

MAKERERE UNIVERSITY



IMPROVING NUTRITIVE QUALITY OF MAIZE STOVER FOR ENHANCING PRODUCTIVITY ON SMALLHOLDER DAIRY FARMS

BY

ATUHAIRE ANDREW MWEBAZE

(B.APTM., MSc. MAKERERE)

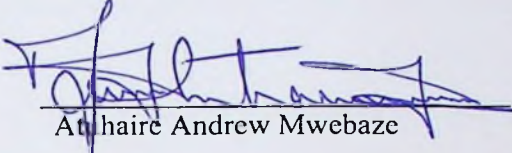
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DECLARATION

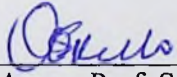
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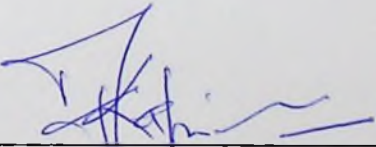
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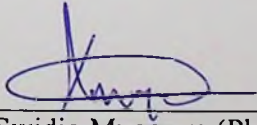
This thesis has been under our supervision and has our approval for its submission.

Signature: 
Assoc. Prof. Samuel Okello (PhD)

Date: 23/02/2017

Signature: 
Assoc. Prof. Fred Kabi (PhD)

Date: 23/02/2017

Signature: 
Dr. Swidiq Mugerwa (PhD)

Date: 24/02/2017

DEDICATION

This thesis is dedicated to my mother who despite being of modest education, has been an inspiration to me all times of my education pursuit. My wife, sons, daughter, brothers, sisters and friends who always encouraged me to keep focused on the mission pertaining to this cause. I am forever indebted to them.

May the almighty God bless you abundantly.

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LIST OF ACRONYMS AND ABBREVIATIONS

%	Percentage
ADFom	Acid detergent fiber
ALA	α -linolenic acid
BCFA	Branched-chain fatty acids
cfu/mL	Colony forming units per millilitre
CI	Confidence Interval
CLA	Conjugated linoleic acid
CO ₂	Carbon dioxide
CP	Crude protein
df	degrees of freedom
DM	Dry matter
E	East
e.g.	for example
<i>et al</i>	and others
FA	Fatty acid
FAME	FA methyl esters
FAO	Food and agriculture organization
g	gravity
GDP	growth domestic product

GPS	Global Positioning System
H ₂ O ₂	hydrogen peroxide
LA	Linoleic acid
Lignin (sa)	Lignin determined by solubilization of cellulose with sulfuric acid
MAAIF	Ministry of agriculture animal industry and fisheries
MFPED	Ministry of finance planning and economic development
mg	milligrams
ml	milliliter
MS Excel	Microsoft excel
NDFom	Neutral detergent fiber
° C	degrees Celsius
<i>p</i>	<i>p</i> -value
pH	hydrogen potential
sp.	specie
spp.	species
SSF	Solid-state fermentation
UBOS	Uganda bureau of statistics
VA	Vaccenic acid
µg/ml	micrograms per milliliter
χ ² Chi Square	

ABSTRACT

Smallholder dairy farms are increasingly being rediscovered as a strategy for improving livelihood to access diversify of food, employment as well as cash income. However, poor feeds remain a big challenge to dairy cattle productivity. While cereal crop residues have been reported as abundant in the Lake Victoria Crescent Agro-ecological zone (LVZ), their utilization by ruminants was still limited. In this research, production characteristics of smallholder dairy farmers, prioritization of available feed resources and optimization of on farm conditions for biological treatment of maize stover were evaluated. One hundred twenty six (126) smallholder dairy farmers were randomly sampled using multi-step simple random procedures. Focus group discussions, structured and semi-structured questionnaires so as to understand production characteristics of dairy farmers, temporal and special availability of alternative feed recourses. In a completely randomised block design, four popular edible *Pleurotus* fungal species namely; *Pleurotus florida*, *Pleurotus ostreatus*, *Pleurotus sajor caju* and *Pleurotus pulmonarius* were subjected to varying temperatures, pH levels, hydrogen peroxide (H₂O₂) concentrations and illumination. Effects of pre-treatment methods of maize stover before solid-state fermentation with *P. sajor caju* including steaming (SMs), soaking in a molasses solution (114 g/kgDM) for 48 h (MMs), soaking in *Lactobacillus plantarum* (*L. plantarum*) (10⁵ cfu/mL) inoculum for 48 h (LMs) and soaking in a mixture of molasses (114 g/kgDM) with *L. plantarum* (10⁵ cfu/mL) for 48 h (MLMs) were examined using *in vitro* and *in vivo* methods. Using *in vitro* methods, maize stover pre-treatments were compared on *in vitro* gas production characteristics. Furthermore, a 4 × 4 Latin square design experiment with four primiporous Holstein-Friesian lactating cows with average body weight of 450 ± 20 kg and in early lactation (60 ± 10 day in milk (DIM)) were subjected to *in vivo* evaluation of pre-treated maize stover (SMs, MMs, LMs and MLMs) based on total mixed ration. Maize stover was ranked as the major cereal crop residue, however, it required qualitative improvement on nutritional quality due to recalcitrance of lignin. Pre-treatment of maize stover with consortia of *Lactobacillus plantarum*, molasses prior to inoculation with *P. sajor caju* (MLMs) resulted into maize stover substrate with the highest (P<0.05) CP (96.6 g/kg DM), IVDMD (752.3 g/kg DM), IVOMD (687.2 g/kg DM) and ME (10.2 MJ/kg DM). Although the maize stover based total mixed ration improved (P=0.03) milk production (23.7 kg/d) and butterfat concentration (44.3 g/kg), there was no significant change in milk fatty acids profile apart from C18 and poly unsaturated fatty acids. Therefore, using consortia of *P. sajor caju*, *L. plantarum* and molasses break the lignin barrier to enhance its nutritional quality for improved productivity on smallholder dairy farm.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

In the 1960s it was generally accepted that the development of the livestock industry of Uganda on a regional basis would be desirable, with the production of milk or dairying centered mainly in the fertile crescent of Lake Victoria, while northern, eastern and western Uganda would be devoted to the production of meat (Jameson, 1970). Ecologically the Lake Victoria littoral was an area where there was plentiful supply of forages available all the year round, while in other areas long and severe dry seasons tended to limit the production and distribution of forages. Besides, the concentration of human population in the Lake Victoria Crescent was such that adequate market for fresh liquid milk would be available. However today with increasing human settlements and urbanization, accessing land for fodder production is a major constraint to dairy farming in Lake Victoria Crescent. Yet, dairy cattle production has become an important source of livelihood by providing milk, manure and creating employment for many resource poor households (Kabirizi *et al.*, 2006). Therefore, upgrading dairy production to 'cut and carry' or stall-feeding system would improve dairy production in Lake Victoria Crescent. In cut and carry system, crop residues that are abundantly available, play vital role as feed resources particularly, in dry season when hardly any fresh pasture is available. Maize stover, a post-harvest material left after the removal of the primary food (grain) forms the bulk of poorly utilized feed resources in Lake Victoria Agro-ecological Zone, Uganda. Maize stover is characterized by high fibre contents of above 770.0 g/kg DM, low crude protein of less than 55.0 g/kg DM and metabolizable energy (ME) of less than 6.0 MJ/kg DM (Mahesh and Mohini, 2013). Therefore, maize stover can be classified as poor quality forage, which translates into low dry matter intake and inadequate nutrient supply for maintenance and production.

Ruminants derive their nutritional needs from breakdown of plants' cell wall polysaccharides (Natsir, 2012). The primary constituents of plant cell walls are cellulose, hemicellulose and lignin polymers that link together to form lignocellulosic structures (Hendriks and Zeeman, 2009). Often, the lignocellulosic structure is extremely difficult to degrade, which reduces the accessibility of cellulose and hemicellulose to cellulolytic enzymes, thus, limiting the potential of maize stover to provide adequate nutrients to ruminants. Therefore, exploiting the locked up potential in the lignocellulosic complex structures of maize stover will solve the outstanding challenge of poor nutritive quality and inadequate ruminant feeds in the region.

1.2 Statement of the problem

Smallholder dairy farms are increasingly being rediscovered in Lake Victoria Crescent, however, quantity and quality of natural forages decrease severely during dry season making it difficult for farmers to provide satisfactory feed to their animals (Kabirizi *et al.*, 2012). Use of crop residues is a well-known strategy for dry season feeding in developing countries (Graminha *et al.*, 2014). However, its potential as alternative feed resource in Uganda has remained unexploited. Therefore, providing feasible quality and quantity alternative feed resource is critical for enhancing productivity of smallholder dairy farms in Lake Victoria Crescent. Although maize stover has been reported as the most abundant non-conventional feed resource, its nutritional quality, palatability and digestibility are inadequate to support dairy cattle productivity. Maize stover is high in fibre, low in nitrogen content with poor digestibility, always burnt or left to rot in the fields yet it is a potential alternative quality feed resource. Burning destroys soil biodiversity and contributes to increased CO₂ emission. Lignin in maize stover forms a lignocellulosic complex, which inhibits rumen microorganism to digest cell wall polysaccharides (Dashtban *et al.*, 2010). Covalent encrustation of lignocellulosic complex in maize stover cell wall with lignin prevents cellulolytic enzymes breakdown and prevents their biodegradation in the rumen. Therefore, removal of lignin barrier in maize stover prior to feeding

could increase nutritive quality with enormous positive outcome on dairy cattle productivity. However, numerous methodological strategies and innovations such as supplementation, chemical and biological treatments have been investigated to increase digestibility, enhance palatability and nutritive quality of maize stover. Supplementation adds nutrients but does not break down lignin barrier, chemical treatments are not feasible to smallholder dairy farmer and biological treatment cause high loss of organic matter and formation of phenolics (Sharma and Arora, 2015). Therefore, providing feasible proven on farm friendly and ecologically sustainable innovative strategy that breaks lignin barrier, preserves cell wall polysaccharides and minimize formation of phenolics will provide quality alternative feed resource. The strategy will solve farmers' immediate challenge of feed scarcity especially in dry season that will stand the test of time.

1.3 Objectives of the study

The overall objective of this study was to evaluate availability, limitation to utilization and effects of feeding biologically processed maize stover on nutritive quality, milk yield and composition.

The specific objectives were

1. To assess production characteristics, temporal and spatial availability of priority crop residues in Lake Victoria Crescent Agro-ecological zone.
2. To optimize on farm biophysical conditions, *Pleurotus* species and pre-treatment options on colonization ability, chemical composition and nutritive value of maize stover.
3. To evaluate the effect of pre-treatment methods of maize stover fermented with *Pleurotus sajor caju* on *in-vitro* gas production characteristics.
4. To evaluate dry matter intake, milk yield and composition of dairy cows fed on pre-treated maize stover fermented with *Pleurotus sajor caju*.

1.4 Justification of the study

Basidiomycetes white rot fungi are the most efficient degraders of lignin in nature (Kasetsart, 2011). Elisashvili *et al.* (2008) reported that basidiomycetes white rot fungi secrete one or more extracellular enzymes that are essential for lignin degradation, such as laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP). Furthermore, they have the ability to bio-transform low value maize stover into high quality alternative feed resource through their extracellular enzyme activities (Kasetsart, 2011). However, high loss of organic matter and formation of small phenolics has remained significantly high necessitating clear understanding of syntropic and synergistic approaches to lignin degradation. The output of this study offered appropriate quality alternative dry season feed resources and contributed to ecologically viable and socially acceptable feed resource pool for dairy cattle.

1.5 Thesis outline

The present thesis is based on the papers that comprise a description of the conducted experimental work, full and summarized paper manuscripts, a broad general discussion of obtained results in relation to the literature, feed evaluation system, overall conclusion and recommendations for future research.

The thesis is organized along the objectives of the study. Chapter 1 gives the general overview of constraints and challenges in smallholder dairy farming system in Lake Victoria Crescent Agro-ecological zone.

Chapter 2 gives the general review of literature on nutritional quality of maize stover, lignocellulosic biomass, and strategic treatments on improving nutritional quality of maize stover, syntropic and synergetic biological treatments on lignocellulosic biomass in maize stover.

Chapter 3 describes the production characteristics and routine practices of smallholder dairy farming in Lake Victoria Crescent Agro-ecological Zone.

Chapter 4 prioritizes and identifies crop residues feedstock in smallholder dairy farming system in Lake Victoria Crescent Agro-ecological zone, their spatial and temporal variability based on farmers' rankings.

Chapter 5 is about optimization of the bio-physical conditions and pre-treatment methods of maize stover fermented with white rot fungus.

Chapter 6 gives the effect of pre-treatment methods of maize stover fermentation with *Pleurotus sajor caju* on nutritive quality and *in vitro* gas production characteristics.

Chapter 7 gives the effect of feeding pre-treated maize stover fermented with *Pleurotus sajor caju* to lactating dairy cows on dry matter intake, body condition, and milk yield and composition.

Chapter 8 is discussion of research results, conclusions and recommendations made in respect to practicing dairy farming in Lake Victoria Agro-ecological Zone.

CHAPTER TWO

LITERATURE REVIEW

2.0 Nutritional quality of maize stover

Maize stover, post-harvest material left after the removal of maize cob is an important and substantial feedstock for ruminants in Lake Victoria Agro-ecological Zone, Uganda. It is characterized by high fibre of more than 770.0 g/kg DM, low crude protein of less than 55 g/kg DM and metabolizable energy of less than 6.0 MJ/kg DM (Mchesh and Mohini, 2013). The voluntary feed intake of maize stover averages 0.12 kg DM/kg live weight, digestibility ranges from 30-45% and as such it cannot adequately support nutrient intake for maintenance and production (Montañez-Valder *et al.*, 2015). Dairy production plays a fundamental role in the livelihood of the majority of smallholder farmers (Tibayungwa *et al.*, 2010). However, most of the smallholder dairy farmers are challenged with feed shortage that fluctuates with seasons. Although maize stover is always abundant and accessible to smallholder dairy farmers, it is always abandoned, burnt or covered in field after grain harvest (Sun *et al.*, 2009). Besides, the wastage of the feed resource, poor disposal method of maize stover causes environmental pollution, CO₂ gases accumulation and soil fertility degradation as a result of heating rich soil biodiversity (Darwish *et al.*, 2012). Therefore, improving nutritive quality of maize stover through biological treatments will not only provide feedstock for ruminants but also it will protect the environment.

2.1 Composition of lignocellulosic biomass in maize stover

Maize stover biomass is comprised of over 60% lignocellulosic biomass that has potential to provide nutrients to ruminants (Tengerdy and Szakacs, 2003). Lignocellulosic material consists mainly three different types of associated polymers, namely cellulose, hemicellulose and lignin (Figure 2.0) (Menon and Rao, 2012). The three different polymers in maize stover are strongly bound by non-covalent forces and covalent cross-linkages (Peréz *et al.*, 2002). By virtue

of these linkages, the accessibility of lignocellulosic polymer in maize stover, which is the source of carbohydrates, is inhibited and significant amounts of energy potential remain locked up (Akinfemi *et al.*, 2010) due to poor digestibility (Elghandour *et al.*, 2014). Furthermore, lignin the most recalcitrant component of lignocellulosic material, acts as a barrier for any solutions or enzymes by linking to both hemicelluloses and celluloses preventing penetration of lignocellulolytic enzymes to the interior lignocellulosic structure (Fengel and Wegener, 1984). Therefore, most of the maize stover is considered a waste (Sánchez, 2009). However, utilization of lignocellulosic material requires physical, chemical or biological delignification (Tengerdy and Szakacs, 2003). Based on the activity of lignin and other lignocellulosic materials, white-rot fungi are characterized as simultaneous and selective degraders. Selective lignin bio-degraders are most attractive white-rot fungi with potential biotechnological applications to remove lignin and provide unprotected carbohydrates for animal nutrition.

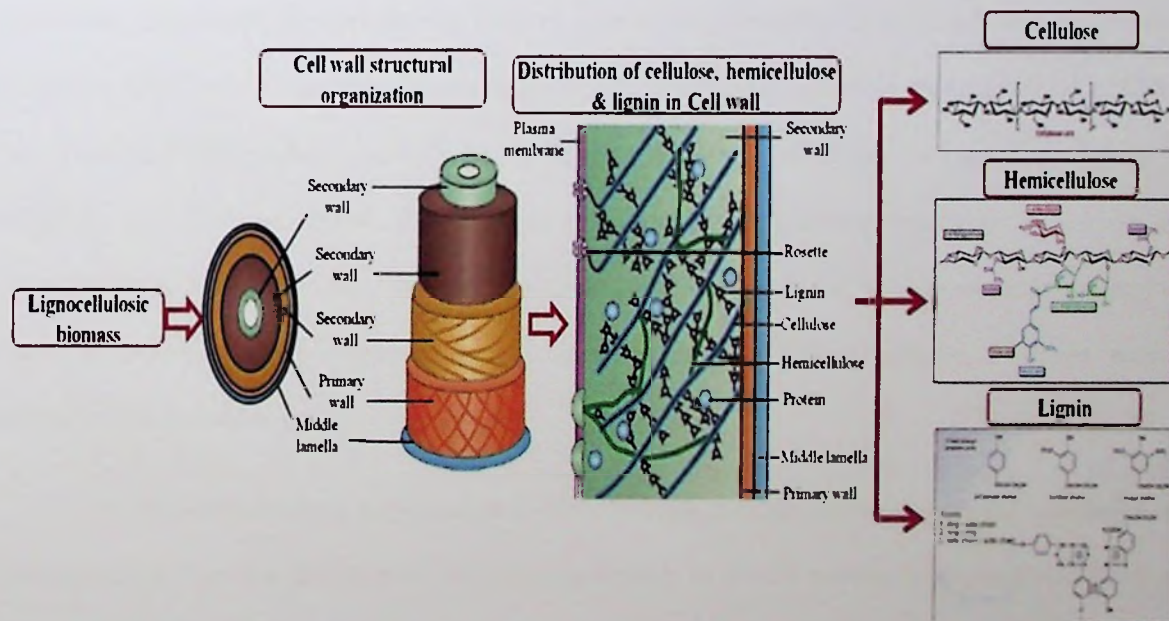


Figure 2.0 Diagrammatic illustration of lignocellulosic material

Adopted from Menon and Rao. 2012.

2.1.1 Cellulose

Cellulose is a highly stable polymer composed of D-glucose subunits linked by β -1, 4 glycosidic bonds forming cellobiose molecules (Himmel *et al.*, 2007; Graminhaa *et al.*, 2008). Cellulose is held together by intermolecular hydrogen bonds in native state and they have a strong tendency to form intra-molecular and inter-molecular hydrogen bonds that increases the rigidity of cellulose making it highly insoluble to most organic solvents (Taherzadeh and Karimi, 2008). The cellulose molecules are 'bundled' together in the form of micro-fibrils. Naturally, cellulose molecules consist of crystalline and amorphous forms of structures where the degree of crystallinity is dependent on polymer containing a hydroxyl residue at the C2, C3 and C6 atoms (Figure. 2.1). The amorphous form is more susceptible to enzymatic degradation (Peréz *et al.*, 2002). Endoglucanases (endo-1, 4- β -glucanases, EGs) hydrolyze internal bonds while cellobiohydrolases (exo-1, 4- β -glucanases, CBHs) act on the existing endoglucanase-generated chain ends. Cellobiohydrolases are the only enzymes that efficiently degrade crystalline cellulose to release cellobiose molecules (Graminhaa *et al.*, 2008). An effective hydrolysis of cellulose also requires β -glucosidases, which break down cellobiose releasing two glucose molecules (Hendriks and Zeeman, 2009). Products of cellulose hydrolysis are available as carbon and energy sources for cellulolytic microorganisms. Stable exo-cellular environment is critical for proper functioning of endoglucanases, exoglucanases and β -glycosidases to form tertiary complex with the substrate.

The endoglucanases have two structural domains: the catalytic domain and the union domain (Hendriks and Zeeman, 2009) with optimum activities in acidic media. Cellobiohydrolases are glycosylated, act synergistically with endoglucanases to solubilize high-molecular-weight cellulose molecules (Peréz *et al.*, 2002). The multiple subunits of the cellulosome are composed of numerous functional domains that interact with each other and with the cellulosic substrate.

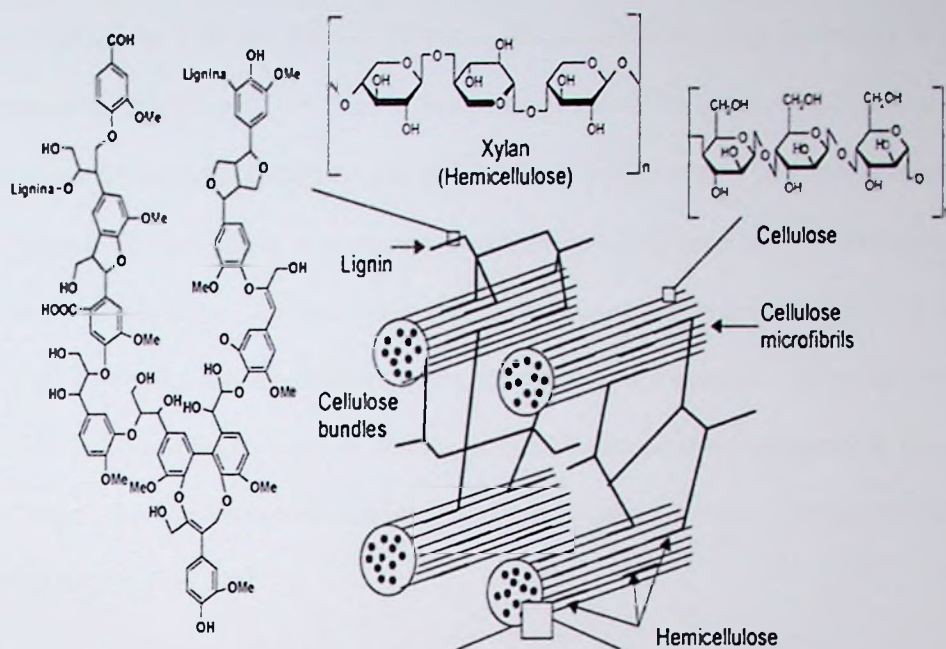


Figure 2.1 Schematic representations of fiber and its component cellulose, microfibrils, hemicellulose and lignin backbone (adopted from Krause et al., 2003)

2.1.2 Hemicellulose

Hemicellulose is a polysaccharide with lower molecular weight compared to cellulose but with a complex carbohydrate structure that consist of D-xylose, D-glucose, D-arabinoxse, D-galactose, D-mannose, D-4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acids linked together by β -1, 4- and occasionally with β -1, 3-glycosidic bonds (Peréz *et al.*, 2002). The main difference between hemicellulose and cellulose are branches in hemicellulose that have a short lateral chains made up of different sugars. Whereas cellulose consists of oligomers, which are easily hydrolysable (Sánchez, *et al.*, 2009), hemicellulose is made up of long linear backbone of one sugar type, branched with side chains, that are composed of acetate and sugars (Sharma and Arora, 2015). Although structurally hemicellulose is complex than cellulose, it does not form packed crystalline structure like cellulose and, therefore, susceptible to enzymatic hydrolysis (Saha *et al.*, 2005). Complete degradation of hemicellulose requires a cooperative action of a variety of hydrolytic enzymes. Endo-1, 4- β -xylanase generates oligosaccharides from the cleavage of xylan, xylan 1,4- β -xylosidase works on xylan oligosaccharides to produce xylose

(Peréz *et al.*, 2002). In addition, hemicellulose biodegradation requires accessory enzymes such as xylan esterases, ferulic and p-coumaric esterases, α -l-arabinofuranosidases and α -4-O-methyl glucuronosidases to act synergistically and efficiently to hydrolyze xylans and mannans (Peréz *et al.*, 2002). Hemicellulose serves as a connection between the lignin and the cellulose fibers and gives rigidity to cellulose-hemicellulose-lignin network (Laureano-Perez *et al.*, 2005). Solubilization of hemicellulose polymers starts at 180°C under neutral conditions however, from 150°C in acid medium it hydrolyses to liberate soluble hemicellulose compounds that follow this descending order: mannose<xylose<glucose<arabinose<galactose that increase with increasing temperature (Garrote *et al.*, 1999).

2.1.3 Lignin

Lignin, the most abundant aromatic heteropolymer, consists of three different phenyl propane precursor units (*p*-coumaryl, coniferyl and sinapyl alcohol) (Figure 2.2) held together with different linkages to both hemicellulose and cellulose (Buranov and Mazza *et al.*, 2008). The main function of lignin is to give a structural support, impermeability and resistance against microbial attack and oxidative stress (Hendriks and Zeeman, 2009).

Lignin is a phenyl-propanoid biopolymer, resistant to most of the microbial enzymatic systems and non-digestible by ruminants as well. The presence of lignin and its hemicellulose-binding matrix affects the accessibility of energy rich polysaccharides (Figure 2.2). Degradation of lignin has got the potential to improve the digestibility of maize stover (Arora and Sharma, 2011). The amorphous heteropolymer is insoluble in water and optically inactivated, which makes degradation robust (Hendriks and Zeeman, 2009). Lignin like hemicellulose normally starts to dissolve in water at 180°C (Wan and Li, 2012). The solubility of the lignin in alkaline or acid environment depends on precursor (*p*-coumaryl, coniferyl and sinapyl alcohol or combination of them) (Grabber, 2005).

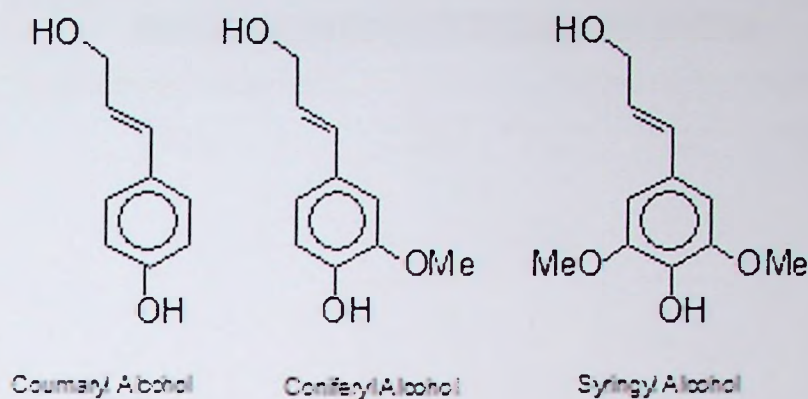
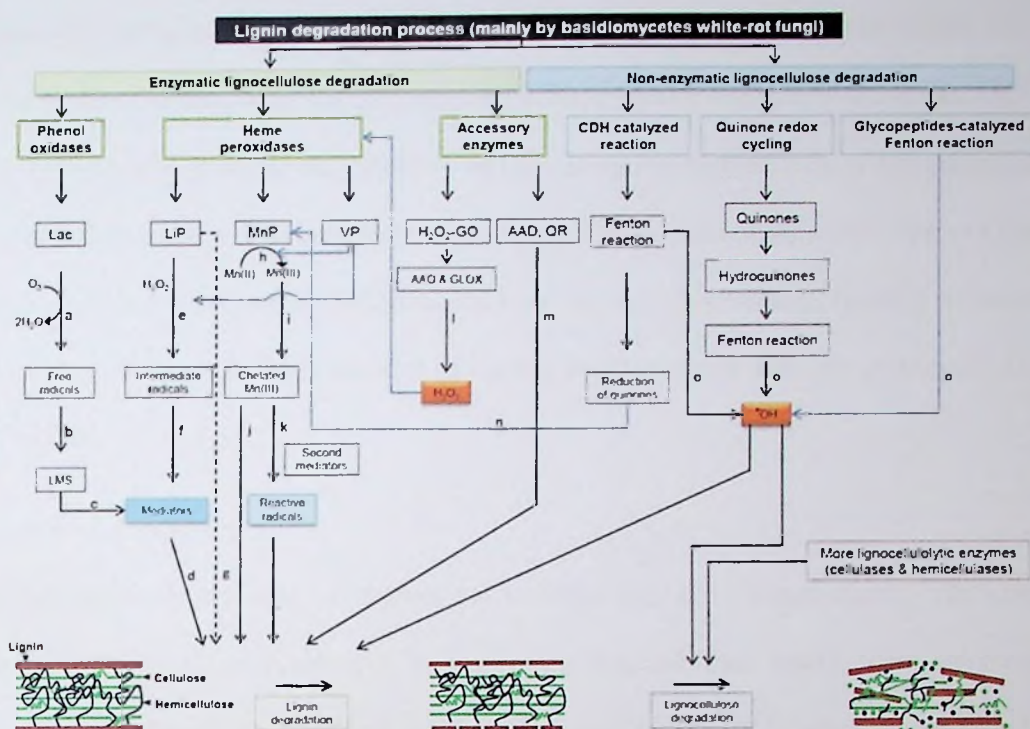


Figure 2.2 Constituent molecules of lignin polymer (Adopted from Krause *et al.*, 2003)

2.2 Lignocellulosic degradation

Several methods of delignification with the potential of releasing tightly bound carbohydrates and removing the lignin fraction have been documented (Figure 2.3) by Alvira *et al.* (2010). Treatment methods for conversion of lignocellulosic material can be categorized into four groups: physical, physico-chemical, chemical and biological. During the treatment process, the crystallinity of cellulose is reduced to increase the amorphous region as this is suitable for hydrolysis of cellulose. The most abundant fraction of hemicellulose is also hydrolyzed, while the lignin fraction is degraded (Sánchez *et al.*, 2009).



Lac: laccase, LMS: laccase-mediator system, LiP: lignin peroxidase, MnP: manganese peroxidase, VP: versatile peroxidase, H_2O_2 -GO: H_2O_2 -generating oxidases, AAO: aryl-alcohol oxidase, GLOX: glyoxal oxidase, H_2O_2 : hydrogen peroxide AAD: aryl-alcohol dehydrogenases, QR: quinone reductases and $OH^{\cdot}$: free hydroxyl radicals.

Figure 2.3 Schematic diagram of lignin degradation by basidiomycetes white-rot fungi
(Adopted from Dashtban *et al.*, 2010)

2.2.1 Physical treatment

Physical treatment of maize stover aims at reduction of the particle size and crystallinity and can be achieved by milling, grinding, chopping, steaming and ionization of lignocellulosic biomass (Kim and Lee, 2005). Milling, grinding and chopping are the commonest physical treatments adopted to increase cellulose surface area for enzymatic action by rumen microorganisms and increase the animals' voluntary feed-intake. Reduction in particle size increases ease of handling, facilitates better storage, reduces wastage, selective eating improve on feed-intake and digestion. Furthermore, reduction in particle size leads to increased relative surface area for ruminal microbial activities and reduces the degree of polymerization (Palmowski and Muller, 1999). The milling causes shearing of the biomass, increase specific

surface area and reduces degree of polymerization. During chopping, lignocellulosic materials are reduced to 10 – 30 mm, whereas grinding and milling reduce the size to 0.2-2 mm (Alvira, *et al.*, 2010). Milling and grinding are effective in increasing the surface area of the material, thus, increasing cellulose exposure to enzymatic hydrolysis. The cost involved in this form of physical degradation is proportional to the final practical size. Physical degradation remains an unpopular treatment method because of high amount of energy input required and low quality of the final product (Sánchez *et al.*, 2009).

2.2.2 Chemical treatment

Several chemicals are used to upgrade the nutritive quality of maize stover. The choice of chemical depends on its effectiveness in improving digestibility, intake, cost of treatment, availability and freedom from chemical residues that could be toxic to animals, and directly or indirectly to humans through products like meat and milk. Although some of chemical are hazardous to handle and corrosive (Owen *et al.*, 2012), others are very expensive and therefore must be purchased in bulk.

Chemical treatment involves de-polymerization of lignocellulosic material with chemical agents like acid, inorganic solvent, alkalis and peroxide. The most successful and thoroughly studied treatment method is use of dilute and concentrated inorganic acids such as sulphuric acid (H_2SO_4) and hydrochloric acid (HCl). Dilute acid hydrolysis is widely used because of its high reaction rates that are achieved with hemicellulose, which significantly improves the availability of cellulose fraction for hydrolysis (Sánchez *et al.*, 2009). The reaction is carried out between 121 - 220°C under pressure. However, the lowest temperature of 121°C is ideal in reducing the formation of inhibitors such as hydroxyl-methyl furfural and minimizing sugar degradation (Saha *et al.*, 2005). The reaction time is dependent on temperature used, where higher temperature requires shorter reaction time (Alvira *et al.*, 2010).

Chemical treatment with sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide ($\text{Ca}(\text{OH})_2$) shows considerable improvement in digestibility of fibrous feedstock (Chandhry, 1988). Alkali treatment involves saponification of ester bonds between xylan hemicellulose, lignin and other hemicellulose with alkali solutions such as sodium hydroxide, potassium hydroxide and ammonium hydroxide (Sun *et al.*, 2002). Alkali treatment is effective at lower temperature, however, this treatment method requires long periods hydrolysis and high alkali concentration (Binod *et al.*, 2010). Although NaOH has been considered as the most effective alkali to treat maize stover, its application and acceptability by farmers was still limited in developing countries (Akinfemi *et al.*, 2008). This is because of its cost, transportation and nature of being corrosive. Although calcium hydroxide is cheap and safe to handle with no problem of calcium residue, it is weaker at delignification compared with NaOH and needs a long time to react depending on the temperature (Hendriks and Zeeman, 2008). Furthermore, poor solubility of calcium hydroxide presents a considerable disadvantage to its use and, renders it less effective. Potassium hydroxide like NaOH is expensive in the pure form. Alkalis are most effective in breaking ester bonds between lignin, hemicellulose and cellulose with low concentration of inhibitors (Gaspar *et al.*, 2007).

Treatment of maize stover with ammonia was documented and carried out in most of the developing countries (Kim and Lee, 2005). It supplies nitrogen to remove lignin in the lignocellulosic biomass of maize stover. Response to ammonia is highly dependent on temperature, stover variety and moisture content (Kim and Lee, 2005). The stover is usually treated in either polythene covered sack or tunnel using aqueous ammonia or an oven using anhydrous ammonia. However, ammonia treatment of maize stover has been reported not practicable in developing countries with low technological base (Kim and Lee, 2005), high cost and scarcity of the reagents (anhydrous and aqueous) brought about by high cost of transportation of gaseous ammonia in special gas tankers, which requires high technical

personnel to handle the potentially hazardous material.

A safer alternative to ammonia in developing countries has been urea, which is available in most areas as fertilizer and precursor of ammonia. The use of urea as a precursor of ammonia has been recommended for developing countries for its simplicity and safety in application, availability in local markets at cheap prices and preservative properties (Schiere and Nell, 1993). The idea of using urea or poultry litter originated from alkali treatment aimed at breaking the lignin bonds in maize stover, thus, releasing the energy contained in them for use by the animal to which these are fed. Gaspar *et al.*, (2007) Observed increased crude protein (CP) from 4.6 percent in untreated rice straw to 11.9 percent in urea-treated straw, an increase of 2.54% urea or about 42% of the quantity of urea used for treatment of straw. However, overuse of urea does not only increase feed costs but also increases on the energy requirement to eliminate the excess N (NRC, 2001) and reduces the efficiency of N utilization. Moreover, N excretion is a potential environmental pollutant ending up as N volatilization or leaching into the ground water (Danes, 2013).

