EFFECTS OF ORANGE (*Citrus sinensis*) PULP-BUFFALO GRASS (*Cenchrus ciliaris*) SILAGE ON DIGESTIBILITY, GROWTH PERFORMANCE AND BLOOD METABOLITES OF WINDSNYER-TYPE AND LARGE WHITE X LANDRACE CROSSED PIGS

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DECLARATION

I, Ramakatana Joseph Glen Kgopong hereby declare that this research dissertation submitted in fulfilment of the requirements for the degree of Masters of Science in Agriculture (Animal Science) at the Department of Animal Science, School of Agriculture, University of Venda by me is of my own work and investigation, and has not previously been submitted for a degree at this or any other university, and in execution and that all reference material contained therein has been duly acknowledged.

Student: ____________________ ___16/04/2019___

Mr. R.J.G. Kgopong Date

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DEDICATION

This dissertation is dedicated to my parents, Miss. Shale Maria Makola and Mr. Nathaniel Kodupo Kgopong, and my beloved brothers Kedibone, Danny and Keletso. I am also dedicating this dissertation to the Lord almighty.

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2. I would like to extend a special thanks to Dr T Nkukwana, who went out of her way to push me to complete my work and supported the project financially when it was in most need. **Dr R Thomas**, who taught me the real meaning of hard work, persistence and consistence. He encouraged me to always look for solutions instead of giving up.

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7. My Life Mottos: Nothing worth having comes easy, stay humble and keep on learning.

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ABSTRACT

The study evaluated the nutritive value of orange pulp- buffalo grass (OPBG) silage as a potential pig feed. In experiment 1, OPBG was ensiled in 58 one-litre jars, split into Renozyme® enzymes (containing α-amylases and β-endo 1, 3; 1, 4 beta glucanase) (denoted OPBGE) and 18 without enzyme (denoted OPBG). Samples were collected from the lab on days 7, 15 and 30 in a three by two factorial design (period by treatment). In Experiment 2, 12 South African Windsnyer-type (SAWIP) (27.2±3.9 kg) and 12 Large White- Landrace crosses (LW x LR) (28±9.8 kg) were fed diets containing different levels of bulk-ensiled OPBG *ad libitum* for 30 days, in a 2 X 3 (breed by level of OPBG (control, low (15% OPBG) and high (30% OPBG))) factorial arrangement. The apparent total tract digestibility (ATTD) of the pigs was measured during week 3 of feeding. The average body gain ABG average daily feed intake (ADFI), average daily gain (ADG), average gain to feed ratio (AG: F) were measured weekly. Serum glucose (GLU) , blood urea nitrogen (BUN), creatinine (CREAT), cholesterol, (CHOL) and triglycerides (TG) were measured during week 4 of the experiment. The levels of CP, EE, NDF, water soluble carbohydrates (WSC), lactic acid (LA) and pH were different ($P<0.05$) for OPBGE and OPBG on day 7. However the OPBGE concentrations of DM, ASH, CP, NDF, HEMI, WSC, LA and pH were higher ($P<0.05$) compared to OPBG. The OPBGE was lower ($P<0.05$) on EE and ADF, compared to OPBG on day 7. The levels of DM, ASH, CP, NDF, ADF, HEMI, LA and WSC were different ($P<0.05$) for OPBGE and OPBG on day 30. However the OPBGE levels of DM, CP, NDF, ADF, LA and WSC were higher ($P<0.05$) compared to OPBG. ASH, EE and HEMI were lower ($P<0.05$) on day 30. There were significant diets x day interactions ($P<0.05$) for DM, ASH, CP, EE, NDF, ADF, HEMI, WSC, and LA, but not for pH. The aerobic stability study was inconclusive. The digestibility levels of OM and NDF were different ($P<0.05$) for LW x LR and SAWIP, where LW x LR ATTD on OM and NDF was higher ($P<0.05$) compared to SAWIP for all diets. The LW x LR ADG was higher ($P<0.05$) compared to SAWIP for all diets. There was a diet x breed interaction effect ($P <0.05$) on ADG whereby the BUN and CREAT were different ($P <0.05$) for LW x LR and SAWIP. The SAWIP BUN was higher ($P<0.05$), but the CREAT and TG were lower ($P<0.05$) compared to LW x LR for all diets fed. In conclusion, OPBG inclusion in pig feed enhanced the quality of feed, digestibility, performance and blood metabolites profile.

Key words: ensiling, exogenous enzymes, palatability, fermentation, fibre, serum metabolites, performance.

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LIST OF ABBREVIATIONS

ARC	:Agricultural Research Council
ADF	:Acid detergent fibre
AOAC	:Association of Official Analytical Chemists
BUN	:Blood Urea Nitrogen
CP	:Crude Protein
CON	:Control
OPBG	:Orange pulp- Buffalo grass
OPBGE	:Orange pulp- Buffalo grass-Enzymes
DM	:Dry Matter
DF	: Dietary fibre
EE	:Ether extract
FCR	:Feed conversion ratio
GIT	:Gastrointestinal tract
30% OPBG	:High silage inclusion
ICP-OES	:Inductively Coupled Plasma- Optical Emission Spectroscopy
15% OPBG	:Low silage inclusion
LAB	:Lactic Acid Bacteria
MRT	:Mean retention time
NSP	:Non-starch polysaccharides
NFE	:Nitrogen free extracts
NDF	:Neutral Detergent Fibre
NSC	:Non Structural Carbohydrates
OM	:Organic matter
SCFA	:Short chain fatty acids
SAWIP	:South African Windsnyer-type Indigenous pigs
TTAD	:Total tract apparent digestibility
WSC	:Water Soluble Carbohydrates

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CHAPTER 1: INTRODUCTION

1.1. Background

Pig production has a high potential to contribute to the economy, both at the local and national levels. The economic advantage of pig farming is in their high fecundity, high feed conversion ratio, early maturing growth pattern, short generation interval, and relatively small space requirements (Lekule and Kyvsgaard, 2003). Pigs provide 44 % of meat in the world market (FAO, 2010). Declining global grain production compounded by increasing demand for human food are increasing the cost of commercial stock feeds, which is a challenge for rural based farmers (Zijlstra and Beltranena, 2013; Woyengo *et al*., 2014). To lower feed costs, research has increasingly focussed on cheaper, unconventional, typically fibrous feed ingredients (Babayemi, 2008; Ayoade *et al*., 2000; Ani and Omeje, 2007; Adeyemi *et al.,* 2009). Fibrous feed ingredients are in abundance and cheap and therefore ideal for farmers in the rural areas of South Africa (Dogari, 1984). In addition to the cheap fermentable energy, increased fermentation improves gut health in non-ruminant animals (Babatunde *et al*., 1975; Isikwenu *et al*., 2000).

Silage making is a dynamic process whereby carbohydrates are transformed by microbes into lactic acid, organic acids and alcohol under an anaerobic environment (Prescott *et al.,* 1996). Exclusion of oxygen to prevent aerobic metabolism is critical during ensiling (McDonald *et al.,* 2011). Tropical grasses are difficult to ensile due to low water-soluble carbohydrate content and due to a high buffering capacity (McDonald *et al.,* 2011). Therefore, steps must be taken to ensure satisfactory ensilage. Options include wilting of very wet grass, the use of acid or inoculant additives, mixing of different grasses and adding molasses or orange residues to ensilage to provide a source of water soluble carbohydrates in order to enhance fermentation (McDonald *et al*., 2011).

Studies have indicated that grasses may have nutritional properties that should allow them to be used in diets for pigs when processed into silage (McDonald *et al*., 2011). This applies to temperate (Lindberg and Andersson, 1998; Reverter *et al*., 1999) as well as to tropical grasses (Phuc *et al*., 2001). Phuc and Lindberg (2000) showed that fermenting forage could be used to improve the dietary protein, carbohydrates and amino acid supply to growing pigs under tropical conditions. It has also been shown that fermented fibrous diets may improve growth performance (Scholten *et al*., 1999) and health (van Winsen *et al*., 2001) of pigs in comparison with non-fermented diets. Pedersen and Lindberg (2003) observed an improve-

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ment in the *in vitro* digestibility of organic matter (OM) and crude protein (CP) due to fermentation. Tropical grass contains low levels of WSC and produce poorer silage due to low pH (Catchpoole and Henzell, 1971; Sujatha *et al.,* 1986; Imura *et al.,* 2001), however orange pulp contains high level of WSC, that is why addition of orange pulp is important, therefore improving the quality of silage (Bureenok *et al.,* 2006).

This study evaluated the effects of orange pulp-buffalo grass silage quality on aerobic stability, nutrient digestibility, growth performance and blood metabolites of South African Windsnyer-type and Large White- Landrace crossed pigs to promote the use of alternative feed ingredients by rural based, resource poor farmers.

1.2. Problem statement

Declining global grain production compounded by increasing demand for human food is increasing the cost of commercial stock feeds. In resource-poor farms, there is a shortage of suitable home grown feedstuffs with an acceptable protein content and quality for the growing or weaned pigs. This is mainly due to the fact that commercial feeds are too expensive. Therefore, there is a need to provide cheaper, but quality feeds which the orange pulp and buffalo grass silage could potentially offer. One way to improve the current situation is to introduce high yielding fodder varieties like the orange pulp and buffalo grass silage. Surplus forage can be ensiled to improve its quality and to preserve it for periods of scarcity.

1.3. Justification

The orange pulp and buffalo grass are readily available and affordable and this silage mixture will limits the competition between human and animals for food. Furthermore ensiled orange pulp and buffalo grass will enhance the intestinal health of the animals. The use of enzymes in diets contributes to improving the silage quality. The results from the research will strengthen farmers' knowledge on the utilization of abundant raw materials, which are cost effective and also the effect of fermented, unconventional pig feeds on nutrient digestibility, growth performance and blood metabolites. The nutritional information will help nutritionists and farmers to formulate low cost diets that are suitable for the indigenous pigs utilising available and affordable feed sources. This will increase the contribution of pork to the gross value of agricultural production significantly.

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1.4. Objectives of the study

1.4.1. Broad objective

The broad objective of the study was to evaluate the nutritive value of buffalo grass-orange pulp silage as an alternative feed to partially replace the expensive conventional ingredients in indigenous and exotic growing pig diets which are increasingly beyond the reach of resource poor farmers.

1.4.2. Specific objectives

The specific objectives of the study were to determine effects of:

a) exogenous enzymes on the quality and aerobic stability of mixed orange pulp-buffalo grass silage.

b) partial substitution of commercial ingredients with orange pulp-buffalo grass silage for the standard growing pig diet on the following in South African Windsnyer-type and Large White- Landrace crossed growing pigs:

    i. Feed intake and nutrient digestibility.
    ii. Growth performance
    iii. Blood glucose (GLU), triglycerides (TG), total cholesterol (CHOL), creatinine (CREAT) and urea nitrogen (BUN)

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1.5. Hypotheses

The null hypotheses were that:

1. Adding exogenous enzymes to the mixed orange pulp-buffalo grass will not improve the quality and aerobic stability of the silage.
2. Adding orange pulp-buffalo grass silage to the standard growing pig diet will not affect feed intake and nutrient digestibility in South African Windsnyer-type and Large White-Landrace crossed pigs.
3. Adding orange pulp-buffalo grass silage to the standard growing pig diet will not affect growth performance in South African Windsnyer-type and Large White- Landrace crossed pigs.
4. Feeding orange pulp-buffalo grass silage will not alter the profiles of blood metabolites that signify changes in overall energy, protein and N metabolism in South African Windsnyer-type and Large White- Landrace crossed pigs.

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CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Pig production plays a critical role in the provision of adequate protein for humans. Pig production is also the main source of cash income for both resource-poor farmers in rural areas and commercial farmers. The exotic pigs are usually kept by commercial farmers under intensive management, as opposed to indigenous pigs which are kept under extensive management. The indigenous pigs therefore remain largely underutilized in the mainstream economies of Southern Africa. The contributory factors include traditional biases in meat and carcass grading systems, prejudice against local pigs, lack of markets and market penetration, and relatively little research that aims at improving the indigenous pigs (Halimani *et al*., 2010). Research on feed utilisation by indigenous pig production systems is limited and confined to samples within specific districts (Mashatise *et al*., 2005; Chikwanha *et al*., 2008; Halimani *et al*., 2008).

Indigenous pigs are known to be adaptable, tolerant to diseases, drought and parasites that are endemic in their areas of production (Lekule and Kyvsgaard, 2003; Zanga *et al*., 2003; Halimani *et al*., 2010). Furthermore, they are better able to utilize fibrous feeds compared to exotic pigs (Kanengoni *et al*., 2002; 2004). The pigs are also more suited for outdoor production in hot environments due to a higher thermotolerance (Styger, 2002; Wilson, 2009). The exact amount of fibre each breed can tolerate or efficiently utilise is unknown; hence there is a need for further fibre utilization studies in pig nutrition.

2.2. The eco-physiology of buffalo grass

Buffalo grass is a tufted tussock-forming perennial grass (Ecoport, 2010; FAO, 2010; Cook *et al.,* 2005). It has a deep, tough rootstock that may go as deep as 2 m. Some varieties are rhizomatous (Ecoport, 2010). The culms are erect or decumbent, reaching up to 2 m in length (Cook *et al.,* 2005). The leaves are linear blades, green to bluish green, slightly 3-30 cm long and 4-10 mm wide (Ecoport, 2010; Clayton *et al.,* 2006). The inflorescence is a spike-like panicle, bearing deciduous spikelets which are surrounded by hairy bristles (SANBI, 2010). The seed is an ovoid caryopsis, 1.4-2 mm long (Ecoport, 2010; Mannetje *et al.,* 1992).

Buffalo grass is commonly found in open bush, woodland and grassland (Ecocrop, 2010). It thrives in arid and semi-arid denuded environments, on light, sandy, rocky and shallow soils as well as in dry calcareous areas. It can be found along roadsides or dry river beds

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and river banks (SANBI, 2010; Quattrocchi, 2006). Buffalo grass grows from 33 °S to 37 °N from sea level up to 2000 m altitude (FAO, 2010; Cook *et al.,* 2005). Optimal growth conditions are an annual rainfall ranging from 375 to 750 mm, with day temperatures ranging from 30 °C to 35 °C, on light, fertile, well-drained soils, and a soil pH between 7-8 (Ecoport, 2010; FAO, 2010).

Buffalo grass is classified as the most drought tolerant of the commonly sown grasses in arid areas and can be found in environments with annual rainfall as low as 100 mm. In such places, it gives the best results under irrigation, if it is available, since it has very high water use efficiency (Osman *et al.,* 2008). It has a moderate tolerance to salinity but is very sensitive to high aluminium and/or manganese levels (Mannetje *et al.,* 1992). Some cultivars have a higher tolerance to salinity (FAO, 2010; Cook *et al.,* 2005). Buffalo grass is tolerant of fire (FAO, 2010; Cook *et al.,* 2005). It does not withstand waterlogging and is killed after 6 days under water (Ecoport, 2010).

2.3. Productivity, nutritive value and utilization of buffalo grass

Buffalo grass is one of the important pasture grasses in the tropics (Ecocrop, 2010) It is cultivated for permanent pastures and leys in Central Africa, East Africa, India and northern Australia where it is widely used as a forage grass (Duke, 1983). It is easy to establish and provides comparatively high value forage, with yields between 2-18 t DM/ha, without fertilizer, and up to 24 t/ha with the addition of a complete fertilizer (Osman *et al.,* 2008; Ecocrop, 2010). Buffalo grass makes reasonable quality hay when cut in the early flowering stage, yielding up to 2.5 t/ha per cut (Osman *et al.,* 2008; Ecocrop, 2010). Buffalo grass is palatable to livestock and, once established it can withstand heavy cutting, grazing and trampling (FAO, 2010; SANBI, 2010). In arid areas, it maintains monogastric and ruminant species during drought periods (Friedel *et al.,* 2006). Some strains also grow well during the wet season (Göhl, 1982). The grass is a valuable stand-over feed for winter grazing if supplemented with urea and molasses (FAO, 2010).

The digestibility of fresh buffalo grass fed to monogastric and ruminant species after 30 to 50 days of regrowth was in the 60-69 % range (Combellas *et al.,* 1972). *In vitro* organic matter (OM) digestibility of standing hay ranged from 53 to 64 % (Jacobs *et al.,* 2004). The digestibility of buffalo grass hay fed to livestock at 56 days of regrowth was in the 49-69 % range depending on the amount of nitrogen (N) fertilizer applied to the crop (0 to 100 kg/ha) (Donaldson *et al.,* 1977). It was found that the nutrient content and digestion parameters of buffalo grass varied among seasons; nutrient digestion was higher during summer and autumn and lower during spring and winter (Ramirez *et al.,* 2001). While live-weight gain was related to digestibility, selecting buffalo grass for higher digestibility would not necessarily identify the highest quality genotypes in terms of animal production (Minson *et al.,* 1995).

