POTATO HASH SILAGE AS AN ALTERNATIVE FEED RESOURCE FOR SMALLHOLDER LIVESTOCK PRODUCTION

BY

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Declaration

I declare that this thesis submitted by me to the University of the Free State for the degree **Doctor of Philosophy in Sustainable Agriculture** is my on independent work and has not previously been submitted by me for a degree at any other University / Faculty. I further cede copyright of this thesis in favour of the University of the Free State.

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B.D. Nkosi

Abstract

Several experiments were conducted to evaluate the ensiling of potato hash (PH) during the period. In the first experiment, a laboratory study was conducted to determine the nutritive value and ensiling potential of PH with poultry litter (PL) and ground hay as absorbents, and whey and molasses as additives. Triplicate samples of PH, PL and hay were collected and sampled for nutritive composition. Mixtures of 800 g PH/kg + 200 g/kg (as is basis) of either PL or hay were produced and treated with: no additive, whey and molasses. The experiment was conducted in a 2 x 3 factorial design (2 absorbents x 3 additives). Mixtures were ensiled in 108 anaerobic jars (1.5L) with 18 jars per treatment, and were stored at 24 - 28°C room temperature. Sampling was done on days 0, 4, 10, 20, 40, 60 and 90 for the determination of fermentation quality and nutritive value of the silage. Further, an aerobic stability test was done on day 90 by exposing silage to air for 5 days.

The results showed that PH had 845 g/kg moisture, 11.4 metabolizable energy (ME) MJ/kg, 105 g crude protein (CP) /kg dry matter (DM) and 704 g starch/kg DM. Ensiling PH with ground hay compared to PL as an absorbent, resulted in a better quality silage as indicated by improved fermentation characteristics and chemical composition. Whey and molasses addition improved the nutritive value and the fermentation quality of PH silage but the aerobic stability was not improved.

In the second experiment, potato hash silage (treated with no additive, whey and molasses) was produced by mixing 800 g PH/kg with 200 g hay/kg (as is basis), and ensiled in 210 L drums for 90 days, and the fermentation quality of the silages was determined thereafter. Diets containing either potato hash silage (PHS) or maize

(Zea mays) silage (MS) were formulated and fed *ad libitum* to 32 South African Dorper lambs (23.5 ± 0.873 kg live weight) for 63 days. A digestibility study was conducted during the last week of the study. Furthermore, digestibility of the 3 PHS were compared using 9 sheep in a 3 x 3 Latin square design. The untreated potato hash silage (UPHS) was poorly fermented as indicated by higher (*P*<0.05) concentration of butyric acid, ammonia-N and pH compared to the other silages. Higher (*P*<0.05) dry matter intake (DMI) and daily gains (218 and 250 g/d) were obtained in lambs fed maize silage diet (MSd) and molasses treated potato hash silage diet (MPHSd) compared to the other diets. Nutrient digestibility was lower (*P*<0.05) in the UPHS diet compared to the other dietary treatments. The fermentation quality of PH was improved with whey and molasses addition. However, the growth performance was improved (*P*<0.05) with the MSd and MPHSd, suggesting that MPHSd can replace MSd in lamb diet at 20 % dietary inclusion level without any adverse effect on animal performance.

In the third experiment, PH was mixed with wheat bran (70:30) as fed basis and ensiled in 210 L drum for 90 days. Three types of PHS : control, *bonsilage forte* (BF) and *Lalsil Fresh LB(LFLB)* were produced. After 3 months, the silos were opened and sampled for fermentation characteristics. Diets were produced by mixing PHS with soybean meal (90:10) as fed basis and a digestibility study was conducted using five South African Mutton Merino rams (37.2 ± 2.21 kg liveweight) per diet. Inoculating PHS with BF and LFLB reduced (*P*<0.05) pH, WSC, butyric acid and ammonia N while increasing the concentration of lactic acid compared to the control. A higher concentration of acetic acid was obtained with LFLB inoculation, which improved the aerobic stability of silage compared to the other silages. Intakes of dry (DM) and organic matter (OM) were not affected. Gross energy (GE) and CP of

silage were improved (*P*<0.05) with BF and LFLB inoculations. Inoculants increased CP, GE and amylase treated neutral detergent fibre (aNDF) digestibility, but did not alter DM or OM digestibility. Inoculating silage with BF improved (*P*<0.05) digestibility of ether extract compared to the other treatments, and both inoculants improved (*P*<0.05) N intake and retention compared to the control. It is concluded that BF and LFLB improved silage fermentation and diet digestibility of CP, aNDF and gross energy. Inoculation with LFLB improved aerobic stability whilst BF inoculation reduced it.