2.2.3 Physical-chemical treatment

Steam explosion (auto-hydrolysis) is by far the most studied physical–chemical treatment technique. It involves the hydrolysis of chopped biomass with high pressure saturation steam for a specific time after, which pressure is quickly reduced, thereby initiating explosive decompression within the material. The temperature used ranges between 160 - 260°C, with higher temperature requiring shorter contact times. In general, lower temperature and longer residence times are employed due to the formation of inhibitors at higher temperature (Sharma and Arora, 2015). This process causes extreme hemicellulosic degradation and lignin transformation, thus, increasing the availability of cellulose fraction (Sun *et al.*, 2002). Up to 90% efficiency of enzymatic hydrolysis can be achieved with this treatment method whereas

only 15% efficiency can be obtained without pretreatment (Cheng *et al.*, 2001). The addition of H_2SO_4 to the process can improve subsequent enzymatic hydrolysis considerably as the digestibility of the substrate is increased due to higher degree of lignin degradation (Sun *et al.*, 2002). Auto-hydrolysis is one of the most cost effective treatment processes for lignocellulosic compounds, however, it is less effective with maize stover because of high percentage of lignin (Shahbazi *et al.*, 2005). Despite the effectiveness of this treatment process, destruction of a portion of xylan fraction occurs that results into the generation of compounds such as furfural that inhibits ruminal microbes.

2.2.4 Biological treatment

Biological treatment is a complex process involving multiple enzymes that are secreted by a number of microorganisms to work synergistically to enhance digestibility of lignocellulosic biomass in maize stover. Biodegradation of lignin is a secondary metabolic process which occurs in the presence of easily digestible carbohydrates in suitable substrate. The main enzymes involved in lignin degradation are extracellular laccases and peroxidases such as lignin and manganese peroxidase. Lignin peroxidase (LiP), in the presence of H_2O_2 , degrades lignin through oxidative cleavage of the carbon-carbon backbone, oxidation and hydroxylation of benzylic methylene groups and oxidation of phenol alcohols. Meanwhile, manganese-dependent peroxidases (MnP) oxidise Mn^{2+} to Mn^{3+} acts as a redox couple oxidizing phenolic substrates (Kim and Lee, 2005). Laccase is a multi-copper polyphenol oxidase that catalyzes the oxidation of a large number of organic and inorganic molecules such as diphenol, polyphenol and aromatic amines via the transfer of electrons (Shahbazi *et al.*, 2005). These enzymes work in synergy to hydrolyze the lignin polymer leaving the hemicellulose and cellulose fraction more exposed to ruminal microbial enzymatic hydrolysis.

Biological treatment of lignocellulosic compounds are usually carried out through solid state fermentation process, it is lignin-specific, with low energy demands, in manageable

environmental conditions and no digestion inhibitors compared to chemical and physico-chemical treatment (Sánchez *et al.*, 2009). However, treatment requires time due to the low rates of hydrolysis, thus, limiting its heavy industrial processes application.

2.3 Lignocellulosic degradation

Lignocellulosic degradation involves utilization of enzymes that synergize each other to hydrolyze the biomass substrate. Ideally, due to the complexity of the lignocellulosic material, selection of three or more different enzyme groups is critical, depending on the final product and degree of hydrolysis targeted (Sarıkaya *et al.*, 1997).

In nature, there are many microorganisms, specifically bacteria as well as fungi that biodegrade lignocellulose. The most efficient decomposers of lignocellulose are the white-rot fungi (Tengerdy and Szakacs, 2003). There are a number of enzymes which function in delivering effective hydrolysis of the lignin polymer, specifically manganese peroxidase, lignin peroxidase and laccase (Sarıkaya *et al.*, 1997). Lignin peroxidase (LiP) has a heme as prosthetic group containing glycoprotein with a molecular mass of between 38 – 48 KDa and plays a central role in the biodegradation of lignin (Peréz *et al.*, 2002). LiPs catalyze the H_2O_2 -dependent oxidative de-polymerization of a variety of non-phenolic lignin compounds. Due to the size of this protein, it cannot enter the plant cell, hence, lignin degradation takes place at the exposed regions of the lumen (Peréz *et al.*, 2002). Unlike the other peroxidases, LiP is able to oxidize non-phenolic aromatic substrates and does not require the participation of mediators due to its high redox potential (Peréz *et al.*, 2002). Manganese-peroxidases (MnP) are molecularly similar to LiPs, that is, they are also glycosylated proteins comprising a heme group, but with higher marginal molecular mass (45 - 60 kDa) (Peréz *et al.*, 2002). The catalytic cycle is also similar, although in contrast to other peroxidases, MnP uses Mn^{2+} which is abundant in lignocellulosic material.

The catalytic cycle of MnP is initiated by the binding of H_2O_2 to a native ferric enzymes with the formation of an iron-peroxide complex. As a result of the donation of two electrons from the heme, MnP complex is formed. MnP also produces phenoxy-radicals which undergo a variety of reactions leading to depolymerisation of the lignin (Peréz *et al.*, 2002). MnP is extremely sensitive to high concentration of hydrogen peroxide, which can result in the inactivation of the enzyme through the formation of other compounds.

2.3.1 Solid state fermentation

Solid-state fermentation defined as the cultivation of a microorganism on a solid substrate in the absence of water but with enough moisture to support the growth and metabolism of the microorganism (Singhania *et al.*, 2009). The ability to mimic natural cycle of the fungi makes it easier to control the morphological cycle (Sharma and Arora, 2015). Superior enzymatic yields with solid-state fermentation tend to have greater stability and require lesser sterility during the process (Hölker *et al.*, 2004).

Although there are significant advantages to solid-state fermentation, a number of drawbacks also exist. These are mainly involved in process control that include: a build-up of gradient temperature, pH control and moisture concentration in the substrate, which are very difficult to control due to lack of moisture in the system (Hölker *et al.*, 2004).

2.3.2 Factors affecting solid state fermentation (SSF) process

There are several factors that affect the use of solid-state fermentation, these factors include; bio-physical conditions such as moisture level, temperature, relative humidity, particle size, form of inoculum, period of cultivation and absence of competing microorganism, white-rot fungi and substrate (Pandey *et al.*, 1999). For efficient and effective solid-state fermentation process, it is desirable that all physico-chemical parameters be optimized since white-rot fungi have inherent variation.

2.3.3 Bio-physical conditions

Temperature affects the growth of the microorganism as well as enzyme secretion. Although fungi can grow over a wide range of temperature, the optimal thermal requirements are specific and differ among fungal species (Bhargav *et al.*, 2008). Temperature has a significant effect on the growth and function of the microorganism in solid-state fermentation. (Zadrazil *et al.*, 1996), revealed that increasing temperature from 22 to 30°C significantly impacted on the rate of decomposition of the substrates as well as the sequence of decomposition. Additionally, Sohail *et al.* (2009) showed that increased incubation temperature during cellulase production had an unflavored effect on endoglucanase and β - glucosidase activity.

Metabolic activities during mycelial growth generate heat, which accumulates in the system as result of low thermal conductivity of solid substrates causing denaturation of the desired enzymes (Pandey, 2003). Furthermore, in the early stages of fermentation process, oxygen transfer remains uniform throughout, however, as the fermentation progresses, oxygen transfer becomes limited resulting in heat formation within the system. Consequently, temperature gradient variation can vary up to 20°C within fermentation processes, thus, requiring efficient monitoring of the substrate to ensure good growth, primary and secondary metabolite formation.

Low moisture content and poor thermal conductivity of the substrate makes it difficult to control the fermentation temperature in SSF. Nevertheless, minimizing the depth of the substrate (reactor bed) is an effective method to reduce heat build-up and allow easy transfer of oxygen and CO₂ removal. Furthermore, system sparge with air can effectively reduce the heat in the system, although large quantities of air are required, far in excess of the amount needed for respiration (Sohail *et al.*, 2009). The use of larger particles can also reduce the heat accumulation, as these larger particles have increased space between them resulting in easier aeration (Bhargav *et al.*, 2008).

Water functions at a cellular level, providing nutrients to the cells, removing wastes and metabolites and works to impart stability to the cell at a molecular and cellular level during SSF. At a molecular level, water stabilizes the biopolymer, proteins and nucleotides while at a cellular level, it stabilizes the lamellar structure of the plasmic membrane, thus, maintaining the membrane permeability (Ferrin and Stanghellini, 2006). Insufficient water content can result in a slowdown of the cellular metabolism due to lack of nutrients, gas or through high concentrations of secondary metabolites, which are normally removed by the liquid. Lack of water affects the metabolic pathway of fungi, as this impacts on the maintenance of the functional properties of the enzymes in the pathway. Water mass transfer is also strongly dependent on other physiochemical parameters such as aeration and temperature (Sohail *et al.*, 2009).

pH aids in providing optimal growth conditions for fungal metabolism and secretion of enzymes. Due to inability of the substrate in SSF processes to uniformly mix the growth matrix, it is difficult to adequately determine the pH of solids (Krishna, 2005). In SSF, the pH of the culture can be reduced as a result of fungal metabolism in which organic acids, such as citric, acetic and lactic acids are secreted. Equally, the pH can be increased due to urea hydrolysis, which results in alkalinisation and organic acid assimilation (Schiere and Nell, 1993). White rot fungi have a broad pH range in which they can grow, however, for optimal growth and enzymatic secretion pH range of 3.8 - 6.0 is optimum (Gowthaman *et al.*, 2001).

Providing oxygen during SSF process removes heat, moisture, volatile metabolites and carbon dioxide (Krishna, 2005). Oxygen requirement is regulated by mycelial growth rate, CO₂ removal and the need to control temperature. The operation parameters including bed depth, porosity and size of the substrate influence the rate of oxygen transfer (Krishna, 2005).

2.3.4 White-rot fungi

White-rot fungi (WRF) are abundant in nature with their natural ability to compete and survive. WRF are the only known organisms that have the ability and potential to degrade lignin material in global carbon cycle, a recalcitrant plant polymer and second most abundant carbon reserve material on earth (Hatakka, 2005). WRF degrade lignocellulosic material during secondary metabolism under low nutrient conditions through methylation process and cocktail of enzymes including lignin peroxidases, manganese peroxidase, laccases, cellulases and hemicellulases (Blanchette, 2000). The ligninolytic enzymes system is non-specific, extracellular and free radical based that allows diverse structural degradation of xenobiotic compounds (Eriksson *et al.*, 1990). WRF are classified into three distinct groups based on their ligninolytic enzyme patterns. The first group of fungi produces the enzymes LiP, MnP and laccase, while the second group produces LiP and laccase and the third group produce MnP and laccase (Hatakka, 2005). Besides, WRF degrade lignin without significant loss of cellulose and hemicellulose fractions (Blanchette, 2000). It must be noted that individual fungi can vary significantly in their ability to degrade specific substrate under the same conditions (Eriksson *et al.*, 1990). The most efficient white rot fungi, which selectively degrade lignin, are of great interest in study as cellulose and hemicellulose are the desired end products of the process.

2.3.5 Substrate

Appropriate substrate avails all the nutrients required for mycelia growth and production of the desired product (Pandey *et al.*, 1999). Rate of substrate colonization, decomposition and mineralization depends on its physical-chemical composition and the availability of digestible carbohydrates for conversion into simpler sugars in fungal primary metabolism (Khattab *et al.*, 2013). Sugars are utilized by the fungus and for secondary metabolism initiated, which consists of the breakdown of structural carbohydrates and lignin by extra-cellular enzymes (Kuhad *et al.*,

2013). The substrate supports fungal mycelial expansion both physically and in supply of nutrients (Pandey, 2003). Maize stover substrate has the advantage of being relatively abundant. However, the substrate is degraded during fermentation, thus, leading to changes in cell wall structure and rigidity (Krishna, 2005). Substrate particle size affects fungal colonization and product formation. Substrates with small particles provide a larger surface area for microbial attack, which is optimal for efficient growth. On the other hand, if the particles are too small, it results into agglomeration, hindering the transfer of air around the substrate and resulting in poor colonization ability. Conversely, coarse material provides a smaller surface area for nutrients release but allows better aeration (Pandey *et al.*, 1999). Therefore, the optimum substrate should be large enough to allow efficient aeration, whilst being small enough to provide sufficient surface area. To achieve a suitable size, substrate may be pre-treated by chopping.

2.4 Lignocellulosic enzymes and their potential use in animal nutrition

There is great potential of using lignocellulosic enzymes to improve the nutritive quality of maize stover a lignocellulosic material to alternative quality ruminant feed. Currently, exogenous enzymes are used to improve the digestibility of the feed, remove anti-nutritional factors and to supplement endogenous enzymes in pigs and poultry (Bedford, 1993; Galante *et al.*, 1998).

Supplementation of enzymes (xylanase and cellulase) in ruminant hay diet has gained research interest after increasing live weight gains up to 35 % and milk production in cattle (Kung *et al.*, 2002; Yang *et al.*, 2000; Beauchemin *et al.*, 2003). Pre-treatment of fibrous feedstock through direct solid-state fermentation with fungi improves the feed digestibility and creates higher quality feed. Several studies have investigated basidiomycetous white-rot fungi use in selective degradation of lignin, leaving cellulose and hemicellulose intact (Karunanandaa *et al.*, 1995; Jung *et al.*, 2009). Results, however, indicated that it is difficult to control selective degradation patterns of most fungi, resulting in partial loss of the cellulose and hemicellulose fractions (Graminha *et al.*, 2008).

Although addition of enzymes to the diet has been investigated, many of these studies were *in vitro* which did not always directly mimic to what happens *in vivo* treatment (Yang *et al.*, 2000; Graminha *et al.*, 2008; Sun *et al.*, 2009). They have, however, indicated that adding lignocellulosic enzymes to the feed increased crude protein and digestibility of the substrate.

Colombatto *et al.* (2003) examined the use of adding enzymes from thermophilic fungi to ruminant diets. Mesophilic fungi tend to produce enzymes with required optimal temperatures of between 45 - 60°C, which can be lower than the temperature found in the later stages of silage processing (Graminha *et al.*, 2008). Enzymes from thermophilic fungi often increase thermostability in comparison to mesophilic enzymes (Colombatto *et al.*, 2003). The mode of action of enzymes in animal feed is not fully understood, although it is thought that the enzymes improves the digestibility, by removing structural barriers thus allowing interaction enzymes and ruminal microorganisms (Colombatto *et al.*, 2003).

White rot fungi are very effective basidiomycetes for biological pre-treatment of maize stover as they degrade lignin extensively. Shojaosadati *et al.* (1999) demonstrated that pre-treatment of fibrous material with WRF increases protein availability by 10-15 %.

2.4.1 Determining the maize stover feed quality

The detergent system is the method commonly used for determining feed quality for ruminant diets (Van Soest, 2006). Forage quality varies significantly depending on factors such as herbage maturity, plant environment, season, variety and geographical location (Buxton, 1996). As a result, the forage quality has to be assessed to determine the quality of forage in the diet. The plant cell wall from the forage provides the fibrous portion of the diet, which is required for normal ruminant function. It is composed of between 40 and 80 % lignin and structural carbohydrates, which can limit the digestibility of the material. Ruminants can only utilize about half of this material. However, degradation can release the contents from within the

cell walls, which can be completely digested (Buxton, 1996). Neutral detergent fibre (NDF) is used to estimate the concentration of cell wall carbohydrates in the forage and is negatively correlated to dry matter intake (Golding *et al.*, 1985), while acid detergent fibre (ADF) is used to assess the concentration of cellulose and lignin in the material (Van Soest and Robertson, 1985). The ADF content is inversely proportional to the digestibility of the substrate where the higher the ADF content is the lower the digestibility of the forage. The NDF concentration in grasses is generally higher than legumes, which is caused mostly by the difference in the structural carbohydrates between grass and legume leaves (Buxton *et al.*, 1989). The optimal NDF concentration in the diet of high producing dairy cows should be between 270 - 290 g/kg DM. Another method used to assess the digestibility of forage is the method introduced by Tilley and Terrey (1963) to determine the *in vitro* dry matter digestibility (IVDMD). This method involves incubation of the forage of interest in rumen fluid, under anaerobic conditions for a defined period of time. It aims to mimic the rumen of the animal so as to determine the true digestibility of the forage.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Description of the study area

Three districts namely Buikwe ($0^{\circ}18'4.32$ N and $33^{\circ}3'6.63$ E), Jinja ($0^{\circ}25'28$ N and $33^{\circ}12'15$ E) and Mayuge ($0^{\circ}27'35$ N and $33^{\circ}28'49$ E) (Figure 3.1) were randomly sampled, using simple random procedure a sample frame of fifteen districts located in Lake Victoria Agro-ecological Zone (LVZ), Uganda. LVZ receives bimodal rainfall pattern, (March to May and September to November), with two dry spells (December to February and June to August). Agriculture is the main economic activity, with mean agricultural area of 529, 601.1 and 603.3 km² for Buikwe, Jinja and Mayuge districts, respectively. Smallholder dairy cattle production is a major enterprise in the three districts where maize is a major food crop.

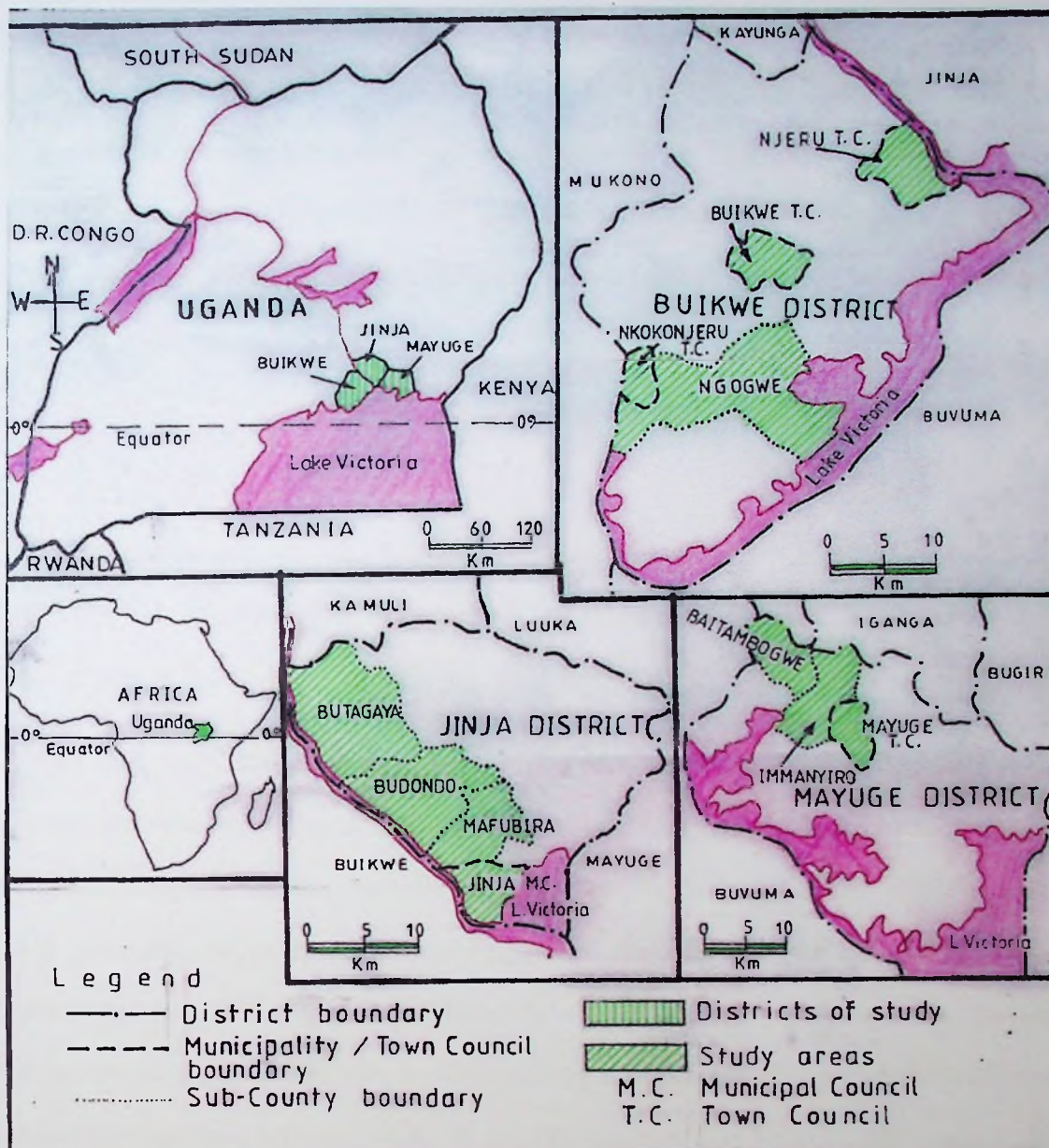


Figure 3.1 Map of Uganda showing area of study

3.2 Study design and collection of data

A cross-sectional study was carried out to investigate the production characteristics, priority non-conventional feedstock and facts associated with dairy cattle nutrition in Lake Victoria Agro-ecological Zone. Three districts were randomly selected using multi-stage sampling procedures. The first stage involved simple random selection of three districts from sample frame of 15 districts in LVZ, the second stage involved simple random selection of three

sub-counties from 21 sub-counties per district and the third stage involved simple random selection of three parishes from 32 parishes per selected sub-county. A list of all smallholder dairy farmers in the selected parish was generated with the help of extension workers and farmers. Farmers were assigned numbers and random selection of smallholder farmers was executed. Sample size was determined following simple random sampling formula as described by Israel, (2009).

$$n = \frac{Z^2_{\alpha/2}}{e^2} p(1 - p)$$

Where n = Sample size, $Z_{\alpha/2}$ = Confidence interval at 95% (Standard value of 1.96) p = 10% of proportion of smallholder dairy farmers in LVZ, Uganda (UBOS, 2012) and e = desired levels of precision at 5%.

With each randomly selected farmer an interview was carried out using structured and open-ended questionnaire (Appendix 2 and 3) administered by trained local research assistants conversant in English and the respective local languages in a one-on-one direct interview. Focused group discussions (one per sub-county) were also held to corroborate the information gathered. The data captured included farmer's awareness on non-conventional feedstock, grazing system used, cattle numbers, feeding method, and the intra and inter-household gender involvement in the cattle management practices. The data was thus based on self-reporting from farmers.

3.3 Experimental materials and treatments

This study was conducted in the Animal Science Laboratory, College of Agricultural and Environmental Sciences, Makerere University, Uganda. Maize stover of open pollinated maize variety Longe 5 was harvested 120 d after planting at National Crops Resources Research Institute (NaCRRI). The crop was planted at spacing of 0.75 m by 0.25 m between and within

row, respectively, in the 4 blocks. Sloping nature of the land formed the basis of blocking. All recommended agronomic practices to establish and manage maize crop, in the area were followed without irrigation practiced across all blocks. Diammonium phosphate (DAP) was applied at a rate of 100 kg/ha at planting time and urea was applied when the plant reached 0.7 m high at a rate of 50 kg/ha, weeding was manually accomplished using a hoe and sickle. Each block was divided into 4 plots and each plot had at least 24 stubbles of maize stover that were randomly harvested. Samples of maize stover were chopped to homogenous length of 2 - 3 cm using a hand forage chopper, washed in flowing tap water, air-dried and subjected to experimental treatments:

Four experimental treatments including: (i) steamed in a batch pasteurizer at 60°C for 30 minutes (SMs), (ii) soaked in liquid molasses (114 g/kg DM) for 48 hours (MMs), (iii) soaked in *Lactobacillus plantarum* (*L. plantarum*) inoculum (10^5 cfu/mL) for 48 hours (LMs), (iv) soaked in a mixture of molasses (114 g/kg DM) and *L. plantarum* inoculum (10^5 cfu/mL) in a ratio of 1:1 v/v for 48 hours (MLMs), all treatments were sun dried before inoculation with pure culture of *P. sajor caju* at rate of 250 g of grain spawn per kg for solid state fermentation and harvested after 14 days. The substrate was sun dried to terminate further colonization after 14 d, packed and stored in cotton-sewn bags for Chemical analysis and *in vitro* gas production.

Pure stock culture of white-rot fungus was procured from MYCELIA, a Belgium-based mushroom culture company. An agar block (approximately 30 mm³) of pure stock culture per species was aseptically transferred to 90 mm sterile petri dishes containing sterile potato dextrose agar (Formedium Hunstanton, England) sterilized at 121°C for 15 minutes. The inoculated plates were sealed with parafilm, incubated at 25°C and mycelia growth from the introduced spawn was allowed to ramify on the agar. At 7 d of fungal ramification, mycelia discs of 8 mm diameter were obtained as secondly mycelia of pure culture that was used in the subsequent experiments.

Molasses used in treatments were obtained from sugar cane factory at Sugar Cooperation of Uganda Limited (SCOUL). *Lactobacillus plantarum* was obtained by harvesting it from yellow apple bananas following procedures as outlined by Dawson *et al.* (1990),

3.3.1 Proximate analysis

Samples were divided into two parts per sample, one part was dried at 60°C for 48 h, milled to pass through a 1-mm screen for subsequent *in vitro* gas laboratory analysis. The other remaining sample was dried at 105°C for 24 h, for analysis of dry matter (DM), organic matter (OM) and crude protein (CP), following methods 930.15, 942.05 and 954.01, respectively (AOAC 1995). Ash content was determined by incinerating samples at 550°C for 5 h (AOAC 1995; method ID 942.05). Neutral detergent fiber (NDF) exclusive of residual ash (NDFom) was determined without addition of α -amylase according to Van Soest *et al.* (1991). The same sample was sequentially analyzed for acid detergent fiber exclusive of residual ash (ADF). Lignin (sa) was determined by solubilization of cellulose with sulfuric acid (12 N) according to the method of Robertson and Van Soest (1981). The concentration of hemicellulose was calculated as the difference between NDF and ADF computed sequentially on the same sample while that of cellulose as a difference between ADFom and Lignin (sa).

3.3.2 *In vitro* digestibility procedures

In vitro digestibility assay to estimate *in vitro* dry matter and organic matter digestibility (IVDMD and IVOMD) was carried out according to the method of Tilley and Terry (1963) as modified by Van Soest and Robertson, (1985). Rumen liquor were obtained from abattoir in thermos flask maintained at 39±3°C, quickly transported to the laboratory, sieved through 3 layers of muslin cloth and flushed with carbon dioxide before incubation. During the first stage finely ground samples (1g) was weighed and placed into flasks. Subsequently, 12 mL of McDougall's buffer (Makkar *et al.*, 1995) and 8 mL of rumen fluid were added. The flasks were closed with rubber stoppers fitted to bunsen valve and incubated under anaerobic conditions for

24 h in an automatic shaker water bath, maintained at 39°C during the process. In the second stage, the residues were incubated with acidified pepsin solution for 24 h at 39°C under aerobic condition. The insoluble residues were filtered off, dried and ignited to obtain ash following method 942.05 (AOAC, 1995). The organic matter of the residue was obtained by subtracting ash from residue dry matter. Blanks (rumen fluid and buffer only without sample substrate) were incubated as described above and served as a correction factor to the DM and OM contents of residuals. The incubation was done in three replicates according to the treatments and two flasks represented each replicate.

3.3.3 *In vitro* gas production procedures

In vitro gas production was carried out in graduated glass syringes (100 mL capacity) following the method described by Menke and Steingass (1988). Each oven-dried sample (500 ± 1 mg) was grounded to pass through 1 mm screen and accurately weighed in 6 replicates into graduated glass syringes (100 mL) fitted with plungers that were lubricated with Vaseline. The airtight graduated glass syringes (100 mL) were filled with 30 mL of buffered rumen fluid consisting of 10 mL of rumen liquor and 20 mL of McDougall's buffer and incubated in a shaking water bath at 39°C (Blümmel and Becker 1997). Rumen liquor was pooled from four slaughtered cattle at the abattoir into a pre-warmed thermos flask, filtered through four layers of cheesecloth and flushed with CO₂. All laboratory handlings were under continuous flushing with CO₂. Six syringes with only buffered rumen fluid were incubated at same time and considered as blanks. Gas production was read immediately and recorded at 2, 4, 8, 12, 16, 24, 28, 32, 36 and 48 h of inoculation. Net gas was determined by subtracting the average blank volume from the observed gas production for each of the test samples. The first round of 15 syringes (three syringes per treatment and three blanks) were terminated after recording the 24 h gas volume. The remaining 15 syringes that included three syringes per treatment and three blanks were terminated after the 48 h gas volume recording. The 24 h gas values were expressed per 500 mg

of DM and the recording were used to determine organic matter digestibility (OMD), metabolizable energy (ME) and partitioning factor (PF) as described by Menke and Steingass (1988). After 24 h, the remaining material was refluxed in neutral detergent fibre solution filtered through sintered crucibles dried and ignited to obtain ash following method 942.05 (AOAC, 1995) to determine NDF_{res} , *in vitro* apparent DM degradability (IVDMD) and *in vitro* apparent organic matter digestibility (IVOMD).

3.3.4 *In vivo* procedures

Four primiparous Holstein cows averaging 454 ± 19.0 kg of body weight and 60 ± 5.3 d in milk were assigned to a 4×4 Latin square design to determine the effect of pre-treatment methods of maize stover fermented with *Pleurotus sajor caju* on feed intake, body condition score, body weight, milk yield and composition. Each experimental period consisted of 14 d of adaption and 7 d of data collection. Experimental cows were individually penned, with adequate floor space in a well-ventilated shade; free to access fresh water and mineral lick all the time. They were ear tagged and dewormed with broad spectrum anthelmintic (10% albendazole at dosage rate of 10 mg/kg body weight) before commencement of the experiment. Every morning before and after milking, routine examination to ascertain any signs of heat or ill health especially mastitis was done on every experimental cow.

Experimental cows were weighted, using electronic weighbridge on day 14 and 21 of each period and the average body weight was used to compute DM intake as a percentage of body weight. The same trained scorer completed body condition scoring on the first day of period 1 and at the end of the sampling week of each experimental period. The body condition score system of Ferguson *et al.* (1994) was used, which works on quarter points based upon spinous processes, spinous to transverse, transverse processes, overhanging shelf (care-rumen fill), tubar coxae (hooks) and tuber Ischii (pins), between the pins and hooks, between the hooks and tail head to pins of the cows. However, when a cow demonstrated characteristics, which made it

difficult to clearly classify her to a specific quarter point for example 2.00 versus 2.25, she was classed as being intermediate (2.125). This resulted in addition of an additional 8th point to the system of Ferguson *et al.* (1994).

All the four pelleted total mixed rations had similar ingredients from well drained solid state fermented chopped maize stover with *Pleurotus sajor caju* for 14 d but differed only in pre-treatments methods that included (i) steaming in a batch pasteurizer at 60°C for 30 minutes (SMs), (ii) soaking in liquid molasses (1 kg of maize stover: 2 litres of molasses 114 g/kg DM) for 48 hours (MMs), (iii) soaking in *Lactobacillus plantarum* (*L. plantarum*) (10⁵cfu/mL) inoculum for 48 hours (LMs) and (iv) soaking in a mixture of *L. plantarum* (10⁵ cfu/mL) inoculum and molasses (114 g/kg DM) (1:1 v/v) for 48 hours (MLMs). The four dietary total mixed ration pellets designated as treatments were: SMs, MMs, LMs and MLMs. Total digestible nutrient and CP content of all diets were formulated to be similar, that is 65% and 17% DM, respectively.

Feed samples were prepared following procedures as described by Ranjit and Kung jr. (2000), stored at 4°C for 24 h in the refrigerator for lactic acid, acetic acid and propionic acid analysis. The slurry mixture was then filtered through 4 layers of cheesecloth and the filtrate used for pH, lactic acid, acetic acid and propionic acid determination following procedures as described by Bolsen *et al.* (1992). The pH was directly measured using a pH meter (Sortorins basic pH meter, Germany). The concentration of lactic acid, acetic acid and propionic acid were analysed with Hewlett Packard 6890 capillary gas chromatograph fitted with a J & W ov 351 capillary column (FFAP phase) 30 m x 0.25 mm (Getachew *et al.*, 1998). Lactic acid was determined using procedures described by Muck (1988). All the chemical analyses studies were conducted in triplicates and the results expressed on DM basis.

3.3.5 Milk fatty acid profile

Milk butterfat, solid not fat, lactose, solids, protein and total solids were measured using milk analyzer (Lactoscan model SA30 version 59 ultrasonic). 200 mg of representative milk sample for FA analysis were prepared every second day from the respective four milking for each individual cow and pooled according to daily milk yield. These aliquots were stored at -20°C . The pooled milk samples were thawed and gently mixed. An amount of 0.5 ml was diluted in 5 ml n-heptane containing tri-undecanoin, tetra-decenoic methylate and tri-valeranol as internal standards. Na-methylate was added for cold trans-esterification to FAME as described by Suter *et al.* (1997). The individual FAME was analyzed with a CP7421 column ($200\text{ m} \times 0.25\text{ mm}$, $0.25\text{ }\mu\text{m}$; Varian Inc., Darmstadt, Germany) using the same GC as for feed analysis. The FAME were injected at a volume of $1.3\text{ }\mu\text{L}$ and a split of 1:30 Hydrogen was used as carrier gas with a flow of 1.7 mL/min , which was kept until the end of the analysis. The initial temperature was 181°C which was held for 60 min. Then the temperature was increased by 5°C/min to 230°C . This temperature was held for another 32 min, and then increased at rate 5°C/min up to 250°C . This final temperature was held for 12 min before cooling down to the initial temperature of 181°C . The FA were identified using a FAME standard (Supelco 37 Component, Supelco Inc., Bellefonte PA, USA) and by comparison with chromatograms for milk lipids resulting from similar determinations (Kramer *et al.*, 2002). In order to correct for the lower response of short-chain FA compared to medium- and long-chain FA, response factors were made from 4:0, 10:0 and 18:0 triglyceride standards that were used to adjust individual FA accordingly.

3.3.6 Statistical analysis

In order to examine if there were statistically significance differences among farmers' response and ranking on crop residues utilization, variability and limitations in utilization of crop residues, farmers' responses were pooled and subjected to non-parametric statistics analysis (Kruskal-Wallis) using (XLSTAT, 2013). Farmers using a scale of 1 to 5 and with five being the

most important crop residues ranked all the crop residues. Also, limiting factors were ranked by farmers using a scale of 1 to 5, with 5 being the most important limitation in utilization of crop residue. The computed sum of ranks and mean of ranks were compared using multiple pair-wise comparisons to examine differences in utilization levels and limitations to utilization of crop residues. Data on chemical composition, IVDMD, IVOMD, OMD, ME and PF of experimental treatments were examined using mixed model procedures of SAS (2010). Statistical model used for the *in vitro* experiment was as follows: $Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$, Where Y_{ij} was the nutritive value, μ was the overall mean, τ_i was the fixed effect of pre-treatment method of maize stover prior to inoculation with *P. sajor caju* ($i = 1, 2, 3, 4$) β_j was the fixed effect of block ($j = 1, 2, 3, 4$) and ε_{ij} is the random residual error. Data obtained were subjected to analysis of variance according to statistical model stated above, tested at $P < 0.05$. When a parameter showed significantly different at $P < 0.05$, the means among various treatments were further tested with Duncan's Multiple Range test. Relationships between various parameters were assessed using principal component analysis (PCA) (Jayanegara *et al.*, 2011).

