, Department of
Animal Sciences P.O. Box,
Egerton, Kenya

Kashongwe OB
(1) Egerton University, Faculty
of Agriculture, Department of
Animal Sciences P.O. Box,
Egerton, Kenya
(2) Leibniz Institute for
Agricultural Engineering and
Bioeconomy, Box 14469,
Potsdam, Germany

King'ori AM
Egerton University, Faculty of
Agriculture, Department of
Animal Sciences P.O. Box,
Egerton, Kenya

Economic implication of feeding *Prosopis juliflora* pod meal with enzyme to grower pigs

Ngugi DM, Kashongwe OB and King'ori AM

Abstract

A feeding trial was conducted to investigate the effect of inclusion of enzyme treated ground *Prosopis juliflora* pod meal (PPM) on the performances of growing pigs. Thirty Landrace x Large white crosses of 20±3 kg with an equal number of barrows and gilts were allotted to five treatment diets in a randomized block design with each treatment having six pigs. The dietary treatments were: Trt1 - containing 0% PPM and 0.035% Enzyme per kg of diet, Trt2 - containing 0% PPM and 0% Enzyme, Trt3 - containing 10% PPM and 0.035% Enzyme, and Trt4 - containing 20% PPM and 0.035% Enzyme, Trt5; diet containing 30% PPM and 0.035% Enzyme per kg of diet, respectively. The multi-enzyme Natuzyme® was added to the dietary treatment according to manufacturer's instructions at a rate of 350mg/kg feed material. Feed intake, feed conversion ratio and body weight gain was collected. For the economics analysis, inclusion of enzyme treated *Prosopis juliflora* pod meal calculation was based on the weight gain and feed intake obtained from feeding trial. The objective was to determine the cost of including treated mature *Prosopis juliflora* pod meal in growing pig's diets. Total feed cost was then calculated as the product of feed intake per pig and the cost per kg of each treatment diet. Total feed cost per kilogram gain of each treatment was calculated as the total feed cost divided by the body weight gain of each pig per treatment diet. Data was analysed using SAS 9.0 (2002) using a one way analysis of variance. Means separation was conducted using Tukey's HSD. From the results Trt5 (124.54 KES/kg) resulted to the lowest feed cost per kilogram gain compared to Trt2, Trt3 and Trt4 (132.20 KES/kg). Enzyme inclusion resulted to a lower feed cost per kilogram gain when Trt1 (111.13 KES/kg) compared to Trt2 (140.80 KES/kg) $p < 0.05$. Inclusion of PPM with Natuzyme® multi-enzyme complex at 30% resulted to the lowest cost of production compared to other diets containing *Prosopis juliflora*. The inclusion of enzyme on the diets lowered the cost per kg weight gain.

Keywords: prosopis pod meal, feed cost, Natuzyme®