In the fourth experiment, totally mixed rations (TMRs) that contained 804 g PH/kg were ensiled in 1.5 L jars with or without *Lalsil Fresh Lactobacillus buchneri* (LB) for 3 months. Jars were opened on days, 0, 3, 7, 10, 21, 45, 60 and 90 of ensiling and sampled for fermentation and chemical composition determinations. Aerobic stability was determined on day 90 of ensiling. Treatments were LB treated TMR (LB-TMR) and untreated TMR (U-TMR). Furthermore, three TMRs that contained 801 g/kg of either maize (280 g DM/kg) or PH (as fed basis) were ensiled for 90 days in 210 L drums for lamb growth and digestibility studies. The ensiled TMRs were: Maize TMR (M-TMR), U-TMR and LB-TMR and were fed to 24 South African Dorper lambs (20± 0.152 kg live weight) that were allocated in 8 lambs per diet. Inoculation with LB decreased (*P*<0.05) pH, butyric acid, NH_3-N, fibre fractions, CO_2 production and yeast population while lactic acid, acetic acid and propionic acid concentrations were increased (*P*<0.05) compared to U-TMR silage. The ensiled LB-TMR was aerobically more stable than U-TMR silage as indicated by lower (*P*<0.05) CO_2 production and yeast population and higher concentrations of acetic acid. Higher (*P*<0.05) feed intake, average daily gain (ADG), nutrient digestibility and N retention occurred in LB-TMR silage compared to the other silages. It was concluded that LB is

effective in producing a better quality PHS, as indicated by improved fermentation, aerobic stability, lamb growth performance and digestibility of LB-TMR silage.

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List of abbreviations

AA	acetic acid
ADF	acid detergent fibre
ADG	average daily gain
ANOVA	analysis of variance
BA	butyric acid
BF	*bonsilage forte*
BW	body weight
Ca	calcium
CO_2	carbon dioxide
Cont	control
CP	crude protein
CPI	crude protein intake
CF	crude fibre
CFU/g	colony forming unit per gram
d	day
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
DoA	Department of Agriculture
EE	ether extract
FAO	Food and Agricultural Organization
FCR	feed conversion rate
FM	fresh matter

g	gram
g DM/d	gram of dry matter per day
g/kg DM	gram per kilogram of dry matter
GDP	Gross Domestic Product
GE	gross energy
h	hour
IVOMD	in vitro organic matter digestibility
kg	kilogram
L	litre
LA	lactic acid
LAB	lactic acid bacteria
LB-TMR	L. buchneri treated total mixed ration
L. buchneri	*Lactobacillus buchneri*
LFLB	*Lalsil Fresh Lactobacillus buchneri*
LSD	least significant difference
m	month
ME	metabolizable energy
MJ/kg	megajoules per kilogram
Ml	mililitre
mm	milimetre
MPHS	molasses potato hash silage
MS	maize silage
M-TMR	maize total mixed ration
N	nitrogen
n	number

aNDF	amylase treated neutral detergent fibre
NH_3-N	ammonia nitrogen
OM	organic matter
P	phosphorus
PH	potato hash
PHS	potato hash silage
PHHS	potato hash hay silage
PHPLS	potato hash poultry litter silage
RPF	resource poor farmer
SEM	standard error of mean
TDN	total digestible nutrient
TMR	total mixed ration
UPHS	untreated potato hash silage
U-TMR	untreated total mixed ration
VFA	volatile fatty acid
WPHS	whey potato hash silage
WSC	water-soluble carbohydrates

CHAPTER 1

GENERAL INTRODUCTION

1.1. Gauteng Province and agricultural production

The Gauteng Province is one of the nine provinces of South Africa, which lies on the elevated plateau of the interior (Highveld) and covers 1.4 % of the country. It is geographically the smallest and most urbanized province with up to 17 % of its land classified as being in urban land uses (Statistics South Africa, 2002), while only 19 % of the total land is for livestock grazing (Provincial Fact Sheet, 1997), which does not support efficient livestock grazing. There has been a 4.1 % per year growth in population since 1996, partly (30 %) attributable to the high number of migrants into the province in search of employment (GPG, 2004). The province has a population of 9 million, with 97 % living in urban areas with 40 % of households earning less than R 1000.00 per month and the province is contributing 33.9 % to the national Gross Domestic Product (GDP) (Statistics South Africa, 2005). Moreover, a considerably new urban population growth in the province is projected to reach 16.5 million by 2010 (Rogerson, 1993).