Data on chemical composition of the TMR pellets, dry matter intake, milk production, milk composition and milk fatty acid were analyzed using a mixed model procedure of SAS (2000) in a 4×4 Latin square design using the model: $Y_{ijk} = \mu + C_i + R_j + P_k + \varepsilon_{ijk}$ Where Y_{ijk} was the observation from lactating cow j receiving TMR i , in period k . μ was the overall mean, R_i was the effect of the different TMR ($i = R1, R2, R3, R4$), C_j was the effect of lactating cow ($j = C1, C2, C3, C4$) and P_k was the effect of the period ($k = P1, P2, P3, P4$) ε_{ijk} was the residual effect. Results were presented as mean values with standard error of the mean. Differences between the treatment means were determined by Duncans New multiple Range test and differences among means with $P < 0.05$ was represented as statistically significant differences.

CHAPTER FOUR

PRODUCTION CHARACTERISTICS OF SMALLHOLDER DAIRY FARMS IN LAKE VICTORIA CRESCENT, UGANDA

This chapter is based on a published paper by:

Atuhair, A. M., Mugerwa, S., Kabirizi, J. M., Okello, S., Kabi, F., Lukwago, G. (2014).
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4.0 ABSTRACT

Smallholder dairy farms are increasingly being rediscovered as a strategy for improving livelihoods for households in Lake Victoria crescent agro-ecological Zone (LVZ), Uganda. The system is faced with challenges of poor productivity of the animals, especially, in dry season when natural forages are scarce. This study was carried out in Buikwe, Jinja and Mayuge Districts with the objective of characterizing smallholder dairy farming and prioritizing the challenges. One hundred and twenty six (126) smallholder dairy farmers were randomly sampled using structured, semi-structured questionnaires and focus group discussions between September and November 2013. Data collected and analysed included household demographics, ranking of challenges, feed resources, labour distribution, milk production and marketing. Households in Buikwe district had relatively larger herd size of 4.29 ± 0.86 head of cattle. Natural pastures were ($P < 0.05$) the main source of feed, smallholder dairy farmers in Jinja ($P < 0.05$) use agro-industrial by-products as an alternative feed resource during periods of scarcity while farmers in Buikwe and Mayuge ($P < 0.05$) use more of crop residues as major feed supplement. Low farm gate price of milk that always fluctuated ($P < 0.05$) with season in all districts was one of the challenges and feed scarcity was ($P = 0.001$) ranked as a priority challenge that should be addressed. The study provided information on production characteristics of smallholder dairy farming system based on farmer's point of view. This evidence-based information would be used to benchmark research interventions to prioritize challenges that affect productivity on smallholder dairy farms in LVZ.

Key words; Dairy cattle, Labour distribution, Smallholder, Uganda

4.1 BACKGROUND OF THE STUDY

Smallholder dairy farming system constitutes an important source of livelihoods to the majority of mixed crop-livestock farmers involved in agricultural production in Uganda (Kabirizi *et al.*, 2006). While smallholder dairy farmers make a shift towards market-oriented dairy production, they are faced with persistent challenges of low productivity coupled with limited labour inputs. This practice has condemned smallholder dairy farmers to subsistence production resulting to low income, low saving and low investment in the dairy sector, thus, triggering vicious cycle of low inputs, low productivity, low technology applications and environmental degradation, which translate into abject poverty (Muia *et al.*, 2011). Uganda's slow growth in the dairy sector is evidenced by declining production yields lower than the estimated potential production growth of about 70% (MAAIF, 2010) considering that over 85% of dairy farmers are smallholders. The annual Gross Domestic Product (GDP) of agriculture in year 2012/13 was at a rate of 1.4% and unstable (MFPED, 2013), yet population growth is estimated at 3.2% per annum and appears to be on the rise (UBOS, 2012). Therefore, it was important, to understand production characteristics of smallholder dairy farming so as to identify their opportunities and strength, and build on their threats and weakness to benchmark on as we build future research processes aimed at getting famers out of abject poverty and extreme hunger.

Dairy production has become increasingly intensive in order for farmers to cope with nutritional needs of exponential human population increase and finite land holdings (Lukuyu *et al.*, 2011). This has led to intensification of smallholder dairy farming adopting stall-feeding (also known as "cut and carry") where one to three heads of cattle are fed indoors instead of *in-situ* grazing (Tibayungwa *et al.*, 2010). Smallholder dairy farming has become an important source of milk and has created employment for many resource poor households in Uganda (Kabirizi *et al.*, 2006), partly due to Uganda's National Development plan (NDP) whose objective is to eradicate poverty through agricultural transformation (MAAIF, 2010).

Smallholder dairy farming system of usually 1 or 2 head of cattle has the highest economic returns compared to other cattle management systems (MAAIF, 2010). However, low productive performance reduces its profitability (Kabi *et al.*, 2013). For example, annual average milk yield per cow per lactation year of 305 days in developed countries is in excess of 8,000 kg while on smallholder dairy farm in Uganda, it is less than 2000 kg per cow per lactation year (Mubiru *et al.*, 2011). Such low milk productivity is to a large extent a result of feed scarcity that leads to poor nutrition (Kabi *et al.*, 2013).

Smallholder dairy farming is based on stall-feeding as a major feeding system because of its efficiency compared to other feeding systems. However, little information is currently available on production characteristics of smallholder dairy farming in Lake Victoria Agro-ecological Zone (LVZ) in Uganda. Moreover, production characteristics of smallholder dairy farming influence decisions on technology dissemination for future profitability, since interaction between production and management revolve mostly around the supply of nutrients and energy through dairy feeds. Therefore, there was a need to characterize smallholder dairy farming system, in order, to predict their performance and benchmark on strategic research innovations to address declining smallholder dairy farmer productivity. However, several studies based on farm characteristics have been conducted among smallholder dairy farmers but the challenge of extreme hunger and poverty has persisted. Scientists, farmers and all stakeholders should all participate in the process of identifying and prioritising of appropriate intervention strategies needed to address challenges of poor productivity. Therefore, the objective of this study was to characterize smallholder dairy farming system from farmers' point of view and prioritize the challenges.

4.2 MATERIALS AND METHODS

4.2.1 Study area

Three districts of Buikwe ($0^{\circ}18'4.32$ N and $33^{\circ}3'6.63$ E), Jinja ($0^{\circ}25'28$ N and $33^{\circ}12'15$ E) and Mayuge ($0^{\circ}27'35$ N and $33^{\circ}28'49$ E) (Figure 4.1) were randomly selected for the study. The mean daily temperature ranges between $16 - 28^{\circ}\text{C}$, $18 - 28^{\circ}\text{C}$ and $17 - 27^{\circ}\text{C}$ for Buikwe, Jinja and Mayuge Districts, respectively. The mean annual rainfall ranges between 1279 to 1544 mm, 1200 to 1500 mm and 1100 to 1500 mm for Jinja, Mayuge and Buikwe, respectively. The three districts are located in the Lake Victoria Crescent Agro-ecological Zone (LVZ).

The districts experience bimodal rainfall pattern, (March to May and September to November), with two dry spells (December to February and June to August). The high rainfall puts the region in one of those ecological zones with the highest potential for crop cultivation, pasture production and intensive livestock production, thus, signifying a huge possibility of integrating crop and smallholder dairy farming for efficient natural resource exploitation and management. According to the Uganda Population and Housing Census (2002), mean estimated population density was 256, 66, and 92.55 people per square kilometre for Buikwe, Jinja and Mayuge districts respectively. Agriculture is the main economic activity but has of late suffered from seasonal unpredictable seasons characterized with unprecedented extremes of weather such as floods and severe droughts that lead to crop failure and increased feed scarcity. The mean arable land is 529, 601.1 and 603.3 km^2 for Buikwe, Jinja and Mayuge districts respectively.

4.2.2 Study design and sampling methods

Using multi-stage simple sampling procedures, the first stage considered a sample frame of 15 districts that make up LVZ. The three districts were randomly selected following simple random procedures. Each of the selected districts formed a homogenous group stratum (Domain

of analysis). The second stage involved simple random sampling of three sub-counties per district, the third stage was sampling of respondents (smallholder dairy farmers) in consultations with the district extension staff and sub-county extension officers. All the smallholder dairy farmers in the sampled sub-county were considered. Selection of households was also by simple random sampling and sample size was determined using formula described by Israel, 2009.

$$n = \frac{Z^2_{\alpha/2}}{e^2} p(1-p)$$

Where n = Sample size, $Z_{\alpha/2}$ = Confidence interval at 95% (Standard value of 1.96) p = 10% of proportion of smallholder dairy farmers in LVZ, Uganda (UBOS, 2012) and e = desired levels of precision at 95%. The chosen sample required 14 respondents in each study site, which was a sub-county, totalling to 42 respondents per district. Altogether, 81 men and 45 women participated in the three districts. Data was obtained using structured and semi-structured questionnaires administered on one-on-one interviews. Focused group discussions (one per sub-county) were also held to corroborate the information gathered in direct interviews. The checklist for focused group discussions were intended to capture information on production characteristics on smallholder dairy farms. The data captured was on household demographics, highest education level of household head, major sources of income into household, herd size, general challenges, available feed resources, labour activities and challenges in milk marketing.

In order to examine differences among farmers' ranking on different parameters, farmers' responses were pooled and subjected to nonparametric statistical analysis (Kruskal–Wallis one-way analysis of variance) (XLSTAT, 2013). Farmers ranked the parameters based on scale of 1 to 5, where 1 scored the least important parameter while 5 scored the most important. The computed mean of ranks was compared using multiple pair-wise comparisons to establish if there were significant differences in the parameters (Dunn, 1964). XLSTAT, 2013 was used to

generate summary statistics (frequencies, percentages and means) for the parameters. Means of ranks of variables were analysed using Chi square and considered different when $P < 0.05$.

4.3 RESULTS

4.3.1 Demographic characteristics of the study population

Across the household stratification, males headed the majority of smallholder households in Lake Victoria Crescent Agro-ecological Zone (LVZ) (Table 4.1).

Table 4.1. Household demographic profiles on the smallholder dairy farm

Household characteristics	Buikwe (n = 42)	Jinja (n= 42)	Mayuge (n=42)
Female headed, %	28.57	66.67	30.95
Male headed, %	71.43	33.33	69.05
Average age of household head (years) Mean± SEM	51± 9	48 ± 11	50 ± 9
Average household size (persons) Mean± SEM	6 ± 2	5 ± 1	6 ± 3
Dairy cattle farming experience (years) Mean± SEM	10 ± 8	12 ± 8	11 ± 7

SEM, standard error of the means

4.3.2 Education level

The educational level of the household heads in smallholder dairy farming system was relatively high in the study districts with majority having reached primary seven and beyond (Figure 4.1).

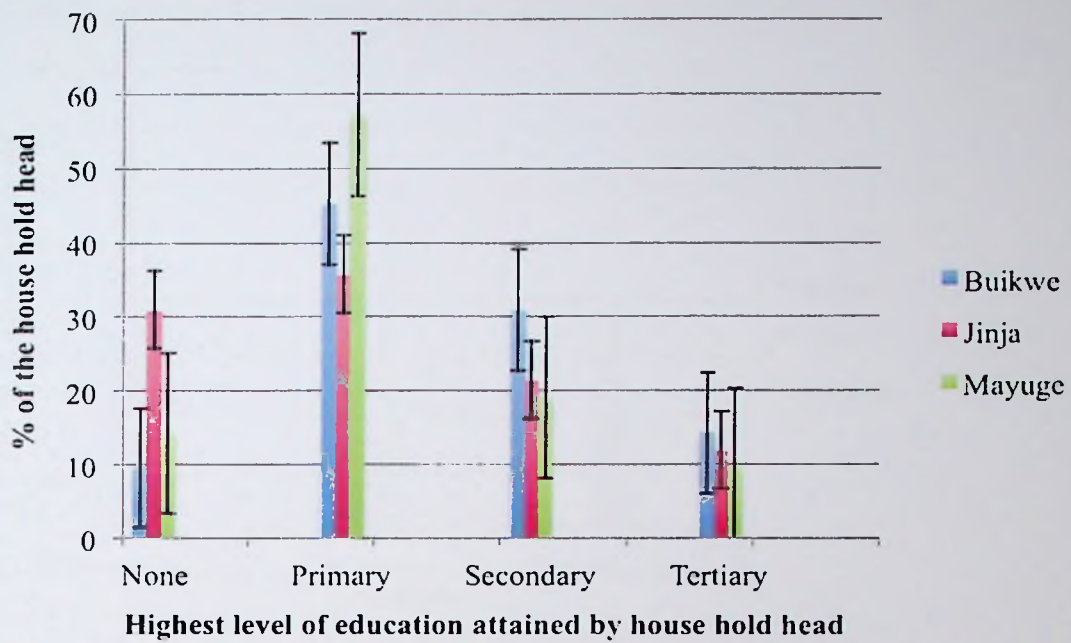


Figure 4.1 Highest educational levels attained by household heads

4.3.3 Source of income of household heads

There was variation in major sources of income of household heads between the three districts. The highest percentages of household heads in Jinja district were full time farmers (68.24%) compared with Buikwe (35.73%) and Mayuge (45%) where over 52.9% of household's heads were engaged in other businesses and formal employment apart from farming as indicated in Figure 4.2.



Figure 4.2 Major income sources of smallholder dairy farm household heads

4.3.4 Herd size and structure on smallholder dairy farms

Smallholder dairy farms households in the districts of Buikwe had a relatively larger dairy herd size (4.29 ± 0.86) compared to Jinja and Mayuge districts, with a herd size of (3.12 ± 1.45 and 2.43 ± 1.12) respectively (Table 4.2). Cows constituted the highest proportion (48.3%) of the herd in Buikwe followed by heifers (22.34%). Smallholder dairy farms in LVZ had breeding bulls.

Table 4.2. Dairy herd structures on smallholder dairy farm in LVZ, Uganda

Herd structure	Number of cattle			SEM
	Buikwe	Jinja	Mayuge	
Mature Bulls	1 ± 0.00	1 ± 0.00	1 ± 0.00	0.00
Cows	2.05 ± 0.44	1.14 ± 0.35	2.14 ± 0.01	0.67
Heifer	1.02 ± 0.16	1.00 ± 0.00	1.00 ± 0.00	0.11
Male calves	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.00
Female calves	1.15 ± 0.37	1.00 ± 0.00	1.00 ± 0.00	0.29

SEM, standard error of the mean

4.3.5 Challenges in smallholder dairy farming system

The farmers' ranking of the challenges encountered in smallholder dairy farming system in LVZ were presented in Table 4.3. Chi square analysis showed that mean ranks of challenges faced within smallholder dairy farming system significantly varied ($P=0.001$) in all the districts. Feed scarcity, which escalated during dry season was highly ranked challenge throughout the zone.

Table 4.3. Farmers' ranking on the challenges encountered.

District	Variable	Sum of ranks	Mean ranks	Rank	(X^2)	P-value
Buikwe	Feed scarcity	2817.0	93.9	1	47.6	0.001
	Inadequate knowledge	2304.0	76.8	2		
	Livestock health	2037.0	70.2	3		
	Limited Labour	1052.5	47.8	4		
	Limited land	567.5	27.0	5		
Jinja	Feed scarcity	1587.5	72.2	1	46.1	0.001
	Livestock health	1217.5	55.3	2		
	Limited land	1173.5	53.3	3		
	Limited Labour	372.5	24.8	4		
	Inadequate knowledge	305.0	20.3	5		
Mayuge	Feed scarcity	2659.0	91.7	1	45.6	0.001
	Livestock health	2114.0	72.9	2		
	Inadequate knowledge	1929.5	68.9	3		
	Limited Labour	1019.5	46.3	4		
	Limited land	534.0	26.7	5		

X^2 = Chi-square

4.3.6 Farmers' ranking on feed resource utilization in smallholder dairy farming system

Chi-square test showed that there was a significant difference ($P=0.001$) among farmers' ranking on utilization of different feed resources on smallholder farms in LVZ. The significant differences in farmers' ranking on utilisation of feed resources were maintained among the districts (Table 4.4).

Table 4.4 Farmers' ranking on feed resources utilization.

District	Feed resources	Sum of ranks	Mean of ranks	Ranking	X ² , df = 4	P-value
Buikwe	Natural pastures	4865.5	121.6	1	28.3	<0.001
	Crop residues	4797.0	119.9	2		
	Legumes	4102.5	105.2	3		
	Fodder pastures	3235.5	83.0	4		
	Agro-industrial by-products	2700.5	67.5	5		
Jinja	Natural pastures	2603.0	76.6	1	9.5	0.049
	Agro-industrial by-products	2700.5	67.5	2		
	Crop residues	1350.0	64.8	3		
	Fodder pastures	1343.5	58.4	4		
	Legumes	1264.5	50.6	5		
Mayuge	Natural pastures	4643.0	129.0	1	47.7	< 0.001
	Crop residues	4133.0	114.8	2		
	Agro-industrial by-products	2741.0	76.1	3		
	Fodder pastures	2458.0	68.3	4		
	Legumes	2315.0	64.3	5		

df; degree of freedom, X²; chi-square.

4.3.7 Availability of Labour on a smallholder dairy farm in LVZ

Major activities performed in the smallholder dairy cattle farming system in LVZ, Uganda, are shown in Table 4.5. There were no distinct age and sex division of labour but, rather, all genders contributed to all farm activities. In Buikwe and Mayuge on average, men contributed more labour (41.4 and 40.9%, respectively) in the dairy unit than women for each district (22.1 and 24.1%, respectively). Women's labour activities were highest in shade cleaning than in any other activity while men's highest labour activities were in chopping and feeding, milking, marketing of milk and spraying against ticks as indicated in Table 4.5.

Table 4.5 Labour activities in smallholder dairy farming system

Activity	Buikwe				Jinja				Mayuge			
	W	H	C	HR	W	H	C	HR	W	H	C	HR
Garden preparation and crop planting	52	21	10	17	63	9	7	21	69	5	8	18
Harvesting and transportation of feed	11	41	14	34	16	23	8	53	19	25	1	55
Chopping and feeding	8	54	7	31	8	38	4	50	5	35	6	64
Water collection and watering animals	47	12	13	28	18	9	6	67	22	26	0	52
Shed cleaning	48	10	10	32	42	8	4	46	51	5	4	40
Milking	2	66	14	18	5	34	5	56	9	77	2	12
Marketing	2	66	14	18	5	34	5	56	9	77	2	12
Spraying the animals	7	61	14	18	5	34	5	56	9	77	2	12
Average	22.1	41.4	12	24.5	20.2	23.6	5.5	50.6	24.1	40.9	3.1	33.1

H=Husband; W=Wife; C=Children; HL=Hired labour

4.3.8 Challenges to milk-marketing on smallholder dairy farm in LVZ

Chi-square test showed how farmers ranked the challenges associated with raw milk marketing in LVZ in the three districts (Table 4.6). Low prices were the top most challenge identified by the farmers while unstable price of milk were ranked the second major challenge across all the districts.

Table 4.6. Smallholder dairy farmer's ranking on challenges in milk marketing.

District	Challenges in milk marketing	Sum of ranks	Mean of ranks	Ranking	X^2 , df = 4,	<i>P</i> -value
Buikwe	Poor price	1633.50	71.0	1	32.8	<0.001
	Fluctuation in price	1296.50	56.4	2		
	Perishable product	1165.50	52.9	3		
	Delayed payments	634.50	37.3	4		
	Long distances to market	320.00	21.3	5		
Jinja	Poor price	579.50	41.4	1	17.5	0.002
	Fluctuation in price	498.50	35.6	2		
	Long distance to market	412.50	31.7	3		
	Perishable product	187.00	18.7	4		
	Delayed payments	152.50	16.9	5		
Mayuge	Poor price	1269.00	63.45	1	33.1	<0.001
	Fluctuation in price	966.00	48.3	2		
	Long distance to market	902.00	47.47	3		
	Perishable product	473.50	31.56	4		
	Delayed payments	217.5	16.73	5		

df, degree of freedom. X^2 ; chi-square.

4.4 DISCUSSION

4.4.1 Demographic characteristics of the households

The explanation for proportionately higher female households heads in Jinja district than in other districts of Buikwe, Mayuge was because of affirmative action by Non-Governmental Organizations (NGO), "Heifer Project International", which operated in the region prior to the study. It targeted women for economic empowerment, especially, widowed by HIV/AIDS pandemic. Almost each widow received an in-calf heifer; hence, this gave opportunity to a

relatively high proportion of women-headed household to own dairy cattle. Average household size in Jinja was also lower than those in Buikwe and Mayuge respectively. Typically, household members comprised of husband, wife and children. The size of household could influence labour availability in dairy farming with Jinja having less labour available to perform dairy activities and relied most on hired labour. Availability of labour in any production system has a significant influence on productivity and since smallholder dairy-farming system is labor-intensive (Njarui *et al.*, 2011). It impacted negatively on productivity.

Buikwe district had more farmers who had completed tertiary institutions of learning (Figure 4.1). This suggests that adoption levels of new innovation in Buikwe can be high compared to other districts. Mugerwa *et al.* (2012) noted that attainment of higher education levels is proportional to level of adoption of agricultural technologies. This was consistent with the general belief that adoption levels are positively correlated with the levels of education attained by the farmer. This was possibly, because education influences the ability of farmers to interpret the technical recommendations that may require some level of education. Furthermore, Kaunda, (2011) reported that literate farmers can comprehend the benefits from extension information and they were aware of the consequences of the prevailing challenges if they are not addressed in time.

Majority of the farmers in Buikwe and Mayuge (Figure 4.2) had other alternative sources of income, while in Jinja most of the farmers relied on dairy farming as their main source of income. This was possibly because of NGOs interventions, a similar observation was made earlier in Masaka district, Uganda (Njarui *et al.*, 2011). Majority of women received animals from NGO (Send-a Cow) had no other alternatives forms of employment and sources of household income. However, smallholder dairy farming in LVZ seemed to be economically unstable venture due to low investment levels, unreliable inputs and lack of infrastructural development such as milk collection centers. Thus, farmers were seeking for alternative sources

of livelihood that could be strategy to compliment on improved livelihoods. This diversification, sometimes, becomes a competitive undertaking, which denies smallholder dairy farming an opportunity for accumulation of more knowledge and capital investment.

4.4.2 Challenges in smallholder dairy farming system

While increased animal productivity has been identified as one of the options to increase household incomes, nutrition and livelihood of the rural people (MAAIF, 2010), feed scarcity was unanimously identified as one of the biggest challenge to productivity in LVZ. The consequence of feed scarcity to smallholder dairy farming system was poor milk yield, distortion of the estrus cycles, poor body condition and long calving intervals (Kaunda, 2011). Farmers, therefore, missed opportunities on proceeds from milk sales and offspring (Lukuyu *et al.*, 2011). High cost of commercial supplementary feeds irrespective of seasons in LVZ, despite the abundance of agro-industrial by-products points to limited investment both in technology development and innovation. This was in agreement with earlier observation by Mubiru *et al.*, (2011) who observed that low milk yield in Uganda was attributed to poor feeding methods resulting from not meeting the right nutritional requirement of dairy cattle. Similarly, limited value addition to highly perishable milk renders it rather difficult to fetch reasonable prices despite its high local demand right at the farm. These findings were consistent with earlier findings that indicated poor milk prices as major challenges to increased dairy productivity in peri-urban areas of East and Central Africa (Mugerwa *et al.*, 2012).

4.4.3 Labour activities in smallholder dairy farming

Most activities were performed daily, implying that dairy farming is a labor-intensive enterprise, an observation that was consistent with (Njarui *et al.*, 2011) who made similar observation in Masaka district, Uganda. There were no distinct age and sex division of labour but all gender contributed to smallholder dairy activities. However, there were disparities in level of

labour contribution between men, women and children contrary to findings of Mubiru *et al.*, (2007). In Buikwe and Mayuge districts, on average, men contributed more labour in the dairy unit than women but in Jinja, men contributed more labour than women. Women contributed labour in shed cleaning than in any other activity while men contributed highest in milking, marketing milk and spraying against ticks a similar observation was made by Kabirizi *et al.* (2006). This suggested that cultural dynamics had influence on dairy farming where inclinations to patriarchal norms where men were seen as household breadwinners may explain why men were responsible for activities involving cash transactions in the dairy enterprise. Similarly, all decision concerning labor activities of the enterprise were unilaterally made by the heads of households the majority of whom were men. The contribution of children to running of dairy unit was insignificant, less than 7% on average of total labour activities. Notably, children did not participate in cutting forages, feeding and watering of the animals. The low contribution of children was primarily because they attended school during weekdays and they were only available during weekends and holidays.

Nonetheless, the family labour was not sufficient to run the dairy unit and significant labour was sourced from outside, particularly, in Jinja. In Jinja, overall hired labour contributed more than half of the total labour required in running of the dairy enterprises. This implies that hired labour was important for the success of dairy farming in the LVZ given the low levels of mechanization. Mugerwa *et al.*, (2012) reported similar scenario where hired employees contributed about 50% of the entire labour requirement on smallholder dairy farm.

4.5 CONCLUSIONS

The conclusions drawn from this study were that lack of knowledge to make timely decisions on available feed resources, limited value addition to highly perishable milk and lack of basic equipment to reduce on hard work contributed to the outstanding challenges that affect productivity. The efficiency of production and marketing of milk should be improved, in order,

to enhance smallholder dairy production in LVZ, Uganda. Therefore, milk productivity could be enhanced through appropriate engagement with the farmer to generate sustainable options needed to improve nutrient supply throughout the year. Highly appreciated and utilized crop residues and agro-industrial by-products should be identified. Limitations to their utilization evaluated and supplementary dairy cattle ration based on highly abundant and agro-industrial by-product and crop residue formulated. Appropriate on-farm feed conservation practices should be promoted that includes biological processing of fibrous crop residues, hay and silage making. Furthermore, it was necessary to conduct on-farm strategic studies in LVZ, Uganda to upgrade and enhance utilization of crop residues and agro-industrial by-products.

4.6 ACKNOWLEDGEMENTS

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CHAPTER FIVE

PRIORITIZATION OF CROP RESIDUES FOR IMPROVING PRODUCTIVITY ON SMALLHOLDER DAIRY FARMS IN LAKE VICTORIA CRESCENT, UGANDA

This chapter is based on a published paper by:

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5.0 ABSTRACT

Poor nutrition of the animals has been identified as a major constraint to productivity in smallholder dairy farming households in Uganda, particularly, in Lake Victoria Crescent Agro-ecological Zone (LVZ). Clarification on nutritional potential of crop residues is central to formulation of sustainable dairy cattle nutrition strategies. Data was collected from 126 randomly selected respondents using structured and semi-structured questionnaires. Farmers' responses on crop residues utilization, handling, spatial and temporal variability were collected. Kruskal-Wallis test showed ($X^2 = 50.4$, $df = 4$, $P = 0.001$) differences on utilization of available crop residues among farmer's rankings. It was established that maize stover was a major crop residues utilized but inadequate knowledge to process it was also ranked as a major limitation. The study provided basic information on the importance of maize stover. Further research studies should focus on improving the nutritive quality of maize stover.

Key words: Haulms; Stover; Nutritive Value

5.1 INTRODUCTION

Because of its contribution to the socio-economic development of rural Uganda in both food security and in-come generation, especially, among women and other disadvantaged groups, smallholder dairy production system has received considerable support from the Government of Uganda as well as non-government organizations (Kabirizi *et al.*, 2006). Moreover, by functioning as a store of wealth (Winrock international, 1992) and supplying manure for crop production (Kabi and Bareeba, 2007), dairy cattle fit very well in integrated crop-livestock systems. In Uganda, dairy cattle play a key role in the nutrition of most households with per capita milk consumption of about 58 litres (MAAIF, 2010) against FAO recommendation of 200 litres per person per year (FAO, 2012). While annual average milk yield per cow per lactation per year of 305 days in developed countries can go above 8000 kg, less than 2000 kg is obtainable from pure dairy breeds, 1000 from cross breeds and 500 kg from

indigenous cows in Uganda (Bahiigwa *et al.*, 2005). These statistics are obviously distressing in light of the rapidly growing human population at a rate of 3.2% annually (UBOS, 2012). Mugerwa *et al.*, (2012), singled out that feed scarcity leads to poor nutrition, which was a key constraint holding down production efficiency and health of the dairy cow on smallholder dairy farms in Lake Victoria Agro-ecological Zone (LVZ). Poor nutrition of dairy cattle is exaggerated by drought-induced feed scarcity attributed partly to change in climate and demographics. As human population increases, demand for milk also increases, crop production expands and availability of land for forage production decreases contributing towards dairy cattle feed scarcity. With projected increase in demand for milk, coupled with declining land size for forage production due to demographic pressure, it seems inevitable for farmers to embrace alternative feed resources. Utilization of crop residues, therefore, seems a logical alternative to address the escalating levels of feed scarcity among smallholder dairy farming systems (Lentes *et al.*, 2010). Efficient utilization of crop residues, however, faces a number of intriguing challenges that include low levels of metabolizable energy and crude protein (Tesfaye and Chairatanayuth, 2007) seasonal variability (Tsopito, 2003), bulkiness (Walli, 2009) and nutritional qualities (Anandan *et al.*, 2010). These challenges should be acknowledged for appropriate technological innovations that prioritize crop residues as alternative feed to supply nutrients to dairy cattle for improved productivity on smallholder farm.

Crop residues have been used as livestock feeds since time immemorial and are readily available feed resources (Njarui *et al.*, 2011), however, their nutritional value is poor (Tesfaye and Chairatanayuth, 2007; FAO, 2012). Considerable research efforts have been devoted to improving their nutritional value through crop management and breeding, physical, biological and chemical treatments as well as supplementation with high protein oil cakes, green fodder and tree leaves (Mugerwa *et al.*, 2012; Preston and Lang, 1987). However, on-farm implementation of these strategic innovations seems unsatisfactory. Furthermore, in Uganda there is scanty

information on crop residue utilization, temporal and spatial variability as well as limitations associated with their utilization on smallholder dairy farms. Unlike in other developing countries, these information a makes a basis for identifying opportunities, for, prioritizing feedstock from crop residues for improved nutrition hence enhanced productivity on smallholder dairy farming household. Thus, this survey was designed to assess crop residues variability, limitations and opportunities in LVZ for future research on developing appropriate dairy feeding systems that utilize crop residues. These would in long run secure smallholder dairy farming from demographic pressure and subsistence farming to improved productivity and sustainable farming systems.

5.2 MATERIALS AND METHODS

5.2.1 Study area

The study was conducted in the Lake Victoria Agro-ecological Zone of Uganda, which hosts the majority of smallholder dairy farmers. Three districts namely Buikwe (0°18'4"N and 33°3'6"E), Jinja (0°25'28"N and 33°12'15"E) and Mayuge (0°27'35"N and 33°28'49"E) were randomly selected from 15 districts in LVZ for the study based on the intensity of smallholder dairy farms (Figure 6.1). The mean daily temperature ranged from 16 - 28°C, 18 - 28°C and 17 - 27°C for Buikwe, Jinja and Mayuge Districts, respectively. The mean annual rainfall ranges between 1279 to 1544 mm, 1200 to 1500 mm and 1100 to 1500 mm for Jinja, Mayuge and Buikwe respectively. The districts experienced bimodal rainfall pattern characterized by two rainy seasons (March to May and September to November) with dry spells (December to February and June to August). According to the Uganda Population and Housing census (2002), the estimated mean population density was 256, 658 and 92.55 per person km⁻² for Buikwe, Jinja and Mayuge respectively. Agriculture is the main economic activity, prolonged droughts leading to crop failure is the main constraint to agricultural production.

5.2.2 Study design and sampling methods

The study was conducted in three districts (Buikwe, Mayuge and Jinja), which were purposively selected based on the high intensity of smallholder dairy farms. Three sub-counties were randomly selected from each district. After consultations with the district extension staff and sub-county extension officers and following all procedures of systematic random sampling selection, fourteen respondents were selected from each sub-county totaling to forty two respondents per district. Data were obtained using pre-tested structured and semi-structured questionnaires administered by way of one-on-one direct interviews. Focus group discussions (one per sub-county) were held to corroborate the information gathered in direct interviews. The questionnaires and focus group discussions were intended to capture information on availability, variability and limitations to utilization of crop residues on smallholder dairy farms.

5.2.3 Statistical analysis

In order to examine if there were statistical significance among farmers' ranking of crop residues utilization, variability and limitations in utilization of crop residues, farmers' responses were pooled and subjected to non-parametric statistical analysis (Kruskal-Wallis one-way analysis of variance) using XLSTAT, (2013). Farmers using a scale of 1 to 5 with five being the most important crop residue ranked five crop residues. Also, five limiting factors were ranked by farmers using a scale of 1 to 5, with 5 being the most important limitation in utilization of crop residue. The computed sum of ranks and mean of ranks were compared using multiple pair-wise comparisons to establish if there were significant differences in utilization levels and limitations to utilization of crop residues. XLSTAT, (2013) was used to generate summary statistics (frequencies, percentages and means) for the variables and later tabulated.

5.3 RESULTS

5.3.1 Farmers' ranking on utilization of crop residues

Kruskal-Wallis test showed that there were significant differences ($P = 0.001$) were maintained with farmers ranking on different crop residues among the districts (Table 5.1). Farmer's ranked maize stover as the most important, yet poorly utilized crop residues in the zone, in Buikwe, Jinja and Mayuge with mean ranks of 168.48, 122.67 and 152.15 respectively. Farmers ranked maize stover (mean of ranks = 152.15, 122.67 and 168.48) and sweet potato vines (mean of ranks = 117.32, 121.65 and 124.42) as the most important crop residue throughout Mayuge, Jinja and Buikwe districts respectively. Sugar cane tops (mean of ranks = 64.56 and 80.37) were ranked as the least important crop residue in Buikwe and Mayuge districts while in Jinja groundnuts haulms (mean of ranks = 69.75) were ranked as the least important crop residue. In Mayuge district, groundnut haulms (mean of ranks = 84.52) were ranked second least important crop residue while in Jinja it was sugar cane tops (mean of ranks = 86.7) and in Buikwe it was banana peels (mean of ranks = 89.33).

Table 5.1 Farmers' ranking on availability of crop residues

District	Crop residue	Sum of ranks	Mean ranks	X ² , P-value, df = 4
Mayuge	Maize stover	6238.0	152.1 ^c	X ² = 54.4, P = 0.001
	Sweet potato vines	4810.0	117.3 ^{bc}	
	Banana peels	3954.5	96.5 ^{ab}	
	G. nut haulms	3465.5	85.5 ^{ab}	
	Sugar cane tops	2647.0	64.6 ^a	
	SEM		9.9	
Jinja	Maize stover	3616.0	122.7 ^{bc}	X ² = 25.8, P = 0.001
	Sweet potato vines	4866.0	121.6 ^{abc}	
	Banana peels	4069.0	101.7 ^{bc}	
	Sugar cane tops	3468.0	86.7 ^{ab}	
	G. nut haulm	2790.0	69.7 ^a	
	SEM		5.56	
Buikwe	Maize stover	7581.5	168.48 ^c	X ² = 54.7, P = 0.001
	Sweet potato vines	5599.00	124.42 ^b	
	G. nut haulms	4608.00	102.4 ^{ab}	
	Banana peels	3616.00	89.33 ^{ab}	
	Sugar cane tops	4020.00	80.37 ^a	
	SEM		7.67	

^{abc} Means in the same column and district without common letter are significantly different ($P < 0.05$), X^2 = Chi-square.