2.4. Improving the value of buffalo grass by ensiling

2.4.1. Grass silage

Ensiling of forage materials is the process of preserving forage, based on spontaneous lactic acid fermentation, under anaerobic conditions (Oude Elferink *et al.,* 2000). Silage is produced by controlled fermentation of fresh forage under anaerobic condition (Obua *et al.,* 2005). Aganga *et al. (*2005) suggested that under wilting conditions, additives may improve fermentation and the nutritive value of the grass silages. Generally, tropical forages are low in water-soluble carbohydrates (WSC), which are important for ensiling. Additions of exogenous WSC to temperate forages increased lactic acid production (McDonald, 1981) by microbial fermentation resulting in production of quality silage.

The silage could supply quality roughage during late winter and early spring periods when the pasture is low in nutritive value, but high in dry matter (DM) concentration (Rethman, 1983; Tainton, 2000). Silage has several advantages over hay in terms of the conservation of fodder. It is routinely used as a feed supplement to increase productivity of animals, and to utilize excess growth of pasture for better management and extended utilization. Silage quality is maintained far longer than hay quality, because hay quality deteriorates during storage, thus silage is better as a fodder bank than hay. It does, however, require a good microbial fermentation process to produce good quality silage. Tropical grass species produce poorer silage due to low pH as well as high acetic acid and ammonia nitrogen (NH_3-N) concentrations (Catchpoole and Henzell, 1971; Sujatha *et al.,* 1986; Imura *et al.,* 2001), that is why addition of WSC is important thus improving the quality of silage.

In silage making, lack of oxygen and the accumulation of lactic acid inhibit its microbial metabolism and preserves nutrients (Ranjit and Kung, 2000). Successful silage fermentation depends on achieving both anaerobic conditions and a low pH. The low pH is usually accomplished through the fermentation of sugars in the grass or crop to lactic acid by lactic acid bacteria (LAB), which decreases plant enzyme activity and prevents proliferation of det-

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rimental anaerobic microorganisms, especially clostridia and enterobacteria (Yang *et al*., 2004).

During ensiling there is a settling process that results from the death and collapse of plant cells, which occurs within initial periods of ensiling in air-tight laboratory silos (Greenhill, 1964 a, b, c). Greenhill (1964 a, b, c) reported that cell breakdown and release of intra-cellular plant juice are prerequisites for the initiation of lactic acid fermentation, and the complete exclusion of fresh air from the silage mass can usually be expected to result in cell breakdown and juice release (McDonald *et al.,* 2011). It has been shown that fermented diets may improve growth performance (Scholten *et al*., 1999) and health (van Winsen *et al*., 2001) in comparison with non-fermented diets.

In the tropical regions, conservation of forage is difficult owing to the short rainy season and high temperatures. Therefore, hay making is difficult and ensilage is often the only option. The silages made from tropical herbages are usually less dense and presumably more permeable, and relativity large quantities of air may be trapped in the forage mass than its temperate counterparts just after ensiling (Alli *et al*., 1985). Tropical pasture forages have often failed to settle during ensiling (Catchpoole and Henzell, 1971).

Buffalo grass is rarely made into silage, as the moisture content of the grass in semi-arid areas is usually low (Göhl, 1982). It also has low concentrations of fermentable carbohydrates, and needs additives to improve the quality of the silage (Iqbal *et al*., 2005). Molasses or orange by-products can potentially enrich the fresh material with carbohydrates and fills the gaseous pores, thereby reducing the influx of oxygen in the silage. Using molasses or corn as additive can increase the amount of fermentation end products due to fermentation of the available sugars (Yakota *et al*., 1992). Addition of fermented juice of epiphytic LAB to buffalo grass improved the quality of the silage (Bureenok *et al.,* 2006).

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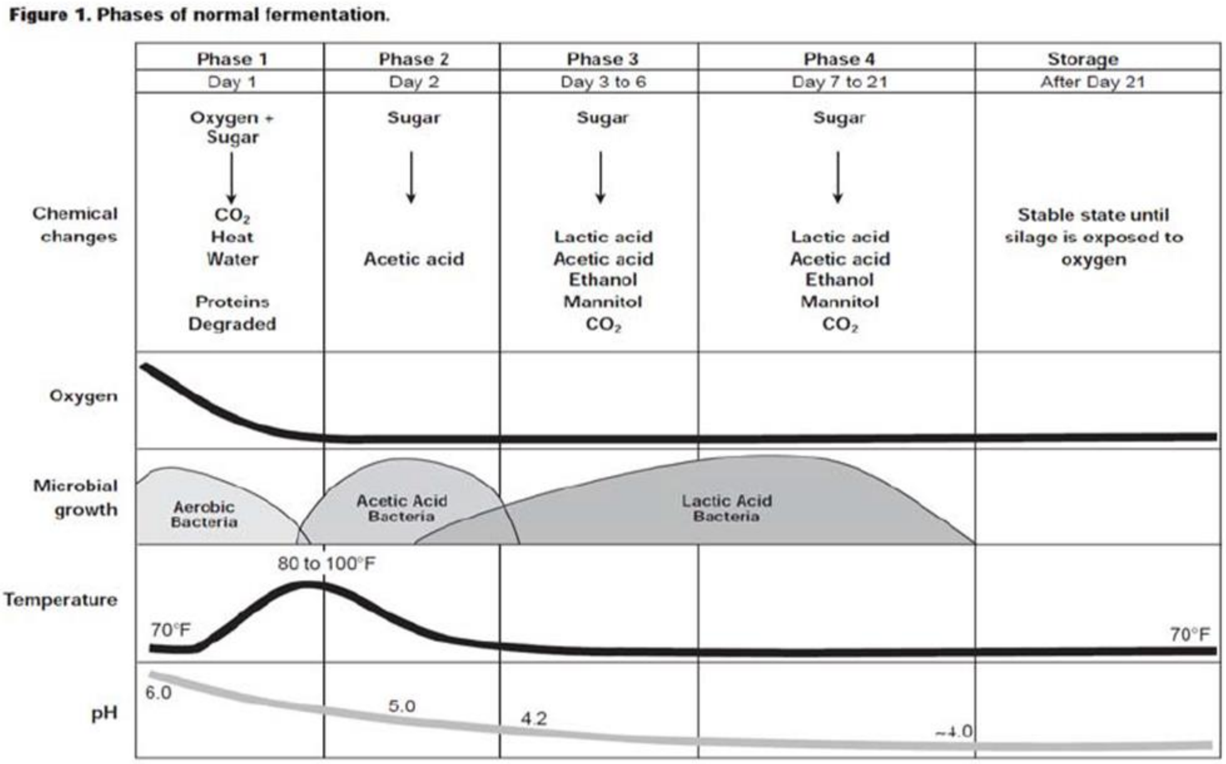

Figure 2. 1: Diagrams of normal fermentation phases (adapted from Ishler *et al*., 1991)

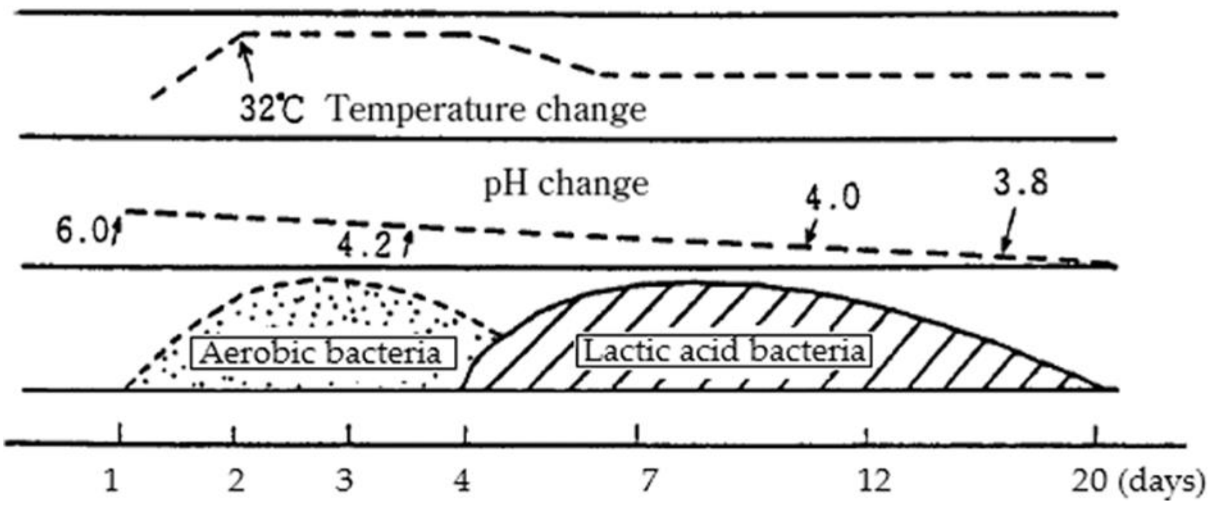

Figure 2. 2: Diagrams of indicating effect of days of ensiling (adapted from Ishler *et al*., 1991)

2.4.2. Orange pulp silage

Orange pulp is a by-product from Orange trees (*sclerocarya birrea*), which are native to Eastern, Southern and Central Africa. Orange pulp contains substantial amounts of nutrients and is an excellent animal feed if further processed to a suitable and easily handled form (Boucque and Fiems, 1988). Orange pulp ensiled without additives gives better results in energy conversion efficiency measured by production of volatile fatty acids (Itavo *et al*., 2000b). Orange pulp is available for ensilage all year-round and also for feeding purposes (Fuller, 2004; Göhl, 1978). However, it is dependent on seasons and geographical locations. The silage has a pleasant odour and is readily eaten by pigs (Fuller, 2004; Göhl, 1978). The ensilage process requires a period of less than 50 days (Fuller, 2004). According to Göhl (1978), it was discovered that before ensiling, the pulp can be pressed or mixed with grass or hay in order to obtain firm silage and also to serve as an absorbent for the wet orange pulp.

2.4.3. Mixed grass-pulp silage

The orange pulp is rich in WSC (Fuller, 2004; Göhl, 1978), however tropical forages (buffalo grass) are low in WSC, which are important for ensiling (Catchpoole and Henzell, 1971). Therefore, additions of orange pulp WSC to temperate forages increased lactic acid production (McDonald, 1981) through microbial fermentation resulting in production of quality silage mixture. Lactic acid bacteria is essential for fermenting the WSC to organic acid mainly lactic acid, which reduces the pH value, thus producing a well-preserved silages with a pH range of 3.8 to 4.2 as reported by McDonald *et al*. (2002). Tropical grass species produce poorer silage due to low pH, as well as high acetic acid and ammonia nitrogen (NH_3-N) concentrations (Catchpoole and Henzell, 1971; Sujatha *et al.,* 1986; Imura *et al.,* 2001) hence the addition of orange pulp with high WSC is important for quality silage mixture.

Tropical pasture forages have often failed to settle during ensiling (Catchpoole and Henzell, 1971). In the tropical regions, conservation of forage is difficult owing to the short rainy season and high temperatures. Therefore, hay making is difficult and ensilage is often the only option. Orange pulp ensiled without additives gives better results in energy conversion efficiency (Itavo *et al*., 2000b). Orange pulp is available for ensilage all year-round and also for feeding purposes (Fuller, 2004; Göhl, 1978).

Ensiling fresh orange pulp with partially dried grass or with legumes that cannot be successfully ensiled on their own is advantageous, since it provides organic acids (particularly malic

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and oxalic acids) and enhances fermentation quality. Furthermore, orange pulp enhances overall silage quantity and quality (i.e. more sugars, more acidic bacteria, lower pH) and reduces the need for acid additives (Crawshaw, 2004). Orange pulp can also absorb moisture from feeds such as brewers' grain and is thus used to reduce effluent risk and nutrient loss from these materials (Crawshaw, 2004; Fuller, 2004), therefore the mixture is very important in the production of good quality silage.

2.4.4. Effects of enzymes on grass-pulp silage quality

The use of additives is common practice implemented to improve the quality of silage by enhancing the fermentative characteristics and nutrients composition of the silage. Renozyme® enzymes are added to silage to partially degrade fibre to fermentable WSC for use by LAB, which cannot use fibre as an energy source (Eun and Beauchemin, 2007). However, exogenous enzyme activities against structural carbohydrates may be inhibited in the presence of LAB (Stokes, 1992; Xing *et al*., 2009). The LAB is essential for fermenting the water-soluble carbohydrates to organic acid mainly lactic acid, which reduces the pH levels. Furthermore well-preserved silages had a pH range of 3.8 to 4.2 as reported by (McDonald *et al*. 2002).

The use of silage additives could help improve the worth of silage as a pig feed ingredient, in standard formulated pig diets. Orange pulps contain substantial amounts of WSC, while buffalo grass contains low level of WSC, which could benefit the ensiling process and revitalize the nutritive value by increasing energy, protein and minerals (Khan *et al*., 2006; Bautista-Trujillo *et al*., 2009; Repetto *et al*., 2011). Furthermore, the addition of Renozyme® enzymes to forage at ensiling has been reported to degrade silage cell wall and increase the availability of WSC that serve as substrate for LAB (McDonald *et al*., 1991; Spoelstra *et al*., 1992; Selmer-Olsen *et al*., 1993; Sheperd and Kung, 1996). Therefore, when LAB is combined with enzymes during ensiling, a stronger effect should be expected because more fermentable sugars are released to produce more lactic acid in comparison with other fermentation products (Kung *et al*., 1991, Chen *et al*., 1994), thus improving the silage quality. In contrast, Stokes (1992) reported reduced enzyme activity in the presence of an LAB inoculant.

Good quality silage can often be made without additive treatment, but LAB present in growing crops can be extremely small (McDonald *et al*. 2002), therefore silage additives can be beneficial in such circumstances (Lundeen, 2002, Weinberg and Munk, 1996). The quality of silage is one of the parameters that will determine successful silage use and inclusion of ad-

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ditives (Shinoda *et al*., 1999). Additives have been found to improve the fermentation quality of grass silage, however if additives are to be used, they need to be selected carefully and accordingly, and applied properly (Weinberg and Munk, 1996). The main purpose of using an additive is to maximize profit from the investment, however it should be decided if the technical or biological benefits that may result from the use of an additive will result in an economic return. Furthermore this should be an additive that is user friendly to the environment and affordable to rural based resource poor farmers.

2.4.5. The aerobic stability of grass-pulp silage

Aerobic stability is a commonly utilised term to define the length of time that silage does not spoil after it is exposed to air. Furthermore the stability of the silage in the presence of oxygen is essential in determining its value and quality. Consequently this is the common challenge facing a lot of high quality silages that start to deteriorate immediately when exposed to air. The deterioration of aerobic stability is through the activity of aerobic micro-organisms mainly yeasts and moulds (Talkington *et al.,* 1981, Woolford, 1990). The aerobic deterioration of silage is characterised by an increase in temperature and pH which is caused by the metabolism of sugar and organic acids by yeast and bacteria (Lundeen, 2002, Oude Elferink *et al.,* 1999).

The primary determinants that have an effect on aerobic stability are air, substrate availability (McDonald *et al*. 2002), temperature (Ashbell *et al.,* 2002) and the availability of acetic acid bacteria. The most common factor that may have a role in aerobic stability of silage is the sealing quality (Kipnis *et al.,* 2001), where a delayed sealing after filling the silos reduced stability (Lundeen, 2002, Mills and Kung, 2002) of the silage. Ashbell *et al.* (2002) reported that the time period of exposure to air has a significant effect on aerobic stability since CO_2 production increases with time.

The period in which silage remains stable upon exposure to air, is essential for the initiate of pH change measurements (Mills and Pitt, 1991), it is also highly valuable among silages (Spoelstra *et al.,* 1988), as it can maintain a range from few hours to weeks (McDonald *et al*. 2002), and also depends on the chemical and microbiological composition of the silage (Ashbell *et al.,* 2002). The stability varies based on the type of raw materials used when making the silage and the type of silage produced thereafter. Mills and Kung, (2002) reported a decrease in WSC concentration, due to prolonged exposure of silage to air, whereas

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ammonia and pH of the silages increased respectively. It is therefore uneconomical to store the silage for more than two days after removal from the silo since its quality will deteriorate.

The most important chemical composition component of a silage is pH, as it can assist in predicting the rate of spoilage. Good quality silage with pH of below 4, tends to be unstable in the presence of air (Woolford, 1990). This is because an effective and rapid reduction to a low pH will restrict the growth of organisms that produce short chains fatty acids (especially butyric acid produced by *clostridia spp)*. Poor quality silage, with a large amount of short chain fatty acids (SCFA) was stable in air whereas good, high quality silage was not (Woolford, 1990).

2.5. Production characteristics of Large white x Landrace cross and South African Windsnyer Indigenous Pigs

2.5.1. Large White x Landrace crossed pigs

Large White x Landrace (LW x LR) pigs are large-framed, having white, pinkish skin colour, which is free from black hair. They are also characterised by moderately long and slightly dished face, and the ears are pricked. The LW x LR crosses are the most popular exotic breed in South Africa (Agricultural Research Council, 2013) due to their fast growth rate potential, and excellent feed conversion efficiency (Mushandu *et al.*, 2005). In addition, LW x LR crosses has been valued for producing good quality pork and bacon products.