Population change exacerbates pressure on resources and service delivery, and in so doing creates pressure on the development of land and contributes to land transformation as more people require space and housing. The percentage of people living below the poverty line is 28 % for urban areas and 58 % for rural areas. Agriculture in the province is geared to provide the cities and towns with fresh products daily, and accounts for 1 % of total employment in the province (GPG, 2002). This sector generates a gross farming income of R3 753 332 000 with animal

and animal products representing 11.7 %, which is higher than the field crops (2.3 %) and horticulture (5.7 %) (GADS, 2006, SAGIS, 2005). Farming in this province competes directly for scarce city space with the pressing demands for shelter for the poor, and is seen as enhancing food security, provides income and employment for both poor and middle-income dwellers, and contributing to an ecologically sound and urban environment (Rogerson, 2003). As a result of high population pressure, biomass yield of the community rangeland in the province becomes insufficient to support the requirements of ruminants.

1.2 Problem statement

Semi-intensive and intensive ruminant production is characterised by a high demand and dependence on mixed cereals. Cereals are imported, requiring foreign exchange, and costs of imported feedstuffs rise steadily especially during times of shortages (Briedenhann, 2008). This increases feed costs which represents 60 – 80 % of the economic inputs in the livestock production system (Henning, 1998). The main problem however, is that human consumptions has priority for the use of cereals and many of South Africa households are not even self-efficient in cereals for human consumption (Watkinson & Makgetla, 2002). Moreover, there is a rapid growing demand for animal products in South Africa (Stroebel, 2004) and continuing interest in reducing the amount of grain fed to ruminants, which are pressuring ruminant producers to find alternative energy-rich feedstuffs.

Limited available land for grazing has been recognized as one of the major constraints to ruminant production under resource poor farmers (RPF) in the Gauteng Province of South Africa. This is because the accelerated rate of urbanization in this

province progressively reduces grazing areas. Given the limitations of land and the expanding of the housing sector in this province, ruminants under RPF production systems are allowed to roam uncontrolled on marginal land where they accentuate land degradation. More-over, there is a bio-security concern that animals end-up ingesting plastic bags and strings (Dreyer *et al*., 1999), and dangers imposed by the animals grazing on the roads or streets (Nzimande, 2005). Chronic sub-clinical malnutrition is one of the prime causes for low productivity in ruminants under these systems (Von Hagen, 2001). Finding sufficient feeds for livestock is often difficult, particularly in the dry season (Smith, 2001), and this reduces the contribution livestock make to poor people's livelihoods (Randolph *et al*., 2007).

There is a large number of food processing factories in South Africa, and most situated in the Gauteng Province. The processing plants are dependent upon agriculture for raw materials, such as sunflower seeds, peanuts, potatoes, maize, among others. This has led to the availability of agro-industry by-products, which have not been exploited commercially, and pose an option to mitigate feed flow problems in this province. These food processors have challenges of by-product disposal, which can be an economical and environmental problem. The South African government does not allow food processors to simply discard these by-products, which are therefore sometimes distributed free or at a small charge. It is however, possible that these by-products could be used effectively by the RPF as part of traditional feeding systems if economic methods could be identified to treat them.

The use of by-products from the food processing industry can be a less expensive source of nutrients suitable for ruminant feeding because of the ruminant's capacity to digest fibre-rich feedstuffs (Boucque & Fiems, 1988). According to

Kajikawa (1996) some by-products have specific properties that might be lacking from grains, and their dietary inclusion might provide a diet with a range of nutrients that could not be supplied by the grain or forage alone. In addition, the use of these by-products can be an alternative for the food industry to diminish dependence of livestock on grains that can be consumed by humans (Bampidis & Robinson, 2006), and to eliminate costs of waste disposal through nutrient cycling of the by-products from numerous urban sources (Rogerson, 1993). However, major constraints in using agro-industry by-products for livestock feeding are the variability in their composition, and high moisture contents (Boucque & Fiems, 1988), which make their handling difficult and favour microbial deterioration (Moon, 1981). This further increases transport costs and limits their use as feed even though they are given free of charge at the processing factory.