5.3.2 Farmers' ranking on spatial and temporal variability of crop residues

Spatial and temporal variability of crop residues in the study area were assessed on monthly basis by asking the respondents to classify abundance of crop residues as highest, moderate or lowest. The orders were then converted to scores in such ways that score 3 was given to the highest in abundance, score 2 moderate and score 1 lowest. Then the percentage score for each crop residue was calculated as its total weighted score divided by the overall total scores. Calculated accordingly, the percent score for variability of crop residues at given point in time by respondents were as represented in Figure 5.2. LVZ has two dry seasons (December to February and June to August) and two wet seasons (March to May and September to November) in a year. Generally, farmers' score indicated that quantities of crop residues vary throughout the year. The highest in abundance was reported to occur towards the beginning of

dry season and least abundant levels were reported to be at the end of dry season. Maize stover was scored highest in abundance in first season, reducing slightly in second season then attaining peak abundance in third season and reducing progressively in fourth season throughout the zone. The abundance levels of sweet potato vines were scored highest in first season reduced in second season, increasing progressively to attain its peak abundance in third season. In fourth season, it decreases then starts increasing again towards the end of the season.

It was noted that most frequently utilized crop residues are highest in abundance in third season and lowest in fourth season. Maize stover was highest in abundance in Jinja district in the first season while the rest of the districts hit their peak abundance in third season. Groundnuts haulms were moderately abundant in fourth season in Jinja district while in the rest of the districts it was lowest in fourth season.

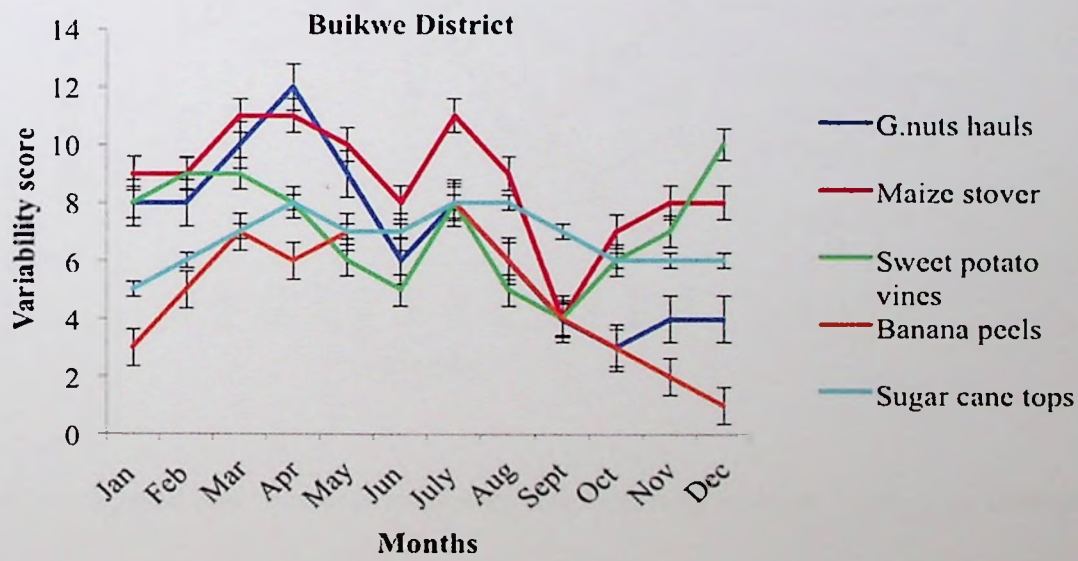


Figure 5.1 Spatial and temporal variability scores of crop residues in Buikwe District

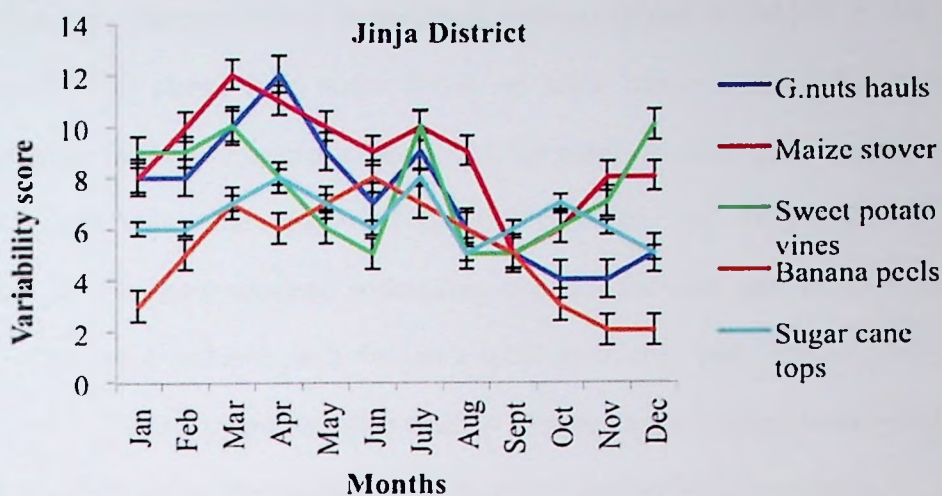


Figure 5.2 Spatial and temporal variability scores of crop residues in Jinja District

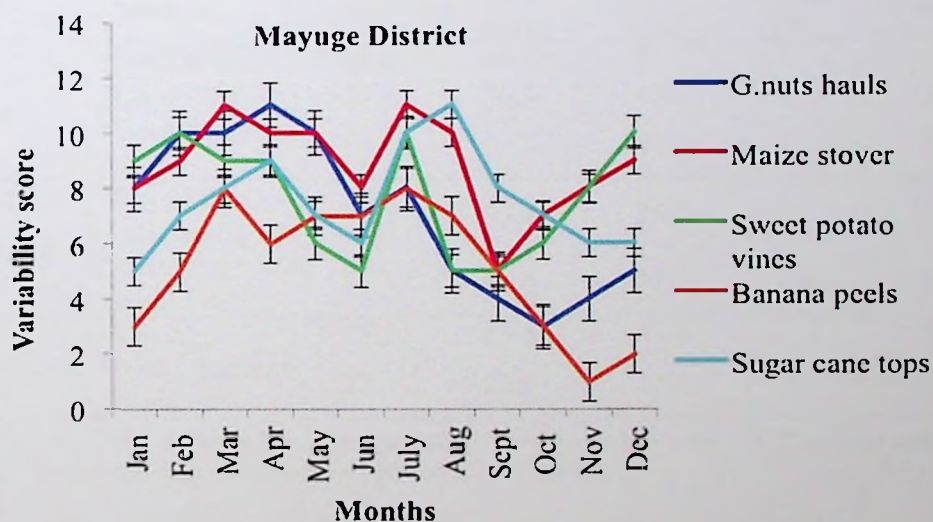


Figure 5.3 Spatial and temporal variability scores of crop residues in Mayuge District

5.3.3 Methods applied by farmers to store, process and preserve crop residues

Table 5.2 shows different methods used by the farmers to store, process and preserve crop residues. It was depicted that storage, processing and preservation had positive effect on improving crop residues intake by the dairy cattle. Percentage of the respondents using different

methods of storage, processing and preservation were as shown in Table 5.2. The majority of the farmers (52.7%) stored their maize stover by home heaps while 7.6% did not use any particular storage facilities. Physical processing (chopping) of maize stover, groundnuts haulms and sweet potato vines were practiced by 71.2%, 60.4% and 88.3% of the respondents respectively was the most common technology applied. Farmers sprayed crop residues with additives that included molasses, salt, brewer's spent grain and yeast (Table 5.2) to improve on dry matter intake. The major preservation method of groundnuts haulms, sweet potato vines and banana peels was by drying represented by 3.6%, 27.9% and 44.1% respectively.

Table 5.2 Methods applied by farmers to store, process and preserve crop residues.

Practice	Percentage score of the farmers undertaking the method					
	Method	MS (%)	GNH (%)	SPV (%)	SCT (%)	BP (%)
Storage	Field heap	26.3	45.1	9		
	Home heaps	54.7	56.3	45.2		98.5
	Communal shade	19.4		47.7		2.1
Processing	Physical processing	71.2	60.4	88.3		
	Spraying with additives*	81.1	41.6	21.7	100	100
Preservation	Drying	75.2	83.8	57.9		100
	Multi-nutrient block	27.4	23.6	42.3		

MS = maize stover, GNH = ground nuts haulms, SPV = sweet potato vines, SCT = sugar cane tops, BP = banana peels, *with table salt, molasses, and brewer's grain/yeast.

5.3.4 Farmers' ranking on limitations to use crop residues as feed resource

The ranking by farmers on limitations to utilization of crop residues in smallholder dairy cattle feeds and feeding system were as presented in Table 5.3. Kruskal-Wallis test revealed $P < 0.05$ differences among farmers ranking. While in Buikwe lack of knowledge to preserve crop residue (mean of ranks = 114.32) was ranked as the major limitation in utilization of crop residues, in Jinja and Mayuge districts lack of knowledge to process crop residues (mean rank = 88.29, 121.24) was ranked as the major limitation. Other limiting factors ranked in descending

order of importance were: limited land, transportation of crop residues from the field and limited labour.

Table 5.3 Farmers' ranking on limitation to use of crop residues

District	Limitation	Sum of ranks	Mean of ranks	(X ² , df =4)	P-value
Mayuge	Lack of knowledge to process	4971.0	121.2 ^c	58.7	0.001
	Lack of knowledge to preserve	4583.0	111.8 ^{bc}		
	Transportation (Bulky)	2268.0	84.1 ^{ab}		
	Limited land	1829.5	55.4 ^a		
	Limited labour	1748.5	52.9 ^a		
	SEM		27.6		
Jinja	Lack of knowledge to process	3443.5	88.3 ^c	12.8	0.012
	Lack of knowledge to preserve	2764.5	83.8 ^c		
	Transportation (Bulky)	1458.0	66.3 ^b		
	Limited land	1639.0	63.0 ^{ab}		
	Limited labour	1572.5	58.2 ^a		
	SEM		8.0		
Buikwe	Lack of knowledge to process	4688.0	114.3 ^b	33.0	0.04
	Lack of knowledge to preserve	4910.5	111.6 ^b		
	Transportation (Bulky)	8262.5	95.9 ^b		
	Limited land	2301.0	67.7 ^a		
	Limited labour	1858.0	59.9 ^a		
	SEM		31.3		

^{abc} Means in the same column and district without common letter are different at $P < 0.05$; df = degree of freedom; SEM = standard error of the mean; X^2 = Chi-square.

5.4 DISCUSSION

Crop residues are fibrous parts of crops that remain after those edible to human beings have been detached. Through their digestive adaptations, which are primarily based on the degradation of fibrous materials by microbes in the rumen (Preston and Leng 1987), ruminant animals have the unique capacity to utilize these, otherwise, useless by-products. This indicates that in dairy cattle feeding system, crop residues could replace roughages in rations, reducing the

competition on cereals between human beings, monogastric and ruminant animals. Crop residues were readily available in LVZ, cheap feed resource because the grain, which is the main marketable product, takes care of the production costs.

The major crop residues available in the study areas were identified as maize stover, sweet potato vines, sugar cane tops, groundnuts haulms and banana peels. Maize stover was ranked a most available crop residue on smallholder dairy farm in LVZ of Uganda. It was also established that with increased crop failures due to prolonged drought as has become more frequent even in the LVZ large acreages of maize crops would be available for conversion into feed for smallholder dairy farms. Uganda currently ranks with the highest potential of maize production for export among the countries in the East, Central and Southern African region where maize is the staple food (Okaboi, 2011). If this potential was exploited, the massive quantities of maize stover generated could be a major feed resource for smallholder dairy farmers.

Furthermore, rankings of spatial and temporal variability of crop residues indicate that first season (December to February) was the main harvest period, which explained the abundance of the crop residues in second season (March to May). This was in line with studies by (Tsopito, 2003) who noted that variations in availability of crop residues were a major constraining factor in their utilization. This further suggested that interventions intended to enhance utilization of crop residues in LVZ should prioritize use of maize stover and enhance its nutritive value focusing on increasing available crude protein, improving digestibility and reducing crude fiber.

While there were prospects to improve the nutritive value of crop residues in LVZ through supplementation, simple treatment, processing and preservation methods, maize stover and sweet potato vines were fed to cattle without improving their nutritive values. The only method

undertaken by number of respondents (71.2% maize stover and 88.3% sweet potato vain) was physical processing by chopping. Feeding crop residues when they were unprocessed or untreated was limiting their intake (Lukuyu *et al.*, 2011). Integrating crop residues with forage legumes improves rumen microbial degradation of crop residues by supplying nitrogen to the rumen microbes hence increasing there digestibility and intake (Smith, 1993). Nitrogen supplementation in the rumen environment deficient of nitrogen leads to increased dry matter digestibility and voluntary feed intake (Mlay *et al.*, 2005). Furthermore, feeding small amounts of naturally occurring high protein supplement such as brewers spent grain also improves the nutritive value of crop residues (NRC, 2001). Biological treatment of maize stover utilizing mushroom fungi through fermentation is another alternative of converting maize stover into high nutritive value feed for dairy cattle (Akinfemi *et al.*, 2009).

Maintaining access to sufficient quality and quantity of nutrition is vital for milk production in the dairy cattle (Lukuyu *et al.*, 2011). Although crop residues are important feed resources, they are low in nutritive value (Tsopito *et al.*, 2003) and poor storage methods (Table 5.2) practiced by farmers predisposes them to rain and sunlight resulting into further deterioration in quality (Njarui *et al.*, 2011). Majority of the farmers (92.4%) interviewed stored maize stover for future use in dairy cattle feeding. Although the majority of the farmers understood very well the importance of storage and practiced the technology it was found that large proportion was left in the field for the animals to graze *in situ*, hence, resulting into inefficient utilization. Besides, where the crop residues were stored during feeding, it was thrown in the cattle shade. This resulted into trampling and wastage. It was further observed that crop residues, especially, maize stover were left to stand in the garden after harvesting of main crop (grain). Even following harvesting and stacking, they tend to be stored outdoors at home in heaps as reported by 52.7% of the respondents resulting into further nutrient losses through leaching. These findings were consistent with earlier studies that mentioned low nutritive value of crop

residues (Tsopito, 2003) and poor handling of maize stover (Akinfemi *et al.*, 2010). Therefore, in order to improve utilization of maize stover, the challenges of handling during harvesting, processing and storage should be addressed. This was vital in enhancing maize stover utilization and improving its intake and nutritive value for improved smallholder dairy farm productivity.

Results of this study further revealed that adequate knowledge needed to process and preserve crop residues was a major limitation in utilization of crop residues. Other limitations in descending order included; difficulty in transportation (bulkiness), seasonal variability, labour and storage facilities. Similar findings were reported by Anandan *et al.* (2010) who cited lack of knowledge and capital by Dejene *et al.* (2009), high labour cost by Ngongoni *et al.* (2006), low nutritive value by Walli, (2009) and difficulty in transportation because crop residues are bulky. All these limitations directly influenced the stability of the nutritive values of crop residues, hence, their utilization. Earlier research interventions on promoting smallholder dairy cattle productivity focused on fodder agronomy and seed production (Mugerwa *et al.*, 2012). Therefore, with increased effects of climate change and reducing household land holdings, emphasis must be shifted to utilization of crop residues. However, their nutritional deficiencies make them unable to support maintenance and production requirements (Preston and Leng 1987), pointing to the need for processing and supplementation research strategy to enhance their nutritional quality (Mubiru *et al.*, 2007). This calls for research innovations aimed at improving on processing, preservation and storage of crop residues, which should be appealing to resource constrained smallholder dairy cattle farmers for sustainable productivity.

5.5 CONCLUSION

Maize stover and sweet potato vines were the major crop residues utilized in the study area. They were not utilized at the optimum period, thus, compromising on their quality and variability. Lack of knowledge, poor quality and transportation were the major limitations. Improving productivity in dairy cattle production system in LVZ should, therefore, target

qualitative nutritional improvement of maize stover. Research thrust should be directed towards nutritive quality improvement techniques both on station and on farm to justify its economic feasibility. Biological processing of maize stover with mushroom fungi may provide a feasible research option for improved utilization of maize stover, in order, to improve smallholder dairy cattle production.

5.6 ACKNOWLEDGEMENTS

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5.7 AUTHORS' CONTRIBUTION

AMA, SM, SO, KOL, FK and JMK conceived and designed this study. AMA carried out data collection, AMA and SM carried out statistical analysis. All authors participated in the manuscript write-up, read the final version and approved it to be submitted for publication.

CHAPTER SIX

OPTIMIZING BIO-PHYSICAL CONDITIONS AND PRE-TREATMENT OPTIONS FOR BREAKING LIGNIN BARRIER OF MAIZE STOVER FEED USING WHITE-ROT FUNGI

This chapter is based on a published paper by:

Atuhaire, A. M., Kabi, F., Okello, S., Mugerwa, S., Ebong, C. (2016). Optimizing bio-physical conditions and pre-treatment options for breaking lignin barrier of maize stover feed using white rot fungi. *Animal Nutrition*. <http://dx.doi.org/10.1016/j.aninu.2016.08.009>.

6.0 ABSTRACT

The greatest limitation to utilization of maize stover by ruminants as a feed is the high concentration of lignin, which limits fibre digestibility. However, ruminants can effectively utilize maize stover if its nutritive value is improved using white rot fungal species. This study was designed to determine optimal bio-physical conditions for mycelial growth and select the most ideal fungal species and pre-treatment options for improving nutritive value of maize stover. This study was designed to determine optimal bio-physical conditions for mycelial growth and select the most ideal fungal species and pre-treatment options for improving nutritive value of maize stover. Four popular edible *Pleurotus* fungal species (viz. *Pleurotus florida*, *Pleurotus ostreatus*, *Pleurotus sajor caju* and *Pleurotus pulmonarius*) were subjected to varying temperatures, pH levels, hydrogen peroxide (H_2O_2) concentration and illumination to establish the extent of mycelial growth rate. Inclusion of H_2O_2 was used to determine optimal levels for preservation and prevention of contamination from other indigenous microbiota. Effects of pre-treatment options on chemical composition and nutritive value of maize stover were also examined. Mycelial growth rate of *Pleurotus* species on potato dextrose agar (PDA) varied ($P<0.05$) with temperature, pH level and H_2O_2 concentration following a quadratic trend. Optimal temperature, pH and H_2O_2 concentration for mycelial growth on PDA were 25°C, 5 and 0.01 mL/L, respectively. Under the different bio-physical conditions, *P. sajor caju* had the highest mycelial density and growth rate. Chemical composition of solid-state fermented maize stover differed ($P<0.05$) among the *Pleurotus* species. Maize stover fermented with *P. sajor caju* had the highest crude protein (CP) of 86.6 g/kg DM, *in-vitro* dry matter digestibility (IVDMD) of 731 g/kg DM, *in-vitro* organic matter digestibility (IVOMD) of 670.4 g/kg DM and metabolizable energy (ME) of 10.0 MJ/kg DM but with the lowest lignin (sa) of 50 g/kg DM. At 25°C, *P. sajor caju* had the highest mycelial growth rate on PDA and highest lignin (sa) breakdown in the maize stover substrate. It was, therefore, selected as the most ideal fungal

species for improving nutritive value of maize stover. Pre-treatment of maize stover with *Lactobacillus plantarum* and molasses under anaerobic condition for 7 days before inoculation with *P. sajor caju* resulted into a substrate with the highest ($P<0.05$) CP (96.6 g/kg DM), IVDMD (752.3 g/kg DM), IVOMD (687.2 g/kg DM) and ME (10.2 MJ/kg DM). However, neutral detergent fiber exclusive of residual ash (NDFom) and lignin (sa) fractions decreased ($P<0.05$) as a result of subjecting maize stover to pre-treatment with *L. plantarum* and molasses prior to fermentation with *P. sajor caju*. Therefore, pre-treatment of maize stover with *L. plantarum* and molasses for 7 days prior to fermentation with *P. sajor caju* for 14 days in darkness at 25°C offered the greatest potential for breaking the lignin barrier.

Keywords: Crop residues, *Lactobacillus plantarum*, Lignin biodegradation, Mycelial growth

6.1 INTRODUCTION

Although the history of lignin and cellulose chemistry is old with voluminous amounts of literature (Barton, 1988), the recalcitrant nature of lignin still limits the availability of nutrients to ruminants. This undermines the utilization of cereal stover and straws as ruminant feeds. Lignin is a phenolic compound of high molecular weight that adds rigidity to cell wall structure but limits the digestibility and availability of cell wall contents to rumen microbes (Chaudhry, 1998). Maize stover is rich in carbohydrates but its voluntary intake by ruminants is limited due to high levels of ligno-cellulosic bondages, which increase with plant maturity. Consequently, many farmers burn maize stover despite its potential as a source of energy for ruminants.

Although ruminants are known to have a highly effective digestive system for fibre, they are incapable of extracting sufficient energy and protein from maize stover to facilitate growth, production and reproduction (Montañez-Valdez *et al.*, 2015). This is because the cell wall contents of maize stover, particularly cellulose and hemicelluloses, are locked into a complex polymeric compound, which is exquisitely constructed to resist biological and chemical hydrolysis (Mahesh and Mohini, 2013). Linkages between lignin with cellulose and hemicellulose inhibit accessibility of rumen microbial enzymes to the structural carbohydrates and, therefore, locking significant amounts of potential energy (Tengerdy and Szakacs, 2003).

Use of white-rot fungi as a biological means to liberate carbohydrates from the ligno-cellulosic bondage has been widely reported as a promising, affordable and environmentally acceptable approach to increasing nutritional value of straws (Chaudhry, 1998; Tengerdy and Szakacs, 2003; Graminha *et al.*, 2008). Furthermore, earlier studies indicated that pre-treatment of crop residues with a consortia of fungi working in a synergistic and syntropic association with other fungi, yeast such as *Saccharomyces cerevisiae* or bacteria (*Lactobacillus plantarum*) would be preferable for better solid state fermentation (Wan and Li., 2012; Owen *et al.*, 2012). Darwish *et al.*, (2012) indicated that maize stover treated with *P. ostreatus* and *S. cerevisiae* at 28°C for 7

- 28 days improved CP content from 36 - 118 g CP/kg DM. Meanwhile, hemicellulose, cellulose and lignin were reported to decrease with increasing incubation time of fermentation process from 260.1 to 141.7 g/kg DM, 410.0 to 200.8 g/kg DM and 114.9 to 58.4 g/kg DM, respectively, when treated with a combination of white rot fungi and yeast. Similarly, Chen *et al.* (1995) observed that solid state fermentation of maize stover for 28 day at 27°C with *Cyathus stercoreus*, *Phledia brevispora* and *Phanerochaete chrysosporium* increased IVDMD from 409 g/kg (untreated maize stover) to 514 g/kg DM (treated with *P. brevispora*) and 523 g/kg DM (treated with *C. stercoreus*) but growth of the white rot fungi *P. chrysosporium* resulted into reduced IVDMD from 409 to 298 g/kg DM. However, Fazaeli (2007) indicated that IVDMD of wheat straw treated with *P. florida* at $22 \pm 5^\circ\text{C}$ for 17 days increased from 281 to 403 g/kg while when treated with *P. ostreatus* IVDMD increased from 281 to 370 g/kg DM. Both *P. florida* and *P. ostreatus* decreased lignin content of fermented wheat straw from 82 g/kg DM to 74 and 72 g/kg DM, respectively.

Ability of white rot *Pleurotus* fungal species to secrete exogenous enzymes that liberate and degrade lignin and yet preserve cellulose (Sharma and Arora, 2015) offers an attractive opportunity to harness the fungal species that secrete oxidative enzymes during mycelial colonisation (Dashtban *et al.*, 2010). However, there is a paucity of information on the appropriate *Pleurotus* species, optimum bio-physical conditions and pre-treatment options that farmers in the tropics can use to improve the nutritive quality of fibrous crop residues including maize stover as livestock feed. The objectives of this study were, therefore, to: (1) determine bio-physical conditions that farmers can use to optimize mycelial growth rate and effectiveness of selected *Pleurotus* species, (2) identify the best *Pleurotus* species that can be used under these condition and (3) determine pre-treatment options that can improve the effectiveness of solid state fermentation of maize stover with the best *Pleurotus* species.

6.2 MATERIALS AND METHODS

6.2.1 Preparation of experimental fungal material

The study was conducted in the Animal Science Laboratory at the College of Agricultural and Environmental Sciences (CAES), Makerere University, Uganda. Pure stock commercial cultures of four white-rot *Pleurotus* species (*Pleurotus* spp.) including; *Pleurotus florida* (*P. florida*), *Pleurotus ostreatus* (*P. ostreatus*), *Pleurotus sajor caju* (*P. sajor caju*) and *Pleurotus pulmonarius* (*P. pulmonarius*) were procured from a Belgium-based mushroom culture company (MYCELIA). The four fungal species are the most popular edible commercial mushrooms in Uganda. An agar block of pure stock culture per species was aseptically transferred to a sterilized 90 mm diameter petri dish containing sterile potato dextrose agar (PDA) (Formedium Hunstanton, England). The aseptic conditions of the petri dishes were achieved by sterilization at 121°C for 15 min in an autoclave. Three petri dishes per fungal species were randomly inoculated with the pure stock culture and sealed with a parafilm to allow uniform mycelial ramification at 25°C. After 7 days of fungal mycelial growth and ramification, a sterile cork borer was used to cut out round mycelial discs of 8 mm diameter. The discs were used in the subsequent experiments as secondary mycelia of the pure cultures for each of the test *Pleurotus* spp.

6.2.2 Determination of fungal growth

The effects of varying temperature, pH, substrate-sterilization with H₂O₂ and illumination regimes on fungal mycelial growth of the four *Pleurotus* spp. were investigated using petri dishes containing sterile PDA substrates. The outside bottom of each petri dish was dissected into four quarters using vertical and horizontal axes and labeled as r1, r2, r3 and r4, respectively (Figure 6.1). The secondary mycelial discs of the different *Pleurotus* spp. were transferred after 7 days of mycelial growth to the center (intersection point) of the petri dishes. Mycelial growth was measured along each radius of the petri dish.

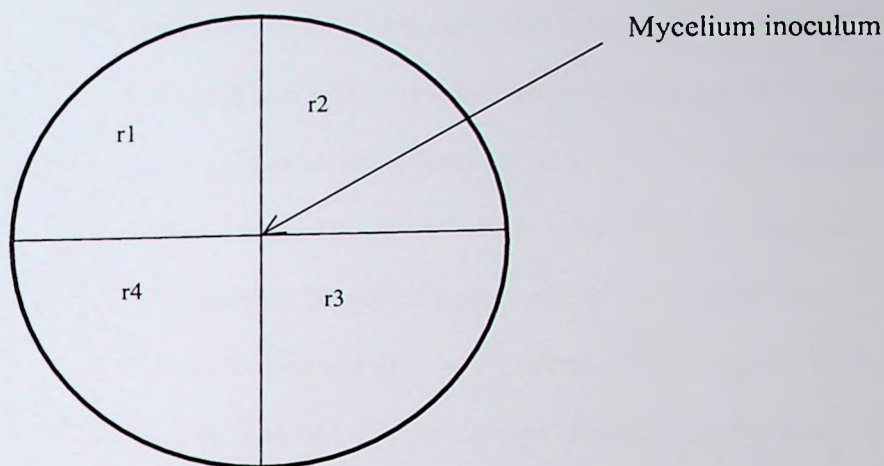


Figure 6.1 Illustration of the four axes along which radial mycelial growth were measured

6.2.2.1 Effects of temperature on fungal mycelial growth

The effects of temperature on mycelial growth rate of the *Pleurotus* spp. were evaluated at four levels including 20, 25, 30 and 35°C. In this experiment, pH was fixed at 5.0 and H₂O₂ concentration at 0.01 m/L (v/v). Secondary mycelial discs of 7 days old from each of the four *Pleurotus* spp. were inoculated in triplicates into the petri dishes for each test temperature. The 12 test petri dishes for each fungal species were randomly allocated to each of the four incubators maintained at different temperatures. Precaution was taken to ensure that each incubator contained twelve petri dishes that comprised three dishes for each *Pleurotus* spp. examined at the assigned temperature.

6.2.2.2 Effects of pH on fungal mycelial growth rate

The effects of pH on mycelial growth rate of the *Pleurotus* spp. was evaluated at five pH levels including 4.0, 5.0, 6.0, 7.0 and 8.0. In this experiment, temperature was fixed at 25°C and H₂O₂ concentration at 0.01 m/L (v/v). The different pH levels were obtained by adding varying proportions of 1.0 mol/L acetic acid and 1.0 mol/L sodium hydroxide solutions to the PDA

media. Adjustments to pH 7 and pH 8 were achieved using calcium carbonate (CaCO_3). Using a pH meter (Activon ACE69 model), which had been calibrated prior to the experiment with a two point buffering system at pH 4 and 8, the pH of the unadjusted medium was initially established at room temperature (25°C). To minimize the impact of temperature on pH readings of the media, the buffer solution used to standardize the pH meter was kept in a water bath at 25°C . Because of the difficulty of maintaining the pH of agar media at the pH extremes of 4 and 8, adjustments to pH 4 and pH 8 were achieved after sterilization. This was possibly because of dissociation rate of the media to H^+ and OH^- ions, which are temperature-dependent. However, agar media for pH 5, 6 and 7 were stable and pH adjustments were achieved prior to sterilization. Same methodological procedures as already indicated in section 6.2.2.1 were followed to assess the effects of pH on the rate of mycelial growth for the different *Pleurotus* spp.

6.2.2.3 Effects of substrate sterilization with H_2O_2 on fungal mycelial growth rate

The effects of chemical sterilization of PDA with H_2O_2 on mycelial growth rate were investigated at five varying concentrations including 0.01, 0.032, 0.1, 0.32 and 1.0 mL/L (Zharare *et al.*, 2010). In this experiment, pH was fixed at 5.0 and temperature at 25°C . Inclusion of H_2O_2 was mainly used to determine optimal levels for preservation and prevention of contamination from other members of the microbiota that lack the peroxidase-mediated antimicrobial system. Each of the four *Pleurotus* spp. was tested in triplicates for each H_2O_2 concentration. The five H_2O_2 concentrations of the media were established at logarithmic intervals using the procedure reported by Zharare *et al.* (2010) in the equation below:

$$ar^{n-1} = b.$$

Where: 'a' is the lowest concentration of H_2O_2 (0.01 mL/L) above zero (control),

'b' is the highest concentration of H_2O_2 lower than 10.0 mL/L fixed at 1.0 mL/L,

'n' is the number of concentration (i.e., $n = 5$) within the range of a - b and 'r' is

the logarithmic constant obtained by solving the equation $0.01r^4 = 1.0$.

6.2.2.4 Effects of substrate illumination on fungal mycelial growth rate

In this experiment, the effects of illumination on mycelial growth rate were evaluated at two conditions including presence and absence of light. Irrespective of illumination conditions, all the PDA substrates in the petri dishes were kept at pH 5 and sterilized with H_2O_2 (0.01 mL/L (v/v)). Under lighted condition, twelve inoculated petri dishes comprising of three petri dishes for each of the four *Pleurotus* sp. were placed in an incubator with artificial light of 322.92 lumens/m² and maintained at 25°C. The dark conditions were obtained by placing twelve inoculated petri dishes comprising of three petri dishes per *Pleurotus* sp. each wrapped with a sterile black piece of paper first and then with an aluminum foil and incubated at 25°C. Visual examination of mycelial density was carried out following the procedure reported by Kanmani *et al.* (2009).

6.2.2.5 Effects of pH and temperature on mycelial growth rate

In this experiment, effects of pH on mycelial growth rate of *Pleurotus* spp. as affected by varying temperatures on the 5th day of mycelial colonization was evaluated at five pH levels of 4.0, 5.0, 6.0, 7.0 and 8.0. The temperature was varied at 20, 25, 30 and 35°C but H_2O_2 concentration was fixed at 0.01 mL/L (v/v). Secondary mycelial discs of 7 days old from each of the four *Pleurotus* spp. were inoculated in triplicates into the petri dishes for each test temperature and pH. The 12 test petri dishes for each fungal species at each pH level were randomly allocated to each of the four incubators maintained at different temperatures.

6.2.3 Calculation of mycelial growth rate

Mycelial growth rate of the different fungal species on PDA was calculated based on the zone of the agar in the petri dish ramified by mycelium. Fungal species with the highest mycelial growth rate was selected as the most suitable for biological treatment of maize stover. The

average mycelial growth rate (mm²/day) was determined using the formula described by Lonergan *et al.* (1993):

$$M = \{[G(d6) - G(d5)] + [G(d5) - G(d4)] + [G(d4) - G(d3)] + [G(d3) - G(d2)]\} / 4 \dots (i)$$

Where: M = average mycelial growth rate per day (mm²/day) for each *Pleurotus* spp.,

G (d6) was the average mycelial growth (in mm) on day 6,

G (d5) was the average mycelial growth (in mm) on day 5,

G (d4) was the average mycelial growth (in mm) on day 4,

G (d3) was the average mycelial growth (in mm) on day 3,

G (d2) was the average mycelial growth (in mm) on day 2.

To compute area of ramification for respective *Pleurotus* spp. on PDA petri dishes, mean radius of mycelial growth (mm/day) was incorporated into the formula πr^2 ($\pi = 3.14$). *Pleurotus* sp. with the highest mycelial growth rate was selected as the most suitable for biological treatment of maize stover.

6.2.4 Sampling and analysis of maize stover fermented with *Pleurotus* spp.

Sample substrates tested were obtained from open pollinated maize (Longe 5), which was planted at a spacing of 0.75 m between rows and 0.25 m between plants within a row in 4 blocks each of one hectare in different locations. Sloping nature of the land from uphill to the valley formed the basis for blocking. In establishment and management of the maize crop, all recommended agronomic practices for maize production in the area were followed in each of the blocks. Diammonium phosphate (DAP) fertilizer was applied at a rate of 100 kg/ha at planting time and urea was applied when the plant reached 0.7 m at a rate of 120 kg/ha. Weed clearing was manually accomplished using a hand hoe and sickle, however, no irrigation was practiced. Harvesting of maize was done per plot per block after 120 days when maize grain was about 175 g/kg DM moisture content. Each block was divided into 8 plots and each plot had at least 12 stubbles of maize stover randomly harvested in 2 batches.

Four bags were used per plot; each accommodating 3 chopped maize stover stubbles, making a total of 32 bags for a block and a total of 128 bags for the four blocks. Maize stover for each bag was separately chopped using a hand forage chopper. The chopper was thoroughly cleaned after every four bags that were obtained per plot. This exercise was repeated to get two batches each of 128 bags from the four blocks. One batch of 128 bags was stored in a cool dry place for later use on the pre-treatment options prior to fermentation with the appropriate fungal species. From one of the batches, all the chopped maize stover harvested from each plot was packed in labeled sisal bags and soaked in a solution of H_2O_2 at a concentration of 0.01 mL/L of distilled water for 24 h in steel water tanks (200 cm × 100 cm × 80 cm size). Although H_2O_2 is commonly used for preservation and prevention of contamination of substrate with indigenous microbiota, it was also used in degradation process of lignin and hemicellulose, which facilitates accessibility of hydrolytic enzymes to crystalline cellulose across all the treatments. The wet samples were drained and sun-dried to a moisture content of 650-700 g/kg (as is) determined by squeeze test following procedures described by Elisashivili *et al.*, (2008).