This breed can be used for commercial production, communal pig production and is commonly used in crossbreeding programs (Taylor and Roese, 2005). Pig breeding programs produce pork for the market that meets consumers’ requirements of low amounts of fat and high levels of lean meat content. In general, pork quality of the imported pig genotypes like the LW x LR crosses, is superior when compared with local genotypes. The LW x LR crosses have been reported to possess superior pork quality measures such as colour, water-holding capacity, pH, and drip loss (Kanengoni *et al.*, 2004); and these are characterised by greater content of intramuscular fat compared to slower growing pigs, thus better tenderness and flavour (Zak *et al.*, 2009).

2.5.2. South African Windsnyer indigenous pigs (SAWIP)

South African Windsnyer indigenous pigs (SAWIP) are small in size, have long noses and have many variations of coat colour, with black and brown being the most common (Halimani *et al.*, 2010). They have a large head, the hams are fairly well developed with sturdy legs

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and strong feet (Holness, 1991), with very short, pricked ears and a squashed face (Crafter and Morton, 2010). The South African Windsnyer indigenous pigs are early maturing, but grow slower than the exotic breeds (Madzimure, 2011). These pigs are tolerant to warm climates, and are often found near rural villages where they are reared as free range or in the backyards. In communal production systems of South Africa, Windsnyer pigs are owned as local genotypes by the community (Halimani *et al.*, 2010). In these areas, indigenous pigs have a potential to increase food security, reduce poverty and improve the livelihood of resource limited farmers (Madzimure, 2011). In addition, they are viewed as a suitable breed for resource limited farmers because of their tolerance to various diseases and adaptability to adverse conditions. These pigs survive under conditions with inefficient breeding management, insufficient veterinary care, inadequate feeds and feeding management (Chimonyo *et al.*, 2005). Halimani *et al.* (2010) reported that all local pigs, the most popular being Mukota and the Windsnyer pig are essentially one genotype based on molecular genetic characterisation.

Local genotype pigs can survive on cheaper high-fibre diets and can convert feed with a low nutrient content very efficiently (Ndindana *et al.*, 2002). Kolbroek pigs produce tasty pork, and have an excellent foraging ability (Chimonyo *et al.*, 2005), which is not highly different from the SAWIP. These are traits of economic importance that make them favorites with communal farmers, and determine the potential profit for the farmers. When compared to imported breeds, local pigs such as the SAWIP fail to meet or achieve good grades for the commercial pork market owing to the grading scheme used (Kanengoni *et al*., 2004). Thus, these pigs tend not to be considered when using pork quality as criteria because of their short carcasses, which cannot be simply prepared into specialized pork portions (Chimonyo *et al*., 2010).

2.5.3. Comparative production characteristics of indigenous and exotic pig breeds

A study conducted by Els (2002) indicates that indigenous pigs are highly fertile under conditions of improved management and nutrition. Indigenous (local) pig breeds also appear to adapt to difficult or specific environments and handling conditions (Campo *et al*., 1999), which may influence the meat quality. However, indigenous pig breeds appear to have substantially more fat than exotic pig breeds (Nicolas, 1999). There is a need to determine the possibility of using South African indigenous pig breeds for the production of pork for fresh consumption.

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The Windsnyer is very hardy and is a good scavenger. It can convert food with a low nutrient content more efficiently compared to exotic Landrace-Large White crossed breeds (South African Indigenous Breeds, 2009). The Windsnyer is also able to survive periods of food shortage, while Landrace-Large White crosses thrive under intensive management. The differences in responses to high fibre diets are due to genetic and physiological determinants of feed intake (Frank *et al.,* 1983).

Differences in total digestibility were observed between indigenous breeds (Ndindana *et al*., 2002; Len *et al*., 2009). This was despite the fact that both the breeds are fed by-products containing high fibre (Ndindana *et al*., 2002; Len *et al*., 2009). Effects of genotype of indigenous pig breeds on the digestibility of nutrients in high fibre diets have also been reported (Len *et al*., 2009a; Len *et al*., 2009b; Borin *et al*., 2005; Ndindana *et al.,* 2002; Fevrier *et al*., 1992). Similarly, Freire *et al*. (2003) found that the native Alentejano indigenous breed in Spain had a higher digestibility of fibrous diets than an improved breed (Duroc x Landrace). However, Ly *et al*. (1998) found that Creole indigenous pigs in Cuba did not have better digestibility of high fibre-diets than improved pigs.

The genotype effect on nutrient digestibility is attributed to the fact that indigenous pigs have higher digestive capacity and higher microbial activity in the hindgut than improved pigs (Freire *et al*., 2000; Jørgensen *et al*., 1996). They have been observed to have undergone anatomical changes which make them better utilizers of fibre (Len *et al*., 2009a; Len *et al*., 2009b; Freire *et al*., 2003; Freire *et al*., 2000). The indigenous Mukota pig was observed to have proportionally longer small and large intestines compared to the exotic Large White pigs (Dzikiti and Marowa, 1997 cited from Ndindana *et al.,* 2002). The extent of the negative effect of fibre level on digestibility is dependent on the breed and the growth stage of the pigs being fed the fibre diets (Noblet and Knudsen, 1991; Anderson and Lindberg, 1997; Laswai *et al.,* 1997; Kanengoni *et al.,* 2002; Len *et al.,* 2009). Len *et al.* (2009) when comparing the indigenous Vietnamese Mong Cai piglets to the Landrace X Yorkshire crosses showed that there was no difference in ileal digestibility between the indigenous Mong Cai and Landrace X Yorkshire crosses. The fibre utilisation be it low or high is dependent on the gastrointestinal tract (GIT) of the breed, the quality of the feed and the hardiness of the breed.

In the monogastric GIT, solubility and viscosity of fibre have profound effects on nutrient digestion, absorption and digesta transit (Guillon and Champ, 2000). The longer large intestine of indigenous pigs could increase the flow of digesta and result in a higher fermentative capacity and therefore a better utilization of diets containing high fibre levels. Differences in digestive ability between pig breeds are also explained due to digesta transit time in the gut (Len *et al*., 2009a; Len *et al*., 2009b; Borin *et al.,* 2005; Ndindana *et al.,* 2002; Fevrier *et al.,* 1992). Pigs with heavier, longer and larger GIT (relative to their BW) usually have longer retention time of digesta in the GIT (Guixin *et al*., 1995). This should contribute to more efficient digestion due to longer contact between digesta, digestive enzymes and absorptive surfaces.

Feeding fibrous diets results in a number of advantages, such as improved well-being of animals, improvement of gut transit time and reduction of stomach ulcers (Low, 1993). However, when included in monogastric diets their high fibre content results in decreased diet digestibility (Nongyao *et al*., 1991; Wang *et al*., 2006) and dilution of dietary nutrients (Schulze *et al*., 1994; Noblet and Le Goff, 2001). Several reports have indicated that indigenous pig breeds can utilize fibre better than exotic breeds (Fevrier *et al*., 1992; Kanengoni *et al*., 2002; Ndindana *et al*., 2002). Furthermore indigenous breeds are considered to be very tolerant when fed diets that are very high in fibre, and of poor quality diets (Rodriguez and Preston, 1996) and are able to digest the fibrous components better than improved breeds (Borin *et al*., 2005) as opposed to exotic pig breeds.

High fibre increases the peristaltic movement in the digestive tract, reduces the flow of digesta (Hansen *et al.,* 1992) and therefore limits the time for microbial digestion and the ability of dietary components to be absorbed. Jørgensen *et al.* (1996) reported a five to six fold increase in the flow of digesta through the terminal ileum of pigs fed high dietary fibre. High fibre in feeds may also trap nutrients, protect them from the action of digestive enzymes and therefore adversely affect absorption in the small intestine. Dissimilar fermentation of fractions of Non-starch polysaccharides (NSP) by pig breeds may differ due to differences in the composition of the microbial populations in the large intestine, resulting in different volatile fatty acid patterns (Morales *et al*., 2002). Moreover, the effect of breed on digestibility also depends on dietary fibre (DF) level, because in some breeds this effect is only seen when they are fed a high-fibre diet (Fevrier *et al*., 1992; Kemp *et al*., 1991).

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2.6. Fibre composition and its functionality

2.6.1. The effects of dietary fibre on feed intake

Pigs demonstrated preferences for feeds based on texture, particle size, nature of feedstuffs, inclusion rate and freshness (Sola-Oriol *et al*., 2009a; 2011; Seabolt *et al*., 2010). Increased feed intake when the fibre level of diets increases is an effort to maintain energy intake (Sterne and Andreson, 2003), because pigs eat to satisfy their energy requirements (Chiba *et al.,* 1991a). Indigenous pigs have been shown to consume relatively more compared to exotic breeds (Ndindana *et al.,* 2002; Kanengoni *et al.,* 2002; Len *et al.,* 2009). The study by Thacker and Haq (2008) also showed that the negative effects of fibre are reduced as the animal weights increase. This indicates that the negative effects of fibre are higher in younger animals compared to mature or older animals.

2.6.2. The effects of dietary fibre on nutrient digestion

The effect of DF on the digestibility of dietary components is variable, and depends on the type of fibre included. In general, soluble fibre is more easily and rapidly fermented than insoluble fibre (Montagne *et al*., 2003; Bach Knudsen and Hansen, 1991). Besides, soluble DF may increase mean retention time (Le Goff *et al*., 2002; Glitsø *et al*., 1998), resulting in greater nutrient absorption from the intestine. The negative effects of DF on nutrient digestion are partly the result of increased digesta flow rate and inhibition of the exposure of digesta to enzymes, and also increased endogenous nitrogen, which is an additional factor reducing apparent nitrogen digestibility (Jørgensen *et al*., 1996; Rainbird and Low, 1986). Inclusion of DF in the diet decreases the digestibility of energy and dietary components in the ileum and in the total tract (Len *et al*., 2009a; Len *et al*., 2009b; Högberg and Lindberg, 2004a; Högberg and Lindberg, 2004b). Different feed sources have different characteristics, such as solubility and degree of lignification (Bach Knudsen and Jørgensen, 2001), thus imputing the differences in fibre effect on digestibility.

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2.6.3. The effects of dietary fibre on gut health and intermediary metabolism

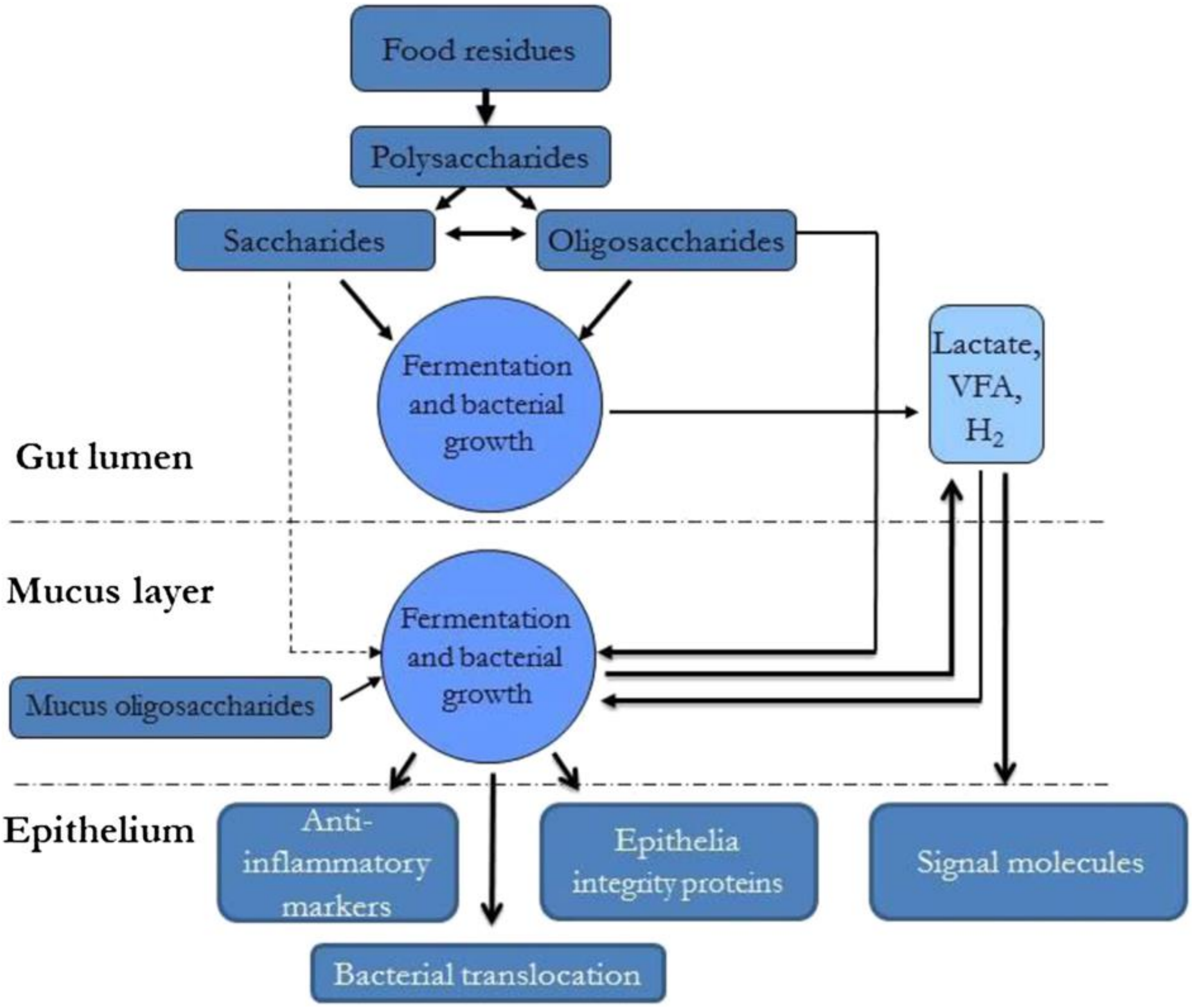

Figure 2. 3: Interactions between dietary fibre, gut environment, gut microbiota and host response with implications on gut health

As shown by some researchers (Jensen, 2001; Simon, 2001; Bach Knudsen *et al*., 1993; Bach Knudsen *et al*., 1991), diets with varying fibre content and fibre properties may lead to changes in the short chain fatty acids (SCFA) due to interactions between the diet and the gut microflora. The main products of fermentation of DF are SCFA, which are mainly acetate, propionate and butyrate, and the gases H_2, CO_2 and CH_4. The energy available to the host animal after microbial fermentation is the energy found in the SCFA and it provides up to 24% of the maintenance requirements for growing pigs (Yen *et al*., 1991) and potentially even more for adult pigs. The SCFA, and especially butyrate, have roles in connection with animal health (Jensen, 2001). Butyrate stimulates the development and growth of the large and small intestine by stimulating epithelial cell proliferation (Montagne *et al*., 2003).

Almost all SCFA are completely absorbed from the lumen of the GIT, leading to the stimulation of resorption of water and sodium from the large intestine (Montagne *et al*., 2003), thus reducing the risk of diarrhoea. Furthermore, SCFA are capable of promoting the proliferation of beneficial bacteria species, which can inhibit the development of some pathogenic species (Bauer *et al*., 2006). The effect of DF on epithelial morphology and cell turnover is variable, and depends on the level and physico-chemical properties of the DF in the diet, the duration of ingestion, the animal species and age, and the site in the intestinal tract (Montagne *et al*., 2003).

In growing pigs, inclusion of 10 % wheat straw in a low-fibre diet has been found to result in longer villi and deeper crypts in the jejunum and ileum, and increased cell division and crypt depth in the large intestine (Jin *et al*., 1994). In contrast, the fibre concentration has no influence on morphological characteristics, and the mitotic counts in the small intestine are lower in pigs fed a high-fibre diet (Hedemann *et al*., 2006). Feeding a diet containing high viscosity carboxy-methyl cellulose reduces the villus height and increases the crypt depth, whereas feeding low viscosity carboxy-methyl cellulose results in villus elongation (McDonald *et al*., 2001).

There are also conflicting results on the effect of soluble fibre on the intestinal morphology of growing pigs (Glitsø *et al*., 1998). It is well known that when pigs are fed a high-fibre diet, the development of the GIT, especially the large intestine, increases relative to that of pigs on a low-fibre diet (Len *et al*., 2009a; Len *et al*., 2009b; Freire *et al*., 2003; Freire *et al.,* 2000). The increase in GIT size is probably due to the prolonged presence of fibre in the gut stimulating an increase in mucosa weight and hypertrophy of the gut, which facilitates the development of bacterial mass (Eastwood, 1992). Alternatively, the increase in GIT size could be due to the production of SCFA, which stimulate epithelial cell proliferation, resulting in growth of the intestine. Source of fibre also influences the length and weight of the intestine (Len *et al*., 2009a; Freire *et al*., 2000), and may be related to fibre properties such as the ratio between soluble and insoluble fibre (Bach Knudsen and Jørgensen, 2001; Freire *et al*., 2000), resulting in the differences in gut health effects.