1.3. Motivation

Potato hash, a by-product from Simba (PTY LTD), (a food processing industry which is based in Isando, Gauteng Province) that derived from the production of snacks and chips, is one of the available by-products that are not efficiently utilized. This by-product contains starch, peels and relatively small amounts of yellow maize and fats. There are currently farmers who are collecting potato hash for feeding their livestock (Vosloo, 2010, personal communication) and there is currently no data on the nutritive value and performance of animals when fed on potato hash. An estimated amount of 50 t per day is produced in South Africa. However, if it is not consumed in a short period of time by animals, it gets mouldy and becomes useless as animal feed. Moreover, feeding the by-product to animals without pre-treatment is prohibited by the South African law (Act 36, 1947) because of the health concerns to animals. Some

studies have demonstrated that treating or processing of agro-industry by-products reduce the health and bio-security concerns that may be involved, and has been recommended to be essential in improving their utilization in livestock feeding systems (Smith *et al*., 1988). Production of meal from potato waste products is technically feasible, but high drying and processing costs are economic deterrents (Charmley *et al*., 2006, Tawila *et al*., 2008). Consequently, ensiling can be considered as an efficient way of preserving high moisture by-products if all essential principles of ensiling are followed (Kayouli & Lee, 1999, Cao *et al*., 2009). For proper ensiling, a material must have high concentrations of water soluble carbohydrates (WSC), low buffering capacity, a dry matter (DM) content of 250 to 400 g/kg and adequate lactic acid bacteria (LAB) prior to ensiling (Wilkinson, 2005). However, potato by-products may contain relatively low DM, WSC and LAB (Nicholson *et al*., 1977, O`Kiely *et al*., 2002, Okine, 2007) due to processing (Moon, 1981). Consequently, silage additives are used to improve the concentrations of WSC and LAB prior to ensiling (McDonald *et al*., 1991).

A good quality well preserved silage has a pH value of less than 4.2 which provides stability of the silage, a value of less than 100 g ammonia-N/kg total N, a value of less than 10 g/kg DM for butyric acid, and efficient conversion of WSC to lactic acid (Kung & Shaver, 2001, McDonald *et al*., 2002). Consequently, silage fermentation aids (e.g. bacterial inoculants) have been used to increase the rate of acidification of ensiled forages in many investigations (Weinberg & Muck, 1996). Generally, potato by-products are ensiled with or without silage additive or bacterial inoculants (Okine *et al*., 2005, Okine *et al*., 2007, Oshita *et al*., 2007) but an increase in temperature of the silage occurs, when silage was exposed to air. Research has shown that heterofermentative lactic acid bacteria (LAB) inoculants improve aerobic

stability of silage through high production of acetic acid and that this subsequently improves animal performance (Driehuis *et al.*, 2001, Ranjit *et al*., 2002).

Some studies have reported improved animal performance when ensiled potato by-products were included in ruminant diets. Aibibula *et al*. (2007) and Okine *et al*. (2005) reported that the high energy digestibility of potato pulp silage was closely associated with high contents of starch, which can be more slowly degraded by rumen micro-organisms than wheat starch (Moteils *et al*., 2002). Hanada *et al*. (2004) found that the daily weight gain of growing steers was satisfactory when corn grain was substituted with potato pulp silage.

Silage making in South Africa has been long practiced mostly by the commercial sector, using high quality crops such as maize, and cultivated pastures. This preservation method relies on heavy equipments, both to dig storage pits and to compress the forage, which makes it difficult to be adopted by the RPF. Consequently, there are methods such as the small-scale silage bag method, whereby forages are stored in large bags made from polythene, and the big drum (210 l drum) which can offer a better solution to the farmers. Plastic bags and drums are relatively inexpensive and ensiling can be done manually by a few workers, and the bag or drum units can be used individually according to feeding requirements. Due to the fact that the RPF in the Gauteng Province do not have facilities for storing feeds, ensiling of feeds in drums or plastic bags may a possible solution. According to Chin (2002), there are several important roles played by silage to smallholder farmers, which are:

1. as feed reserve for future utilization: some farmers in South Africa use silage as a method for fodder conservation to overcome feed shortages in the dry

season. This practice is very rare under RPF systems due to lack of knowledge, finance, labour, etc.

2. as routine feed to increase productivity of animals: silage is also routinely fed to increase the productivity of high producing animals (e.g. beef and dairy cattle) by providing nutrients necessary to nutritionally balance existing diets. Many commercial dairy operations in South Africa produce and feed maize silage to dairy herds. However, producing maize for silage production purposes is a difficult option to be made by the RPF because maize is grown soley for home consumption. Moreover, the lack of land for the cultivation of maize is another factor limiting RPF in producing high quality silage.

3. as means to utilize excess growth of pasture for better management and utilization: ensiling is a good option to utilize the excess forage if stocking density is not increased and hay making particularly during the rainy period is also not practical. Harvesting excess growth for ensiling enables proper management of these pastures as well.

4. as a way of storing and enabling extended use of potentially unstable material: ensiling enables storage of food by-products that are perishable and unstable which, unless dehydrated or ensiled, can only be for immediate or at most very short term use. Since many of these by-products are high moisture content, sun-drying is difficult especially in the tropical wet areas and artificial drying may be costly or unavailable.