After H_2O_2 treatment, maize stover samples (10 kg DM/bag) were transferred into a black polyethylene bags (35 cm × 40 cm; 100 μ m thickness) and inoculated with 7-day old secondary mycelial discs (10 discs per bag). Each of the 4 popular commercial *Pleurotus* spp. (viz; *P. florida*, *P. sajor caju*, *P. pulmonarius* and *P. ostreatus*) was randomly assigned to ferment one of the four H_2O_2 -treated maize stover substrate from each plot. Each *Pleurotus* spp. was inoculated on 8 sample bags of maize stover from each of the four blocks. From each of the 32 bags randomly allocated to a single fungal species samples of the original maize stover treated with H_2O_2 were also transferred into black polyethylene bags without fungal inoculation and designated as the control. Thereafter, the control bags were subjected to similar conditions as those inoculated with the different *Pleurotus* spp. After inoculation, the bags were tightly

sealed with nylon ropes to allow controlled aeration but with a headspace to ensure gaseous exchange. Limited aeration was earlier recommended for effective selectivity of lignin degradation over cellulose and hemicellulose by white rot fungi (Wan and Li, 2012). The substrate was then subjected to solid-state fermentation for 14 days at 25°C in a dark room. During the second week of solid-state fermentation, when mycelial running started, all the sides of the bags were punched with pinholes to provide aeration necessary for the highly oxidative lignin degradation process (Fazacli, 2007; Wan and Li, 2012). Thereafter, the substrate was sun-dried to terminate colonization and then preserved in cotton-sewn bags for subsequent laboratory analyses.

Analyses of dry samples were done in triplicates for dry matter (DM), crude protein (CP) and ash according to procedures of 930.15, 954.01 and 942.05, respectively (AOAC, 1995). Neutral detergent fiber (NDF), exclusive of residual ash (NDFom), was determined without addition of α -amylase or sodium sulphite according to the method (973.18; AOAC, 1995). The same sample was sequentially analyzed for acid detergent fiber exclusive of residual ash (ADFom) and Lignin (sa) was determined by solubilization of cellulose with sulphuric acid (12N) according to the method of Robertson and Van Soest (1981). The concentration of hemicellulose was calculated as the difference between NDFom and ADFom analyzed sequentially on the same sample, while cellulose was computed as the difference between ADFom and Lignin (sa). Estimation of *In vitro* digestibility was the proportions of dry matter (IVDMD) and organic matter (*in vitro* organic matter digestibility [IVOMD]) disappearance using Tilley and Terry method (1963) as modified by Goering and Van Soest *et al.* (1970). Metabolizable energy (ME MJ/kg DM) values were estimated from *in vitro* organic matter digestibility (IVOMD) data using the equation:

$$ME_{(IVOMD)} = 0.81 \times 0.184 \times IVOMD \text{ (ARC, 1980).}$$

6.2.5 Pre-treatment of maize stover prior to fermenting with *P. sajor caju*

Effects of four pre-treatment options of maize stover prior to fermentation with *P. sajor caju* were randomly selected and investigated. The pre-treatment options included (1) steaming chopped maize stover in an autoclave at 121°C for 5 min used as the control, (2) thoroughly mixing and soaking the chopped maize stover with molasses solution (11 g/kg DM) for 7 days, (3) thoroughly mixing and soaking chopped maize stover in *Lactobacillus plantarum* (*L. plantarum*) solution (10^3 cfu/mL) for 7 days and (4) thoroughly mixing and soaking chopped maize stover in a mixture of *L. plantarum* (10^3 cfu/mL) and molasses (11 g/kg DM) for 7 days. Each of the 4 pre-treatment options was conducted in triplicates. The substrates were kept under anaerobic conditions by tightly tying the sample bags with a nylon rope for 7 days before inoculating it with *P. sajor caju* and fermenting for 14 days.

The *L. plantarum* was obtained by harvesting it from yellow apple bananas following procedures as outlined by Dawson *et al.* (1990). Bioinformatics analysis of putative extracellular proteins of the lactic acid-forming bacterium was carried out in a molecular laboratory and *L. plantarum* was identified as a domain composition of the sample (Teusink *et al.*, 2006).

6.2.6 Statistical Analysis

Data to establish optimal growth performance of the *Pleurotus* species on PDA was analyzed as a completely randomized design using mixed model procedures of SAS (2002). The model included the different bio-physical conditions as fixed factors, while the four *Pleurotus* spp. were considered as random variables. Effects of bio-physical conditions on mycelial growth rate with increasing temperature, pH levels and H₂O₂ concentration were examined using linear and quadratic contrasts. The data on mycelial growth rate of the different *Pleurotus* spp. on the

5th day and the relationship between pH levels, temperature and how this influenced mycelial growth rate were illustrated using graphical means.

The effects of substrate illumination on mycelial growth rate were subjected to a *t*-test while data on the effects of fermenting maize stover with 4 different *Pleurotus* spp. on chemical composition, and effect of pre-treatment option on nutritive value were subjected to a one-way analysis of variance using a completely randomized block design. The treatment combinations were replicated 4 times as represented by the number of block but repeated 8 times in each of the four blocks. The total numbers of observations were: 4 (fungal species tested on each of the four stover bag per plot) × 8 (plots per block) × 4 (blocks) × 3 run replicates in the laboratory. After averaging the 8 plot results for the respective white rot fungal species from each block and the laboratory run replicates, the remaining 16 observations were randomly distributed in the 4 blocks and subjected to separation of the treatment means using Duncan's multiple range test at a significance level of $P < 0.05$.

6.3 RESULTS

6.3.1 Mycelial growth rate

Effects of bio-physical conditions on mycelial growth rate of *Pleurotus florida* (*P. florida*), *Pleurotus sajor caju* (*P. sajor caju*), *Pleurotus pulmonarius* (*P. pulmonarius*) and *Pleurotus ostreatus* (*P. ostreatus*) were as presented in Table 6.1. Increasing temperature from 20°C increased mycelial growth rate of the *Pleurotus* spp. ($P < 0.05$) at a decreasing rate with the highest growth observed at the intermediate temperature. The optimum mycelial growth rate was observed with *P. sajor caju* at 25°C. Moreover, *P. sajor caju* maintained the highest mycelial growth rate across all the four incubation temperatures.

Mycelial growth rate of all *Pleurotus* spp. increased quadratically with increasing levels of pH apart from that of *P. sajor caju* ($P = 0.06$) (Table 6.1). The highest mycelial growth rate of 1,133.74 mm²/day was attained at pH 5 and was recorded among petri dishes with *P. sajor caju*. At pH 5, *P. ostreatus* had the lowest mycelial growth rate of 979 mm²/day compared to the other species.

The mean mycelial growth rate on PDA sterilized with different concentrations of H₂O₂ varied among the white rot fungal species in the order of *P. sajor caju*, *P. pulmonarius*, *P. florida* and *P. ostreatus* (Table 6.1). As H₂O₂ concentration increased above 0.01 mL/L, an inverse relationship appeared between mycelial growth rate and H₂O₂ concentration across all the *Pleurotus* spp. At the highest H₂O₂ concentration (1.0 mL/L), mycelial growth rate of *P. pulmonarius* was the lowest.

Table 6.1 Effect of bio-physical conditions⁽¹⁾ on mycelial growth rate of *Pleurotus* spp⁽²⁾.

Treatment	Mycelial growth rate, mm ² /day			
	<i>Pleurotus florida</i>	<i>Pleurotus sajor caju</i>	<i>Pleurotus pulmonarius</i>	<i>Pleurotus ostreatus</i>
Temperature °C				
20	655.6	888.6	755.4	540.3
25	970.4	2,082.8	1,918.1	1,256.4
30	638.2	658.6	422.0	635.9
35	199.3	542.2	205.9	188.0
SEM	71.0	71.5	71.8	71.4
Linear	<0.001	0.01	0.04	0.03
Quad	0.03	0.04	0.04	0.04
pH level				
pH 4	980.9	992.8	962.7	913.1
pH 5	992.8	1,133.7	1,119.1	979.4
pH 6	571.1	638.2	638.2	638.2
pH 7	305.7	418.8	418.8	307.7
pH 8	195.3	307.7	307.7	195.3
SEM	72.2	72.2	72.0	72.0
Linear	0.23	0.24	0.17	0.86
Quad	0.01	0.06	<0.001	<0.001
H₂O₂, mL/L of distilled water				
0	563.5	915.1	913.1	418.8
0.01	561.5	1,133.7	1,107.4	571.1
0.032	418.8	563.5	561.6	396.0
0.1	307.7	195.3	202.0	211.2
0.32	202.0	181.5	174.2	173.4
1.0	151.8	174.5	139.2	163.0
SEM	70.7	71.1	71.1	70.7
Linear	0.2	0.14	0.2	0.2
Quad	0.02	<0.001	<0.001	0.03

⁽¹⁾ Temperature, pH level, H₂O₂ concentration. ⁽²⁾ *Pleurotus florida*, *Pleurotus sajor caju*, *Pleurotus pulmonarius*, *Pleurotus ostreatus* (n = 12. Significant (P<0.05), SEM = standard error of the mean

Mycelial growth rates of *P. sajor caju* and *P. pulmonarius* were influenced (P<0.05) by illumination (Table 6.2). Generally, all *Pleurotus* species in petri dishes incubated in the darkness had relatively higher mean mycelial growth rate than those incubated under light. Also, very thick mycelium was recorded in petri dishes incubated in the dark condition while thin mycelium was observed in plates incubated under light conditions.

Table 6.2 Effect of illumination on mycelial growth rate of *Pleurotus* spp.

<i>Pleurotus</i> species	Mean mycelial growth rate, mm ² /day			
	Light	Dark	SEM	<i>P</i> -value
<i>Pleurotus florida</i>	1,170.2	1,176.0	11.09	0.08
<i>Pleurotus sajor caju</i>	2,038.4	2,067.7	10.26	0.02
<i>Pleurotus pulmonarius</i>	2,053.4	2,087.2	10.29	0.02
<i>Pleurotus ostreatus</i>	1,178.9	1,187.2	10.82	0.06
Mycelial density ⁽¹⁾	+	++		

⁽¹⁾ + thin mycelial density, ++ thick mycelial density (Kanmani *et al.*, 2009).

The relationship between pH and mycelial growth rate for all the *Pleurotus* species exhibited a quadratic trend and was also graphically shown to depend on temperature (Figure. 6.2). The optimal temperature was 25°C in all *Pleurotus* species and mycelial growth rate of *P. sajor caju* was almost 2 times higher than that of *P. florida* under similar bio-physical conditions. Furthermore, mycelial growth rate of *P. sajor caju* was observed even beyond pH 8 at 25°C.

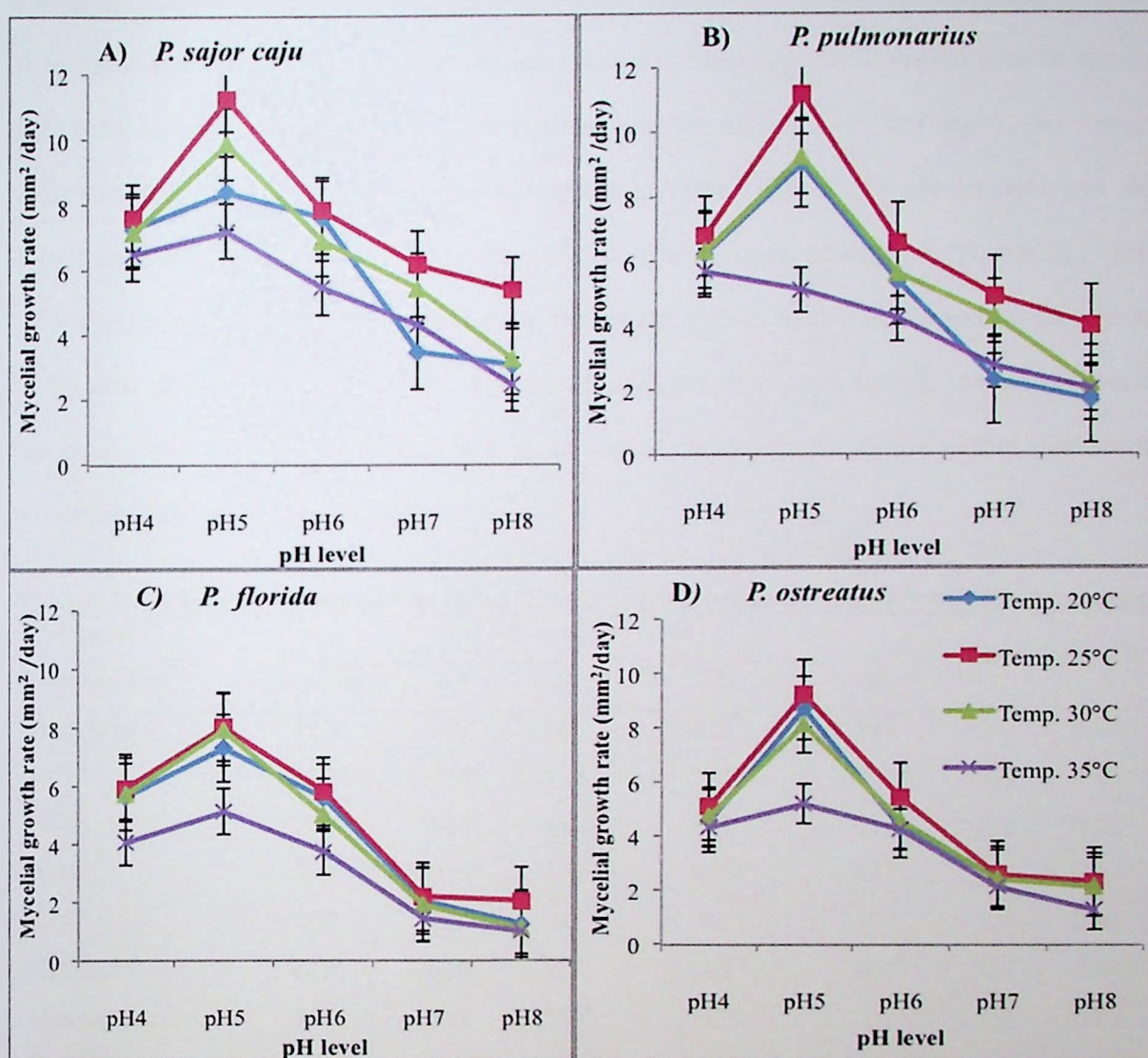


Figure 6.2 Influence of pH and temperature on mycelia growth rate (mm^2/day) of (A) *P. sajor caju* (B) *P. pulmonarius* (C) *P. florida* and (D) *P. ostreatus*.

6.3.2 Chemical composition of maize stover fermented with *Pleurotus* spp.

Chemical compositions and *in-vitro* digestibility of maize stover sterilized with 0.01 mL/L H_2O_2 concentration at 25°C, pH 5 and inoculated with *Pleurotus* spp. for 14 days were as shown in Table 6.3. There were differences ($P < 0.05$) in DM, CP, NDFom, ADFom and lignin (sa) among maize stover samples fermented with different *Pleurotus* spp. Maize stover substrate fermented with *P. sajor caju* had the highest CP (86.6 g/kg DM) but with the lowest ADFom

(419 g/kg DM) and lignin (sa) (50 g/kg DM) content compared to substrates fermented by other fungal spp. and the control. Although *P. sajor caju* exhibited superior mycelial growth rate on both PDA and maize stover substrate, it resulted into the highest ($P<0.05$) dry matter loss of fermented maize stover. However, fermenting maize stover with *P. florida* resulted into the lowest DM loss. The highest IVOMD (670.4 g/kg DM) and, consequently, ME (10.0 MJ/kg DM) were also observed in maize stover substrate fermented with *P. sajor caju*. Based on the results of mycelial growth rate at 25°C on PDA, rate of lignin (sa) breakdown and ME content of maize stover, *P. sajor caju* was identified as a more suitable white rot fungi for further evaluation during pre-treatment of maize stover.

Table 6.3. Chemical composition (g/kg DM) of fermented maize stover with *Pleurotus* spp

Chemical composition ⁽¹⁾	<i>Pleurotus</i> species					SEM	P-value
	Control	<i>Pf</i>	<i>Ps</i>	<i>Pp</i>	<i>Po</i>		
DM (g/kg)	913 ^a	887 ^b	865 ^c	877 ^b	883 ^b	7.9	0.02
CP ¹	54.2 ^b	81.2 ^a	86.6 ^a	85.9 ^a	80.1 ^a	6.0	0.03
NDF ¹	772 ^a	625 ^b	619 ^b	612 ^b	627 ^b	6.4	0.02
ADF ¹	458 ^a	426 ^b	419 ^b	425 ^b	427 ^b	6.9	0.04
ADL ¹	83 ^a	65 ^b	50 ^c	53 ^c	67 ^b	5.9	0.02
Cellulose ¹	375 ^a	360 ^c	372 ^a	369 ^b	361 ^c	4.7	0.01
Hemicellulose ¹	314 ^a	198 ^b	200 ^b	187 ^c	201 ^b	4.3	0.01
IVDMD ¹	385.5 ^c	698.2 ^b	731 ^a	717.3 ^a	682.3 ^b	4.9	0.01
IVOMD ¹	310.6 ^c	638.9 ^b	670.4 ^a	656.9 ^b	618.6 ^b	6.7	<0.001
ME (MJ/kg DM)	4.6 ^c	9.5 ^a	10 ^a	9.8 ^a	9.2 ^b	0.9	<0.001

¹g/kg DM; ^{abc}Least square mean in the same row followed by different superscript differ ($p<0.05$); ⁽¹⁾g/kg DM; *Pf* = *Pleurotus florida*, *Ps* = *Pleurotus sajor caju*, *Pp* = *Pleurotus pulmonarius* *Po* = *Pleurotus ostreatus* CP=crude protein = (N*6.25); NDF=Neutral detergent fibre; ADF=Acid detergent fibre; ADL=Acid detergent lignin; IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility; ME= metabolisable energy; SEM = standard error of the mean.

6.3.3 Pre-treatment options of maize stover prior to fermentation with *P. sajor caju*

Chemical composition of pre-treated maize stover varied ($P < 0.05$) with pre-treatment options (Table 6.4). Chopped maize stover pre-treated with *L. plantarum* and molasses before inoculation with *P. sajor caju* had the highest CP content (96.6 g/kg DM). The NDFom and lignin (sa) contents of maize stover varied ($P < 0.05$) with the pre-treatment options. Pre-treating maize stover with *L. Plantarum* and molasses prior to inoculation with *P. sajor caju* resulted into the highest reduction in lignin (sa) from 61.0 to 45.01 g/kg DM. Subsequently, IVDMD, IVOMD and ME content of maize stover significantly improved ($P < 0.05$) to 752.3, 687.2 g/kg DM and 10.2 MJ/kg DM, respectively.

Table 6.4 Effect of pre-treatment options on nutritive value of maize stover substrate fermented with *P. sajor caju*

Nutritive value ⁽¹⁾	Pre-treatment options				SEM	P-value
	SMS	MMs	LMs	MLMs		
DM, g/kg DM	887.0 ^a	883.0 ^a	877.0 ^a	865.0 ^b	7.9	0.05
CP ⁽¹⁾	81.2 ^b	80.1 ^b	95.9 ^a	96.6 ^a	7.7	0.04
NDFom ⁽¹⁾	625.0 ^a	627.0 ^a	602.0 ^b	591.0 ^c	9.8	0.03
ADFom ⁽¹⁾	407.0 ^a	419.0 ^a	412.0 ^a	398.0 ^a	7.4	0.07
Lignin (sa) ⁽¹⁾	61.0 ^b	68.0 ^a	51.1 ^c	45.0 ^c	7.7	<0.001
Cellulose ⁽¹⁾	346.0 ^b	351.0 ^b	361.0 ^a	353.0 ^b	3.5	0.04
Hemicellulose ⁽¹⁾	218.0 ^a	208.0 ^b	190.0 ^c	193.0 ^c	5.9	0.03
IVDMD ⁽¹⁾	698.1 ^b	602.4 ^c	728.4 ^b	752.3 ^a	6.9	0.02
IVOMD ⁽¹⁾	649.3 ^b	615.8 ^c	666.5 ^b	687.2 ^a	9.8	<0.001
ME, MJ/kg DM	9.7 ^{ab}	9.2 ^b	9.9 ^a	10.2 ^a	0.5	0.05

⁽¹⁾g/kg DM; ^{a,b,c} Least square means in the same row within chemical composition with different superscript differs significantly ($P < 0.05$); DM, ADFom = acid detergent fibre expressed exclusive of residual ash; CP = crude protein (N*6.25); DM = dry matter; IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility; Lignin (sa) = lignin determined by solubilization of cellulose with sulphuric acid; ME = metabolizable energy; NDFom = neutral detergent fibre not assayed with α -amylase expressed exclusive of residual ash; SMS = steamed maize stover at 121 °C for 5 minutes; Mol, soaked maize stover in molasses, 11.4 g/L for 7 d; Lp, soaked maize stover in *L. plantarum* 10³ cfu/ml inoculum for 7 d; Lpm, soaked maize stover in mixture of molasses, 11.4 g/L and *L. plantarum*, 10³ cfu/ml for 7 d.

6.4 DISCUSSION

6.4.1 Bio-physical conditions for mycelia growth

Although bio-delignification of maize stover with *Pleurotus* species often results in increased IVOMD (Karunanandaa and Varga, 1996), identifying the best fungal species and optimizing its bio-physical and pre-treatment conditions is essential for better mycelial growth rate and lignin break down. Arguably, mycelial growth rate is one of the ways of comparing colonization ability and enzyme production potential of different fungal species under different bio-physical conditions (Novotný *et al.*, 2004). Mycelial growth rates of *Pleurotus* species spawned on PDA media subjected to varying temperature, pH level, H₂O₂ concentration and illumination varied among species but were similar to earlier reported values (Khushairi and Zainol, 2011; Dulay *et al.*, 2012). Quadratic mycelial growth rate observed across all *Pleurotus* species with varying temperature, pH levels and H₂O₂ concentration suggested that these bio-physical parameters had an inhibitory effect on mycelial growth beyond optimal levels of 25°C, pH 5, and 0.01 mL/L, respectively. The observed mycelial growth rate of 542.2 mm²/day in petri dishes inoculated with *P. sajor caju* at 35°C possibly indicates its tolerance and resilience to higher tropical temperatures compared to other *Pleurotus* species. Relationships in Figure 6.2 further demonstrate that pH effects on mycelial growth rate were heavily dependent on temperature for all the *Pleurotus* spp. Moreover, the highest growth rate of *P. sajor caju* on PDA at pH 5 and 25°C on the 5th day of fermentation agree with earlier results (Dulay *et al.*, 2012).

Mycelial growth rate of *Pleurotus* spp. on PDA under light and dark conditions were higher than what was earlier reported (Dulay *et al.*, 2012). However, presence of light had negative influence on mycelial growth rate and vigor, especially, for *P. sajor caju* and *P. pulmonarius*. This was consistent with earlier findings by Khushairi and Zainol (2011) who indicated superiority in mycelial growth rate of *Pleurotus* spp. in darkness. This was an indication that

farmers may need black polyethylene bags of the right gauge to cut off light for better colonization ability of *P. sajor caju* when fermenting maize stover as a feed for ruminants.

6.4.2 Chemical composition of fermented maize stover

The profile of chemical components (Table 6.3) of maize stover fermented with different *Pleurotus* species were in agreement with earlier studies (Chen *et al.*, 2009; Montañez-Valdez *et al.*, 2015) and depicts inherent variations among *Pleurotus* species. Badarinaa *et al.* (2013) reported that cellulose, hemicelluloses and lignin were the main sources of carbon and energy for the growth of *Pleurotus* spp. Therefore, mycelial proliferation by all the *Pleurotus* species on maize stover was possibly due to their saprophytic ability to solubilize structural carbohydrates and their ability to extract fibre bound nitrogen for their metabolism and growth. Different *Pleurotus* species are known to differ in their ability to secrete both hydrolytic and oxidative extracellular enzymes (Rodriguez Couto and Sanroman, 2005; Olfati and Peyvast, 2008; Mahesh and Mohini, 2013). Based on results of this study, it was possible that changes in DM, different enzymes secreted by the different *Pleurotus* species influence CP and fibre components of maize stover substrate. Hemicellulose and lignin are the two cell wall structural components that are metabolized during primary growth stage of the fungi in almost the same proportions (Montañez-Valdez *et al.*, 2015). Therefore, a significant reduction of lignin (sa) from 83.0 g/kg DM in the control to 50.0 g/kg DM in maize stover fermented with *P. sajor caju* indicates increased fibre solubility and improved nutritive value of maize stover. Moreover, it has been documented that mycelial growth rate and substrate colonisation are the most critical parameters that influence bio-degradation of ligno-cellulosic materials (Rodrigues *et al.*, 2008). Furthermore, Perez *et al.* (2002) reported that the ability of *Pleurotus* spp. to produce extracellular lignin-modifying enzymes such as phenol oxidases (laccase) or heme peroxidases (lignin peroxidases; manganese peroxidases and versatile peroxidases) varies among white rot fungi.

Maize stover substrate fermented with *P. sajor caju* for 14 days at 25°C had the highest amounts of CP (86.6 g/kg DM) and exhibited superiority of IVDMD (731.0 g/kg DM) and IVOMD (670.4 g/kg DM). This was possibly due to supremacy in colonisation ability of *P. sajor caju* that allowed lignin breakdown and transportation of nitrogen and iron in form of heme peroxidases needed to modify ligno-cellulosic complexes through depolymerisation of cellulose and lignin oxidation using the Fenton system (Fe^{2+} and H_2O_2). Therefore, the dominance exhibited by *P. sajor caju* in mycelial growth rate on PDA and colonization ability that resulted into the highest DM loss of the maize stover compared to other *Pleurotus* species was possibly an indication of its unique lignolytic system. This was in agreement with earlier findings of Rahman *et al.* (2011) who indicated that preferential degradation of lignin depends on fungal species. This is also consistent with earlier results obtained by Chen *et al.* (1995) who observed increase in IVDMD, of maize stover treated with *Phlebia brevispora* at 27°C for 28 days, from 409.0 to 514.0 g/kg DM. Similarly, Fazacli (2007) indicated that IVDMD of wheat straw treated with *P. florida* at 22±5°C for 17 days increased from 281.0 to 403.0 g/kg DM while when treated with *P. ostreatus*, IVDMD increased from 281.0 to 370.0 g/kg DM. Such feedstock improvement was far lower than values obtained in the current study under similar conditions possibly because of the different fungal species, substrates and days of solid-state fermentation.

Generally, irrespective of the *Pleurotus* species used, white rot fungi decreased lignin (sa), ADFom and NDFom contents of fermented maize stover but increased CP content, IVDMD and IVOMD, which was consistent with earlier results observed in wheat straw and maize stover (Chen *et al.*, 1995; Adamović *et al.* 1998; Fazacli, 2007). However, the relatively high ADFom observed in maize stover substrates fermented by the *Pleurotus* species was probably due to chitin content of the fungal mycelia known to interfere with digestibility in ruminants (Hendriks and Zeeman, 2009). The high chitin content ascribed to fungal mycelia may lead to a rise in phenolic intermediates during lignin metabolism by *P. sajor caju* thus reducing the *in vivo*

digestibility and utilisation of fermented maize stover (Owen *et al.* 2012). This, therefore, necessitates evaluation of fermented maize stover substrate using both *in-vitro* and *in-vivo* experiments to validate the high IVDMD and IVOMD in this study. Owen *et al.* (2012), however, contends that lignin-degradation products are unlikely to be harmful to rumen microbes because lignases produced by white rot fungi are active *in vitro*, but arguably concedes that a consortia of fungi, yeast and bacteria would be preferable for solid state fermentation.

6.4.3 Pre-treatment of maize stover prior to fermentation with *P. sajor caju*

Effects of the different pre-treatment options on enhancement of the nutritive value of maize stover fermented with *P. sajor caju* is consistent with earlier observations by Pérez *et al.* (2002) who indicated that enhancement of nutritive value of maize stover fermented with white rot fungi requires multi-step process that may be synergistic in order to optimise enzymatic hydrolysis and oxidation. If ruminants are to optimally utilize maize stover as a feed, biomass pre-treatment with heat, fungal enzymes, physical chopping or use of additive is one of the additional steps needed for effective liberation of cellulose and hemicelluloses from lignin (Wan and Li, 2012; Sharma and Arora, 2015). Since pre-treatment of maize stover with *L. plantarum* and molasses before fermentation with *P. sajor caju* improved CP content from 81.2 to 96.6 g/kg DM, IVDMD from 698.1 to 752.3 g/kg DM and IVOMD from 649.3 to 687.2 g/kg DM, it is possible that homofermentation of water soluble carbohydrates to lactic acid provided a conducive environment for fungal colonisation. This was consistent with the findings of Gupta *et al.* (2011), which showed increased ME content when hay was pre-treated with yeast and *L. plantarum*. The *L. plantarum* inoculant is not only known to produce cellulolytic enzymes, such as cellulases, cellobiohydrolase, endoglucanase and exoglucanase that act on the surface of insoluble ligno-cellulosic complexes, but also known to produce enzymes that hydrolyse pectin (Malik *et al.*, 2015). Therefore, pre-treatment of maize stover with *L. plantarum* and molasses possibly synergized the action of *P. sajor caju* to biodegrade lignin to its lowest value of 45.0

g/kg DM by initiating production of a series of enzymes including pectin-hydrolysing enzymes. Such pectinases hydrolyse pectin that binds hemicellulose to cellulose microfibrils and lignin, thereby unlocking the lignin bondage. A series of hydrolytic enzymes have also been reported to play an important role of providing easily digestible hemicellulose-derived sugar such as xylose, mannose, galactose, rhamnose and arabinose as main source of carbon for selective ligninolysis needed for fungal metabolism and growth (Sharma *et al.*, 2012).

The hydrolytic enzymes produced by *L. plantarum* possibly accelerated homofermentation of water-soluble carbohydrates to lactic acid and acetic acid, which shortens the time for pH stability. The low pH provides a favorable environment for mycelial growth and high colonization ability of *P. sajor caju* resulting into higher solid state fermentation reflected by the elevated IVDMD, IVOMD and ME. Although Wan and Li (2012) argued that pretreatment process is quintessential in overcoming biomass recalcitrance, the role of lignolytic enzymes during the early stage of solid-state fermentation needs further elucidation.

There was no evidence of correlation between presence of oxidative enzyme and lignin degradation by *Ceriporiopsis subvermispora* as was reported during the early stages of fungal colonization (Wan and Li, 2012). Possibly, pre-treatment of maize stover with *L. plantarum* and molasses induced formation of not only H_2O_2 but also a series of low molecular mass intermediates within the glyoxylate cycle that include glyoxylic acids, oxalic acid, and several unsaturated fatty acids, which initiate depolymerisation of crystalline cellulose and facilitate the penetration of lignin-degrading enzymes. Moreover, such low molecular mass agents have been suggested as a mechanism by which delignification and cellulose depolymerisation is induced (Msimango and Fon, 2016) by the Fenton system. It can, therefore be argued that mineralization of lignin occurs after lignin modification in early stages of pretreatment, which is indicative of a series of syntropic and synergistic reactions in the lignin degradation (Wan

and Li, 2012). Consequently, improved diffusion of the oxidative enzymes possibly explained why a consortium of *P. sajor cajor* working in concert with *L. plantarum* and molasses resulted into the highest IVDMD, IVOMD and ME but with lowest lignin (sa) and NDFom. The importance of *P. ostreatus* working in a consortium with *S. cerevisiae* when used for solid state fermentation of maize stalks for 14 day at 28°C was earlier illustrated with the improved IVDMD and IVOMD from 153.5 to 480.0 g/kg DM and 292.5 to 720.0 g/kg DM, respectively, coupled with reduction of lignin from 114.9 to 58.4 g/kg DM (Darwish *et al.*, 2012).

Although *L. plantarum* is a facultative organism that can also use oxygen for respiration, it has no electron transport chain (ETC) to convert oxygen to water but instead it ultimately metabolises it to H_2O_2 (Ranjit and Kung, 2000), which is used as a weapon to exclude other competing microorganisms on the substrate that would otherwise be contaminants. Molasses in consortium with *L. plantarum* was, therefore, used as a source of carbon to the bacterium and in production of organic H_2O_2 , which was essential in the Fenton system necessary for manufacturing extracellular phenolic ligninases such as Lignin peroxidase and in cellulose depolymerisation (Novotný *et al.*, 2004). Pre-treatment of maize stover with *L. plantarum* and molasses, therefore, negated the need by farmers to treat maize stover with inorganic H_2O_2 to sterilize the substrate. However, if applied in excessive quantities, molasses may supply extra nitrogen, sulphur and readily available carbon that may stimulate release of enzymes by *P. sajor caju* that hydrolyse cellulose and hemicellulose at the expense of selective lignin oxidation by peroxidases (Tripathi and Yadav, 1992; Rahman *et al.*, 2011). Therefore, biological pre-treatment of maize stover with a combination of *L. plantarum* and molasses prior to inoculation with *P. sajor caju* enhances lignin bio-degradation and enriches nutritive value of maize stover. However, before promoting the innovation, *in vivo* and *in situ* studies with ruminants are recommended. For safety of ruminants from mycotoxins, care should be taken to ensure that the

maize stover pre-treated with *L. plantarum* and molasses prior to solid-state fermentation with *P. sajor caju* should not be of dark colour and foul in smell due to production of butyric acid.

6.5 CONCLUSION

Pre-treatment of maize stover with a mixture of *L. plantarum* and molasses prior to fermentation with *P. sajor caju* offers a promising option for breaking lignin barrier and improving its nutritive value. The potential of *P. sajor caju* to break down lignin barrier at a temperature of 25°C, pH 5, and in the absence of light was demonstrated. However, to rule out anti-nutritional effects that may arise from high fungal chitin and phenolic intermediates of lignin break down, there was a need for *in vitro* and *in vivo* experimentation studies to evaluate productive performance of dairy and beef cattle fed on this ration.