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2.6.4. The effects of fibre on gut microbes and fermentation

As ingested feed remains in the stomach only a short period of time, the pig as a non-ruminant has a smaller population of micro-organisms in the stomach than in the lower parts of the digestive tract, leading to limited microbial activity. In the lower part of the small intestine and particularly in the large intestine, an increased number of micro-organisms can be found. The total number of bacteria in the pig GIT ranges from 10^7 to 10^9 viable bacteria per gram digesta in the stomach and small intestine, and from 10^{10} to 10^{11} viable bacteria per gram digesta in the large intestine (Jensen and Jørgensen, 1994; Bach Knudsen *et al*., 1993). Dietary fibre that escapes digestion in the upper part of the GIT is potentially available for bacterial fermentation in the large intestine (Jensen, 2001).

Microbial growth depends on the availability of substrates that can be metabolised (Wenk, 2001). The NSP are the main energy source for microbial fermentation in the large intestine (Bach Knudsen and Jensen, 1991; Bach Knudsen *et al*., 1991), and the amounts and chemical and structural composition of the DF are important factors for the microbial activity in the digestive tract. In a study by Jensen and Jørgensen (1994), greater microbial activity was found in the stomach, caecum and colon in pigs fed a high-fibre diet than in pigs fed a low-fibre diet. Moreover, the amount of digested carbohydrates in the large intestine is correlated to microbial activity (Bach Knudsen *et al*., 1991). Through modifying the diet, the composition of the microflora can be altered and although bacteria numbers appear unchanged, the dominant strains or species of bacteria may vary (Conway, 1994).

2.6.5. Effects of fibre on serum metabolites

The use of serum metabolite has been a common practice in evaluating the physiological responses to diets and diseases in animals (Kaneko, 1989; Varghese *et al.,* 2012), these have generally encompassed metabolic markers, (glucose, cholesterol, creatinine, urea triglycerides). Frank *et al*. (1983) reported a linear decrease in glucose levels and an increase in urea levels in crossbred pigs fed diets with incremental levels of fibre, irrespective of the sex of the animals. The glucose decrease was enhanced by low levels of starch ingredients in the diets, which were included in the diet at the expense of cereal sources. The increase in urea levels could be the cause of increased ammonia production by intestinal microbes. Mashatise *et al*. (2005) reported no differences in glucose, urea and creatinine levels in Mukota and Mukota x Large White gilts fed a control and a high fibre diet in contrast with a report by Frank *et al*. (1983).who reported the opposite. Breed of pig and level of fibre affects serum energy metabolites, depending on the type of fibre utilised and raw materials in the

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diet as reported by Weber and Kerr, (2012). Serum metabolites assist in outlining response of the pigs to different diets fed.

2.7. Summary

Indigenous pigs in southern Africa are largely kept in resource-poor households by vulnerable groups in marginal areas (Chikwanha *et al*., 2007; Chiduwa *et al*., 2008) as opposed to exotic breeds mostly kept in commercial farms and are better able to utilize fibrous feeds compared to exotics (Kanengoni *et al*., 2002; 2004). Buffalo grass is a highly variable, tufted tussock-forming perennial grass (Ecoport, 2010; FAO, 2010; Cook *et al.,* 2005). Buffalo grass is more rarely made into silage, as the moisture content of the grass in semi-arid areas is usually low as reported by (Göhl, 1982). The grass also has low concentrations of fermentable carbohydrates, but the addition of additives such as orange pulps can however improve the quality of its silage (Iqbal *et al*., 2005). Additive could be essential for enhancing the quality of silage.

Differences in digestive ability between pig breeds may also be credited to the size of the GIT and digesta transit time in the gut as reported by (Kanengoni *et al*., 2002; 2004). It has been indicated that local indigenous pigs pig breeds have greater GIT size compared with improved breeds, resulting in higher digestibility of dietary components, in particular when pigs are fed a high-fibre diet (Len *et al*., 2009a; Len *et al*., 2009b; Freire *et al*., 2003; Freire *et al*., 2000). The effect of DF on the digestibility of dietary components is different, and depends on the primary source, the feed forms and type of fibre included. The diet fed has an effect on the blood metabolites such as cholesterol, urea and glucose levels of pigs (Mashatise *et al.,* 2005), thus dietary effects both on digestibility, performance and metabolism are dependent on the nature of the breed type, the type, feed form, amount of fibre in a diet and the size of the gastrointestinal tract and the nutrient composition of the diet fed. Serum metabolites assist in determining response of the pigs to different diets fed.

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CHAPTER 3

THE FERMENTATION DYNAMICS, NUTRIENT COMPOSITION AND AEROBIC STABILITY OF THE ENSILED ORANGE PULP (*CITRUS SINENSIS*) AND BUFFALO GRASS (*CENCHRUS CILIARIS*) MIXTURE SILAGE

Abstract

The study was conducted to assess effects of Renozyme® enzyme (α-amylases and β-endo 1, 3; 1, 4 beta glucanase) on the fermentation dynamics, nutrient composition and aerobic stability of ensiled Buffalo Grass (*Cenchrus Ciliaris*) and Orange Pulp (*Citrus Sinensis*) at a ratio of 80:20 (Crawshaw, 2004). Two treatments evaluated by ensilage in 1.5 L anaerobic glass jars over 30 days i) control (orange Pulp and Buffalo Grass without Renozyme® enzyme (OPBG); ii) Orange Pulp and Buffalo Grass with Renozyme® enzyme (OPBGE) at 1 g/kg of the mixture, as per package recommendation. There was a (P<0.05) day and treatment effect on ASH, CP, EE, NDF, ADF, HEMI, WSC, LA, and pH for OPBG and OPBGE. The DM, HEMI, WSC and pH were lower on day 30 compared to day 7 for both treatments. By day 30, the neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations in OPBG were lower than OPBGE. At day 30, OPBG pH (3.8) and OPBGE silage pH (3.8) was lower than the pH values of OPBG (4.2 and 4.4) and OPBGE (4.3 and 4.4) silages at day 0 and 7, indicating a (P<0.05) day and treatment effect on pH. There were significant (P<0.05) diets and day interactions for DM, ASH, CP, EE, NDF, ADF, HEMI, WSC, and LA, but not for pH. During the aerobic stability test, the enzyme did not affect silage pH (P>0.05) between day 30 and day 35 for both treatments (OPBG and OPBGE) respectively. The OPBG silages produced more carbon dioxide than OPBGE silages. The OPBGE silages produced higher pH readings than OPBG silages. In conclusion, overall, treatment with exogenous enzymes did not improve fermentation characteristics, nutrient composition and aerobic stability of the silage, but reduced the fibre fractions of the silage**.**

Key words: silage, fermentation, fibre, aerobic stability, exogenous enzymes.

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3.1. Introduction

The tropical grass species produce poorer silages due to high pH of the silage as well as high acetic acid and ammonia nitrogen (NH_3-N) concentrations (Imura *et al.,* 2001). Generally, tropical forages are low in water-soluble carbohydrates (WSC), which is an essential ingredient for the production of lactic acid. Addition of exogenous WSC to silages of temperate forages increased microbial fermentation resulting in higher lactic acid production and improved silage quality (McDonald, 1981), depending on the type of raw materials ensiled , the ensiling method used and the period of ensiling.

Buffalo grass is difficult to ensile (Catchpoole and Henzell, 1971), as tropical regions conservation of forage is difficult due to the short rainy season and high temperatures. Therefore it is rarely made into silage but mostly made into hay, as the moisture content of the grass in semi-arid areas is low (Göhl, 1982). Furthermore it contains low levels of fermentable carbohydrates, which makes additives inclusion essential to improve the quality of the silage (Iqbal *et al*., 2005). Orange pulp ensiled without additives gives better results in energy conversion efficiency (Itavo *et al*., 2000b) and it is available for ensilage all year-round and also for feeding purposes (Fuller, 2004; Göhl, 1978). Furthermore its silage has a pleasant odour and is readily eaten by pigs (Fuller, 2004; Göhl, 1978). The ensiling process requires a period of less than 50 days, as reported by (Fuller, 2004). Göhl (1978) reported that before ensiling, the pulp can be pressed or mixed with grass or hay in order to obtain firm silage. This is due to the high level of moisture and fermentable carbohydrates it contains in the pulp, which can enhance the quality of the dry buffalo grass.

Ensiling fresh orange pulp with partially dried grass forage that cannot be successfully ensiled on its own is advantageous. The pulp provides organic acids (particularly malic and oxalic acids), moisture, fermentable carbohydrates and enhances fermentation quality (Itavo *et al*., 2000b). Furthermore, orange pulp enhances overall silage quantity and quality (i.e. more sugars, more acidic bacteria, lower pH) and reduces the need for acid additives (Crawshaw, 2004). Orange pulp can also absorb moisture from feeds such as brewers' grain and is thus used to reduce effluent risk and nutrient loss from these materials (Crawshaw, 2004; Fuller, 2004). Sheperd and Kang.(1996), Meeske *et al*.(1999;2002) and Colombatto *et al*.(2004) indicates that cell wall degrading enzymes reduces fibre content in forage at ensiling. Ensiling high fibre roughages with exogenous enzymes to improve silage quality should be explored further. The aim of the study was to evaluated effect of mixing orange pulp with buffa-

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lo-grass on the quality of the mixed silage and the effect of exogenous enzymes on the quality of mixed orange pulp-buffalo grass silage.

3.2. Materials and methods

3.2.1. Experimental site

The study was carried out at the Agricultural Research Council (ARC) Pig Nutrition Section at Irene Gauteng province. Irene is situated about 15 km South of Pretoria on the Old Olifantsfontein road and lies at an altitude of 1526 m. Its coordinates are 25º 53' 59.6" S and 28º 12' 51.6" E. The average daily outdoor and indoor temperatures are 13.4 ^{0}C and 22 ^{0}C respectively.

3.2.2. Sample collection

3.2.2.1. Buffalo grass

The dry buffalo grass was acquired from the Towoomba Pasture Research Station in Limpopo province at Towoomba village in Waterberg municipality. It is located at an elevation of 1.109 m above sea level. Its coordinates are 24^{o}54’0’’S and 28^{o}19’60’’E. Maximum dry matter production occurs between 42 and 56 days of plant age and stem-leaf ratio increases rapidly with plant maturity and it has been proposed that buffalo grass should be cut during this time (Garcia *et al.,* 1980). The grass was cut to 7 cm height, at 6 to 8-week intervals.

Table 3.1: Nutrient composition of buffalo grass

PROXIMATE	**Buffalo grass (g/kg)**
DM	92.0
MOISTURE	8.0
ASH	131.0
PROTEIN	110.0
FAT	26.0
NDF	69.0
ADF	49.0
WSC	31.5
LA	0.8

(Source: Gohl (1981)

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3.2.2.2. Orange pulp

Wet orange pulp was purchased from Letaba orange farm, Letsie village, which is situated in the western sides of the Tzaneen town, 20 km from the Tzaneen town. It is located at an elevation of 1316 m above sea level. Its coordinates are 25^{o}73'03''S and 28^{o}29'33''E

Table 3. 2: Nutrient composition of orange pulp

PROXIMATE	Orange pulp (g/kg)
DM	86.7
MOISTURE	13.3
ASH	2.5
PROTEIN	3.1
FAT	54.0
NDF	105.5
ADF	85.2
WSC	83.5
LA	1.1

(Source: Akpata *et al.,* 1999)

3.3.3. Treatment, experimental design and measurements

Two treatments namely i)Orange pulp and buffalo grass mixture without enzyme (OPBG) which served as the control and, ii) Orange pulp and buffalo grass mixture with Renozyme® enzyme (containing α-amylases and β-endo 1, 3; 1, 4 beta glucose) (OPBGE) which was the test treatment were used in the study. The orange pulp and buffalo grass were mixed at a ratio of 80:20. The enzyme was added at a concentration of 1 g/kg mixture as per package recommendation. These mixtures were ensiled in forty-eight 1.5 L anaerobic glass jars equipped with lids, rubber rings and steel clamps to enable gas release and keep the jars airtight in a 2 x 3 (Treatment (OPBG, OPBGE) by period in days (7, 15, 30)) factorial design. Jars were stored out of sunlight and kept at 24^{o} - 28 ºC. Each jar was filled with approximately 1116 ± 5.8 g (wet weight) orange pulp and buffalo grass mixture without a headspace. Six jars per treatment were opened on days 7, 15 and 30 of ensiling, and sampled to determine pH, dry matter (DM) Ash, crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF) WSC, and lactic acid (LA). Representative silage samples from day 30 of all treatments were subjected to an aerobic stability test, in which samples were exposed to air for five days and carbon dioxide (CO_2) production and pH were determined following the procedure of Ashbell *et al*. (1991).

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3.3.4. Chemical analysis

Buffalo grass, orange pulp and their ensiled mixtures were analyzed for DM, ash, EE, CP according to standard procedures of AOAC (2000). A 40 g sample of ensiled material was collected from each jar and mixed with 360 mL distilled water in a stomacher bag, blended, and left for 24 hours at 10 ºC (Suzuki and Lund, 1980). It was then homogenized for 4 min and filtered through a Whatman No. 4 filter paper (G.I.C. Scientific, Midrand, South Africa). The extract was then used to determine pH, WSC (according to Dubois *et al*. 1956) and LA (Pryce, 1969). The DM of silage was determined by drying the samples at 60 ºC until a constant mass was achieved. This value was corrected for loss of volatiles with the equation of Porter and Murray (2001). Dry samples were ground to pass through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, Phil, USA) prior to being used for chemical analyses. The NDF was determined following the procedures of Van Soest *et al*. (1991) using heat stable α-amylase, and the ADF was determined with Fibretec System equipment (Tecator LTD., Thornbury, Bristol, UK). Separate samples were used for ADF and aNDF analysis and both included residual ash. Crude protein (ID 968.06), ash (ID 942.05) and EE (ID 963.15) were determined according to the procedures of AOAC (2000).

3.3.5. Mathematical calculations

CO_2 was calculated as:

$$\mathbf{CO2\ g/kgDM} = \frac{(\boldsymbol{TxVx0.044x1x1000})}{(\boldsymbol{AxFmxDM}\%/\mathbf{100})}$$

T = ml in 1N HCL used to lower pH 8.1 to 3.6.

V = Total volume of 20% KOH in beaker.

A = Total volume of 20% KOH solution used during titration.

FM = Kg fresh material placed in bottle.

DM = Dry matter content of the silage.

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3.3.6. Statistical analysis

The PROC MIXED procedures of SAS software, version 9.3 (SAS, 2010) was used to analyze the fermentation characteristics (WSC, LA, pH), nutrient concentrations (DM, Moisture, Ash, CP, EE, NDF, ADF) and aerobic stability (pH, CO_2) of the silage.

The following was used model;

$$\mathbf{Y_{ijk} = \mu + G_i + T_j + (G \times T)_{ij} + \varepsilon_{ijk}}$$

Where:

Y_{ijk} = Observation - DM, Moisture, Ash, CP, EE, NDF, ADF WSC, LA, pH and CO_2

μ = Overall mean

G_i = Effect of the i^{th} days (ensiling time period)

T_j = Effect of the j^{th} inclusion of enzyme in diet

$(G \times T)_{ij}$ = Interaction between days and diet

ε_{ijk} = Random error

Turkey's procedure was used to compare means of fermentation characteristics and nutrients concentration, ($P<0.05$) significant differences occurred among the effects of days (ensiling time period) and diets (inclusion of enzyme).

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3.4. Results

Table 3.3 Indicates the effects of ensiling days and enzyme renozyme on the nutrient composition and fermentation characteristics of ensiled treatments (OPBG and OPBGE). The CP, EE, NDF, ADF, HEMI, LA and pH were not difference between treatment (P>0.05) on day 7. The DM, ASH and WSC were difference (P<0.05) for OPBG and OPBGE on day 7. The OPBG levels of ASH, CP, NDF, ADF and LA were higher (P<0.05), but the DM, HEMI and WSC levels were lower (P<0.05), compared to OPBGE on day 7. The EE, LA and pH were not affected by treatment (P<0.05) on day 15. The concentrations of DM, ASH, CP, NDF, ADF, HEMI and WSC were affected by difference in treatments (P<0.05) on day 15. The OPBG levels of DM, ASH, EE, CP, NDF, ADF, HEMI, LA and WSC were higher (P<0.05), but the pH was lower (P<0.05) compared to OPBGE on day 15. The EE and pH were not affected by treatment (P<0.05) on day 30. The DM, ASH, CP, NDF, ADF, HEMI, LA and WSC were difference (P<0.05) for OPBG and OPBGE on day 30. However the OPBGE levels of DM, CP, NDF, ADF, LA and WSC were higher (P<0.05), but the ASH, EE and HEMI were lower (P<0.05), compared to OPBG on day 30. There were treatment x day interactions (P<0.05) on DM, ASH, EE, CP, NDF, ADF, HEMI, LA and WSC concentration.

No treatment x day interaction observed for pH.