Due to the high moisture content of potato hash, its ensiling requires materials with absorbent properties (e.g. chopped barley straw, sugar beet pulp, cereal grains),

which have been successful added to various high moisture forages at ensiling to reduce DM losses and improve nutritive value (Jones *et al*., 1990, Ferris & Mayne, 1994, Khorvash *et al*., 2006). However, these materials are not readily available to farmers in South Africa, and alternative materials that are accessible to the farmers are required. Therefore poultry litter (PL), *Eragrostis curvula* hay and wheat bran can be used as absorbents for ensiling potato hash. Poultry litter has been shown to improve the crude protein (CP) content of maize silage (Fontenot *et al*., 1975) and sorghum silage (Al-Rokayan *et al*., 1998), and is often used by the RPF as a CP supplement for their livestock. However, its use in animal nutrition is prohibited in South Africa and poultry litter must be processed before it may be considered as an animal feed source (Act 36, 1947). Research has proved that ensiling improves the quality of PL by reducing pathogenic agents through fermentation (Al-Rokayan *et al.,* 1998).

In addition, other two by-products (i.e. whey and sugarcane molasses) were selected as additives for ensiling PH. Molasses, a waste product of sugar production, has been widely used as a silage additive (Weinberg *et al*., 2008, Kwak *et al*., 2009, Nkosi *et al*., 2009a). Whey from cheese production, contains large amounts of LAB and lactic acid, and has also been used for silage making (Dash *et al*., 1974, Bautista-Trujillo *et al*., 2009, Zobell *et al*., 2004).

Furthermore, preparing total mixed rations (TMR) silage is one practice whereby food by-products are stored and utilized as animal feeds, and has been reported to also improve the aerobic stability of by-product silage (e.g. Nishino *et al*., 2003). This practice can also avoid energy costs associated with drying, and may improve odours and flavours of unpalatable feed resources through fermentation in a silo. Total mixed rations (TMR) containing 800 g/kg potato hash were formulated, ensiled, and fed to lambs in comparison with TMR ensiled with maize. In addition,

microbial inoculants such as Lalsil fresh *Lactobacillus buchneri* (LFLB), which had shown promising results in improving the fermentation quality and aerobic stability of maize silage in South Africa (Nkosi, 2009b), has been tested on potato hash silage. In addition, *bonsilage forte* (BF) was also tested.

Data on the nutritive value of potato hash, its ensiling with either molasses or liquid whey as additives, and PL and hay as absorbents is limited. Furthermore, microbial inoculants such as Lalsil Fresh *Lactobacillus buchneri* (heterofermentative LAB) and *bonsilage forte* (homofermentative LAB) were also selected to improve the fermentation and aerobic stability of ensiled potato hash.

1.4. Study objectives

Driven by the facts that: i) there is currently no data pertaining the nutritive value and the beneficial effects of feeding ensiled potato hash to livestock, ii) there is a general lack of awareness of the possible uses of potato hash as livestock feeds and iii) ensiling is the most affordable method to be adopted by the farmers for preserving potato hash, it was therefore imperative to conduct research on the use and preservation of potato hash. The objectives of this study were therefore:

1. to determine the chemical composition of potato hash.

2. to evaluate the potential nutritional value of silage obtained by ensiling potato hash and poultry litter as a protein source, with and without the use of whey and molasses (silage additives/fermentation stimulants).

3.   to determine the effect of adding combinations of hay, poultry litter, molasses or whey to potato hash on silage quality, and aerobic stability.

4.   to evaluate the effect of a heterofermentative LAB, *Lalsil Fresh LB* and a homofermentative LAB, *bonsilage forte* (BF) on the fermentation and aerobic stability of potato hash silage.

6.   to determine the growth performance and nutrient utilization by lambs fed potato hash silages treated with either whey or molasses.

7.   to compare the growth performance and nutrient utilization by lambs fed ensiled total mixed rations (TMR) that contained potato hash with that contained maize.

8.   to determine the effect of Lalsil Fresh LB on the fermentation quality of ensiled TMR containing potato hash

1.5. Hypothesis

The following hypotheses are tested:

a)   the best storage method for potato hash would be anaerobic ensiling to avoid the respiration losses and inhibit the growth of the putrefactive micro-organisms.

b)   the addition of poultry litter and hay to potato hash at ensiling will improve the DM content and facilitate a lactic acid fermentation.

c)   additives such as molasses, whey and Lalsil Fresh *LB* and *bonsilage forte* will improve the fermentation and aerobic stability of potato hash silage, the growth performance and nutrient digestibility in lambs.

d)   using ensiled potato hash compared with maize silage in ruminant systems under RPF systems will significantly increase lamb productivity, nutrient use efficiency and, thereby the sustainability of these systems.