6.6 ACKNOWLEDGEMENTS

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CHAPTER SEVEN

EFFECTS OF PRE-TREATMENT METHODS OF MAIZE STOVER FEEDSTOCK FERMENTED WITH *PLEUROTUS SAJOR CAJU* ON *IN VITRO* GAS PRODUCTION CHARACTERISTICS

This chapter is based on a published paper by:

Andrew.M. Atuhaire, Fred Kabi, Samuel Okello, Cyprian Ebong, Swidiq Mugerwa

7.0 ABSTRACT

Although maize stover has been reported as abundant and carbohydrate-rich residue, its utilization as a source of dietary energy for ruminants is still limited. The objective of this study was to compare the effect of pre-treatment methods of maize stover before fermentation with *P. sajor caju* on chemical composition and *in-vitro* gas production characteristics. In a completely randomised block design experiment, maize stover was harvested from 16 plots in four blocks at 120 days post planting and subjected to four treatments in three replicates that included: SMs (steamed chopped maize stover, sun dried, fermented with *Pleurotus sajor caju* (*P. sajor caju*) for 14 d), MMs (chopped maize stover, soaked in molasses (114 g/kgDM) for 48 hours, sun-dried, fermented with *P. sajor caju* for 14 d), LMs (chopped maize stover, soaked in *Lactobacillus plantarum* (*L. plantarum*) (10^5 cfu/mL) inoculum for 48 hours, sun dried, fermented with *P. sajor caju* for 14 d), MLMs (chopped maize stover, soaked in a mixture of molasses (114 g/kgDM) and *L. plantarum* (10^5 cfu/mL) for 48 hours, sun dried, fermented with *P. sajor caju* for 14 d), respectively. Maize stover substrates were analysed for proximate composition, metabolisable energy (ME), organic matter digestibility (OMD) and neutral detergent fibre digestibility (NDFd). Rumen fermentation characteristics and efficiency of energy utilisation were examined by determining the partitioning factor (PF). Gompertz model was used to describe the kinetics of gas production. *In vitro* dry matter and organic matter disappearance (IVDMD and IVOMD) were determined after 48 h of incubation. Results indicate variations ($P < 0.05$) among treatments on chemical composition, OMD, ME and NDFd. Also, PF (mg TOMD/mL), gas production (mL), rate of gas production (mL/h), and LAG time differed ($P < 0.05$). IVDMD and IVOMD varied ($P < 0.05$) following the order of effectiveness; MLMs>LMs>MMs>SMs. Significant variation in DM, NDFom, *in vitro* dry matter and organic matter digestibility were exhibited among treatments. Maize stover pre-treated with the mixture of molasses and *L. plantarum*, prior to fermentation with *P. sajor caju* showed superiority in

lactic acid concentration, crude protein and metabolisable energy. This observation suggests that bio-technological approach of treating maize stover with consortia of molasses, *L. plantarum* and *P. sajor caju* has the potential to enhance nutritive quality of maize stover feedstock. However, *in vivo* study on methodological procedure with lactating dairy cows was recommended for a secured approval of the technology.

Key Words: Fibrous, *Lactobacillus plantarum*, Lignocellulosic material, Ruminant feed.

7.1 INTRODUCTION

Livestock production depends on the accessibility and quality of feed offered. For ruminant animals, forages such as grasses and legumes are the main source of feeds needed to satisfy their nutritional requirement for maintenance, production and reproduction. Forages are however, scarce especially during the dry season, thus, providing quality alternative feedstock is critical for smallholder dairy farms. Accordingly, maize is one of the most important economic crops in Uganda cultivated by over 90% of Agricultural households (UBOS, 2014), especially, the smallholder dairy farmers. Maize stover is the most abundant non-conventional feedstock available throughout the dry season when maize grain is being harvested and has great potential for use as the quality feed resource for ruminants (Methu *et al.*, 2001; Tang *et al.*, 2006; Atuhaire *et al.*, 2014). However, compared to other forages, maize stover is of poor nutritive quality, has low crude protein content, deficient in vitamins and minerals, poor palatability and digestibility all because of its high lignin content (Mahesh and Mohini, 2013). Lignin a phenyl-propanoid bio-polymer, is resistant to most of the microbial enzymatic systems and non-digestible to ruminants as well (Sharma and Arora, 2015).

Natural degradation of plant biomass is mainly by different fungal and bacterial species. Basidiomycetes white-rot fungi have the ability to selectively and efficiently degrade lignin in nature using their extracellular ligninolytic enzyme system (Kholif *et al.*, 2014). Elisashvili *et al.*

(2008) reported that basidiomycetes white-rot fungi using their ligninolytic enzyme system comprising of laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP) have the potential to degrade lignin and liberate polysaccharides that can easily be utilized by rumen microflora, thus, improving on ruminant digestibility of fibrous feed resources.

Use of selective ligninolytic fungi *P. sajor caju* on maize stover improves CP, IVOMD and IVOMD (Atuhaire *et al.*, 2016). However, high loss in total organic matter observed, limits the practical use of maize stover as feedstock necessitating an innovative strategy to improve effectiveness of solid-state fermentation. Thus, use of fungal biotechnology to improve the nutritive quality of maize stover can only be feasible if delignification is achieved, hemicellulose and cellulose preserved for ruminant feeding and formation of phenolic inhibitors minimised (Tuyen, *et al.*, 2012). The objective of this study was therefore, to compare the different pre-treatment methods of maize stover prior to fermentation with *P. sajor caju* on nutritional quality improvement of maize stover. Chemical composition and *in vitro* gas production characteristics were used as indicators of nutritional quality.

7.2 MATERIALS AND METHODS

7.2.1 Maize stover production and processing

Maize stover of open-pollinated maize variety longe 5 was harvested after 120 d post planting at National Crops Resources Research Institute (NaCRRI) Namulonge, Uganda. Maize was spaced at 0.75 m between rows and 0.25 m between plants within a row in 4 blocks each of one hectare in different locations. Sloping nature of the land from uphill to the valley formed the basis of blocking. All recommended agronomic practices of establishment and management of the maize crop in the area were followed without irrigation practiced across all blocks. Diammonium phosphate (DAP) was applied at a rate of 100 kg/ha at planting time and urea was applied when the plant reached 0.7 m high at a rate of 50 kg/ha. Weeding was manually

accomplished using a hoe and sickle. Each block was divided into 4 plots and each plot had, at least, 24 stubbles of maize stover that were randomly harvested. Four bags each weighing 10 kgs (as is) was obtained per plot making a total of 16 bags per block. Samples of maize stover were chopped (2-3 cm) using a hand forage chopper, washed in flowing tap water and air-dried in preparation for treatment.

Pure stock of commercial edible *Pleurotus sajor caju* (*P. sajor caju*) was procured from a Belgium-based mushroom culture company (MYCELIA). The culture was maintained on potato dextrose agar (PDA) slants at $27\pm 2^{\circ}\text{C}$, and sub-cultured every 7 d. During preparation of spawn, sorghum grain was washed in flowing water, autoclaved at 121°C for 15 min, supplemented with 3.5% (w/w) CaCO_3 and 1.3% (w/w) CaSO_4 and packed (250 g) in polyethylene bags of size 10×15 cm. Each spawn bag was inoculated with 6 pieces of 8 mm diameter 7 days old actively growing mycelium of *P. sajor caju* on PDA that was evenly mixed with the substrate and incubated at $27\pm 2^{\circ}\text{C}$ for 14 days to allow full mycelial colonization. *P. sajor caju* was cultivated on maize stover substrate following the method developed by Rajarathnam and Bano (1989) with slight modification.

The water-soluble microbial additive based on lactic acid bacteria inoculant Biomate (*Lactobacillus plantarum* MA 18/5U, SI forage inoculant, Chr. Hansen's Laboratory, Inc. Malwankee, WI, USA) was maintained as frozen stock at -20°C in distilled water and 20% (v/v) glycerol and propagated twice on Man Rogosa Sharp (MRS) broth (Oxoid Ltd UK) at $27-30^{\circ}\text{C}$ before use at a dose of 0.01g/kg substrate following manufactures' instruction.

7.2.2 Experimental design

In vitro incubation experiments were conducted to measure gas production characteristics of pre-treated maize stover before fermentation with *P. sajor caju*. Four experimental treatments including steamed chopped maize stover in a batch pasteurizer at

60°C for 30 minutes and sun dried (SMs), chopped maize stover, soaked in liquid molasses (114 g/kgDM) for 48 hours and sun-dried (MMs), chopped maize stover, soaked in *Lactobacillus plantarum* (*L. plantarum*) inoculum (10^5 cfu/mL) for 48 hours and sun-dried (LMs), chopped maize stover, soaked in a mixture of molasses (114 g/kgDM) and *L. plantarum* inoculum (10^5 cfu/mL) in a ratio of 1:1 v/v for 48 hours and sun-dried (MLMs). All the pre-treated maize stover substrates were spawned with *Pleurotus sajor caju* (*P. sajor caju*) at rate of 250 g of grain spawn per kg, fermented and harvested after 14 days of fermentation, sun-dried to terminate further colonization, packed and stored in cotton sewn bags for chemical analysis and *in vitro* gas production. Each of the four fixed experimental treatment methods of maize stover appeared twice per each plot.

7.2.3 Chemical analyses

Experimental samples were divided into two parts per sample, one part of the samples was dried at 60°C for 48 h, milled to pass through a 1 mm screen for subsequent *in vitro* gas laboratory analysis. The other remaining samples were dried at 105°C for 24 h for analysis of dry matter (DM), organic matter (OM) and crude protein (CP) following methods 930.15, 942.05 and 954.01, respectively (AOAC 1995). Ash content was determined by incinerating samples at 550°C for 5 h (AOAC 1995; method ID 942.05). Neutral detergent fiber (NDF) exclusive of residual ash (NDFom) was determined without addition of α -amylase or sodium sulphite according to Van Soest *et al.* (1991). The same sample was sequentially analyzed for acid detergent fiber exclusive of residual ash (ADFom) and Lignin (sa) was determined by solubilization of cellulose with sulfuric acid (12 N) according to the method of Robertson and Van Soest (1981). The concentration of hemicellulose was calculated as the difference between NDFom and ADFom computed sequentially on the same sample while that of cellulose as a difference between ADFom and Lignin (sa).

Samples for lactic acid, acetic acid and propionic acid were prepared as described by Ranjit and Kung. (2000) and stored at 4°C for 24 h in the refrigerator. The slurry mixture was then filtered through 4 layers of cheesecloth and the filtrate used for pH, lactic acid, acetic acid and propionic acid determination following procedures described by Bolsen *et al.* (1992). The pH was directly measured using a pH meter (Sortorins basic pH meter, Germany). The concentration of lactic acid, acetic acid and propionic acid were analysed with Gas Chromatography equipment (Hawlet Packard 6890N series Plus 11, Avondale, PA, USA) according to Playne (1985). All the chemical analyses studies were conducted in triplicates and the results expressed on DM basis.

7.2.4 Gas production, IVDMD and IVOMD measurements

In vitro gas production was carried out in graduated glass syringes (100 mL capacity) following the method described by Menke and Steingass (1988). Each oven-dried sample (500 ± 1 mg) was grounded to pass through 1 mm screen and accurately weighed in 6 replicates into 100 mL graduated glass syringes fitted with plungers that were lubricated with Vaseline. The airtight graduated glass syringes (100 mL) were filled with 30 mL of buffered rumen fluid consisting of 10 mL of rumen liquor and 20 mL of McDougall's buffer and incubated in a shaking water bath at 39°C (Blümmel and Becker 1997). Rumen liquor was pooled from four slaughtered cattle at the abattoir into a pre-warmed ($39 \pm 3^\circ \text{C}$) thermos flask, filtered through four layers of cheesecloth and flushed with CO₂. All laboratory handlings were under continuous flushing with CO₂. Six syringes with only buffered rumen fluid were incubated at same time and considered as blanks. Gas production was read immediately and recorded at 2, 4, 8, 12, 16, 24, 28, 32, 36 and 48 h of inoculation. Net gas was determined by subtracting the average blank volume from the observed gas production for each of the test samples. The first round of 15 syringes (three syringes per treatment and three blanks) were terminated after recording the 24 h gas volume. The remaining 15 syringes that included three syringes per treatment and three blanks were terminated after the 48 h gas volume recording. The 24 h gas values were expressed

per 500 mg of DM and the recording were used to determine organic matter digestibility (OMD), metabolizable energy (ME) and partitioning factor (PF) as described by Menke and Steingass (1988). After 24 h, the remaining material was refluxed in neutral detergent fibre solution ashed to determine NDF_{res} , *in vitro* apparent DM degradability (IVDMD) and *in vitro* apparent organic matter degradability (IVOMD).

7.2.5 Calculations and statistical analysis

OMD and ME were estimated according to Menke et al. (1979) Eqs (1) and (2).

$$OMD \text{ (g/kg DM)} = 148.8 + 8.89GP_{24} + 4.5CP + 0.651XA \quad (1)$$

$$ME \text{ (MJ/kg DM)} = 2.2 + 0.136GP_{24} + 0.057CP + 0.0029CP^2 \quad (2)$$

Where; GP_{24} is the net gas production (ml/500 mg) at 24 h after inoculation.

XA is the ash content (g/kg DM) and CP is the crude protein (g/kg DM).

NDFd was estimated based on Goering and Van Soest (1970) equation

$$NDFd \text{ (g/KgDM)} = \frac{1000 \times (NDF_{feed} - NDF_{res})}{NDF_{feed}} \quad (3)$$

Where: NDF_{feed} is the neutral detergent fibre in the feed

NDF_{res} is the neutral detergent fibre in the residue after refluxing in the neutral detergent solution.

Partitioning factor (PF) was calculated based on equation.

$$PF \text{ (mg/ml of gas volume)} = TOMD/IVGP_{24} \text{ (Blümmel and Becker, 1997)} \quad (4)$$

Where: TOMD is the true organic matter digestibility after reflexing and ashing the residue and

$IVGP_{24}$ is the *in vitro* gas production at 24 h of incubation.

Results were computed using least square means of frequency procedure of SAS (2010). Chemical composition, IVDMD, IVOMD, OMD, ME and PF of experimental treatments were examined using mixed model procedures of SAS (2010).

To describe the dynamics of *in vitro* gas production over time, Gompertz function model was used (Schofield et al., 1994) as follows:

$$GP = A \times \exp \left\{ -\exp \left[1 + \frac{be}{A} (LAG - t) \right] \right\} \quad (5)$$

Where: GP is cumulated gas production (mL) at time t, A is the theoretical maximum of gas production (mL), b is the maximum rate of gas production (mL/h), LAG (h) is the lag time defined as the time-axis intercept of a target line at the point of inflection, t is the incubation time (h), and e is the Euler constant 2.718 (base of the natural logarithm). The parameters A, b and LAG were estimated by the nonlinear regression analysis procedure and data were subjected to statistical analysis as completely randomised block design experiment using mixed model procedure of SAS (2010) was employed. Statistical model used for the *in vitro* experiment was as follows: $Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$,

Where Y_{ij} was the gas volume, μ is the overall mean, τ_i is the fixed effect of treatment ($i = 1, 2, 3, 4$), β_j is the fixed effect of block ($j = 1, 2, 3, 4$), and ε_{ij} is the random residual error. Data obtained were subjected to analysis of variance according to statistical models above, tested at $p < 0.05$. When a parameter showed significantly different at $p < 0.05$, the means among various treatments were further tested with Duncan's test. Relationship between parameters was assessed using principal component analysis (PCA) (Jayanegara et al., 2011).

7.3 RESULTS

7.3.1 Chemical composition of pre-treated maize stover fermented with *P. sajor caju*

There were significant differences ($P < 0.05$) in chemical composition, pH, lactic acid and acetic acid composition among treatments (Table 7.1). Pre-treatment of maize stover with a mixture of *L. plantarum* and molasses prior to fermentation with *P. sajor caju* was ($P < 0.05$) superior in CP content (116.6 g/kg DM) and inferior in NDFom (591 g/kg DM), ADFom (398 g/kg DM) and lignin (45 g/kg DM). Steamed maize stover generated ($P < 0.05$) highest NDFom (772 g/kg DM), ADF (458 g/kg DM) and Lignin (sa) (83 g/kg DM), with lowest CP (54 g/kg DM), Ash (97 g/kg DM) and lactic acid (55.2 g/kg DM).

Table 7.1. Chemical composition of pre-treated maize stover fermented with *P. sajor caju*

Chemical composition	Pre-treatment methods				SEM	P-value
	Steamed	Molasses	<i>L. plantarum</i>	<i>L. plantarum</i> and molasses		
DM (g/kg)	898 ^a	863 ^b	876 ^b	881 ^c	4.92	0.03
Ash ¹	65 ^c	78 ^b	74 ^b	81 ^a	2.31	0.01
CP ¹	54.4 ^c	81.4 ^b	94.7 ^b	116.2 ^a	4.69	0.02
NDFom ¹	763.6 ^a	725.1 ^b	701.9 ^b	680.7 ^c	3.87	0.02
ADFom ¹	457.2 ^a	417.6 ^b	414.3 ^b	397.9 ^c	4.38	0.01
Lignin (sa) ¹	83 ^a	61 ^b	51 ^c	45 ^c	5.66	0.01
Hemicellulose ¹	306.4 ^a	307.5 ^a	287.6 ^b	282.8 ^b	2.89	0.04
Cellulose ¹	374.2 ^a	356.6 ^b	363.3 ^c	352.9 ^c	3.52	0.03
pH	4.47 ^a	4.41 ^a	4.05 ^b	4.03 ^b	0.02	0.04
Lactic acid ¹	54.3 ^d	60.6 ^c	62.2 ^b	63.4 ^a	0.77	0.02
Acetic acid ¹	27.2 ^a	23.2 ^b	19.9 ^c	21.5 ^d	0.56	0.02
Propionic acid ¹	0.84 ^a	0.83 ^b	0.81 ^c	0.78 ^d	0.07	0.04

¹ g/kg DM; ^{abcd} Means in the same row with similar superscripts are significantly not different at $P < 0.05$, ADFom; acid detergent fibre expressed exclusive of residual ash, g/kg DM. CP; crude protein (N x 6.25), g/kg DM. DM; dry matter, g/kg. *L. plantarum*; *Lactobacillus plantarum*. Lignin (sa) determined by solubilisation of cellulose with sulphuric acid, g/kg DM. NDFom; acid detergent fibre not assayed with α -amylase expressed exclusive of residue ash, g/kg DM. NM; not measured.

7.3.2 *In vitro* gas production characteristics

The *in vitro* cumulative gas production curves of different pre-treatment methods of maize stover before fermented with *P. sajor caju* are shown in Figure 7.1, which clearly demonstrates the significant difference in cumulative gas production among pre-treatment methods. Maize stover pre-treated with a mixture of molasses and *L. plantarum* produced more gas ($P < 0.05$) than other pre-treatment methods over 48 h incubation periods. Steamed maize stover produced the least gas compared with other treatments.

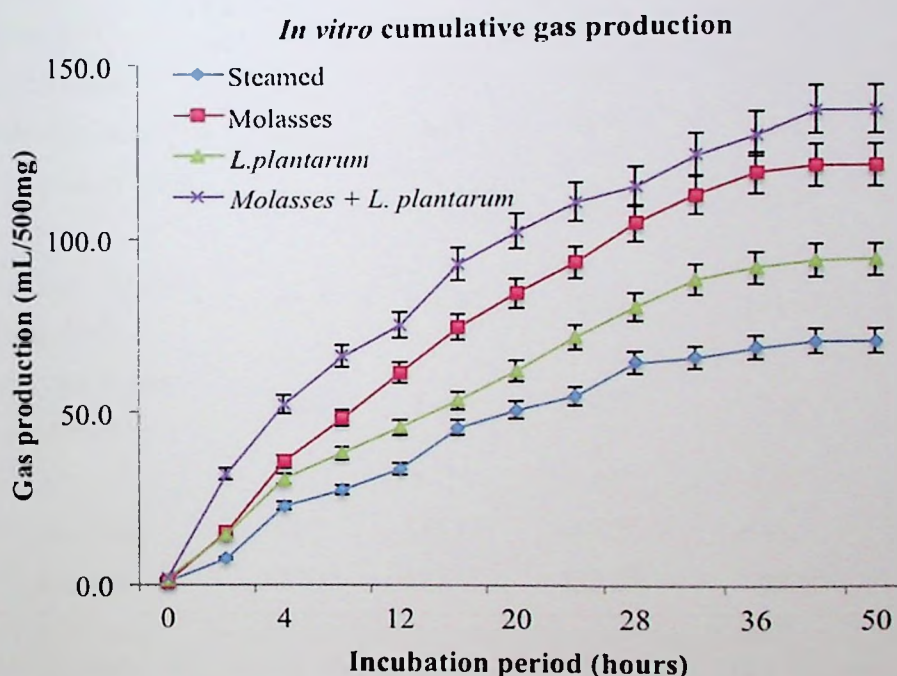


Figure 7.1 *In-vitro* cumulative gas production of different pre-treatment methods of maize stover fermented with *P. sajor caju*.

The parameters of the Gompertz model, IVDMD and IVOMD at 48 h incubation for SMs, MMs, LMs and MLMs are shown in table 7.2. The maximum gas volume was higher ($P = 0.04$) for MLMs than SMs, MMs and LMs. The maximum rate of gas production was highest ($P = 0.02$) for MLMs and greater in MMs than SMs and LMs. The lag time was lowest ($P = 0.01$) in

SMs and highest with MLMs. IVDMD values of MLMs were higher ($P = 0.03$) than MMs, LMs and SMs. ME was highest ($P = 0.03$) in MLMs and lowest in SMs.

Table 7.2 Effect of pre-treatment methods of maize stover on *in-vitro* gas production parameters

<i>In vitro</i> gas production parameters	Pre-treatment method				SEM	<i>P</i> -value
	Steamed (SMs)	Molasses (MMs)	<i>L. plantarum</i> (LMs)	<i>L. plantarum</i> and molasses (MLMs)		
OMD (g/kg DM)	544 ^c	724.5 ^a	643.7 ^b	769.9 ^a	14.41	0.001
NDFd (g/kg DM)	430 ^b	414.9 ^c	397.4 ^c	517.5 ^a	8.21	0.024
ME (MJ/kg DM)	5.22 ^d	6.30 ^c	6.64 ^b	9.53 ^a	7.01	0.003
PF (mg TDOM/mL)	4.5 ^a	3.4 ^b	4.0 ^b	3.1 ^c	3.53	0.032
Microbial efficiency (%)	48.8 ^c	65 ^b	55.5 ^c	71.1 ^a	5.4	0.046
Microbial protein yield (g/kg DM)	124.9 ^a	109.3 ^b	125.2 ^a	97.9 ^b	6.7	0.47
Maximum gas volume (mL/500 mg DM)	67.2 ^c	113.9 ^b	89.4 ^b	131.3 ^a	0.83	0.041
Maximum rate of gas production (mL/h)	1.45 ^c	2.41 ^b	1.87 ^b	2.8 ^a	0.03	0.002
Lag time (h)	0.41 ^c	1.12 ^b	1.14 ^b	1.64 ^a	0.04	0.001
IVDMD (g/kg DM)	335 ^c	533.1 ^b	467.2 ^b	645.3 ^a	11.75	0.03
IVOMD (g/kg DM)	327.3 ^c	481.5 ^b	455.2 ^b	608.6 ^a	11.53	0.04

^{abc}Means in the same row with the similar superscripts are significantly not different at $P < 0.05$. IVDMD; *In vitro* dry matter digestibility, g/kg DM. IVOMD; *In vitro* organic matter digestibility, g/kg DM. OMD; organic matter digestibility g/kg DM. NDFd; Neutral detergent fibre digestibility g/kg DM. ME; Metabolisable energy MJ/kg DM. PF; Partitioning factor, mg TDOM/mL. *L. plantarum*.

Correlations between gas volume (mL/g OM) and organic matter digestibility, metabolizable energy and partitioning factor of pre-treated maize stover prior to fermentation with *P. sajor caju* were as indicated in Figure 7.3. The organic matter digestibility (OMD) and metabolizable

energy (ME) showed a high and positive correlation with gas volume but partitioning factor (PF) showed a negative curvilinear relationship.

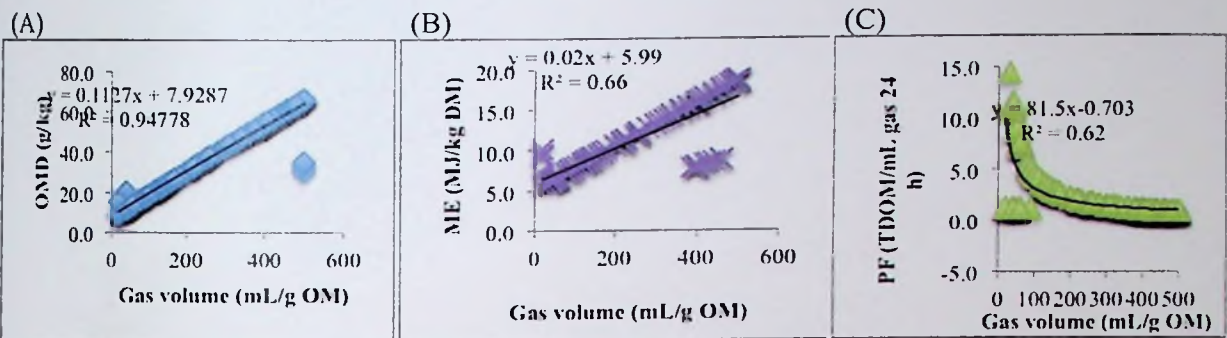


Figure 7.2 Relationships between gas production volume (mL/g OM) and (A) organic matter digestibility, (B) metabolisable energy and (C) partitioning factor of pre-treated maize stover before inoculation with *P. sajor caju*.

In order to describe the relationship among the parameters of nutritive value and chemical composition of pre-treated solid-state fermented maize stover with *P. sajor caju*, all were subjected to principal component analysis. Loading plot of principal component 1 (PC1) and principal component 2 (PC2) described correlated factors in pre-treated solid-state fermented maize stover substrate. Total variation explained by both principal components was 83.3% in which PC1 and PC2 contributed 73.10% and 10.20%, respectively (Figure 7.2). Based on variability explained, crude protein and metabolisable energy closely related lactic acid, ash, IVDMD and IVOMD were indicated as determinant parameters and negatively correlated to propionic acid, NDF and lignin contents.

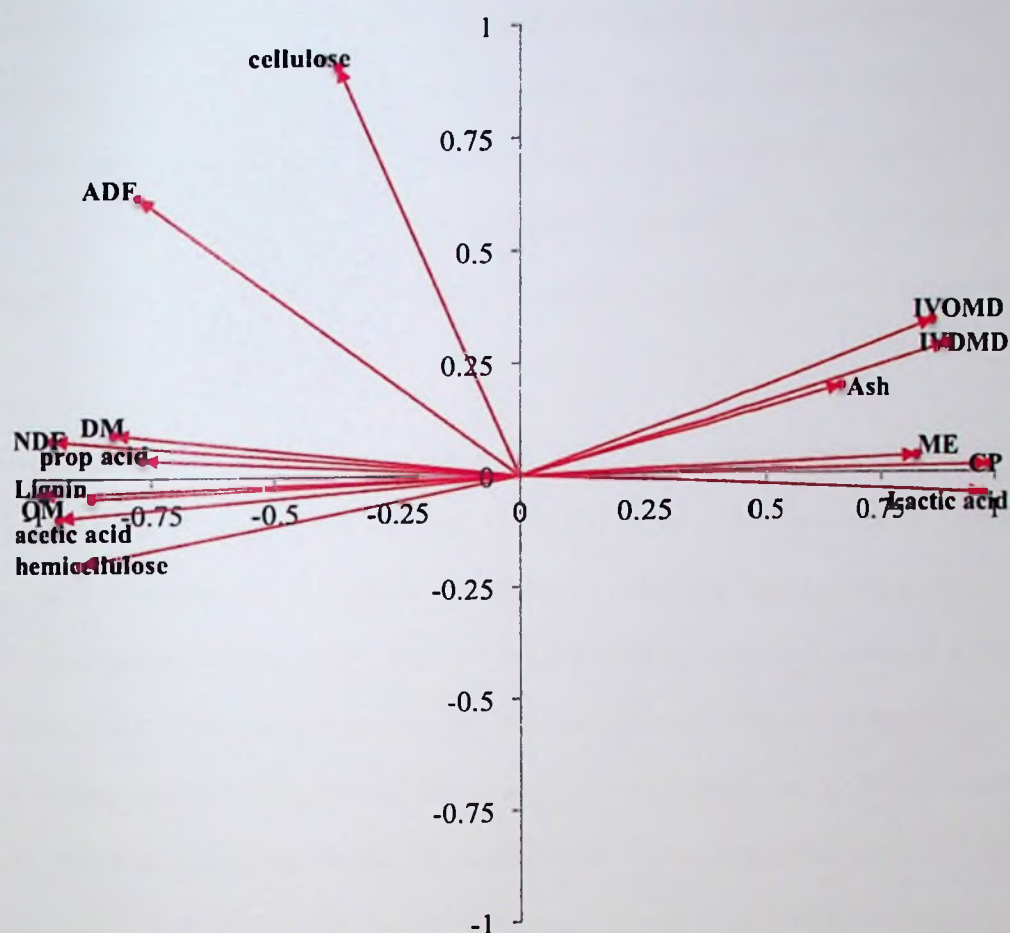


Figure 7.3 Principal component 1 (PC1, 73.10%) and principal component 2 (PC2, 10.20%)

7.4 DISCUSSION

Chemical composition of untreated maize stover (control) obtained in this study was within the common range reported by other authors (Tang *et al.*, 2006; Atuhaire *et al.*, 2016). Pre-treatment reduces the physical strength of lignocellulosic material to enable degradation of lignified cuticle barrier and cleavages of lignin-carbohydrate bonds (Van Soest, 2006). Consequently, digestibility of pre-treated maize stover increases as indicated by higher IVOMD and IVDMD values compared to the controlled substrate. Furthermore, increase in CP content of pre-treated maize stover could be attributed to fungal mycelial colonization ability, which is high

degradability. Therefore, pre-treatment of maize stover with molasses and *L. plantarum* before solid-state fermentation with *P. sajor caju* was more effective and resulted in high gas productions compared to other pre-treatment methods. However, the partitioning factor was poorly related to lignin loss the discrepancy that might be related to adaptation of the rumen microbiota. Generally, such conditions cause massive fiber degradation as confirmed by significant decreased in NDF and ADF contents of pre-treated maize stover as well as an increase in IVDMD and IVOMD. Lower ADF content of maize stover pre-treated with consortia of *L. plantarum*, molasses and *P. sajor caju* observed in this study was in agreement with Karp *et al.* (2012), who reported that adding molasses to sugar cane bagasse decreased ADF by 8.2%. Moreover, H₂O₂ produced from molasses was utilized by peroxidase mediated anti microbial system (Jung *et al.*, 2009) and delignification through the Fenton system (Fe²⁺ and H₂O₂). While Lactic acid produced by *L. plantarum* was utilized in stepwise homofermentation control (Zalán *et al.*, 2005).

The high and positive correlations between gas production rate (mL/24 h gas volume), OMD, ME but and negative curvilinear relationship between gas production rate (mL/24 h gas volume) and PF (Figure 2) was in agreement with Moya-Rodrigués *et al.* (2002) who described the relationship between lignin and cell wall degradation of fibrous feedstock as negatively correlated. However, the PF of SMs was likely overestimated due to interference of secondary phenolic compounds in solid-state fermented maize stover, which could not be validated using gravimetric methods, hence, the curvilinear relationship. The high partitioning factor and low microbial efficiency in SMs may be an indication of non-structural carbohydrates being less affected by pre-treatment method which influenced the equilibrium in the carbonate buffer, by binding H⁺ ions and holding back the release of CO₂ (Cone *et al.*, 1999).

Principal component analysis (PCA) (Figure 3) indicated a positive correlation between digestibility, CP and ME, and a negative correlation between digestibility and fiber fractions.

Such relationships were in agreement with results obtained from the study conducted by Karabulut *et al.* (2007), confirming the already established relationships among the respective parameters. Though pre-treatment of maize stover influenced structural modification, it also provided additional source of nitrogen, which may be utilized by rumen microbes for their microbial protein synthesis and for subsequent utilization of the protein by the host animals (Hendriks and Zeeman, 2009). Efficiency of energy utilisation for microbial growth depends on the synergy of carbon and nitrogen availability for microbial biosynthesis and gas production, an indicator of microbial growth. Thus, differences in chemical composition and *in-vitro* gas production characteristics among pre-treatment methods partly related to the variations in configurations of cell-wall polysaccharides (Pala *et al.*, 2012). Based on the results of this study, it was established that utilizing the consortia of *L. plantarum*, molasses and *P. sajor caju* in modifying chemical composition and digestibility of maize stover could enhance its poor nutritional quality. Nevertheless, additional studies to estimate the extent of *in vivo* interactive effect and understand effectiveness of pre-treatment methods was recommended.

7.5 CONCLUSIONS

This study has adequately demonstrated that *P. sajor caju* was an efficient decomposer of the lignin fraction in maize stover. Apart from modifying structural carbohydrates of maize stover, *P. sajor caju* treatment may provide an additional source of nitrogen, which can be utilized by rumen microbes for their microbial protein synthesis, and subsequent utilization of these proteins by the host animal. Furthermore, treatment of maize stover with consortia of molasses, *L. plantarum* and *P. sajor caju* has enormous potential to benefit both maize and livestock farmers in Uganda. The optimized biotechnological procedures are socially acceptable and ecologically sustainable therefore, recommended for transforming maize stover generally disposed off through burning into high nutritive quality animal feed. However, validation studies to optimize

biotechnological treatment of maize stover fermented with *P. sajor caju* focusing on lactating dairy cows need to be done.

7.6 ACKNOWLEDGEMENTS

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CHAPTER EIGHT

EFFECT OF PRE-TREATMENT METHODS OF MAIZE STOVER FERMENTED WITH *PLEUROTUS SAJOR CAJU* AND *LACTOBACILLUS PLANTARUM* ON MILK PRODUCTION AND COMPOSITION

This chapter is based on a manuscript by

Atuhaire, Andrew Mwebaze., Kabi, Fred., Okello, Samuel., Ebong, Cyprian., Mugerwa Swidiq.

8.0 ABSTRACT

Maize stover is abundant on dairy farms, but its utilization as feedstock is limited. The greatest limitation is high levels of lignin, which limits fibre digestibility and dry matter intake. Biological treatment of maize stover with consortia of fungal and bacterial organisms unlocks the lignin barrier, rendering nutrients available for rumen fermentation and post rumen digestion, which improves on utilization efficiency and performance. The objective of this study was to evaluate the effect of feeding lactating cows on maize stover treated with consortia of *Lactobacillus Plantarum*, molasses and *Pleurotus sajor caju* on feed intake, milk yield and composition. Chopped maize stover was subjected to different pre-treatment processes that included; steaming (SMs), soaking in molasses for 48 hours (MMs), soaking in *Lactobacillus plantarum* inoculum for 48 hours (LMs) and soaking in a mixture of *Lactobacillus plantarum* inoculum and molasses (MLMs) for 48 hours before solid-state fermentation with *P. sajor caju* for 14 days. Maize stover based total mixed ration (TMR) pellets were formulated centering on the pre-treatment methods. The experiment was a 4×4 Latin square design with 21 days periods using four primiporous Holstein Friesian dairy cows with average body weight of 450 ± 20 kg in early lactation (60 ± 10 days in milk). Formulating a TMR pellets based on SMs, LMs, MMs and MLMs maize stover created treatments. Although the formulated total mixed ration pellets (MLMs) improved ($P=0.03$) milk production (23.7 kg/d) and butterfat concentration (44.3 g/kg), there was no significant change in milk fatty acids profile apart from C18 isomers and polyunsaturated fatty acids. Results of this study are interpreted to mean that consortia of *P. sajor caju*, *L. plantarum* and molasses breaks lignin barrier to improve the nutritional quality of the maize stover for improved milk yield and quality.