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Table 3.3: The nutrient, fibre composition in (g/kg) and fermentation characteristics of ensiled treatments of orange pulp and buffalo grass without enzyme (OPBG) or with enzyme (OPBGE) from 7-30 days.

Silage mix		**Nutrient And Fibre Concentrations** (g/kg)							**Fermentation Characteristics** (g/kg)		
		DM	ASH	CP	EE	NDF	ADF	HEMI	WSC	LA	pH
	Days										
OPBG	7	869^{g}	53^{b}	69^{c}	12^{d}	320^{b}	213^{c}	110^{bcd}	42^{f}	0.9^{d}	4.4^{a}
	15	907^{a}	66^{a}	78^{a}	15^{ab}	430^{a}	280^{a}	153^{a}	61^{c}	0.9^{cd}	3.6^{e}
	30	895^{d}	50^{c}	56^{f}	16^{ab}	300^{f}	210^{cd}	93^{d}	30h	1.4b	3.8d
OPBGE	7	875^{f}	47e	68^{c}	12d	319^{b}	194^{cd}	124^{b}	58^{d}	0.7^{d}	4.4^{a}
	15	880^{e}	48^{d}	75^{b}	14^{bc}	320^{c}	210^{cd}	110^{bcd}	50^{e}	0.8^{d}	3.8e
	30	899^{c}	43^{f}	62^{e}	15^{ab}	310^{d}	245^{b}	63^{e}	32^{g}	2.2^{a}	3.8^{d}
	SEM	0.092	0.009	0.001	0.017	0.022	0.179	0.1781	0.118	0.005	0.077
P- Values	Silage Mix	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.01**	0.00**	0.00**	0.00**
	Days	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
	Silage X Days	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.07

$^{a-h}$ Means with different superscripts in a column differ significantly (*P* <0.05). *: significant and **: highly significant. N: 18 bottles of OPBG and N: 18 bottles of OPBGE. SEM: standard error of mean. DM: dry matter; CP (N x 6.25): crude protein; EE: ether extract; αNDF: α-amylase treated neutral detergent fibre; ADF: acid detergent fibre. HEMI: hemicellulose (calculated as the difference between NDF and ADF). WSC: water-soluble carbohydrate; LA: lactic acid; pH. Control (buffalo grass and orange pulp mixture without enzyme (OPBG); Buffalo grass and orange pulp mixture with exogenous enzyme (OPBGE).

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Table 3.4 indicates the volume of carbon dioxide produced (CO_2 g/kg DM) and pH of treatments (OPBG and OPBGE) silages after five days of aerobic exposure. The pH and CO_2 values were not affected by the difference in treatments on day 30, however the OPBGE pH levels on day 30 were higher compared to OPBG pH on day 30. The pH values were not affected by the difference in treatments on day 35, however the OPBGE pH was higher compared to OPBG pH on day 35. The pH was affected by treatments. The CO_2 values were difference on day 35 for OPBG and OPBGE, however the OPBG CO_2 was higher compared to OPBGE CO_2 on day 35. The CO_2 levels were not affected by treatments.

Table 3.4: Carbon dioxide produced (CO_2 g/kg DM) and pH of OPBG and OPBGE silages after five days of aerobic exposure.

PARAMETERS	**TREATMENT**	
	OPBG	**OPBGE**
CO_2 g/kg DM day 30	0.63	0.55
CO_2 g/kg DM day 35	0.41	0.34
pH DAY 30	3.77	3.81
pH DAY 35	3.63	3.65

Control (buffalo grass and orange pulp mixture without enzyme (OPBG);
Buffalo grass and orange pulp mixture with exogenous enzyme (OPBGE).

3.5. Discussion

Wet orange pulp and dry buffalo grass (OPBG) were mixed with exogenous enzymes to reduce dry matter content, improve the water-soluble carbohydrate content and break down the fibre matrix. When silage DM content is less than 300 g/kg, conditions for clostridial activity are favourable, resulting in high losses and silage of low nutritional value (McDonald *et al*., 1991). This was however not the case with OPBG (895 g/kg DM) and OPBGE (899 g/kg DM), thus restricting the high loss of silage of low nutritive value, due to unfavorable conditions for clostridial activity. The DM content of freshly chopped orange pulp was 210 g/kg DM, ideal for ensiling with dry buffalo grass as an absorbent.

The crude protein values of the orange pulp at before ensiling was in agreement with values reports by (Hernandez *et al*., 1998, Karabulut *et al*., 2007, Aregheore and Siasan, 2008), as increase in CP of the treatment OPBGE was observed, after the addition of enzyme in this silage mixture, compared to the OPBG with no enzyme inclusion. The pH was reduced from 4.4 to < 3.8 after 30 days of ensiling. Therefore pH level was low enough for efficient preser-

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vation since it should fall between 3.8 and 4.2 according to (Kung and Shaver, 2001). This was observed from day 0 to day 30 of ensiling between the treatments, where the pH ranged between 4.3±0.012 and 3.8±0.013 for both treatments, which is essential for successful silage preservation.

Water-soluble carbohydrates are regarded as essential substrates for growth of LAB for proper fermentation (McDonald *et al*., 1991), and low levels may restrict LAB growth. The WSC concentration of orange pulp before ensiling was 83.5 g/kg DM, which was higher than the minimum of 60 g/kg required for efficient fermentation (Jaakkola *et al*., 1991). After 30 days of ensiling, silage had lower residuals of WSC for both treatments OPBG and OPBGE, indicating that more sugar was utilized by LAB to produce LA as it increased both treatments OPBG and OPBGE between day 7 and day 30 of ensiling. Well preserved silage should contain 80 to 120 g/kg DM of LA concentrations as reported by (De Brouwer *et al*., 1991). Similarly (Zobell *et al*., 2004) also stated that good silage has lactic acid levels ranging from 30 to140 g/kg and the present study had lower concentrations than the threshold, which implies that the silage was poorly fermented thus poorly preserved.

The increase in fibre levels for OPBGE at day 30 compared with day 0, suggests enhanced fermentative processes of highly fermentable non-starch polysaccharide components by the enzyme. The results agreed with the reports that cell wall degrading enzymes reduced fibre content in forage at ensiling (Sheperd and Kung, 1996; Meeske *et al*., 1999; 2002; Colombatto *et al*., 2004). Furthermore, there was a decrease in fibre of the treatment after the addition of enzyme in this study were OPBGE hemicellulose decreased drastically compared to the OPBG hemicellulose with no enzyme inclusion. The results are supported by reports that cell wall degrading enzymes reduced fibre content in forage at ensiling (Sheperd and Kung, 1996; Meeske *et al*., 1999; 2002; Colombatto *et al*., 2004). The extent to which this occurs is difficult to measure accurately, because ensiling probably solubilizes part of the non-starch polysaccharide fraction, so that polysaccharides that are recovered in NDF and ADF fractions would differ before and after ensiling, as observed by De Vries *et al*. (2012).

The aerobic stability study was inconclusive. The CO_2 production obtained in this study is an indication of DM losses and the extent of aerobic stability (McDonald et al., 1991). The aerobic stability of the OPBG improved with the addition of enzymes. However, there were similar decreases in pH in all silages after exposure to air, which is indicative of good aerobic stability. Ensiling with exogenous enzymes could help improve aerobic stability.

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3.6. Conclusion

The results of this study suggest that enzymes are necessary to improve the, nutrition, fibre concentration and fermentation characteristics of the orange pulp silage, thus improving the silage quality. The results reject the null hypothesis which states that, adding exogenous enzymes to the mixed orange pulp-buffalo grass will not improve the quality and aerobic stability of the silage. The results of the study indicate that the enzyme inclusion improved the quality and aerobic stability of the mixture. The OPBG was selected as the silage to be bulked as it’s in line with aim of the study, thus to provide cheaper, but quality feeds.

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CHAPTER 4

EFFECTS OF ENSILED ORANGE (*ORANGE SINENSIS*) PULP-BUFFALO-GRASS (*CENCHRUS CILIARIS*) SILAGE ON NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF SOUTH AFRICAN WINDSNYER-TYPE (SAWIP) AND LARGE WHITE X LANDRACE (LW X LR) CROSS BRED PIGS

Abstract

The study was conducted to determine the effects of buffalo grass-orange pulp silage on nutrient digestibility of South African Windsnyer-type (SAWIP) and Large White- Landrace (LW x LR) crossed pigs. Three treatments; T0 CON (NO OPBG), T1 15% OPBG (15% OPBG) and T2 30% OPBG (30% OPBG) were evaluated. Apparent total tract digestibility (ATTD) was assessed on 24 pigs, 12 SAWIP and 12 LW x LR pigs weighing 28.0 ± 9.8 kg and 27.20 ± 3.9 kg respectively in a 2 (breed) X 3 (diets) factorial arrangement. There was no ($P < 0.05$) diet and breed effect on DM and CP for LW x LR and SAWIP on all diets (CON, 15% OPBG and 30% OPBG). There was a ($P < 0.05$) breed effect on ATTD of OM, where LW x LR ATTD on OM was better compared to the SAWIP. Furthermore there was a ($P < 0.05$) breed and diet effect on ATTD of NDF, where LW x LR ATTD on NDF was higher compared to SAWIP on all diets (CON, 15% OPBG and 30% OPBG). There was a ($P < 0.05$) diet effect on ATTD of ADF and HEMI, were the LW x LR ATTD on ADF and HEMI was higher compared to the SAWIP. There were no ($P > 0.05$) breed x diet interactions throughout the experiment. The breed and diet did not have a significant ($P<0.05$) effect on ABW, ADFI and AG: F during the experiment. However there was a significant ($P<0.05$), difference observed on ADG across the diets. Furthermore there was a significant ($P<0.05$) breed x diet interaction observed during the experiment on ADG across the treatment. In conclusion, high fibre diets improved average daily gain and the gain to feed ratio of both breeds, therefore both breeds can utilize high fibre diets productively. There was no ($P < 0.05$) diet effect on ADG of both breeds, were the LW x LR ADG was higher compared to SAWIP for all diets. There was a ($P < 0.05$) diet x breed interaction effect on ADG, were the LW x LR ADG was higher compared to SAWIP when fed 30% OPBG diet, compared to the CON and 15% OPBG diets. There was no ($P < 0.05$) diet x breed effect on ABW, ADFI and AG: F for LW x LR and SAWIP on all diets (CON, 15% OPBG and 30% OPBG). Ensiled OPBG added to pig diets at 150g/kg and 300 g/kg respectively improved digestibility of nutrients and growth performance.

Key words: fibre, digestibility, utilization, fermentation, breeds

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4.1. Introduction

Silage is the acid fermented product of an aerobic fodder fermentation of greater than 50 % moisture content (McDonald *et al*. 2002), with the aim of preserving summer crops for winter feeding. Ensiling is primarily practiced to preserve and enhance the quality of a crop, furthermore it reduces non-starch polysaccharide (NSP) levels in ingredients ensiled (Meeske *et al*., 1999; Khan *et al*., 2006; Rezaei *et al*., 2009). This technology can be easily introduced to rural based resource poor farmers. Ensiling is also expected to improve palatability and hence quality in feedstuffs from the volatile fatty acids (VFA) and organic acids produced.

Digestion is the breaking down of the large insoluble molecules before they can pass through the mucous membrane and be absorbed (McDonald *et al*. 2002). The effect of dietary fibre (DF) on the digestibility of dietary components is variable, and depends on the type of fibre included and the raw materials used in the diets. Montagne *et al*., (2003) and Bach Knudsen and Hansen, (1991) indicated that soluble fibre is more easily and rapidly fermented than insoluble fibre. Furthermore Guillon and Champ, (2000) reported that in monogastric gastro intestinal tract (GIT), solubility and viscosity of fibre have effects on nutrient digestion, absorption and digesta transit. This implies that longer large intestine of indigenous pigs could increase the flow of digesta and result in a higher fermentative capacity and therefore a better utilization of diets containing high fibre levels. The differences in digestive ability between pig breeds are expected due to digesta transit time in the gut, caused by difference in body size (Len *et al*., 2009a; Len *et al*., 2009b; Borin *et al.,* 2005; Ndindana *et al.,* 2002; Fevrier *et al.,* 1992). The size of an animal can also affect its ability to utilise fibrous diets.

The extent of the negative effect of fibre level on digestibility is dependent on the breed and the growth stage of the pigs being fed the fibre diets (Noblet and Knudsen, 1991; Anderson and Lindberg, 1997; Laswai *et al.,* 1997; Kanengoni *et al.,* 2002; Len *et al.,* 2009). Feeding fibrous diets results in a number of advantages, such as improved well-being of animals, improvement of gut transit time and reduction of stomach ulcers (Low, 1993). However, when included in monogastric diets their high fibre content results in decreased diet digestibility (Nongyao *et al*., 1991; Wang *et al*., 2006) and dilution of dietary nutrients (Schulze *et al*., 1994; Noblet and Le Goff, 2001). Several reports have indicated that indigenous pig breeds can utilize fibre better than exotic breeds (Fevrier *et al*., 1992; Kanengoni *et al*., 2002; Ndindana *et al*., 2002). Thus indigenous breeds are considered to be very tolerant of poor quality diets (Rodriguez and Preston, 1996) and able to digest the fibrous components better than improved breeds (Borin *et al*., 2005) as opposed to exotic pig breeds.

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South African commercial pig farmers commonly keep exotic pig breed like the Large White-Landrace cross bred pigs, as opposed to South African resource poor farmers keeping indigenous breeds. The South African Windsnyer-type Indigenous pigs (SAWIP) thrive under marginal conditions and they are often fed fibrous diets and nothing has been documented of their feeding behavior except for anecdotal evidence. The objective of the current study was therefore to assess average body gain, average daily feed intake, average daily gain and gain to feed ratio when ensiled orange pulp and buffalo grass were included in indigenous and commercial pig diets.

4.2. Materials and methods

4.3.1. Ensiling process and diets

The buffalo grass was acquired from the Towoomba Pasture Research Station in Limpopo province at Towoomba village in Waterberg municipality. Fresh orange pulp was purchased from Letaba orange farm, Letsie village, which is situated on the northern, north-eastern and western sides of the Tzaneen town, which is 20 km from the Tzaneen town. The ensiling process was performed at Agricultural Research Council - Animal Production Institute (ARC-API, Irene, Gauteng, South Africa), and both the orange pulp and buffalo grass were ground to pass through a 10 mm sieve. Fresh orange pulp was mixed with buffalo grass (*Cenchrus Ciliaris*) at a ratio of 80:20 and ensiled by compacting in 210 L drums lined with a plastic bag, and closed with a rubber lid to prevent damages to the bags by rodents **(Table 4.1)**. The drums were stored at between 22 and 25^{0}C. After 3 months of ensiling, drums were opened and sampled for the determination of fermentation characteristics and chemical compositions as described in Chapter 3. The silage was then used to formulate diets with inclusion levels of 15 % and 30 % of diet (as fed). The diets were formulated to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg DM and 11.6 g lysine /kg which meet and exceed the requirements of growing pigs (NRC, 1998) **(Table 4.2).** This resulted in three treatments namely; control diet without OPBG (CON), diet containing 15 % OPBG diet (Low Fibre Inclusion - 15% OPBG), and diet containing 30 % OPBG (High Fibre Inclusion - 30% OPBG) **(Table 4.3)**. The silage silo drums were opened weekly as the experimental diets were mixed to prevent spoilage **(Table 4.2)**. The effect of these diets on Apparent Total Tract Digestibility (ATTD) parameters dry matter (DM), organic matter (OM), ether extract (EE), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) in growing pigs were evaluated. The experimental procedures described in the study were ap-

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proved by the Animal Ethics Committee of the Agricultural Research Council, Animal Production Institute (ARC-API).

Table 4.1: The fermentation characteristics and nutrients composition (g/kg DM), of ensiled OPBG at 30 days.

Nutrients (g/kg)	**OPBG**
DM	893
Ash	52.8
CP	62
EE	1.5
αNDF	338
ADF	228
Fermentation characteristics (g/kg)	
WSC	50.8
LA	1.0
pH	4.0

DM: dry matter; CP (N x 6.25): crude protein; EE: ether extract; αNDF: α-amylase treated neutral detergent fibre; ADF: acid detergent fibre. WSC: water-soluble carbohydrate; LA: lactic acid; pH. Control (buffalo grass and orange pulp mixture (OPBG).

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Table 4.2: Composition of experimental diets; control (CON), low fibre inclusion (15% OPBG) and high fibre inclusion (30% OPBG) fed to growing South African Windsnyer-type indigenous (SAWIP) and Large White x Landrace (LW x LR) pigs.

Ingredients (kg)	**CON**	**15%OPBG**	**30%OPBG**
Maize meal	554	456	357
Hominy chop	100	100	100
Wheat bran	100	75	50
Soyabean oil cake	208	180	152
OPBG silage	0.0	150	300
Lysine HCL	14	14	14
Methionine	0	0	0
Feed lime	10	9	8
Monocalcium phosphate	13	15	16
Salt	0	0	0
Vitamin and mineral premix	2	2	2
Total (kg)	**1000**	**1000**	**1000**
Laboratory Analysis g/kg DM			
DM	945	942	938
CF	56	58	60
CP	181	180	179
EE	35	33	34
DE(MJ/kg)	15	14	16
αNDF	188	193	201
ADF	61	61	60
Ca	9	9	8
P	7	7	7

DM - dry matter; DE - digestible energy; CF- crude fibre; CP (N x 6.25) - crude protein; EE - ether extract; αNDF- α-amylase treated neutral detergent fibre; ADF - acid detergent fibre; Ca-calcium ;P-phosphorous.