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CHAPTER 2

Reviewed literature

2.1. Agro-industry by-products as alternative feed sources for livestock

Feeding costs for livestock production represent between 60 and 80 % of the total costs (Henning, 1998). Cereals may have to be imported, requiring foreign exchange, and human consumptions has priority for the use of cereals (Briedenhann, 2008). In addition, there is a decline in the production of feeds worldwide (Leng, 2008) which makes feed costs to be high. It is therefore essential to reduce the cost of feeding by utilizing food by-products. By-products are an economical alternative for feeding livestock, with relatively lower costs than the cost of cereals. A by-product is by definition, a secondary product obtained during harvesting or processing of a principal commodity and has a value as an animal feed (Grasser *et al.*, 1995). A large proportion of the by-product may be culls, trimmings, or raw product which is inferior in some way and unfit for packing. These by-products may still contain substantial amounts of nutrients (Table 2.1) and might make an excellent animal feed if further processed to a suitable and easily handled animal feed source (Boucque & Fiems, 1988). The use of by-products and alternative feeds has increased substantially in recent years (Griffiths *et al*., 2004). In the past, by-products have been more common used as supplements to fibrous, low quality roughages, especially during droughts. However, with more widespread use of feed-mixer wagons and total mixed rations, and a better understanding of their nutritive value, by-products are now more commonly used in full production rations.

Table 2.1 Nutritive value of a range of by-product silage (mean values with range in brackets)

By-product	n	DM content (%)	CP (% DM)	ME MJ/kg DM
Citrus pulp	26	15.2 (9.4 - 23.8)	8.7 (6.0 – 12.9)	12.5 (9.9 - 14.1)
Citrus pulp silage	3	15.6 (15.1 - 16.5)	9.5 (8.9 – 9.8)	11.9 (10.5 - 13.1)
Brewers` grains	27	25.4 (13.9 – 33.0)	21.7 (16.9 - 25.2)	10.7 (9.7 - 11.9)
Brewers` grains silage	3	29.7 (27.9 - 33.0)	22.0 (20.7 - 23.3)	10.6 (9.9 – 11.1)
Grape marc	3	35.8 (28.1 – 46.4)	17.9 (11.7 - 23.3)	8.1 (4.3 – 11.1)
Apple pomace	3	24.5 (21.0 – 27.6)	7.1 (6.0 – 8.0)	9.6 (8.4 – 11.1)
Tomato pulp	8	27.0 (16.6 – 30.2)	20.5 (17.7 - 22.4)	7.7 (4.8 – 9.5)
Potato mash	45	23.1 (10.9 – 62.3)	11.2 (6.7 – 25.8)	13.3 (10.8 - 14.8)
Orange pulp		13	7.5	12.6
Sweet corn trash silage		32	7.7	10.6
Potato tuber silage		25	7.6	13.6

Adapted from Griffiths *et al*. (2004), n; number of samples

2.2 Utilization of potato by-products in ruminant nutrition

The production of potato food products generates large amount of by-product material which has potential to be used as feed sources for ruminants. Approximately 35 % of the total processed potato crop is discarded as a waste during processing, and accounts for 12 million ton per year world wide (Tawila *et al*., 2008). Potato wastes are a generic description for a heterogeneous mixture of potato components that varies depending on the nature of the processing method (Schroeder, 1999). They may contain varying amounts of inedible spoiled potatoes, chips, peels and fats. However, they contain < 400 g DM/kg, crude protein (CP) content that range between 40 and 143 g/kg DM, and crude fibre (CF) that ranged between 16 and 175 g/kg DM (Onwubuemeli *et al*., 1985, Charmley *et al*., 2006). These wastes ferment rapidly and add to the pollution problem if not properly utilized.

The use of potato by-products in livestock diets had been examined for lactating dairy cows (Okamoto *et al*., 2004) beef cattle ((Nelson *et al*., 2000, Duncan *et al*., 1991, Aibibula *et al*., 2007) and small ruminants (Gado *et al*., 1998, Okine *et al*., 2005). According to Aibibula *et al*. (2007) observations arising from these studies are that large quantities of potato by-products can be consumed by ruminants and

degraded in the rumen. Potato wastes are primarily energy sources, containing approximately 13 MJ ME/kg DM (Rooke *et al*., 1997) and contain fat content that ranged between 50 to 100 g/kg DM (Duynisveld & Charmely, 2002). Consequently, potato wastes can be used as an energy source for ruminants without negatively affecting the animals. Stanhope *et al*. (1980) reported that the digestible energy content of potato by-product was similar to that of barley when the by-product was included in cattle diets 30 to 60 % of dietary dry matter. Furthermore, Sauter *et al*. (1980) and Crickenberger and Miller (1983) reported that potato by-products could be used in feedlot diets at 25 % dietary dry matter without reducing the performance or affecting carcass traits in cattle. Gado *et al.* (1998) reported an increased dry matter digestibility and N balance in goats fed concentrates containing 250 g/kg DM potato wastes. Not only could the by-product be utilized as a source of nutrients for ruminants, but using them to replace imported commercial feedstuffs could save energy in transportation, and possibly reduce the environmental impact of burning or burying them as landfill.