Key Words: Fibrous, *Lactobacillus plantarum*, Lignocellulosic material, Ruminant feed

8.1 INTRODUCTION

Although Maize stover is rich in cell wall polysaccharides with high energy content, but it represents low quality forages, with high neutral detergent fiber (NDF, 844 g/kg dry matter (DM) (Methu *et al.*, 2001) and lignin (98.1 g/kg DM) (Kabi *et al.*, 2006) contents. Besides, maize stover is low in crude protein (CP, 54.2 g/kg DM) (Atuhaire *et al.*, 2016) and deficient in vitamins and minerals (Kholif *et al.*, 2014). Therefore, maize stover is disposed off through burning, which destroys rich soil biodiversity; increases CO₂ emission leading to global warming (Vibha and Sinha, 2005) yet, there is scarcity of feed for animal.

Cellulose, hemicellulose and lignin in maize stover are organized into microfibril structure of lignocellulosic biomass via ester linkages that mediate stability in the plant cell to resist degradation due to cross-linkages (Yan and Shuya, 2006; Xiao *et al.*, 2007). Structural formation in maize stover disrupts enzymatic cellulolytic activity and fiber digestion in the rumen (Tang *et al.*, 2008). Therefore, enriching nutritive quality of maize stover can only be achieved either by suppressing the undesirable structures or biodegrading of lignin to enhance digestibility, improve the rate of enzyme hydrolysis, hence, yielding quality nutritional feedstock (Mosier *et al.*, 2005; Hendriks and Zeeman, 2009). Solid-state fermentation of lignocellulosic residue with white rot-fungi (WRF) is considered to be a promising treatment process (Okano *et al.*, 2005). However, previous studies have focused on biodegradation of undesirable structures in maize stover with fibrolytic enzymes secretion from white rot fungi but not considering enrichment of desirable components that influence animal performance (Yu *et al.*, 2005).

Furthermore, increased organic matter (OM) losses and production of phenolic compounds during lignin degradation seem to curtail the strategic biological treatment process (Raj *et al.*, 1989; Singh *et al.*, 1990). Therefore, pre-treatment of lignocellulosic biomass, with syntropic and synergistic microorganism may effectively break lignin barrier at same time preserve hemicellulose and cellulose to yield maximum short chain fatty acids for quantity and quality

milk production. Besides, fibers protected against ruminal biohydrogenation increase proportions of unsaturated fatty acids in “protected” fibers, which usually increase milk fat (Ashes *et al.*, 1997). Protection of fibers against ruminal biohydrogenation can also improve digestibility and milk yield (Neves *et al.*, 2009).

Milk fat is an important determinant of milk’s nutritional quality and its composition is increasingly becoming of concern in animal product consumers (Dewhurst *et al.*, 2006). The major isomer of conjugated linoleic acid (CLA) in ruminant’s milk is rumenic acid (cis-9, trans-11 and 18:2), which exhibits anti-carcinogenic and anti-thaerogenic properties (Wahle *et al.*, 2004). Besides, milk fatty acid profile can be improved by lignosulfonate treatment that increases concentrations of cis9, trans11 CLA in milk fat (Neves *et al.*, 2009), which may improve milk fatty acid composition from animal products consumers’ perspective. Therefore, the objective of this study was to evaluate the effect of feeding lactating cows on biologically pre-treated maize stover with consortia of *Lactobacillus Plantarum*, molasses and *Pleurotus sajor caju* on feed intake, milk yield, quality and milk fatty acid profile.

8.2 MATERIALS AND METHODS

8.2.1 Cows and ration

Four primiparous Holstein cows averaging 450 ± 20 kg of body weight and 60 ± 10 days in milk were assigned to a 4×4 Latin square design to determine the effect of pre-treatment methods of maize stover fermented with *Pleurotus sajor caju* on feed intake, body condition score, body weight, milk yield and composition. Each experimental period consisted of 14 d of adaption and 7 d of data collection. Experimental cows were individually penned in well-ventilated shade 3.5 m high, with floor space of 8×3 m and freedom to access fresh water and mineral lick all the time. They were ear tagged and dewormed with broad spectrum anthelmintic (10% albendazole at dosage rate of 10 mg/kg body weight) before commencement of the

experiment. Every morning before and after milking, routine examination to ascertain any signs of heat or ill health, especially, mastitis using a strip cup was done on every experimental cow.

The four total mixed ration pelleted diet (Table 8.1) consisted of chopped maize stover; (i) soaked in boiled water for 3 h (SMs), (ii) soaked in liquid molasses (1 kg of maize stover: 2 litres of molasses 114 g/kg DM) for 48 h (MMs), (iii) soaked in *Lactobacillus plantarum* (*L. plantarum*) (10^5 cfu/mL) inoculum for 48 h (LMs), (iv) soaked in a mixture of *L. plantarum* (10^5 cfu/ml) inoculum and molasses (114 g/kg DM) (1:1 v/v) for 48 h (MLMs) in all the treatments the substrates were drained over night before spawning with *P. sajor caju* at rate of 250 g per kg substrate and solid-state fermented for 14 d. The four dietary treatments were designated as SMs, MMs, LMs and MLMs.

The formulated total mixed ration will target nutrient requirement of 450 kg body weight lactating cows, producing 25 kg/d of milk that contains 39 g/kg of fat (NRC, 1989). The ration targeted CP 17%, NDF 30% and ME of 11.3 MJ/kg DM.

Table 8.1. Chemical composition of chopped maize stover subjected to different pre-treatment options prior to fermentation with *P. sajor caju*.

Chemical composition	Pre-treatment methods of maize stover			
	SMs	MMs	LMs	MLMs
DM (g/kg)	927.0	883.0	877.0	835.0
Ash (g/kg DM)	67.0	74.9	75.6	84.5
CP (g/kg DM)	51.2	80.1	95.9	116.6
NDFom (g/kg DM)	755.0	627.0	602.0	571.1
ADFom (g/kg DM)	447.0	417.1	412.3	398.3
Lignin (sa) (g/kg DM)	84	63	55	42
Ether extract (g/kg DM)	67.0	70.2	73.1	76.3
ME (MJ/kg DM)	8.7	9.2	9.9	10.2
pH	4.43	4.29	4.08	4.04
Lactic acid (g/kg DM)	54.2	60.7	62.1	65.4
Acetic acid (g/kg DM)	28.4	23.5	21.3	19.8
Propionic acid (g/kg DM)	0.84	0.83	0.81	0.78
Calcium (g/kg DM)	29	28.3	30	32
Phosphorus (g/kg DM)	7.1	6.9	7.3	7.8

SMs; steamed chopped maize stover, MMs; soaked maize stover in molasses, MLS; soaked maize stover in *Lactobacillus plantarum* inoculum, MLMs; soaked maize stover in a mixture of *Lactobacillus plantarum* inoculum and molasses.

8.2.2 Experimental materials and treatments

The ingredients in total mixed rations (TMR) (Table 8.2) were similar for all treatment groups with the primary difference related to pre-treatment of maize stover fermented with *Pleurotus sajor caju* (Table 8.1). Each treatment diet was formulated to provide similar amount of CP, NDF and ME (Tables 8.3). Therefore, the treatment variables were the pre-treatment methods of maize stover before fermented with *Pleurotus sajor caju*, which were SMs, MMs, LMs and MLMs. Each treatment diet was fed as a total mixed ration (TMR). During the adaptation period, the animals were fed on treatment diet to determine average dry matter intake for each of the experimental animals. To ensure that experimental cows had *ad libitum* access to

treatment diet, the amount offered was assessed on the dairy basis with aim of producing 3-5% refusal.

Table 8.2. Chemical composition of the ingredients used to formulate TMR pellets

Chemical composition	Ingredients in TMR					Vitamin-mineral mix ^b
	Whole maize	Soya bean meal	Cotton seed cake	<i>Calliandra</i> leaf hay	Molasses	
DM (g/kg)	902.0	890.0	880.0	863.0	11.4	
Ash (g/kg DM)	71.0	70.0	74.2	94.5	1.8	
CP (g/kg DM)	113.0	430.0	347.4	204.3	37.8	
NDFom (g/kg DM)	122.3	248.0	237.3	477.0	nd	
ADFom (g/kg DM)	32.0	47.0	139	283	nd	
Ether Extract (g/kg DM)	31.7	29.6	89.0	61.7	nd	
ME (MJ/kg DM)	13.8	14.8	12.5	9.7	nd	
Calcium (g/kg DM)	1.7	5.3	3.2	4.6	nd	220
Phosphorus (g/kg DM)	5.5	6.4	14.1	4.7	nd	55

^bDM: 55 g of P; 220 g of ca; 105.5 g of cl; 70 g of Na; 35 g of Mg; 22 g of S; 1,500 mg of Mn; 500 mg of Co; 40 mg of I; 20 mg of Se; 90,000 iu of vitamin A; 75,000 iu of vitamin D3; 1000 iu of vitamin E; Non fiber carbohydrates = 1000 - (NDF + Ash + CP + Ether Extract)

8.2.3 Experimental procedure

Four total mixed ration pellets as dietary treatments; SMs, MMs, LM and MLMs, were all iso-nitrogenous and offered three times in a day (5.00, 12.00, 17.00). To ensure *ad libitum* feeding 3-5% refusals were targeted and composite samples of feed refusal per period were pooled, mixed systematically and sub-sampled for chemical analysis. Experimental cows were weighted at the same time of the day (following the a.m. milking) on the 2 final days (day 14 and 21) of each experimental period, using an electronic weighbridge and average body weight was used to compute DM intake as a percentage of body weight.

Cows were milked twice every day at 06:00 and 15:00 hours, milk yield was measured and recorded in kg at every milking per cow. Milk samples were obtained during the first milking in the morning (06:00 - 07:00 h) for four consecutive milking on day 15, 16, 17 and 18 of each experimental period. A representative sample was collected in the milk-sampling flask and drawn after stirring the milk using milk plunger. Milk samples were pooled per cow throughout the period to obtain one proportional composite milk sample per cow per period and transported to the laboratory for milk composition and chemical analysis. Milk fat was isolated from individual milk samples that were collected on the last day of milk sampling period, centrifuged for 15 min at $822 \times g$ and immediately stored at -15°C until FA profiling analysis was done.

Table 8.3. Composition of ingredients used in pelleted TMR.

Ingredients	Total mixed rations			
	SMs	MMs	LMs	MLMs
SMs (g/kg DM)	460	-	-	-
MMs (g/kg DM)	-	465	-	-
LMs (g/kg DM)	-	-	470	-
MLMs (g/kg DM)	-	-	-	475
Cotton seed cake (g/kg DM)	130	130	130	130
Soya bean meal (g/kg DM)	40	35	30	25
Maize grain (g/kg DM)	185	185	185	185
<i>Calliandra</i> leaf hay (g/kg DM)	140	135	130	125
Molasses (g/kg DM)	35	40	45	50
Premix (g/kg DM)	5	5	5	5
Salt (g/kg DM)	5	5	5	5
Nutritive value				
Dry matter (kg DM)	12.05	12.09	11.87	11.71
CP (kg DM)	2.05	2.06	2.02	1.98
NDF (g/kg DM)	3.62	3.62	3.56	3.51
ME (MJ/kg DM)	15.6	15.6	15.7	15.6

Each kg of TMR will contain 270 g/kg of ca, 80 g/kg of P, 20 g/kg of S, 2200 mg/kg of Fe, 2800 mg/kg of Zn, 800 mg/kg of Cu, 801 mg/kg of F, 60 mg/kg of I, 50 mg/kg of Co, 40 mg/kg of Se, 216,000 iu/kg of vitamin A, 67600 iu/kg of vitamin D3 and 500 iu/kg of vitamin E.

8.2.4 Chemical Analysis

Dry matter of the TMR was determined in a forced-air oven according to procedure 930.15 of AOAC (1995). TMR were ground to pass a 1 mm screen in a Wiley mill before analysis of N, ether extract, ADFom and NDFom. Crude protein (CP) were determined following methods 954.01 (AOAC 1995) and ash was determined by incinerating samples at 550°C for 5 h (AOAC 1995; method ID 942.05). Neutral detergent fiber (NDF) exclusive of residual ash (NDFom) was determined without addition of α -amylase or sodium sulphite according to Van Soest *et al.* (1991). The same sample was sequentially analyzed for acid detergent fiber exclusive of residual

ash (ADFom) and Lignin (sa) was determined by solubilization of cellulose with sulphuric acid (12 N) according to the method of Robertson and Van Soest (1981). The concentration of hemicellulose was calculated as the difference between NDFom and ADFom computed sequentially on the same sample while that of cellulose as a difference between ADFom and Lignin (sa).

Samples for lactic acid, acetic acid and propionic acid were prepared following procedures described by Ranjit and Kung jr. (2000), stored at 4°C for 24 h in the refrigerator. The slurry mixture was then filtered through 4 layers of cheesecloth and the filtrate was used to determine pH, lactic acid, acetic acid and propionic acid following procedures described by Bolsen *et al.* (1992). The pH was directly measured using a pH meter (Sortorins basic pH meter, Germany), while concentration of lactic acid, acetic acid and propionic acid were analysed using Hewlett Packard 6890 capillary gas chromatograph that was fitted with J & W ov 351 capillary column (FFAP phase) 30 m x 0.25 mm (Getachew *et al.*, 1998). Determination of lactic acid concentration followed the procedures described by Muck (1988). All the chemical analysis studies were conducted in triplicates and the results expressed on DM basis. Butterfat, solid not fat, lactose and protein in the milk were determined using ultrasonic milk analyzer (Lacto scan SA30 version 59).

Fat in the milk was separated by centrifugation as described by Murphy *et al.* (1995) and fatty acids were methylated according to method 5509 of ISO (1978) using KOH/methanol (Synth[®] São Paulo, Brazil) and n-heptane (Vetec[®], Rio de Janeiro, Brazil). Fatty acids methyl ester profiles were measured at a split ratio of 1:80 with GLC on Varian chromatography (Palo Alto, CA, USA) using G1315A auto sampler that was equipped with a flame ionization detector and a CP-7420 fused silica capillary column that was maintained at 65°C for 40 min and increased to 50°C per min up to 240°C then held for 28.5 min. Injector and detector temperatures were set at 220 and 235°C, respectively. The carrier gas hydrogen was flowing at 1.4 ml/min,

and the detector was set at 30 ml/min, airflow at 300 ml/min and flow of make up gas (N₂) was set at 30 mL/min. The fatty acid peaks were identified using pure methyl ester standard (Synth[®] São Paulo, Brazil).

Milk fatty acid analysis, was effected by sampling 200 mg of milk fat layer into culture tubes, and milk lipids extracted according to the procedure of Folch *et al.* (1957). Solvents were then evaporated under N, and FA methyl esters prepared by base-catalyzed trans-esterification with sodium methoxide (Christie, 2004).

8.2.5 Statistical analysis

Data on chemical composition of the pelleted TMR, dry matter intake, milk production, milk composition and milk fatty acid were subjected to mixed model procedure of SAS (2010) using the model:

$$Y_{ijk} = \mu + C_i + R_j + P_k + \varepsilon_{ijk}$$

Where Y_{ijk} was milk yield and composition observed from lactating cow j receiving TMR i , in period k . μ was the overall mean, R_i was the effect of the different TMR ($i = R1, R2, R3, R4$), C_j was the effect of lactating cow ($j = C1, C2, C3, C4$) and P_k was the effect of the period ($k = P1, P2, P3, P4$) ε_{ijk} was the residual effect. Multiple comparison tests used Duncan's multiple-range test (Snedecor and Cochran, 1980). Correlations among parameters and prediction of milk yield and composition from pre-treated maize stover based TMR pellets used Pearson correlation.

8.3 RESULTS

8.3.1 Ration evaluation

There were no variations on DM intake, CP, ADFom and NDFom (Table 8.4). However, there was a significant change in body weight and cows fed on maize stover pretreated with a mixture of molasses and *Lactobacillus plantarum* had superior body weight change.

Table 8.4. Body weight gain, dry matter intake and nutrient intake of Holstein Friesian cows fed on TMR pellets based

Parameter	Total mixed ration pellets				SEM	P-value
	SMs	MMs	LMs	MLMs		
Body weight (kg)						
Initial	454	454	454	454	19.1	0.13
Final	465 ^c	477 ^b	471 ^b	486 ^a	7.2	0.042
Change	11 ^c	23 ^b	17 ^b	32 ^a	3.4	0.031
Intake (g/Kg BW ^{0.75} /d)	28.2	30.4	30.7	30.6	0.83	0.32
Intake (kg/d)						
DM	13.5	13.9	13.8	13.9	1.02	0.21
OM	12.6	12.8	12.8	12.9	0.13	0.19
CP	2.5	2.6	2.6	2.5	0.04	0.45
EE	1.0 ^b	1.2 ^a	1.1 ^b	1.0 ^b	0.04	0.01
ADFom	3.3	3.2	3.2	3.3	0.05	0.87
NDFom	5.4	5.5	5.5	5.3	4.89	0.16

BW, body weight; DM, dry matter; OM, organic matter; N, nitrogen; ns, non-significant; NDF, neutral detergent fiber; ADF, acid detergent fiber. Different superscripts within the same row are significantly different at p<0.05.

There were variations among the total mixed ration pellets on milk production (p = 0.03), milk CP yields (p = 0.04), fat (p = 0.03) and lactose (p = 0.05). There was no variation (p = 0.23) on somatic cell scores (SCC) (Table 8.5). Cows fed on MMLs had numerically higher milk fat concentration and lactose than those fed on other rations.

Table 8.5. Milk production and composition of cows fed on pelleted TMR based on different pre-treatment methods of maize stover fermented with *P. sajor caju*.

	Treatment (TMR)				SEM	P-value
	SMs	MMs	LMs	MLMs		
Production (kg/d)						
Milk	16.7 ^c	18.9 ^b	19.6 ^b	23.7 ^a	1.54	0.03
Crude protein	0.58 ^b	0.58 ^b	0.61 ^b	0.68 ^a	0.17	0.04
Fat	0.54 ^c	0.65 ^b	0.56 ^c	1.05 ^a	0.64	0.03
Lactose	0.81 ^c	0.83 ^c	0.89 ^b	1.01 ^a	0.72	0.05
Components (g/kg)						
Crude protein	34.73 ^a	30.69 ^b	31.12 ^b	28.69 ^c	1.23	0.04
Fat	32.33 ^b	34.39 ^b	28.5 ^c	44.3 ^a	2.11	0.02
Lactose	44.3 ^a	44.5 ^a	44.3 ^a	42.61 ^b	0.78	0.04
Total solids	118.7	118.9	118.7	118.9	1.01	0.16
Somatic cell count ('000)	2.66	2.58	2.66	2.68	0.0	0.23

Variations on fatty acid profiles tended to be significant ($p = 0.05$) among formulated rations on milk fatty acid profile with the exception of *cis*6-18:2, *cis*3-18:3, 20 and PUFE (Table 8.6).

Table 8.6. Fatty acid profile (g/kg of fatty acids) of milk of dairy cows fed different pre-treated methods of maize stover fermented with

Fatty acids (g/kg total fatty acids)	Treatment				SEM
	SMs	MMs	LMs	MLMs	
4.0	3.9	4.2	4.3	5.0	0.33
6.0	4.4	4.6	4.6	5.5	0.32
8.0	3.7	3.9	3.9	4.5	0.22
10.0	10.7	11.1	11.0	12.1	0.64
12.0	13.9	13.7	13.6	14.6	0.62
14.0	76.1	73.2	74.9	74.8	2.49
<i>cis</i> 9-14:1	7.7	7.4	7.7	7.4	0.23
<i>cis</i> 7-14:1	7.3	6.9	7.3	7.2	0.31
<i>cis</i> 11-16:1	1.9	2.0	2.1	2.3	0.31
<i>cis</i> 9-16:1	197.8	198.7	198.5	196.3	3.12
<i>cis</i> 7-16:1	16.7	15.7	16.5	15.8	0.48
17:0	4.4	4.7	4.6	4.3	0.13
<i>cis</i> 7-17:1	3.9	3.9	3.9	3.9	0.10
18:0	1.6	1.8	1.6	1.7	0.11
trans 11-18:1	198.1 ^c	201.1 ^b	201.8 ^b	213.4 ^a	6.70
<i>cis</i> 6-18:2	44.8 ^a	30.0 ^d	42.4 ^b	37.2 ^c	0.67
<i>cis</i> 9 trans 11-18:2	331.3	340.2	329.6	334.5	4.87
<i>cis</i> 12 trans 10-18:2	15.3	15.7	15.3	16.9	0.56
<i>cis</i> 6-18:3	6.5	5.7	6.4	5.8	0.45
<i>cis</i> 3-18:3	1.9 ^a	1.1 ^c	1.9 ^a	1.5 ^b	0.14
20.0	2.6 ^b	3.2 ^a	2.6 ^b	3.5 ^a	0.19
MUFA	3.8	3.9	3.7	3.7	0.16
PUFA	0.8 ^b	1.0 ^a	0.7 ^c	0.9 ^b	0.05
SFA	0.4	0.5	0.4	0.4	0.03

Total trans 18:1 Isomer; Total trans 18:2 Isomer, MUFA; monounsaturated fatty acids, PUFA; Polyunsaturated fatty acids, SFA; Saturated fatty acids.

8.4 DISCUSSION

The chemical composition of TMR pellets met all nutrient requirements of lactating dairy cows producing 25–30 kg milk/d (NRC, 1989) (Table 8.3). Results from pre-treated maize stover showed that it had a much higher CP but lower NDF content than untreated maize stover. However, crude protein composed of rumen degradable protein, a fraction that escapes microbial rumen degradation to be available to the cow in the small intestines for direct use in milk synthesis (McDonald *et al.*, 2002). Although there were no differences in the DM, CP, fat and NDF content among experimental treatments, confirming iso-proximate nutrient rations, the treatments differed only in the pre-treatment process of maize stover before fermentation with *P. sajor caju*. Total mixed ration based on solid-state fermented maize stover increased the efficiency of milk production as a result of increased nutrient supply for milk production. These responses were attributable to the increase in polysaccharide digestion in the rumen that increases propionic acid production a major gluconeogenic precursor in milk synthesis (Chen *et al.*, 1995). Theurer *et al.* (1999) reported that feeding dairy cows with steam-flaked sorghum enhanced uptake of glucose and amino acids by the mammary gland, increased ruminal polysaccharide digestion and microbial protein synthesis, which improved milk and milk protein yields. Therefore, maize stover pre-treatment enhanced glucose and amino acids uptake by mammary glands, as a result of increased ruminal polysaccharide digestion.

The efficiency of milk production was significantly greater in cows fed on MLMs ration than in those fed on other TMR pellets. Schingoethe *et al.* (1999) reported that cows fed solid-state fermented fibrous feed resources (45% of TMR) increased milk production to the same extent as that achieved when cows were fed a high-concentrate (55%) diet without the enzymes. Such results suggests that solid-state fermentation of fibrous feed resources with high fiber proportions can be fed without jeopardizing milk production, therefore, lowering the cost of the feed and reducing the risk of acidosis. Although solid-state fermentation of fibrous feed

resources improved utilization of cell wall polysaccharides, it may indirectly depress ruminal pH and predispose cows to ruminal acidosis (Eun and Beauchemin, 2005). Hristov *et al.* (1996) also noted that solid-state fermentation of fibrous feed resources might decrease both chewing time and saliva output thereby, increasing the risk of acidosis. However, the risk of acidosis was minimized during formulation of maize stover based TMR to contain the recommended 30% NDF hence, minimizing the risk of acidosis.

Pre-treatment of maize stover with a mixture of molasses and *Lactobacillus plantarum* prior to inoculation with *P. sajor caju* decreased the acetate: propionate ratio ($P = 0.04$; 3.09 vs. 2.87), implying improved efficiency of energy utilization in the rumen. Furthermore, acetate: propionate ratio decreased ($P = 0.004$) when feeding MMs total mixed ration, implying increased ruminal energetic efficiency. Molasses-mediated digestibility in this study could have occurred through increased microbial colonization of maize stover or by direct cell wall hydrolysis (Chen *et al.*, 1995; Yang *et al.*, 2000). Eun and Beauchemin (2005) reported that feeding more concentrates had no effect on molar acetate proportion but increased short chain fatty acid concentration and molar propionate proportion. Maize stover based TMR pellets influenced ruminal degradation of rations which is in agreement with Krueger *et al.* (2008) who reported similar findings with *Bermuda grass* based total mixed ration. Treatment of maize stover with consortia of molasses, *Lactobacillus plantarum* and *P. sajor caju* increased total-tract digestibility but did not affect *in situ* ruminal degradability, which suggested that pre-treatment increased post-ruminal digestion of the TMR. Sutton *et al.* (2003) also reported similar findings that solid-state fermentation increased post-ruminal and total-tract digestion of TMR but did not increase ruminal digestion.

8.4.1 Milk fatty acid

The dietary effects on milk fatty acid (FA) profiles were as given in Table 8.4. MLMs had only a minor, but significant effect on milk FA. Milk fat is an important determinant of milk's nutritional quality. For example, the major isomer of conjugated linoleic acid (CLA) in ruminant milk is rumenic acid (cis9, trans11-18:2) and it exhibits anti-carcinogenic and anthiatherogenic properties (Wahle *et al.*, 2004). Rumenic acid is produced from dietary linoleic acid and by delta 9-desaturase from the trans11- 18:1 in animal tissues (Shingfield *et al.*, 2008). Linolenic acid (cis3-18:3) also contributes as precursors of CLA and trans11-18:1 (Chilliard *et al.*, 2007).

It has been shown that soaking maize stover in water before fermentation with *Pleurotus sajor cajo* (SMs) decreased the sum of C18 FA, mostly derived from diet or from mobilization of adipose tissue reserves from 46.8- 44.1 % of total FA. Because body fat mobilization is not expected to be of great importance in cows averaging 60 days in milk, the reduction of C18 FA could be explained by a lower intake of these FA (He *et al.*, 2005). Natural pasture is rich in linolenic acid and cows on pasture have been shown to have higher concentrations of CLA in milk fat than those consuming conserved forages (AbuGhazaleh *et al.*, 2007). Thus pelleting may have increased release of linoleic acid in MLMs total mixed ration and adversely impacted on ruminal fermentation and milk production, as was shown by earlier studies on sunflower seed (Chouinard *et al.*, 2001), another rich source of linoleic acid.

Milk PUFA concentration averaged 4.2% of total FA, and it was affected ($P = 0.01$) by treatments. Together, 18:2n-6 and 18:3n-3 constituted 41% of milk PUFA, and this proportion was unaffected by treatments, in spite of the significant effects observed on 18:2n-6. Overall, these minor effects could be justified by the small differences between the FA compositions among TMR pellets (Table 8.2). However, previous study had shown that lignosulfonate

treatment of oilseeds rich in linoleic acid, such as full-fat soybeans, tended to increase concentrations of CLA in milk fat (Neves *et al.*, 2007).

Fatty acid composition of milk produced by cows fed on MMLs has consistently higher content of unsaturated FA, particularly of CLA cis-9, trans-11, 18:1 trans-11, and 18:3n-3. The significant variation observed in CLA cis-9, trans-11 and 18:1 trans-11 concentrations in milk fat of microbial FA could reflect an improvement in rumen fermentation, hence higher availability of short chain fatty acids. The large variation observed in CLA cis-9, trans-11 and 18:1 trans-11 concentrations in milk fat of individual cows has been observed in other studies (Kelly *et al.*, 1998; Rego *et al.*, 2004) and has been attributed to individual differences in the activity of delta 9-desaturase in animal tissues (Salter *et al.*, 2007).

8.5 CONCLUSIONS

This study shows clear evidence on benefits of pre-treating maize with consortia of molasses, *Lactobacillus plantarum* and *Pleurotus sajor caju* on dry matter intake, milk production and composition. These favorable effects were attributed primarily to improved nutrient digestion and improved ruminal energy utilization. Biological treatment of maize stover with consortia of molasses, *L. plantarum* and *P. sajor caju* enhanced digestion and ruminal energy utilization. It influenced milk production and composition, improved the efficiency to utilize dietary N and rumen microbial biomass. Pre-treatment of maize stover before fermentation with *P. sajor caju* influenced significantly the composition of C 18 and polyunsaturated fatty acids in the milk suggesting improved dietary quality of milk fat for the final consumer. Results of this study are interpreted to mean that consortia of *P. sajor caju*, *L. plantarum* and molasses played a recommendable role to break lignin barrier and improve nutritional quality of the maize stover for enhanced dairy farm productivity.

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CHAPTER NINE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

9.1 DISCUSSION

Degradation of maize stover biomass is limited by several factors that include degree of polymerization, moisture content, available surface area, crystallinity of cellulose and lignin content (Chang and Holtzapple, 2000; Laureano-Perez *et al.*, 2005). Elisashvili *et al.* (2008) reported that decreased particle size and increased available surface, rather than, crystallinity affect the rate and extent of the hydrolysis. It is documented that the pore size of the substrate in relation to the size of the enzymes is the main limiting factor in the enzymatic hydrolysis of lignocellulosic biomass (Cheng *et al.*, 2001; Thompson *et al.*, 1992). Removal of hemicellulose increases the mean pore size of the substrate and, therefore, increases the probability of the cellulose to get hydrolyzed (Palonen *et al.*, 2004; Sharma and Arora, 2015). However, dry lignocellulosic material can cause a collapse in pore structure, which results into a decreased enzymatic hydrolysis (Wang *et al.*, 2012). White-rot fungi *P. sajor caju* has the capability to produce lignin-degrading enzymes like lignin peroxidases and manganese peroxidases (Singh and Chen, 2009; Dashtban *et al.*, 2009; Sharma and Arora, 2015).

Crude protein content of untreated maize stover obtained in this study was within the range reported by other authors (Chen *et al.*, 1995; Kabirizi, 2006; Tang *et al.*, 2008; Montañez-Valdez, *et al.*, 2015). The improved CP content of the treated maize stover compared to that of control (Table 6.3) could be attributed to mycelial growth of the fungi, which is high in protein (Ghorai *et al.*, 2009). Though there was a strong relationship between concentration of lignin in a feedstock and poor digestibility (Vadiveloo *et al.*, 2009; Sarnklong *et al.*, 2010, Sharma and Arora, 2015), it was possible that protein increase per unit dry matter could due to fungal growth

(Zadrazil *et al.*, 1996; Misra *et al.*, 2007). Therefore, removing lignin barrier that shields degradable polysaccharides improves on the digestible parts of the substrate (Chang *et al.*, 2001).

Dry matter loss due to biological treatment of maize stover with *P. sajor caju* compared to control was 5.4% while the loss in other white-rot fungal treatments was between 2.9 and 3.9%. Although treatment with white-rot fungi may increase digestibility significantly (Karunanandaa *et al.*, 1995) a challenge with such treatment is loss of biomass since white rot fungi utilized part of nutrients for their metabolic activities. Loss of biomass is pronounced when the substrate is rich in highly fermentable components such as sugar, starch and protein but less biomass loss when the substrate is rich in fibrous materials. Therefore, one of the benefits of pre-treatment on maize stover prior to fermentation with *P. sajor caju* was to avail the easily digestible carbohydrates to be converted into simpler sugars during primary metabolism. The fungus utilized the sugars generated during primary metabolism, in order, to initiate secondary metabolism that consists of the structural breakdown of carbohydrates and lignin from substrates by extracellular enzymes (Kholif *et al.*, 2014). Accordingly, *P. sajor caju* is enabled to degrade the aromatic structure of lignin and release cellulose and/or hemicellulose attached to lignin and facilitates its utilization by rumen microbes and their host animals.

Structural modification of cell wall components due to *Lactobacillus plantarum* (*L. plantarum*) and molasses option prior to solid state fermentation with *P. sajor caju* appears to facilitate fiber-degrading microbes in the rumen to degrade fibrous components more intensively to improve on the digestibility of maize stover. Earlier Vadiveloo *et al.* (2009) reported that incubating rice husks with *P. sajor-caju* at 25°C for 25 days significantly improved its *in vitro* digestibility. Fazaeli *et al.* (2002) also observed increased digestibility of DM and OM in cattle when wheat straw was treated with *P. florida*. However, it was reported that White-rot fungi degrade highly digestible polysaccharides and consume relatively more lignin and hemicellulose than cellulose in its primary metabolism while in it's secondly metabolism more hemicellulose

are degraded than lignin (Zadrazil *et al.*, 1996). Altogether, these conditions cause massive fiber degradation as confirmed by a decrease in NDF and ADF contents of maize stover as well as an increase in IVDMD and IVOMD.

Pre-treatment of maize stover with a mixture of *L. plantarum* and molasses before solid-state fermentation with *P. sajor caju* appear to be effective in improving the nutritive quality. Although Pan *et al.* (2012) observed that the effective rumen degradability of DM and OM of solid fermented cottonseed hull *P. ostreatus* was significantly higher on the spent mushroom substrate.

It has been shown that there was effective rumen degradability of NDF and ADF were increased by biological treatment of maize stover with *P. sajor caju* and improved its nutritive quality. Díaz and Sánchez (2002) reported that proportion of DM ruminal disappearance was higher in maize stover treated with *P. ostreatus* compared to untreated maize stover. They also reported higher digestible and degradation values in treated maize stover as found in this study.

Lactic acid forming bacteria in presence of molasses releases H_2O_2 required for peroxidase mediated antimicrobial system (Jung *et al.*, 2009). Such treatment improves on the feeding value of maize stover, interrupts the lignified cuticle barrier and causes cleavages of some crystalline cellulosic bonds (Schiere and Nell, 1993; Van Soest, 2006). Furthermore, the relationship of chemical composition in the treated maize stover was further confirmed by principal component analysis (PCA) with positive correlation between digestibility metabolisable energy and CP. Such relationships were in agreement with results obtained from other studies (Barahona *et al.*, 2003; Karabulut *et al.*, 2007).

Performance of lactating dairy cows fed on pre-treated maize stover with *L. plantarum* and molasses before inoculation with *P. sajor caju* was a true reflection of effective improvement of chemical composition and digestibility of quality maize stover. High dry matter intake in dairy

cattle was beneficial in increasing productivity as shown by higher average milk yield and better composition, which may be a result of fermentation process. Nutrient supply for the dairy cows fed on pre-treated maize stover fermented with *P. sajor caju* rations was higher due to the increased dry matter intake. Improved nutrient supply also provided sufficient substrate for rumen microbes to grow and yield more microbial protein from their biomass (Clark *et al.*, 1992; Bach *et al.*, 2005), which was observed in this current study. As a result of active and abundant rumen microbes, an elevated short chain fatty acid concentration was observed as the end product of microbial fermentation in the rumen (Kamra, 2005). Increasing rumen microbial concentrations of dairy cows fed on pre-treated maize with a mixture of *L. plantarum* and molasses fermented with *P. sajor caju* were probably due to the tendency of higher N intake in the corresponding treatments.

Furthermore, pre-treatment of maize stover with either *L. plantarum* or molasses only and fermented with *P. sajor caju* apparently increased the proportion of rumen degradable protein over the un-degradable protein, thus contributing to the increased ammonia concentration which could lead to high absorption of NH₃-N from rumen and converted into urea. The synthesis of urea in the liver requires expenditure of energy (Van Soest, 1996).