Digestible Energy (DE) was calculated as:

$$\text{DE (MJ/kg)} = 4151 - (122 \text{ x } \%\text{ Ash}) - (64 \text{ x } \%\text{ CF}) + (38 \text{ x } \%\text{ EE}) + (23 \text{ x } \%\text{ CP});$$

(Perez, 1993).

4.3.2. Animals, diets, housing and experimental design

Twelve LW x LR (28.0 ± 9.8 kg) and 12 SAWIP (27.20 ± 3.9 kg) pigs, were randomly selected from the ARC-Irene pig breeding units and used for the digestibility and growth performance trial. The pens for the LW x LR measured 2 x 1.5 m and the SAWIP's were 1.5 × 0.9 m in naturally ventilated house with the temperature ranging from 22 to 25 °C. The pigs stayed individually in pens containing one feeder. The feeders were checked and adjusted twice each day to ensure constant access to fresh feed and minimize any possible wastage. One feeder in each pen contained a diet without OPBG (CON), 15% OPBG (15% OPBG) or 30% OPBG (30% OPBG) ensiled mixture. There were 4 replications for each comparison. The study was carried out in 30 days. The diets CON, 15% OPBG (15% OPBG) and 30% OPBG (30% OPBG) were allocated to pigs in a completely randomised design (CRD) with a 2 X 3 (breed X diet) factorial arrangement of the treatments. The pigs were fed *ad libitum* for 30 days. The diets included 2 g chromium oxide (Cr_2O_3) per kg as indigestible marker to estimate nutrient digestibility. The pigs were adapted to the Cr_2O_3 diets for the first week of the experiment over five days, prior to faecal collection, and continued on diets without the Cr_2O_3 marker post the faecal collection for two weeks prior to the conclusion of the experiment. Pig digestibility and growth performance data was collected weekly, after one week of adaptation. Water was freely available through low- pressure nipple drinkers in each pen.

4.3.2.1. Feed and faecal sampling procedures

Representative feed samples from each diet were taken each time diets were mixed and stored at -20 °C for laboratory analyses. Faeces were collected by rectal grab sampling on day 6, post five days of Cr_2O_3 adaptation, from 08h00 to 10h00 from each pig and stored at -20 °C for subsequent laboratory analyses. At the end of the collection period, the faeces were thawed overnight and oven dried at 60 °C for 48 h before analysis. The 5-day faecal samples for each pig were combined and mixed after the drying period and then a representative sample analysed for nutrient digestibility. Dry faecal samples were ground to pass through a 1-mm screen to ensure good mix (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, Phil, USA) prior to being used for chemical analyses.

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4.3.2.2. Mathematical calculations

Apparent total tract digestibility (ATTD) was calculated as:

$$\mathbf{ATTD = 1 - \frac{NF\ XCD}{ND\ X\ CF}\ X\ 100}$$

Where:

ATTD = apparent total tract digestibility coefficient

NF = nutrient concentration in feces (% DM)

ND = nutrient concentration in diets (% DM)

CF = chromium concentration in feces (% DM)

CD = chromium concentration in diets (% DM)

4.3.3. Performance Measurements

The pigs were weighed weekly from the start to the end of the trial. The pigs consumed feed *ad libitum*. Feed intake was measured weekly. Refusals were not analysed but they were visually examined to see if the pigs were selecting against the OPBG silage mixture. Selection was high in the first week after which the pigs consumed all feed offered. Average daily gain (ADG), average daily feed intake (ADFI), average body gain (ABG), average gain to feed ratio (AG: F) were calculated over the period each group of animals were in the trial from the metabolic weight and feed intake values. The trial was terminated after 30 days of the growth performance experiment. ABW: average body weight, ADFI: average daily feed intake, ADG: average daily gain, AG: F: average gain to feed ratio, calculated as:

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4.3.3.1. Mathematical calculations

$$\text{ABG} = \text{Final body weight(kg)} - \text{Initial body weight(kg)}$$

$$\text{ADFI} = \frac{\text{Feed intake (kg)}}{\text{Number of days}}$$

$$\text{ADG} = \frac{\text{Total weight gain (kg)}}{\text{Number of days}}$$

$$\text{AG: F} = \frac{\text{Average daily gain (kg)}}{\text{Average weekly body weight(kg)}}$$

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4.3.4. Chemical analyses

The DM of the feed and faecal samples was determined by drying the samples at 60 ºC for 48 hours according to the procedure of AOAC (1990). After drying, samples were ground through a 1 mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA, USA) for chemical analyses. Following the procedures of Van Soest *et al*. (1991), the neutral detergent fibre (αNDF) concentration was determined using heat stable α-amylase (Sigma-Aldrich Co. LTD., Gillingham, UK, no. A-1278) with sodium sulphite and the ADF concentration was determined using the Fibretec System equipment (Tecator LTD., Thornbury, Bristol, UK). Separate samples were used for ADF and αNDF analysis and both included residual ash. The nitrogen (N) content of the feed was determined with a N analyzer (FP-428, Leco Corp., St Joseph, MI) using a combustion method (990.03; AOAC, 1997). Crude protein was calculated by multiplying the N content by 6.25. Crude fat was measured using AOAC Soxhlet method (ID 960.39; AOAC, 1997). Ash (ID 942.05) and ether extract (EE, ID 963.15) were determined according to the procedure of AOAC (1990). The gross energy (GE) was determined with bomb calorimetry (MS-1000 modular calorimeter, Energy Instrumentation, Centurion, South Africa). Chromium was determined following the procedure described by Fenton and Fenton (1979).

4.3.5. Statistical analyses

The ATTD of DM, OM, EE, CP, NDF, ADF and HEMI and ABW, ADFI, ADG, AG: F were analysed according to a factorial arrangement of treatments with two breeds (SAWIP and LW x LR) and three diets (CON, 15% OPBG, 30% OPBG) using ANOVA in the GLM procedures of the statistical package of SAS (SAS Inst. Inc., Cary, NC). The following model was used:

$$\mathbf{Y_{ijk} = \mu + G_i + T_j + (G \times T)_{ij} + \varepsilon_{ijk}}$$

Where:

Y_{ijk} = Observation – DM, OM, EE, CP, NDF, ADF, HEMI, ABW, ADFI, ADG and AG: F
μ = Overall mean
G_i = Effect of the i^{th} breed
T_j = Effect of the j^{th} diet
$(G \times T)_{ij}$ = Interaction between breed and diet
ε_{ijk} = Random error

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Turkey’s procedure was used to compare means of apparent total tract digestibility and growth performance of growing pigs where significant differences occurred among the diets and breed

4.4. Results

Pig ATTD and growth performance on the different diets are presented in **Table 4.3**. The digestibility of DM, CP and EE were not different (P>0.05) between diets and breeds. The digestibility of OM and NDF were affected by the different in breed (P<0.05) LW x LR and SAWIP. However SAWIP digestibility of OM and NDF on CON and 15% OPBG were higher, but lower on 30% OPBG, compared to LW x LR. The digestibility of fibre components (NDF, ADF, HEMI) were different (P<0.05) CON and 30% OPBG. However, LW x LR digestibility of NDF, ADF, HEMI on the 30% OPBG were higher compared to SAWIP (P<0.05), but lower on CON. The growth performance on ABG, ADFI and AG: F was not different (P>0.05) between diets and breeds. The growth performance on ADG was different (P<0.05) for 30% OPBG and 15% OPBG. However LW x LR performance on ADG of 30% OPBG diet was higher (P<0.05), but SAWIP had lower (P<0.05) ADG on 15% OPBG diet. There was a diet x breed interaction on ADG (P<0.05).

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Table 4.3: Apparent total tract digestibility (ATTD) of nutrients (g/kg), growth performance (kg) in control (CON) and diets containing ensiled OPBG at low (15% OPBG) and high (30% OPBG) fibre inclusion levels fed to South African Windsnyer-type Indigenous pigs (SAWIP) and Large White x Landrace (LW x LR) pigs.

Breed		**Digestibility (g/kg)**							**Growth Performance**			
	Diet	DM	OM	CP	EE	NDF	ADF	Hemi	ABG (kg)	ADFI (kg)	ADG (kg)	AG:F(kg)
	CON	62	68^{ab}	61	70	71^{ab}	50^{bc}	61^{ab}	32.7	1.5	0.6^{ab}	0.3
LWXLR	15% OPBG	62	73^{a}	61	71	85^{a}	60^{ab}	72^{a}	31.6	1.6	1.7^{ab}	0.3
	30% OPBG	59	68^{ab}	56	72	83^{a}	67^{a}	75^{a}	34.3	1.5	0.7^{a}	0.2
	CON	72	52^{b}	72	60	61^{b}	41^{c}	51^{b}	31.3	1.6	0.5^{ab}	0.2
SAWIP	15% OPBG	63	60^{ab}	63	62	76^{ab}	58^{ab}	67^{ab}	32	1.4	0.5^{b}	0.2
	30% OPBG	59	59^{ab}	63	75	78^{ab}	65^{ab}	72^{a}	31.9	1.4	0.5^{ab}	0.2
	SEM	4.319	3.846	4.013	4.698	4.435	3.536	3.906	4.187	0.112	0.05	0.038
	Diets	0.218	0.246	0.262	0.210	0.006**	0.000**	0.001**	0.942	0.653	0.050*	0.155
P- Value	Breeds	0.301	0.001**	0.057	0.199	0.041*	0.174	0.075	0.751	0.609	0.073	0.089
	Diets X Breeds	0.456	0.683	0.585	0.278	0.852	0.554	0.754	0.944	0.861	0.050*	0.062

$^{a\text{-}c}$ means with different subscripts in a row differ significantly ($p<0.05$). *: significant and **: highly significant. SAWIP n=12; LW x LR n = 12; SEM: Standard error of mean; DM - dry matter; OM – organic matter; CP – crude protein; CF – crude fibre; NDF- neutral detergent fibre; ADF - acid detergent fibre; HEMI: hemicellulose (calculated as the difference between NDF and ADF); ABG - average body gain; ADFI - average daily feed intake; ADG - average daily gain; G: F - gain to feed ratio; 15% OPBG – Low fibre inclusion at 15% inclusion of OPBG; 30% OPBG – High fibre inclusion at 30% inclusion of OPBG; CON - control diet with 0% inclusion of OPBG; LW x LR - Large White and Landrace crosses; SAWIP - South African Windsnyer-type Indigenous Pig).

4.5. Discussion

4.5.1. Apparent Total Tract Digestibility

The apparent digestibility of DM, CP and EE were not different between diets and breeds, however a significant difference was observed on OM and NDF digestibility between LW x LR and SAWIP. This can be attributed to the fact that indigenous pigs and exotic pigs digest feed differently irrespective of the sex of the breeds. Kanengoni *et al*. (2002; 2004) reported that differences in digestive ability between pig breeds may also be credited to the size of the GIT and digesta transit time in the gut, which is in agreement with the results of the current study. Kyriazakis (2011) also reported that the capacity of pigs' caecum and colon to ferment fibre can increase at high fibre levels. In addition to adaptation and increased substrate for fermentation in the colon with increased fibre levels, the poor performance in digestibility can also be attributed to poor ensiling process, as ensiling reduces the fibre content and improves the nutrient composition of the ensiled feed material. This is in contrast with a report by Jørgensen *et al*. (2010) who reported improvements in ileal digestibility of DM, OM and energy after fermentation and the total tract digestibility also improved post consumption of fermented diets. Similarly, Lyberg *et al*. (2006) found an increase in OM digestibility of 9 % units at the ileal level and 2 % for the total tract in pigs fed fermented diets compared to the same fed as dry feed. Jørgensen *et al*. (2010) then suggested that fermenting ingredients, especially those with low digestibility and fermentability, can be a strategy to improve their energy value for pigs.

The digestibility differences on fibre components (NDF, ADF, HEMI) between diets CON and 30% OPBG can be attributed to the fibre levels in each diets. Stanogias and Pearce (1985); Kanengoni *et al*. (2002), Ndubuisi *et al*. (2008) reported that digestibility on low fibre diet was higher compared to high fibre diets, which is in agreement with findings of the current study. The 30% OPBG observation indicated that ATTD of NDF in LW x LR, was higher than in the SAWIP which is inconsistent with the results from Kanengoni *et al*. (2002), who reported that Mukota pigs, digestibility of NDF decreased by 16 % while in the LW x LR it decreased by 21 % when the inclusion level of fibre in the diet was increased from 100 to 300 g/kg. Similarly a range 150 g/kg- 300 g/kg inclusion levels was utilized in 15% OPBG and 30% OPBG diets respectively. Possible explanations for the breeds' differences in digestibility of nutrients in high fibre diets are that gut capacity and intestinal microbial populations differ among age groups and breeds (Kyriazakis and Emmans, 1995; Whittemore *et al*., 2003; Thacker and Haq, 2009).

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4.5.2. Growth Performance

The growth performance parameters indicated no difference on ABG, ADFI and AG: F between breeds and diets. However the similarity was observed on ADG and this was caused by the diet effect between 30% OPBG fed to LW x LR and 15% OPBG fed to SAWIP. The similarity implies that the LW X LR can utilize a high fibre diet effectively and efficiently as the SAWIP fed a low fibre diet to maintain a similar ADG. This is surprising but, can be attributed to the differences in fibre content levels in each diet and differences in utilisation of diets containing fibrous content. Stanogias and Pearce (1985), Kanengoni *et al*. (2002), Ndubuisi *et al*. (2008) reported that digestibility of low fibre diet was higher compared to high fibre diets. However the results indicate that feeding high or low fibre diets will yield similar results in ADG depending in the level of ADFI, irrespective of the breed, which is surprising.

Whittemore *et al*. (2003) and Thankers and Haq, (2009) reported that the SAWIP growers' consume more feed per body gain than the LW x LR growers which may be a reflection of an adaptation to survive under marginal nutritional resources. This is inconsistent with the current study as it indicated similarities in ADG, despite the differences in ADFI. According to Frank *et al.* (1993), the LW x LR would normally consume more feed than SAWIP growers because of their bigger body size and gut capacity, which is in agreement with the results as the LW x LR consumed more feed than SAWIP, however yielding similar results in ADG. SAWIP are slow growing pigs and have a small frame compared to LW x LR, therefore the lower body gains are expected. Surprisingly it was not the case in the current study as ADG values were similar. Reduction in average daily gain and feed intake as high silage inclusion increased was reported by Ndindana *et al*. (2002) and Kanengoni *et al*. (2004). There were no similar reductions in this study which implies that the breeds utilized the high fibre and low fibre diets similarly. The difference between previous studies and the current study could be credited to ensiling method and ensiled material although other factors such as the different experimental conditions.

4.6. Conclusion

The results of the study indicate that inclusion of OPBG silage to the standard growing pig diet different inclusion levels had a positive impact as it enhanced growth performance and nutrient digestibility in the pig breeds. The results reject the null hypothesis. Ensiled OPBG

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inclusion in the standard growing pig diets enhanced growth performance and digestibility of both exotic and indigenous pigs.

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CHAPTER 5

THE EFFECT OF FEEDING DIETS CONTAINING ENSILED ORANGE PULP (*CITRUS SINENSIS*) AND BUFFALO GRASS (*CENCHRUS CILIARIS*) MIXTURE ON SERUM METABOLITES OF SOUTH AFRICAN WINDSNYER-TYPE (SAWIP) AND LARGE WHITE X LANDRACE (LW X LR) CROSSES

Abstract

A study to compare serum metabolites blood urea nitrogen (BUN), cholesterol (CHOL), creatinine (CREAT), glucose (GLU), phosphorus and triglycerides (TG), responses in indigenous pigs (SAWIP) and commercial pigs (LW x LR) fed diets containing ensiled orange pulps- buffalo grass (OPBG) silage at different inclusion levels was conducted. This was to evaluate the effects of buffalo grass-orange pulp on the composition of serum metabolites comparatively on the South African Windsnyer-type and Large White x Landrace cross pig breeds. Twelve South African Windsnyer-type indigenous pigs and 12 Large White x Landrace crossed pigs where assessed in the study. They were fed CON (control with no buffalo grass-orange pulps silage), 15% OPBG (low silage inclusion with buffalo-orange pulp silage at 15% of the diet) and 30% OPBG (high silage inclusion with buffalo-orange pulp silage at 30% of the diet) in a 2 (breed) X 3(diets) factorial arrangement, in a completely randomized design. Blood urea nitrogen (BUN) and creatinine (CREAT) concentrations were greater ($P < 0.05$) in the SAWIP than LW x LR. Triglycerides (TG) values were greater ($P < 0.05$) in LW x LR than in the SAWIP. There was no ($P <0.05$) diet effect on BUN, CHOL, CREAT, GLU, and TG for both breeds on all diets. There was ($P <0.05$) breed effect on BUN and CREAT, were SAWIP concentration were higher on BUN, but lower CREAT on compared to LW x LR for all diets fed. There was no ($P <0.05$) breed x diet interactions effect on serum metabolites. There were differences in serum and serum metabolite levels that were diet and breed related. Serum metabolite assays can still be effectively utilized as the main tool to assess physiological responses to diets and diseases in animals. The serum metabolites responses in different breed on high fibre further investigation to understand the relationship or interactions involved.