The effects of different dietary inclusion levels of potato by-products on ruminant performance were summarized in Figure 2.1 by Charmley *et al*. (2006). The researchers showed that higher (> 200 g/kg DM) dietary inclusion levels of potato by-products depressed DM intake, and animals required more time to adapt to the ration. However, digestibility of the rations was improved at these inclusion levels.
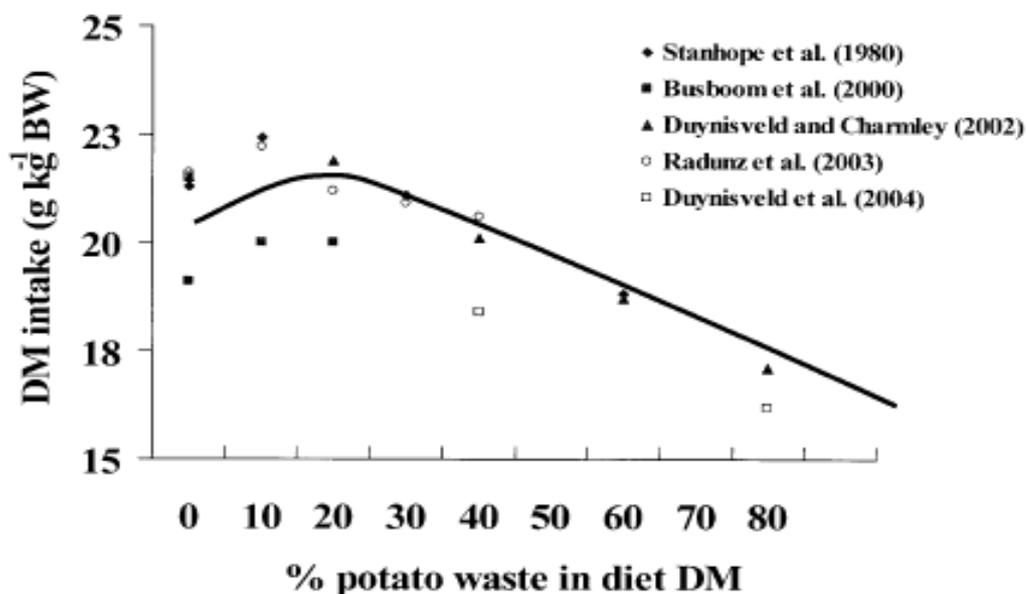
Figure 2.1 Relationship between inclusion of potato processing by-products in the diet and DM intake by finishing cattle (adapted from Charmley *et al.,* 2006).

2.3. The ensiling of forages or agro-industry by-products

Production of meal from high moisture by-products is technically feasible, but high drying and processing costs are economic deterrents (Charmley *et al*., 2006, Tawila *et al*., 2008). Ensiling can be considered as an efficient way of preserving high moisture by-products if all essential principles of ensiling are followed (McDonald, *et al*., 1991, Kayouli & Lee, 1999, Cao *et al*., 2009). Farmers have been preserving forages and by-products by ensiling them for several thousand years. The principles in the ensiling of forages and agro-industry by-products are the same (McDonald, *et al*., 1991, Kayouli & Lee, 1999). Procedures of preserving forages have now evolved to the point where it is known that there are at least three characteristics of forage materials necessary to ensure a good silage (Wilkinson, 2005): adequate level of fermentable substrate, a relatively low buffering capacity, and a DM content of 250 to 400 g/kg. These characteristics in combination with anaerobic storage conditions, promote effective fermentation. Anaerobic conditions are needed to reduce the

activity of respiratory enzymes in forage material. Such enzymes tend to promote heat build up and reduce both total DM and nutritional value of silage if left unchecked.

There is also competition between lactic acid producing bacteria (LAB) and lactic acid utilizing bacteria in the silo. Lactic acid producing bacteria are facultative anaerobes that ferment sugars (mainly glucose and fructose) to produce lactic acid. If the LAB prevail, the silo pH will be ideally reduced to 4.0 over a period of several days and plant material will be well preserved (McDonald *et al*., 2002). Acidic conditions discourage lactic acid utilizing bacteria, such as *Clostridia* bacteria that degrade amino acids to products of poor nutritive value, but the higher the moisture content of the silage, the lower the pH *Clostridia* can remain active.