Based on the results obtained, it was concluded that poor nutritional quality of maize stover can be overcome through pre-treatments of maize stover with molasses and *L. plantarum* and fermented with *P. sajor caju*. Pre-treatment of maize stover shows a comparative advantage over other biological treatments. Apart from modifying structural carbohydrate of maize stover, *L. plantarum* and molasses pre-treatment followed by solid state fermentation with *P. sajor caju* provided additional source of nitrogen, which was utilized by rumen microbes for their microbial protein synthesis, and subsequent utilization of the amino acids by the host animals. Animals receiving maize stover rations pre-treated with *L. plantarum*, molasses and *P. sajor caju* responded positively over the steamed maize stover group in terms of average milk yield and

composition, body condition score and a number of rumen fermentation parameters. These outcomes were due to compositional changes that occur in maize stover after being treated. Treatment of maize stover using white rot fungi species like *P. sajor caju*, nevertheless, needs a special attention since, apart from its effectiveness in degrading lignin aromatic structure as well as cellulose or hemicellulose attached to the substance, it consumed part of the nutrients in feed hence biomass loss.

Maize stover, a lignocellulosic biomass represents a rather unused source for quality animal production. Lignin content limits the digestibility of the hemicellulose and cellulose present in the maize stover. Pre-treatment improved the digestibility of the maize stover. Each pre-treatment has its own effects on the cellulose, hemicellulose and lignin.

9.2 CONCLUSIONS

Conclusions drawn from this study are:

1. Farmers in Lake Victoria agro-ecological zone appreciate the importance of feed scarcity especially in dry season and availability of unexploited non-conventional feedstock.
2. Farmers identified maize stover as a major non-conventional feedstock that was well distributed across the agro-ecological zone and available to all farming households during dry season but un-utilized on dairy farms.
3. Farmers identified lack of knowledge, skill and strategy to preserve, process and make timely decisions on efficient use of crop residues especially maize stover.
4. Biological treatment of maize stover with consortia of molasses, *L. plantarum* and *P. sajor caju* has adequately demonstrated as appropriate treatment to break lignin barrier in maize stover for improved CP, IVDMD, IVOMD and ME. *P. sajor caju* can optimally break down lignin barrier at 25°C, pH 5, and in absence of light.

5. Apart from modifying structural carbohydrates of maize stover, consortia of molasses, *L. plantarum* and *P. sajor caju* provided an additional source of nitrogen, which could be utilized by rumen microbes for their microbial protein synthesis, and subsequent utilization of the protein by the host animal, hence, improving the efficacy of rumen and post rumen digestion and quantity of milk fat for final consumer.
6. The innovation enhances nutritional quality of the abundant maize stover feedstock by breaking down lignin, preserving cell wall polysaccharides and minimizing formation of anti-nutritional factors.

9.3 RECOMMENDATIONS

1. The innovation of treating maize stover with consortia of molasses, *Lactobacillus plantarum* and *P. sajor caju* should be validated on farm and up scaled to address dry season feed scarcity challenges on smallholder dairy farms in Lake Victoria Crescent
2. The study has provided evidence based information for improved nutritive quality of maize stover using ecologically sustainable treatment, therefore, recommended for policy formation that will enhance maize stover utilisation as an alternative quality feed resource.
3. Molecular characterisation of synergistic enzymes created by a consortia of *Lactobacillus plantarum*, molasses and *P. sajor caju*.
4. This study focused on maize stover, however it is recommended that similar studies on nutritional enhancement other abundant fibrous cereal crop residues like sorghum stover be considered.

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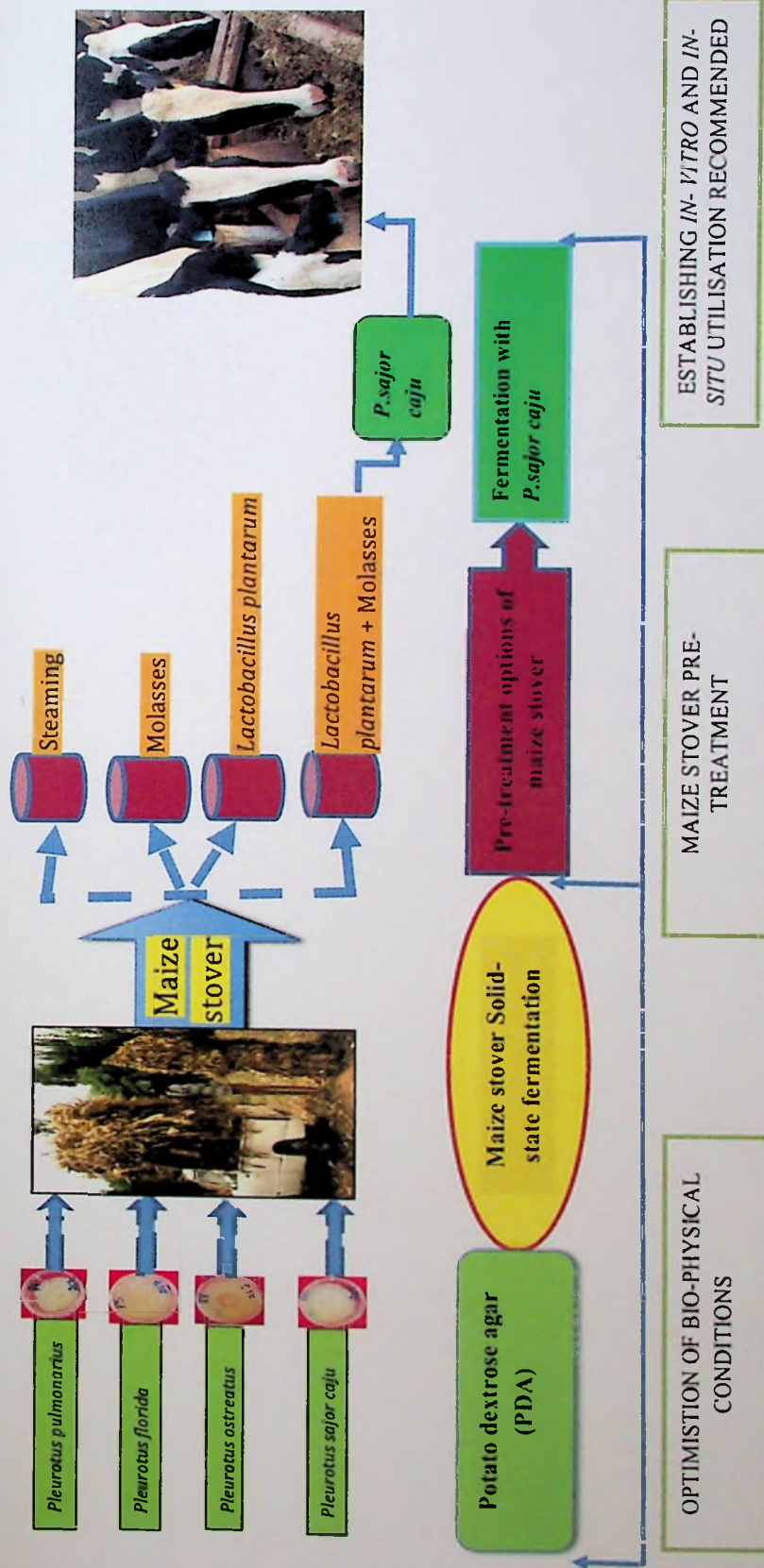
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Appendix 1



Appendix 2

Crop residue/agro-industrial by-products utilization in smallholder crop-livestock systems

The objective of this survey is to obtain a better understanding on the extent/level of utilization of crop residues and agro-industrial by-products in smallholder crop-livestock systems in Uganda. This survey is part of the EAAPP project, which seeks to enhance agricultural productivity and profitability in the East African region. The National Livestock Resources Research Institute carries out this survey. Data collected here will be confidentially kept and reports will not make reference to individual cases explicitly.

Enumerator name: _____ Date: _____

HOUSEHOLD GENERAL DATA

Identification: if possible, please add the coordinates of the homestead.

a. District _____ b. Sub-county _____ c. Village _____
d. Coordinates homestead _____ e. Altitude _____ masl

Household head: main information of the household head

a. hh head name _____ b. hh head father's name _____
c. hh head gender _____ Male (1) Female (2)

Household members: number and age of member including household head.

	1. Female	2. Male		1. Female	2. Male
< 6 years old			6 – 9 years old		
10 – 15 years old			15 - 60 years old		
> 60 years old					

Farm labour availability: number of people farming and keeping livestock in the farm.

	1. Female	2. Male		1. Female	2. Male
Family full-time			Family part-time*		
Hired permanent			Hired temporal (man/days)		

* Part-time means less than 70% of the year working on the farm.

Water access: main sources (please locate each item in the resource flow map).

Purpose	1. Type	2. Ownership?	3. Frequency of access	4. Distance
				km
				km
				km

(1) drinking (2) Irrigation (3) livestock
(1) Open well; (2) canal (3) tubewell /borehole; (4) river (5) lake/pond; (6) other _____
(1) Owned (2) shared (3) common (4) Government supply (5) other _____
(1) Daily (2) weekly (3) monthly; (4) seasonally; (5) annually

Market access: main input and output markets, including traders

Main markets	1. Name place	2. Channel	2. Distance	3. Main transport	4. Travelling time	5. Frequency
Crop inputs			Km		Hours	
Crop outputs			Km		Hours	
Livestock inputs			Km		Hours	
Livestock outputs			Km		Hours	
Livestock traders						
Crop traders						

(1) Auction
(2) Village market
(3) Shop
(4) other _____

(1) Walking
(2) Public trasp.
(3) Owned trasp.
(4) Other _____

(0) Never;
(1) Daily;
(2) Weekly;
(3) Monthly;
(4) Seasonally
(5) Annually

Credit access: does the household have access to credit? ____ (0) No; (1) Yes. If yes:

1st source: ____

Hh member ____

2nd source: ____

Hh member ____

(1) Formal;
(2) Informal;
(3) Other ____

(1) Hh head; (2) leading female
(3) other ____

(1) Formal; (2) informal;
(3) Other ____

(1) Hh head; (2) leading female
(3) other ____

Saving strategies: Is the household engaged in savings? ____ (0) No; (1) Yes. If yes, how? ____

(1) Banks; (2) livestock; (3) property, land (4) other way ____

LAND & CROPS

Agricultural seasons:

Duration of season	1 st Season	2 nd Season	3 rd Season	4 th Season
First – last month	-	-	-	-

Plots/management units

managed by hh: general information (use with the resources flow map)

ID	Name	1. Size	2. Ownership	3. Current use	5. Productivity	6. Distance from home	Slope	
							7. Level	8. Length
1								
2								
3								

Unit: ____ (1) Owned;
(a) acre (2) Shared;
(h) hectae (3) Rented;
(o) other (4) Other

(1) Idle/fallow;
(2) Crops;
(3) Fodder;
(4) Pasture;
(5) Other ____

(1) Good;
(2) Average;
(3) Low.

Unit: ____ (1) Flat;
(2) Mild;
(3) Steep.

Unit in km: Unit in m:

Use of main crop products: data to be collected in % or absolutes ____ (1) %; (2) absolutes

Crop (List)	Season	Product name	Use product					Total*
			Eaten	Sold/ bartered	Seed	Livestock	Others	
								100%
								100%

* If % percentages are used

Access to information (0) No; (1) Yes

Type of information	1. Family, friends or farmers	2. Government/ extensions	3. Private sector/NGOs	4. Other
For information on new crop varieties?				
For information on crop inputs/outputs price?				
For information on other crop technologies?				

1 st Season	2 nd Season	3 rd Season	4 th Season

Extension: how many times do you meet crop extensionist?

CROP RESIDUE MANAGEMENT

CR allocation: for the year 2013

	Crop 1		Crop 2		Crop 3	
Where CR is allocated:	Name:					
	Season:					
	Technology (only SAsia) man com		Man com		Man com	
In field						
Left in the field (mulch)	%	%	%	%	%	%
Stubble grazing own animals	%	%	%	%	%	%
Stubble grazing by others	%	%	%	%	%	%
Burnt	%	%	%	%	%	%
From field taken for:						
Sold village members	%	%	%	%	%	%
Sold other people	%	%	%	%	%	%
Given as payment in kind	%	%	%	%	%	%
Collected by others for free	%	%	%	%	%	%
Taken home for:						
Stall feeding	%	%	%	%	%	%
Household fuel	%	%	%	%	%	%
Roofing/construction	%	%	%	%	%	%
Selling later	%	%	%	%	%	%
Other:	%	%	%	%	%	%
	100%	100%	100%	100%	100%	100%

CR exchange:

	Name	1 st Season	2 nd season	3 rd season	Unit	Unit in kg
Amount sold CR1						
Amount bought CR1						
Amount sold CR2						
Amount bought CR2						

Access to information: Have you heard about: (0) No; (1) Yes

Type of information	Knowledge and use	If yes, from whom?
Chemical treatment of CR?		
Use of CR for mulching?		
Composting of CR?		
Improved storage methods?		
Chopping/cutting CR?		
Varieties with improved straw quality?		

(0) I haven't heard about it
 (1) I've heard but I never practiced it
 (2) I'm practicing it;
 (3) I practiced it before, but I stop it

(1) Family/friends/farmers
 (2) Government/extensionist;
 (3) Private sector/NGO
 (4) Other _____

Perceptions on crop residues

Statements	Strongly disagree	Disagree	Neither agree or disagree	Agree	Strongly agree	Not applicable
Tillage is needed to obtain a good crop yield	-2	-1	0	1	2	-8
The incorporation of CR improves soil quality	-2	-1	0	1	2	-8
The use of CR as mulch is a waste of feed	-2	-1	0	1	2	-8
CR are a vital feed source for my livestock	-2	-1	0	1	2	-8
Feeding CR to livestock improves the profit of my farm	-2	-1	0	1	2	-8
No CR should be left on field before next tillage	-2	-1	0	1	2	-8
If I leave CR in the soil, I don't need to use fertilisers	-2	-1	0	1	2	-8
<i>Region specific</i>	-2	-1	0	1	2	-8
<i>Region specific</i>	-2	-1	0	1	2	-8

LIVESTOCK

Information access (0) No; (1) Yes

	1. Family, friends or farmers	2. Government/ extension staff	3. Private sector/NGOs	4. Other _____
On new breeds				
On feed requirements of animals				
On animal health				
On other livestock technologies				
On marketing livestock products				

Extension: how many times do meet livestock extensionist?

1 st Season	2 nd Season	3 rd Season	4 th Season

Perceptions on livestock

Statements	Strongly disagree	Disagree	Neither agree or disagree	Agree	Strongly agree	Not applicable
Manure is essential to grow my crops	-2	-1	0	1	2	-8
To keep livestock is not economically profitable	-2	-1	0	1	2	-8
I don't have enough land to grow green fodder	-2	-1	0	1	2	-8
The more livestock, the higher status in my village	-2	-1	0	1	2	-8
Livestock is vital as cash income	-2	-1	0	1	2	-8
Livestock is a vital saving strategy	-2	-1	0	1	2	-8
Feed shortage is a major constraint for my farm	-2	-1	0	1	2	-8
<i>Region specific</i>	-2	-1	0	1	2	-8
<i>Region specific</i>	-2	-1	0	1	2	-8

Livestock structure and dynamics: species fed and taken care of the household. Initially, just list all livestock/breeds kept by household to help with filling the table

	Species/breeds (use codes listed below)											
Structure												
Adult males – castrated												
Adult males – intact												
Adult females – in milk												
Adult females – dry												
Young males												
Young females												
Calve/lamb/kid												
Total kept in household												
Total owned by hh												
Trend 10 years	↑=↓	↑=↓	↑=↓	↑=↓	↑=↓	↑=↓	↑=↓	↑=↓	↑=↓	↑=↓	↑=↓	↑=↓
Trend 1 st reason-code												
Born last year												
Bought last year												
Sold/bartered last year												
Eaten last year												
Given away last year												
Death last year												
Manure animal/day												
Milk female/day (average)												
Species and breeds 1 = Indigenous cattle (Zebu, N'dama etc) 2 = Cross-bred cattle (Ind. x Exotic) 3 = Buffalo 4 = Indigenous goat breed 5 = Cross-bred goat breed 6 = Indigenous sheep breed 7 = Cross-bred sheep 8 = Donkeys 9 = Horse 10 = Poultry 11 = Other 12 = Camel												
Trend main reason 1=More/less grassland 2=More/less feed 3=More/less labour available 4=More/less disease 5=More/less market 6=More/less drought 7=Other												

Feeding strategies: select the main livestock species/breeds (max 3). Please try first with absolute, if it fails switch to %.

	1 st season	2 nd season	3 rd season
1 st livestock specie/breed (use codes of the previous page) _____			
Grazing grass			
Grazing stubbles of _____			
Dry fodder 1 _____			
Dry fodder 2 _____			
Green fodder			
Supplements 1 _____			
Supplements 2 _____			
Total feed intake	100 %	100 %	100 %
Overnight keeping (code below)			
2 nd livestock specie/breed: (use codes of the previous page) _____			
Grazing grass			
Grazing stubbles of _____			
Dry fodder 1 _____			
Dry fodder 2 _____			
Green fodder			
Supplements 1 _____			
Supplements 2 _____			
Total feed intake	100 %	100 %	100 %
Overnight keeping (code below)			
3 rd livestock specie/breed: (use codes of the previous page) _____			
Grazing grass			
Grazing stubbles of _____			
Dry fodder 1 _____			
Dry fodder 2 _____			
Green fodder			
Supplements 1 _____			
Supplements 2 _____			
Total feed intake	100 %	100 %	100 %
Overnight keeping (code below)			

Overnight keeping codes (1) stall; (2) homestead; (3) other on-farm; (4) off-farm; (5) other

Grassland access: percentage of grass and browse intake

	1 st season	2 nd season	3 rd season	Trend 5 yrs	If change, main reason
Open communal land	%	%	%	↑ = ↓	
Communal grass. Reserves	%	%	%	↑ = ↓	
Private land	%	%	%	↑ = ↓	
Along road/rivers	%	%	%	↑ = ↓	
Other _____	%	%	%	↑ = ↓	
Total access	100 %	100 %	100 %	↑ = ↓	

Shortage periods

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Dry fodder												
Green fodder												
Grazing												

(0) No shortage, (1) low shortage, (2) shortage, (3) considerable shortage, (4) extreme shortage

Livestock product allocation of the two main livestock species/breeds

	1. Species code	2. Production	3. Self-consumption	4. Sold	5. Other _____
Milk		l/day	%	%	%
Meat			%	%	%
Milk		l/day	%	%	%
Meat			%	%	%

Manure allocation

	1 st season	2 nd season	3 rd season	Trend 5 yrs	If change, main reason
Fuel	%	%	%	↑ = ↓	
Manure/organic fertiliser	%	%	%	↑ = ↓	
Sold	%	%	%	↑ = ↓	
Other _____	%	%	%	↑ = ↓	
Not used	%	%	%	↑ = ↓	
Total dung	100 %	100 %	100 %	↑ = ↓	

Household income for the year 2012.

Activity	1. Revenue	2. Trend 5 yrs	3. If change, main reason
On-farm			
Crops	%	↑ = ↓	
Crop residue	%	↑ = ↓	
Other feed or forage	%	↑ = ↓	
Livestock	%	↑ = ↓	
Milk	%	↑ = ↓	
Others	%	↑ = ↓	
Off-farm			
Agricultural labour	%	↑ = ↓	
Other non-agric. labour	%	↑ = ↓	
Regular employment	%	↑ = ↓	
Business/self-employed	%	↑ = ↓	
Remittances	%	↑ = ↓	
Others	%	↑ = ↓	
Total revenue 2010	100 %	↑ = ↓	

Expenditure of the household for the year 2012

Item	1. Expenditure	2. Do you use specific sources to cover it (max. two sources)?
Food	%	
Education	%	
Health	%	
Social events/leisure	%	
Personal transport	%	
Housing	%	
Hired labour	%	
Crop inputs	%	
Livestock inputs	%	
Others	%	
Total expenditure 2010	100 %	(0) No (1) Crop & CR sells; (2) livestock sells; (3) off-farm job; (4) remittances; (5) savings (6) other

I thank you for the information and your time well spent. God bless you.

Appendix 3

Survey to document existing technologies and constraints on utilisation of maize stover and brewers spent grain in smallholder dairy farms in Lake Victoria Crescent agro-ecological zone

Dear esteemed respondents, the objective of this survey is to obtain better understanding of the available technologies and constraints on utilization of maize stover and brewers spent grain as feedstuff in smallholder dairy production system. This survey is part of the EAAP project, which seeks to promote and enhance agricultural productivity and profitability in the East African region. The generated data on available technologies and constraints on utilization of maize stover and brewers spent grain will be used in subsequent research study. Therefore, this questionnaire will guide the collection of data in the selected sites. We are kindly requesting you to cooperate with us in availing this very useful information, which will help in developing dairy industry in this region. National Livestock Resources Research Institute (NaLIRRI) Tororo is carrying out the survey. Please note that all the answers given will be treated as confidential and collectively used to promote livestock productivity.

1. General information

1.1 Classifying information	
Date of interview	
District	
Sub-county	
Village	
GPS coordinates of residence	
Household ID	

1.2 Respondent and general household information

Name of respondent (optional)	
Respondent gender	1 = Male, 2 = Female

1.3 Household Composition and age of household head

Age group	Male	Female	Head of household
Less than 5 years			
5- 10 years			
11- 20 years			
21-40 years			
41-59 years			
Over 60 years			

1.4 Highest level of education attained

Primary school level and below	
Secondary school level or equivalent	
Tertiary level education or equivalent	
University education or equivalent	

2.0 Land ownership and tenure

2.1 How many pieces of land do you have? ____

2.2 Size of land (acres)	2.3 Land ownership 1 = Freehold 2 = Rented 3 = Communal 4 = Traditional 5 = Others	2.4 Mode of acquisition 1 = Purchase 2 = Inherited 3 = Government allocation 4 = Rented 5 = Others	2.5 Main land use 1 = Crop production 2 = Livestock 3 = Grazing land 4 = Planted pasture 5 = Homestead 6 = Kitchen garden 7 = Woodlot/forestry 8. Others (Specify)
< 1			
1-4			
5-9			

10-14			
> 15			
Total			

3.0 Labour use and costs

Labour use	Cost
3.1. Type of labour used in livestock production. 1 = Family labour, 2 = Hired, 3 = Both family and hired, 5= Family part time* 4= Others (specify)....	
3.2. If hired, average payment per day/month	
3.3 Gender involvement in the following Dairy activities 1= Males, 2 = Female, 3 = youth, 4 = Children, 5 = Others (specify)	
3.4 Feeding /grazing	
3.5 Milking and milk handling	
3.6 Health management (de-worming, Castration etc)	
3.7 Calf feeding	
3.8 Breeding	
3.9 Milk marketing	

* Part-time means less than 70% of the year working on the farm

4.0 Livestock inventory and ownership

4.1 Type of Livestock	4.2 Number currently owned	4.3 Type 1 = Indigenous 2 = Exotic 3 = Crosses
a) Cattle		
b) Cows		
c) Breeding bulls		
d) Oxen		
e) Heifers		
f) Calves		
g) Sheep		
h) Goats		

Maize stover Management

5.0 Maize stover allocation

	Season 1	Season 2	All the year
Field and post field allocation of maize stover			
In field			
Left in the field (mulch) %			
Stubble grazing own animals (%)			
Stubble grazing by others (%)			
Ploughed in the soil (%)			
Burnt (%)			
From field			
Sold (%)			
Stall feeding (%)			
Given as payment in kind (%)			
Collected by others for free (%)			
Household fuel (%)			
Roofing /construction (%)			
Preservation (%)			
Processing into animal feeds (%)			
Others (%)			

Season 1= January to March; 2= April to June; 3= July to September; 4= October to December

6.0 Processing done on maize stover

6.1 Type of information on processing technologies	6.2. Knowledge and use 1=Have heard about it, 2= Have never heard about it, 3= I am practicing it, 4= I practiced it before but I stopped	6.3 If yes who trained you? 1= Family/friend/farmer 2= Government agent /extension 3= NGO/church/ private sector 4=others (specify) _
Chemical treatment		
Composting		

Improved storage methods		
Chopping/cutting and feeding		
Plant varieties with improved stover quality		
Integration into other feed resources		
Other processing technologies		

7.0 Maize stover utilization as dairy cattle feed

	7.1 Sources in descending order starting with the major source 1 = Own production 2 = Purchased 3 = Other	7.2 Quantity produced (acres)	7.3 Quantity purchased (acres / bundles)	7.4 Buying price per unit (acre/bundle)	7.5 Value addition 1 = Yes 2 = No	7.6 If Yes, type of value addition 1 = Molasses 2 = Urea 3 = Brewers waste 4 = Brewer's yeast 5 = Others	7.7 Average cost (per kg) of value addition material (local currency)
1.							
2.							
3.							
4.							

7.8 Any other information on methods or technology used to conserve/add value to maize stover? --1= yes 2=No

7.9 If yes, how are they done /added value? __ 1= silage 2= drying 3= others specify __

8.0 What are the major challenges of maize stover on utilization as dairy cattle feed?

Challenges	8.1 Rank on a scale of 1-5 1=highest priority, 5 = lowest priority	8.2 What can be done about this constraint
Crop diseases		
Quality of maize stover		
Quantity of maize stover		
Lack of varieties that give good stover		
High costs of maize stover		
Access to knowledge on preservation methods		
Access to machinery		
Drought		
Floods		
Others (specify)		

8. Brewers spent grain management

8.1 Brewers marsh allocation

Allocation of brewers marsh	Season		All the year	
From factory taken for:				
Sale to village members				
Sale to other people				
Given as payment in kind				
Collected by others for free				
Taken home for:				
Stall feeding				
Processed and incorporated in feeds				
Processed and sold				
Other:				

8.2 Access to information on brewers mash processing

Type of information	8.2.1 Knowledge and use 1 = Have heard about it. 2 = Have never heard about it. 3 = I am practicing it. 4 = I practiced but stopped.	8.2.2 If yes from whom? 1 = Family/friend/farmer. 2 = Gov't /extension agent. 3 = NGO/church/private sector. 4 = Others (specify)
Chemical treatment		
Feed directly to livestock		
Processing and incorporation methods		
Mixed with fodder pastures		
Dried and storage methods		
Mixed with maize stover		
Others (specify)		

8.3 Brewers mash utilization

	8.3.1 Sources 1 = Own production 2 = Purchased 3 = Other specify	8.3.2 Quantity utilised month (kg)	8.3.3 Quantity purchased per month (kg)	8.3.4 Buying price per unit (kg)	8.3.5 Value addition before feeding 1 = Yes 2 = No	8.3.7 If Yes, type of value addition material 1 = CR 2 = Urea 3 = Others	8.3.8 Average cost (per kg) of value addition material (local currency)
1.							
2.							
3.							

8.4 Have you heard about any method or methods used to conserve brewer's mash? 1= yes 2=No

8.4.1 If yes, how is it done and perfected? __ 1= silage; 2= Haylage 2= drying; 3= others specify ____

8.5 What are the constraints to brewers mash utilization as dairy cattle feed?

Constraint	Rank on a scale of 1-5 1=highest priority, 5 = lowest priority	What can be done about this constraint
Shortage		
Lack of knowledge to improved its utilization		
High costs of brewers mash		
Poor conservation and storage methods		
Lack of proper methods for treatment		
Access to brewers mash		
Preservation method		
Others (specify)		

8.6 Perceptions and preference of brewers' mash as dairy cattle feed

	Strongly disagree	Disagree	Neither agree or disagree	Agree	Not applicable
Hi tech techniques are required					
The incorporation of maize stover improves its quality					
Brewers mash are important feed resource of a dairy cow					
Feeding brewers mash to livestock improves the productivity and profit					
Brewers mash is region specific					

9. Smallholder dairy production systems and management practices

9.1	Production systems 1 = Zero grazing/cut and carry; 2 = Semi-intensive; 3 = Extensive; 4 = Roadside grazing; 5 = Tethering	
9.2	Type of livestock structures: 1= Traditional; 2 = Improved	
9.3	Livestock housing and structures used 1 = Cattle shed; 2 = Bomas; 3= Calf pens; 4 = Others specify	
9.4	Indicate if these husbandry practices are done: 1 =Yes; 2 = No	
9.4.1	Endo-parasite control (Routine drenching)	
9.4.2	Eco-parasite control (Spraying/dipping)	
9.4.3	Routine vaccination	
9.4.4	Castration and Method of castration 1=open/surgical, 2= closed	
9.4.5	Age at castration (months)	
9.4.6	Debudding / dehorning	
9.4.7	Age at dehorning/ debudding	
9.4.8	Method of identification: 1 = Ear tagging, 2 = Notching, 3= Branding	
9.4.9	Milking methods used: 1 = Hand milking, 2 = Machine milking	
9.4.10	Are your animals registered? 1 = Yes, 2 = No	
9.4.11	Do you keep milk production records? 1 = Yes, 2 = No	
9.4.12	What is average milk production per cow? (Litres)	
9.4.13	Type of services 1 = natural service 2 = AI	
9.4.14	Average age at first service	
9.4.15	Calving interval	
9.4.15	Average weight at birth	
9.4.16	Method of calf rearing 1 = bucket feeding 2 = natural suckling	
9.4.17	Average age at weaning	

10. Marketing of dairy products

10.1. Sale of dairy cattle during the last 12 months

10.1.1 Livestock	10.1.2 Number sold	10.1.3. Where is it sold 1 = Farm gate 2 = local Market 3 = Secondary market 4 = Cross border	10.1.4 Distance to the market (km)	10.1.5 Farm gate price/head (local currency)	10.1.6 Market price/head (local currency)	10.1.7 Purpose for selling
Improved cattle						
Breeding heifers						
Cull cows						
Breeding bulls						
Castrates						
Local cattle						
Breeding heifers						
Cull cows						
Breeding bulls						
Castrates						

10.2. Sale of dairy products during the last 12 months

10.2.1. Product	10.2.2. Qty sold	10.2.3. Units of sale 1 = Kgs 2 = Litres 3 = Bottles 4 = Wheelbarrows 5 = Acres 6 = Others	10.2.4. Where they are sold 1 = Farm gate 2 = Local Market 3 = Secondary market 4 = Cross border	10.2.5. Farm gate price per unit (local currency)	10.2.6. Market price per unit (local currency)
Fresh Milk					
Butter					
Manure					
Grazing land rental					
Hay					
Silage					
Fresh fodder					
Biogas					
Others					

10.3 Access to market

Products	10.3.1 How is marking done? 1 = individual 2 = Group 3 = Others Specify _____	10.3.2 Who sets market price? 1= Farmer 2=Trader 3= Farmer in consultation with the trader 4 = Market 5= Government 6=others specify__	10.3.3 Distance to the market (km)	10.3.4 Access to market 1=Walking 2=Public transport 3=Owned transport 4=Others specify _____	10.3.5. Type of buyers 1= Neighbors 2= Hawkers 3 = Cooperatives 4 = Processors 5 = Others
Fresh Milk					
Butter					
Manure					
Grazing land rental					
Hay					
Silage					
Fresh fodder					
Biogas					
Others					

10.4 Do you have contract arrangements with the buyers? ____ 1 = Yes 2 = No

10.5 If yes, what type of contracts? ____ 1 = Signed contract 2 = Informal/word of mouth, 3 = other specify ____

10.6 What are the major marketing constraints in smallholder dairy production system?

Constraint to dairy products marketing	Rank on a scale of 1-5 1=highest priority, 5 = lowest priority
Low quality products	
Low market prices	
Lack of market	
Lack of market information	
Difficulties in processing	
Difficulties in storage	
Transport to the market	
Unorganized marketing systems	
Difficulties in setting prices	
Seasonal variations	
Others.....	

11. Decision making in the household

11.1. Resources	11.2. Ownership 1=Husband, 2=Wife 3=Children, 4= All	11.3. Decision making on buying selling and use of the items: 1=Husband, 2=Wife, 3=Children, 4=All		
		Buying	Selling	Utilization
Assets				
Land				
Livestock				
Credit				
Farm equipment				
Inputs				
Professional fee				
Seeds				
Fertilizers				
Livestock drugs				
Labour				
Outputs				
Cash crop products				
Food crop products				
Dairy products sales				
Livestock products				

12. Membership to organized group(s) and collective information sharing

12.1 Provide the following information on household involvement in organized group activities

Attribute
12.1.1. Does any member of your family belongs to any organized group? 1= Yes, 2= No
12.1.2. Main objectives/activity of group 1=Crop Production, 2=Processing, 3=Marketing, 4=Dairy production, 5=Dairy goat production, 6= Others (specify)_____
12.1.3. Number of female family members belonging to group(s) _____
12.1.4. Number of male family members belonging to this group(s) _____

13. Access to information and services

13.1 Do you access agricultural production and market information? ____ 1 = Yes, 2 = No

13.2 If yes, please indicate the source, frequency, distance and its perception on effectiveness

13.2.1. Sources 1= Yes 2= No	13.2.2. Frequency of use 1= Never used 2 = Rarely 3 = Regularly	13.2.3. Distance to the information source	13.2.4. Perception on effectiveness 1 = Not effective 2 = Effective 3 = Very effective
a) Other Farmers			
b) Extension officer			
c) Research organizations			
d) Farmer field schools			
e) Agricultural shows			
f) Family and friends			
g) Mass media (Radio/TV)			
h) Traders			
i) Market place			
j) Print materials (Posters, newspapers etc)			

13.3. Do you have access to credit facilities? ____ 1 = Yes, 2 = No

13.4. if Yes, please provide the following details

13.4.1 Source	13.4.2 Have you ever borrowed	13.4.3 Purpose 1 = Purchase of food 2 = Purchase household assets 3 = Payment of fees 4 = Cover medical costs 5 = Buying crop inputs 6 = Buying livestock inputs 7 = Cover educational costs 8 = Other (specify)
a) Relative / friends		
b) Informal savings and credit group		
c) Money lender		
d) Government credit schemes		
e) NGO/Church		
f) Bank or micro-finance institution		
g) Others.....		

14. Income

14.1. Details on the household income sources in the last one-year

14.1.1 Source of income /activity	14.1.2. Do you get income from this source 1 = Yes 2 = No	14.1.3. Who gets it? 1=Husband 2= Wife 3 = Both 4 =Other members	14.1.4. Estimated amount from this source in the last 12 months	14.1.5 What was the trend 1 = up wards 2 = down wards 3 = no change
On-farm				
a) Cash crops				
b) Food crops				
c) Crop residue				
d) Other feed or forage				
e) Livestock				
f) Milk				
g) Others				
Off-farm				
a) Agricultural labour				
b) Other non-agric. Labour				
c) Regular employment				
d) Business/self-employed				
e) Remittances				
f) Others				

15. Expenditure for the year 2012

Item	Expenditure (%)	15.1 Do you use specific sources to cover it? 1 = No; 2 = Crop & CR sells, 3 = livestock sells; 4 = off-farm job 5 = remittances 6= saving 7 = other
a) Food		
b) Education		
c) Health		
d) Social events/leisure		
e) Personal transport		
f) Housing		
g) Hired labour		
h) Crop inputs		
i) Livestock inputs		
j) Others		
Total expenditure 2012		

16. Other technological and production constraints affecting dairy production

16.1 Constraint	1= Yes, 2= No	16.2 Rank on a scale of 5 = lowest priority
a) Low soil fertility		
b) Pests and diseases		
c) Lack of improved forage varieties		
d) Poor access and high cost of inputs		
e) High climate variability		
f) Small land holding		
g) High cost of labour		
h) Inappropriate/poor breeds		
i) Poor access to appropriate knowledge		
j) Lack of professional guidance		
k) Inadequate grazing land		
l) Poor access to markets		
m) Insufficient information		
Inadequate access to financial services		
Poor feed quality		
Others (specify)_____		

Thank you