Key words: fibre, serum metabolites, fermentation, breeds.

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5.1. Introduction

Metabolites are the intermediate products of metabolic reactions catalyzed by various enzymes that naturally occur within cells. This term is usually used to describe small molecules, although broader application is often practiced, therefore there is a need to assess how differences in feed intake and digestibility of high fibre diets present at the level of cellular biology. Kaneko (1989) and Varghese *et al*. (2012) reported that serum metabolites have been largely used as the main tool analyzing physiological responses to diets and diseases in animals. These metabolites have generally encompassed metabolic markers, (glucose, cholesterol, triglycerides) and other serum markers (α-amylase, blood urea nitrogen, calcium, creatinine, phosphorus). The use of serum to identify metabolites is common practice as it’s affordable.

High fibre diets are likely to have a direct impact on serum and liver metabolites and proteins, but since these are not homogenous tissues, there are no easily reliable for identification of dietary-induced changes in metabolites. In this study, it was hypothesized that a diet containing high levels of ensiled OPBG modifies serum metabolites differently in South African Windsnyer-type indigenous pigs (SAWIP) and Large White x Landrace (LW x LR) crosses. The objective of the study was to compare serum metabolite changes in SAWIP and LW x LR crosses fed diets containing ensiled OPBG at varied inclusion levels.

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5.2. Materials and methods

5.2.1. Pigs, housing, and experimental design

The experimental procedures described in this study were approved by the Animal Ethics Committee of the Agricultural Research Council, Animal Production Institute (ARC-API). The pigs, housing conditions and diets are described in Chapter 4. Measurements of weight gain and feed intake were taken in 12 LW x LR and 12 SAWIP, fed on the three diets (CON, 15% OPBG and 30% OPBG) as part of the serum metabolites study. Each pig was allocated feed daily in the morning based on their ability to finish their daily allocation. Refusals were weighed in weekly and subtracted from feed offered to determine intake. The feeders were checked and adjusted twice each day to ensure constant access to fresh feed and minimize any possible wastage. Water was freely available through nipple drinkers.

5.2.2. Sampling procedures

At the end of the growth study, post 08h00 feeding period, the pigs' blood was drawn from the jugular vein at 1000. During collection 10 ml blood samples were collected in plain tubes and left to clot for about 1 h after which they were centrifuged for 10 min at 2 000 x g at room temperature to separate the serum as supernatant. An aliquot of the serum was then kept at -20 °C for biochemical analysis.

5.2.3. Serum analysis

Determination of serum metabolite levels was done using IDEXX Vettest® Chemistry Analyzer (IDEXX Laboratories, Inc., Westbrook, ME. USA). The IDEXX Vettest® Chemistry Analyzer employs dry-slide technology that uses a potentiometric end-point. The analyte in the sample catalyzes a reaction sequence to yield products that absorb light at wavelengths in various regions (340-680 nm), diffuses into an underlying layer, and are monitored by reflectance spectrophotometry. The dry slide technology minimizes interferences from lipemic, icteric and hemolyzed samples. The General Health Profile (GHP) evaluated comprised blood urea nitrogen (BUN), cholesterol (CHOL), creatinine (CREAT), glucose (GLU) and triglycerides (TG).

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5.2.4. Statistical analysis

Serum metabolites (BUN, CHOL, CREAT, GLU, and TG) were compared between the breeds and among the diets using ANOVA in the GLM procedures of the statistical package of SAS (SAS Inst. Inc., Cary, NC). The following model was used:

$$\mathbf{Y_{ijk} = \mu + G_i + T_j + (G \times T)_{ij} + \varepsilon_{ijk}}$$

Where:

Y_{ijk} = Observation - BUN, CHOL, CREAT, GLU, and TG
μ = Overall mean
G_i = Effect of the i^{th} breed
T_j = Effect of the j^{th} diet
$(G \times T)_{ij}$ = Interaction between breed and diet
ε_{ijk} = Random error

Turkey's procedure were used to compare means of serum metabolites of growing pigs where significant differences occurred due to the effects of diet and breed.

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5.2.5. Results

Table 5.1: Serum metabolite measurements (mg/dL) in South African Windsnyer-type Indigenous pigs (SAWIP) and Large White x Landrace (LW x LR) fed diets containing ensiled OPBG low (15% OPBG) and high (30% OPBG) silage inclusion levels.

		Parameters				
		GLU mg/dL	**CHOL mg/dL**	**BUN mg/dL**	**CREAT mg/dL**	**TG mg/dL**
Breed	**Diet**	85-150	28-48	21-64	1.60-2.70	52-157
	CON	101.6	93.8	28.5^{c}	0.9^{ab}	39.8
LWXLR	15%OPBG	129.7	120.8	33.3^{bc}	1.0^{ab}	59.1
	30%OPBG	114.3	128.6	31.2^{bc}	1.1^{a}	58.6
	CON	112.3	107.3	52.0^{a}	0.7^{b}	39.2
SAWIP	15%OPBG	109.3	94.7	45.9^{ab}	0.8^{ab}	36.1
	30%OPBG	99.1	105.4	38.5^{abc}	0.9^{ab}	35.6
	SEM	17.1	9.03	3.302	0.403	6.145
	Diets	0.698	0.217	0.227	0.165	0.313
P- Value	Breeds	0.557	0.123	0.00	0.001	0.006
	DietsxBreeds	0.629	0.076	0.068	0.807	0.139

$^{a-c}$ Means with different letters in a row differ significantly (P<0.05). *: significant and **: highly significant. SAWIP - n=12; LW x LR - n = 12; SEM - standard error mean. GLU - glucose; BUN - blood urea nitrogen; CREAT - creatinine; CHOL - cholesterol; TG – triglycerides.

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5.3. Discussion

The study aimed to evaluate the effects ensiled orange pulp-buffalo grass in the standard growing pig diet on blood glucose, triglycerides, total cholesterol creatinine and urea of South African South African Windsnyer-type and Large White- Landrace crossed pigs growing pigs. Metabolites are the intermediate products of metabolic reactions catalysed by various enzymes that naturally occur within cells. This term is usually used to describe small molecules, although broader application is often practiced.

The GLU levels are more stable after feeding, hence the blood was collected at 10h00 two hours after 08h00 feeding, ensuring stable glucose levels as opposed to when collection is done when pigs are hungry as that can largely influence the GLU levels, which is ideal for blood collection. Re´ rat, (1996) reported that glucose levels of sows were more stable, not only immediately after feeding, but also several hours later. The metabolites GLU and CHOL were similarly affected by the diets and the breeds. The GLU levels were within the normal range **Table 5.1** (85-150 mg/dL), however CHOL levels were above the normal range **Table 5.1** (28-48 mg/dL) which is concerning, as high levels of cholesterol my lead to an increased risk heart disease. (Ziemer *et al*., 2012) reported that pigs fed high fibre diet are likely to have high CHOL levels from increasing availability and absorption a high GLU concentration resulting from increased availability of sugars from the fermentation of fibre. However GLU levels in the current study were within the normal range and the responses of LW x LR and SAWIP in regulating the GLU levels were similar, which opposes finding from the previous study. Furthermore (Mashatise *et al*., 2005) reported no differences in GLU in Mukota and Mukota x Large White gilts fed a control and a high fibre diet, which agrees with the GLU results of the current study. As OPBG inclusion levels in the diet increased, there was no change in GLU levels, which is in contrast with the results of Frank *et al*. (1983) who reported a decrease in GLU levels as fibre levels increased.

The SAWIP tend to lay fat easily compared to the LW x LR, therefore in theory have higher serum CHOL levels. However, CHOL levels in the LW x LR and SAWIP were higher than the normal range **Table 5.1** (28-48 mg/dL). These results are in corroboration with those of Ferna’ndez-Fi’gares *et al*. (2007) who reported no differences in CHOL serum levels from Iberian and Landrace gilts, which is consistent with finding of the current study. Similarly there were no differences between lean and obese pigs in serum CHOL levels as reported by Mersmann *et al*. (1982). Pond *et al*. (1986) however, reported that CHOL increased dramati-

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cally in pigs fed low fibre diets than in pigs fed high fibre diets, which in contrast with findings from the current study as fibre inclusion has no significant effect on CHOL levels. Furthermore (Ferna’ndez-Fi’gares *et al*., 2007) reported an inverse relationship between dietary fibre levels and CHOL, which was not the case in the current study. The outcomes of the current study could be attributed to the GLU levels which were within the normal range for both the SAWIP and LW x LR, therefore the high cholesterol level could be attributed to the increased availability and absorption of high GLU concentration resulting from increased availability of sugars from the fermentation of fibre as reported by (Ziemer *et al*., 2012).

Blood urea and creatinine were affected by the difference in breeds (SAWIP and LW x LR) from the current study. According to (Rassin and Bhatia, 1992; Ferna’ndez-Fi’gares *et al*., 2007) CREAT is released from muscle in amounts proportional to muscle mass. Higher CREAT levels in the LW x LR therefore relate to their increased lean mass and lower levels relate to decrease in lean mass, as it was the case with SAWIP. The CREAT levels were lower than the normal range **Table 5.1** (1.60-2.70 mg/dL) between both breeds. However LW x LR had higher CREAT levels compared to the SAWIP and this can be attributed to ability of the breed to utilize high fibre diets efficiently as reported by (Faulkner and King, 1982).

(Kohn *et al*., 2005) indicated that Urea, a principal product of the catabolism of protein and the levels of BUN can act as indicators of body protein status and efficiency of renal function. The BUN levels for pigs on all treatments in this study were within the normal range (21-64 mg/dL) and only the SAWIP pigs on the CON diet had higher levels than on all other treatments. Blood urea nitrogen levels decreased with dietary increases in fibre. Since BUN levels have been used to determine protein requirements and lean tissue growth rates in pigs (Chen *et al*., 1995; Coma *et al*., 1995) thus results from the current study could be used to make dietary recommendations for the SAWIP. Frank *et al*. (1983) on the other hand reported an increase in urea levels in crossbred pigs fed diets with incremental levels of fibre and attributed it to increased ammonia production by intestinal microbes, which is in contrast with results of the current study, were BUN levels decreased with an increase in fibre for LW x LR pigs on 30% OPBG (31.2 mg/dL) compared to the CON (52.0 mg/dL). Berschauer *et al*. (1983) reported that a reduction in BUN levels can be associated with an increase in the efficiency of nitrogen utilization, however this was not the case in the current study as BUN decreased with the increase in dietary fibre. This suggests that the increase in ensiled OPBG in

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the diet in the both breeds did not improve the efficiency of nitrogen utilization. BUN levels may also be largely influence by fermentation processes in the hind gut.

Triglycerides (TG) were similarly affected by the breed factor, however TG values were greater in LW x LR than in the SAWIP. The effect of high fibre diets on TG was observed to be greater in exotic breeds more than the indigenous pigs. The CHOL increased with an increase in TG levels, therefore there is a direct proportional relationship between CHOL and TG. TG levels in the LW x LR were within normal range (52-157 mg/dL), however SAWIP TG levels were below the normal range when fed fibre diets. These results are in contrast with those of Ferna'ndez-Fi'gares *et al*. (2007) who found no differences in CHOL serum levels from Iberian and Landrace gilts, which implied that the TG levels between the breeds will not differ. However this was not the case in the current study as Triglycerides (TG) values were greater in LW x LR than in the SAWIP. Similarly the results are in contrast with (Mersmann *et al*. 1982) who reported that there was no difference between lean and obese pigs in serum CHOL.

5.4. Conclusion

The results of the study indicated that feeding OPBG silage altered the profile of blood metabolite profiles of metabolites that signify changes in overall energy, protein and N metabolism in the breeds. The results are rejecting the null hypothesis, given that the metabolites we maintained within the normal range to satisfy changes in energy, protein and N metabolism. Therefore ensiled OPBG inclusion in the standard growing pig diets can alter and maintain the blood metabolites within the normal range in exotic and indigenous pigs.

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1. Overall discussion

The overall study aimed to evaluate the nutritive value of buffalo grass-orange pulp silage as alternative feed to partially replace the expensive conventional ingredients in commercial growing pig diets which are increasingly beyond the reach of resource poor farmers. The overall results indicate that wet Orange pulp and dry buffalo grass mixed with exogenous enzymes to reduce dry matter content, improved fermentation characteristics such as the water-soluble carbohydrate content and break down the fibre matrix. When silage DM content is less than 300 g/kg, conditions for clostridial activity are favourable, resulting in high losses and silage of low nutritional value (McDonald *et al*., 1991). The DM content of freshly chopped orange pulp was 210 g/kg DM, ideal for ensiling with dry buffalo grass as an absorbent. The crude protein of our orange pulp at pre-ensiling was in agreement with those reported from previous studies (Hernandez *et al*., 1998, Karabulut *et al*., 2007, Aregheore and Siasan, 2008).

The pH was reduced to < 3.8 after 30 days of ensiling, and it was low enough for efficient preservation since it was with the range 3.8 and 4.2 according to (Kung and Shaver, 2001). Water-soluble carbohydrates are regarded as essential substrates for growth of LAB for proper fermentation (McDonald *et al*., 1991), and low levels may restrict LAB growth. The WSC concentration of orange pulp at pre-ensiling was 83.5 g/kg DM, which was higher than the minimum of 60 g/kg required for efficient fermentation (Jaakkola *et al*., 1991). Well preserved silage should contain 80 to 120 g/kg DM of LA concentrations (De Brouwer *et al*., 1991). Zobell *et al*. (2004) also stated that good silage has lactic acid levels ranging from 30 to140 g/kg, and those of the present study were lower than that threshold, which implies that the silage was poorly fermented thus poorly preserved.

The results on enzyme inclusion are supported by reports that cell wall degrading enzymes reduced fibre content in forage at ensiling (Sheperd and Kung, 1996; Meeske *et al*., 1999; 2002; Colombatto *et al*., 2004). The extent to which this occurs is difficult to measure accurately because ensiling probably solubilizes part of the non-starch polysaccharide fraction, so that polysaccharides that are recovered in NDF and ADF fractions would differ before and after ensiling, as observed by De Vries *et al.* (2012). The aerobic stability study was conclusive. The CO_2 production (0.41 – 0.34 g/kg DM) obtained in this study is an indication of DM

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losses and the extent of aerobic stability (McDonald *et al*., 1991). Ensiling with exogenous enzymes could help improve aerobic stability.

The results on apparent digestibility were in agreement with findings by (Stanogias and Pearce 1985; Kanengoni *et al*., 2002; Ndubuisi *et al*., 2008), which suggested that increase in fibre content in the diets will not improve digestibility. However that was not the case for LW x LR as showed an improvement in digestibility, but it was true for SAWIP. The poor performance of SAWIP in digestibility can also be attributed to poor ensiling, as it reduces the fibre content and improves the nutrient composition of the ensiled feed material. Jørgensen *et al*. (2010) suggested that fermenting ingredients, especially those with low digestibility and fermentability, can be a strategy to improve their energy value for pigs. Urriola and Stein (2012) suggested that the superiority of the Meishan pig (an indigenous Chinese pig) to digest insoluble fibre over the Yorkshire could be a result of differences in the intestinal microbial populations or types. The main indicator of the extent of fermentation is the quantity and quality of fermentation products produced. The inclusion of OPBG silage in a standard pig diet can improve digestibility of the pigs.

The results on growth performance measurements indicate extend of an effective utilisation of the feed offered by the pigs. The SAWIP growers' ability to consume more feed per body weight than the LW X LR growers may be a reflection of an adaptation to survive under marginal nutritional resources. The LW X LR would normally consume more feed than SAWIP growers because of their bigger body size and gut capacity (Whittemore *et al*., 2003; Thankers and Haq, 2009).Frank *et al.* (1993), Ndindana *et al*. (2002) and Kanengoni *et al*. (2004) reported a reduction in average daily gain and feed intake as high fibre inclusion increased. There were similar reductions in this study. The difference between these studies and the current study could be attributed to ensiling although other factors such as the difference experimental conditions and the differences in genotypes cannot be discounted. The observation that the LW x LR had a higher performance on the CON-CON and 15% OPBG-15% OPBG combination in the overall compared to the SAWIP was expected, however the observation that the LW x LR had a higher performance on the 30% OPBG-30% OPBG combination overall compared to the SAWIP was unexpected given the assertion that indigenous pigs digest and utilize high fibre diets better than the LW x LR crosses (Kanengoni *et al*., 2002; Ndindana *et al*., 2002). Feeding diets with and ensiled OPBG can improve growth performance.

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The results on blood metabolites indicate that glucose (GLU) is a component of many carbohydrates, therefore pigs fed high fibre diet are likely to have high CHOL levels from increasing availability and absorption a high GLU concentration resulting from increased availability of sugars from the fermentation of fibre (Ziemer *et al*., 2012). In contrast to these findings however, Pond *et al*. (1980) reported lower GLU levels in obese pigs than in lean or contemporary pigs. As OPBG level in the diet increased, there was no change in GLU levels, in contrast to the results of Frank *et al*. (1983) who reported a decrease in GLU levels as fibre levels increased.

Cholesterol (CHOL) in high proportion in the blood of low-density lipoprotein (which transports cholesterol to the tissues) is associated with an increased risk heart disease. The SAWIP tend to lay fat easily compared to the LW x LR, therefore in theory have higher serum CHOL levels. However, CHOL levels in the LW x LR and SAWIP were higher than the normal range .These results are in corroboration those of Ferna’ndez-Fi’gares *et al*. (2007) who found no differences in CHOL serum levels from Iberian (an obese genotype) and Landrace (a lean genotype) gilts. Ferna’ndez-Fi’gares *et al*. (2007)’s reports of an inverse relationship between dietary protein levels and CHOL agree with these findings. The outcomes could be attributed to the GLU levels which were within the normal range for both the SAWIP and LW x LR, therefore the high cholesterol level could be attributed to the increasing availability and absorption of high GLU concentration resulting from increased availability of sugars from the fermentation of fibre as reported by (Ziemer *et al*., 2012).

Creatinine (CREAT) is an energy substrate used by muscle and is a biomarker for renal function evaluation along with BUN (Faulkner and King, 1982). Creatinine is released from muscle in amounts proportional to muscle mass (Rassin and Bhatia, 1992; Ferna’ndez-Fi’gares *et al*., 2007). Higher CREAT levels in the LW x LR therefore relate to their increased lean mass. Urea, a principal product of the catabolism of protein and the levels of BUN can act as indicators of body protein status (Kohn *et al*., 2005) and efficiency of renal function. The BUN levels for pigs on all treatments in this study were within the normal range and only the SAWIP pigs on the CON diet had higher levels than on all other treatments. Blood urea nitrogen levels increase with dietary increases in protein. Since BUN levels have been used to determine protein requirements and lean tissue growth rates in pigs (Chen *et al*., 1995; Coma *et al*., 1995) thus results from the current study could be used to make dietary recommendations for the SAWIP.

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Triglycerides (TG) are an ester formed from glycerol and three fatty acid groups. Triglycerides are the main constituents of natural fats and oils. The CHOL increased with an increase in TG levels, there is direct proportionality between CHOL and TG. TG levels in the LW x LR were within normal range, however SAWIP TG levels were below the normal range when fed fibre diets. These results are in contrast with those of Ferna'ndez-Fi'gares *et al*. (2007) who found no differences in CHOL serum levels from Iberian (an obese genotype) and Landrace (a lean genotype) gilts, which implies that the TG levels between the breeds will not differ, which was not the case as Triglycerides (TG) values were greater ($P < 0.05$) in LW x LR than in the SAWIP. This can also be attributed to the increasing availability and absorption of high GLU concentration resulting from increased availability of sugars from the fermentation of fibre as reported by (Ziemer *et al*., 2012). Ensiled OPBG had a positive impact on the blood profiles of the pigs.

6.2. Overall conclusions

The results of this study indicate that mixing orange pulp with buffalo-grass improved quality of the mixed silage at 80:20 ensiling ratio. Exogenous enzymes are necessary to enhance the fermentation characteristics of the orange pulp silage, thus improving the silage quality therefore good quality orange pulp silage can be produced when treated with exogenous enzymes. The inclusion of orange pulp-buffalo grass in the standard growing pig diet at different levels improved feed intake, nutrient digestibility growth performance and blood metabolites profiles in South African Windsnyer-type and Large White- Landrace crossed growing pigs. The exotic LW x LR pigs utilised diets containing orange pulp-buffalo grass mixed silage efficiently compared to the SAWIP pigs. The orange pulp and buffalo-grass can be utilised as an alternative feed to partially replace the expensive conventional ingredients in commercial growing pig diets which are increasingly beyond the reach of resource poor farmers.

6.3. Recommendations

Based on the results of the study, orange pulp-buffalo-grass silage mixture can be introduced to rural based farmers as an alternative feed for seasons of feed scarcity on indigenous and exotic pigs at low inclusion levels in formulated pig grower diets. This mixture can also be utilized for exotic pigs at high inclusion as it was proven by the measured performance of the pigs in terms of improved feed intake, nutrient digestibility growth performance and blood metabolites profiles. The exotic breeds proved that they can better utilize fibre diets than the indigenous pigs.

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Further investigation on the mixture and its effect on a large scale are necessary:

I. To identify optimum orange pulp-buffalo-grass mixture with different inoculants.
II. To identify optimum orange pulp-buffalo-grass mixture at different ratios.
III. To explore different inclusion levels and their impact on rate of passage, carcass traits and blood metabolites response.
IV. To evaluate the microbial population response of pigs fed diets containing the optimum mixture.
V. To test the mixture at different feed forms and feeding methods.

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6.5. Appendices

Appendix 1: Analysis of fermentation dynamics, nutrient composition and aerobic stability of the ensiled Buffalo Grass (*Cenchrus Ciliaris*) and Orange Pulp (*Citrus Sinensis*) mixture silage (%).

DAYS	TRT	WSC	LA	pH	DM	MOISTURE	ASH	PROTEIN	EE	NDF	ADF	HEMI
0	1	7.14	0.08	4.24	89.99	10.01	4.26	4.51	1.69	30.07	20.22	9.85
0	1	7.12	0.07	4.22	89.95	9.99	4.25	4.49	1.68	30.05	20.19	9.86
0	2	9.31	0.12	4.32	90.04	9.96	4.33	5.37	1.39	30.64	19.05	11.59
0	2	9.28	0.11	4.31	90.03	9.8	4.3	5.35	1.37	30.62	19.04	11.58
7	1	4.19	0.09	4.42	86.97	13.03	5.35	6.97	1.22	31.99	21.31	10.68
7	1	4.17	0.08	4.39	86.95	12.99	5.33	6.95	1.21	31.98	21.29	10.69
7	2	5.87	0.07	4.44	87.49	12.51	4.7	6.82	1.2	31.88	19.44	12.44
7	2	5.85	0.07	4.42	87.48	12.49	4.68	6.79	1.19	31.87	19.42	12.45
15	1	6.1	0.09	3.59	90.76	9.24	6.58	7.82	1.16	42.96	27.69	15.27
15	1	5.99	0.07	3.58	90.74	9.21	6.55	7.82	1.15	42.94	27.65	15.29
15	2	4.68	0.09	3.63	88.04	11.96	4.84	7.54	1.48	31.54	20.64	10.9
15	2	4.66	0.08	3.61	88.01	11.95	4.81	7.53	1.46	31.53	20.63	10.9
30	1	2.9	0.14	3.77	89.48	10.52	4.96	5.61	1.61	30.35	22.07	8.28
30	1	2.89	0.12	3.76	89.47	10.49	4.95	5.6	1.6	30.34	22.04	8.3
30	2	3.16	0.22	3.80	89.86	10.14	4.34	6.23	1.5	30.8	24.53	6.27
30	2	3.14	0.21	3.79	89.84	10.14	4.32	6.21	1.49	30.77	24.51	6.26

TRT= Treatment.

1= OPBG (Orange pulp + buffalo grass with no enzyme)

2= OPBGE (Orange pulp + buffalo grass + enzyme)

Appendix 2: Analysis of ensiled orange (*Orange Sinensis*) pulp-buffalo-grass (*Cenchrus Ciliaris*) silage on nutrient digestibility (%) of SAWIP (South African Windsnyer pigs) and LW x LR (Large White-Landrace) crossed pigs.

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BREED	SEX	Diet	DM	OM	CP	EE	NDF	ADF	HEMI
LWXLR	F	T1 15%	67.81	62.64	61.92	57.62	81.88	58.85	70.37
LWXLR	F	T2 30%	67.58	54.51	62.61	73.13	71.27	58.02	64.64
LWXLR	F	T2 30%	53.57	68.38	54.84	69.51	89.40	73.17	81.28
LWXLR	F	T2 30%	54.37	76.83	53.51	73.29	83.40	70.77	77.08
LWXLR	F	T3 CON	63.78	63.67	63.29	79.83	66.53	50.47	58.50
LWXLR	M	T1 15%	57.96	82.71	59.48	78.39	81.22	58.52	69.87
LWXLR	M	T1 15%	63.57	70.25	58.90	69.98	87.93	58.15	73.04
LWXLR	M	T1 15%	58.38	77.74	61.57	78.75	88.52	63.14	75.83
LWXLR	M	T2 30%	59.61	73.68	53.82	69.93	89.15	66.04	77.59
LWXLR	M	T3 CON	59.78	60.66	63.05	71.55	72.60	47.79	60.20
LWXLR	M	T3 CON	62.51	72.61	59.46	65.32	70.48	51.37	60.93
LWXLR	M	T3 CON	59.96	73.44	56.76	64.79	75.50	50.42	62.96
SAWIP	F	T1 15%	58.00	66.85	56.50	74.19	87.92	66.80	77.36
SAWIP	F	T1 15%	80.80	53.17	79.20	50.40	56.78	40.24	48.51
SAWIP	F	T1 15%	57.18	58.89	55.98	68.76	78.64	60.77	69.70
SAWIP	F	T1 15%	57.43	59.38	60.40	55.15	81.44	64.32	72.88
SAWIP	F	T2 30%	56.13	60.01	70.42	59.52	75.07	62.20	68.63
SAWIP	F	T2 30%	58.27	58.86	60.61	77.42	76.11	64.31	70.21
SAWIP	F	T2 30%	65.17	49.11	68.09	85.14	77.85	65.80	71.82
SAWIP	F	T3 CON	78.74	46.96	77.24	55.19	54.50	38.23	46.36
SAWIP	F	T3 CON	58.93	53.91	59.60	57.29	76.66	50.62	63.64
SAWIP	F	T3 CON	59.43	60.07	62.80	77.01	66.90	47.72	57.31
SAWIP	M	T2 30%	54.65	69.41	51.38	79.26	83.54	67.52	75.53
SAWIP	M	T3 CON	89.22	47.11	86.85	51.44	46.87	29.08	37.98

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Appendix 3: Analysis of orange (*Citrus Sinensis*) pulp-buffalo-grass (*Cenchrus Ciliaris*) silage inclusion levels on the growth performance of SAWIP and LW x LR crossed pigs.

BREED	Diet	ADFI(kg/day)	ADG(kg/day)	AG:F (kg/day)	ABG(kg/day)
LWXLR	T1 15%	1.41	0.53	0.20	36.8
LWXLR	T1 15%	1.44	0.57	0.25	33.5
LWXLR	T1 15%	1.74	0.64	0.21	28.5
LWXLR	T1 15%	1.57	0.77	0.36	32.0
LWXLR	T2 30%	1.12	0.60	0.40	30.5
LWXLR	T2 30%	1.56	0.66	0.28	37.0
LWXLR	T2 30%	1.77	0.81	0.32	30.5
LWXLR	T2 30%	1.74	0.85	0.36	28.3
LWXLR	T3 CON	1.64	0.43	0.08	38.3
LWXLR	T3 CON	1.50	0.43	0.11	40.5
LWXLR	T3 CON	1.59	0.44	0.09	30.0
LWXLR	T3 CON	1.13	0.56	0.39	28.5
SAWIP	T1 15%	1.41	0.45	0.15	23.6
SAWIP	T1 15%	1.58	0.54	0.18	39.0
SAWIP	T1 15%	1.55	0.58	0.22	42.1
SAWIP	T1 15%	1.68	0.59	0.19	20.7
SAWIP	T2 30%	1.20	0.40	0.17	23.2
SAWIP	T2 30%	1.32	0.43	0.16	37.3
SAWIP	T2 30%	1.52	0.53	0.19	23.7
SAWIP	T2 30%	1.71	0.72	0.27	43.9
SAWIP	T3 CON	1.17	0.45	0.24	19.2
SAWIP	T3 CON	1.32	0.49	0.22	27.1
SAWIP	T3 CON	1.39	0.52	0.21	34.7
SAWIP	T3 CON	1.80	0.63	0.19	46.8

ABW: average body weight, ADFI: average daily feed intake, ADG: average daily gain, AG: F: average gain to feed ratio.

Appendix 4: Analysis of weekly pig performance

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			Weeks				Average performance
Parameter	**BREED**	**Diet**	**1**	**2**	**3**	**4**	
Body Weight	LWXLR	T1 15%	29.88	31.88	34.63	38.25	34.06
	LWXLR	T2 30%	30.25	33.63	36.25	41.63	35.94
	LWXLR	T3 CON	29.88	31.75	33.00	35.00	32.44
	SAWIP	T1 15%	29.00	30.88	32.63	35.63	32.31
	SAWIP	T2 30%	29.50	31.38	33.13	35.38	32.44
	SAWIP	T3 CON	29.66	31.91	33.75	36.91	33.28
Daily gain	LWXLR	T1 15%	0.40	0.29	0.39	0.52	0.63
	LWXLR	T2 30%	0.21	0.27	0.25	0.43	0.54
	LWXLR	T3 CON	0.46	0.48	0.38	0.77	0.73
	SAWIP	T1 15%	0.32	0.27	0.25	0.32	0.52
	SAWIP	T2 30%	0.13	0.27	0.18	0.29	0.46
	SAWIP	T3 CON	0.28	0.32	0.26	0.45	0.56
Feed intake	LWXLR	T1 15%	1.55	1.46	1.46	1.69	1.54
	LWXLR	T2 30%	1.57	1.59	1.40	1.65	1.55
	LWXLR	T3 CON	1.62	1.56	1.41	1.60	1.55
	SAWIP	T1 15%	1.48	1.47	1.32	1.49	1.44
	SAWIP	T2 30%	1.48	1.44	1.42	1.52	1.47
	SAWIP	T3 CON	1.54	1.52	1.39	1.56	1.50
Feed conversion ratio	LWXLR	T1 15%	0.25	0.19	0.27	0.31	0.25
	LWXLR	T2 30%	0.12	0.17	0.18	0.26	0.18
	LWXLR	T3 CON	0.26	0.31	0.28	0.50	0.34
	SAWIP	T1 15%	0.20	0.18	0.19	0.22	0.20
	SAWIP	T2 30%	0.16	0.19	0.13	0.19	0.17
	SAWIP	T3 CON	0.19	0.21	0.19	0.29	0.22

Appendix 5: Analysis of Serum metabolites of South African Windsnyer-type (SAWIP) and Large White x Landrace (LW x LR) cross fed diets containing ensiled OPBG mixture.

BREED	Diet	GLU	CHOL	UREA	CREAT	TRIGLY
LWXLR	T1 15%	87.55	100.53	26.25	0.87	45.14
LWXLR	T1 15%	117.48	116.00	34.05	0.98	65.49
LWXLR	T1 15%	108.83	123.73	41.26	0.97	51.33
LWXLR	T1 15%	204.86	143.06	31.53	1.19	77.88
LWXLR	T2 30%	164.68	112.13	35.14	0.96	49.56
LWXLR	T2 30%	119.46	127.59	27.99	1.02	60.18
LWXLR	T2 30%	78.02	131.46	26.97	1.00	53.10
LWXLR	T2 30%	95.14	143.07	34.71	1.22	71.69
LWXLR	T3 CON	90.81	69.60	20.00	0.64	30.98
LWXLR	T3 CON	94.23	85.06	28.05	0.95	41.60
LWXLR	T3 CON	103.06	108.26	32.31	1.15	41.60
LWXLR	T3 CON	118.38	112.13	33.81	1.01	45.14
SAWIP	T1 15%	87.02	77.33	55.44	0.66	24.78
SAWIP	T1 15%	83.95	85.06	41.86	0.76	21.24
SAWIP	T1 15%	98.38	104.40	43.06	0.84	41.60
SAWIP	T1 15%	167.73	112.13	43.06	0.79	60.18
SAWIP	T2 30%	107.39	88.93	37.84	0.76	31.86
SAWIP	T2 30%	92.25	108.26	42.88	0.86	39.83
SAWIP	T2 30%	118.02	112.13	34.95	0.79	40.71
SAWIP	T2 30%	78.56	112.13	38.20	1.10	30.09
SAWIP	T3 CON	117.12	81.20	46.61	0.64	37.17
SAWIP	T3 CON	87.03	96.66	53.87	0.66	44.25
SAWIP	T3 CON	92.25	108.26	41.56	0.68	20.36
SAWIP	T3 CON	152.79	143.06	66.13	0.86	54.87

GLU mg/dL - glucose; BUN mg/dL - blood urea nitrogen; CREAT mg/dL - creatinine; CHOL mg/dL - cholesterol; TG mg/dL – triglycerides.

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