2.3.1. Silage additives

Normally during ensiling the fodder undergoes an acid fermentation in which bacteria produce lactic acid, and to a lesser extent, acetic acid from WSC present in the raw material. The net result is a reduction in pH, which prevents the growth of spoilage micro-organisms (McDonald, 1981). The natural population of LAB occurring in plant tissues varies between 100 and 100 million bacteria per gram of wet forage (Weinberg & Muck, 1996). The vast fluctuations in LAB population have led to the belief that the addition of inoculants containing LAB to silages would eliminate the possible lack of such populations and, therefore, be beneficial. In order to reduce the dependence of the ensiling process on epiphytic lactic acid bacteria (LAB) and on chemical additives, inoculants containing selected strains of LAB have been developed (Weinberg & Muck, 1996). The addition of LAB inoculants as a means of controlling fermentation has met with varying results regarding the ability of the

inoculants to achieve rapid acidification and low pH. By achieving this, it is believed that silage of superior nutritional value will be obtained.

Silage additives are according to Kaiser (2004) and Tauqir (2004) classified into five categories, based on their mode of actions as shown in Table 2.2. These include: i) stimulants which encourage lactic acid fermentation (e.g. whey, sugarcane molasses, enzymes, etc), ii) fermentation inhibitors, which partially or completely restrict microbial growth, iii) aerobic deterioration inhibitors, which prevent the deterioration of silage during feed out phase, iv) nutrients, to enhance the nutritive value of the crop after ensiling, and v) absorbents for preventing effluent loss by raising the DM content of silage.

2.3.1.1 Microbial inoculants

2.3.1.1.1. Fermentation

Microbial LAB inoculants are applied to forage at the time of ensiling to accelerate the decline of pH, and to preserve plant carbohydrates and proteins through fermentation and by decreasing proteolysis and deamination (Seale, 1986). Thus, inoculated silages are expected to improve feed intake, dry matter digestibility (DMD) and organic matter digestibility (OMD), resulting in improved animal performance (Chamberlain, 1982, Bolsen *et al*., 1996). The principle of microbial inoculation was first adopted in 1909 by Bouillant and Crolbois when they applied lactic acid inoculants to beet pulp to improve fermentation (Watson & Nash, 1960). Later in 1934, Rushmann and Meyer (1979, cited by Fish, 1991) documented that the rate of acidification during silage fermentation is dependent on epiphytic bacteria found on forages. At the present time, there are several silage inoculants available on the market. Bacterial inoculants are used to enhance the ensiling process and have been reported to occasionally result in improvements in animal performance (Muck, 2010).

Table 2.2 Classification of silage additives based on their mode of action (adapted from Kaiser, 2004)

Additive class	Potential response*	Examples of additives
Fermentation stimulants		
a) Fermentable carbohydrates		
Sugar sources	A,B,C	Molasses, sucrose, glucose, citrus pulp, pineapple pulp, sugar beet pulp
b) enzymes **	A,B	Cellulases, hemicellulases, amylases
c) Inoculants **	A,B,C	Lactic acid bacteria (LAB)
Fermentation inhibitors		
a) Acids and organic acid salts	A,B,C,D	Minerals (e.g. hydrochloric acid), formic acid, acetic acid, lactic acid, acrylic acid, calcium formate, propionic acid, propionates
b) Other chemical inhibitors	A,B,C,D	Formaldehyde, sodium nitrite, sodium metabisulphite
Aerobic spoilage inhibitors	B,C,D	Propionic acid, propionates, acetic acid, caproic acid, ammonia, some inoculants
Nutrients	C	Urea, ammonia, grain, minerals, sugar beet pulp
Absorbents	B	Grain, straw, bentonite, sugar beet pulp, polyacrylamide, hay

Potential responses:

A - improved fermentation quality; B – reduce in-silo losses; C – improve nutritive value; and D – reduce aerobic spoilage

* Not all additives listed are consistently effective

** Inoculants and enzymes are also referred to as 'biologicals'

Bolsen (1978) described silage inoculants as those products that supply lactic acid bacteria and enzymes and/or micro-organisms that increase the availability of carbohydrates and other nutrients to lactic acid bacteria (LAB).

Most products include one or more homofermentative lactic acid bacterial (LAB) species. *Lactobacillus plantarum*, other *Lactobacillus* species, *Enterococcus faecium*, and various *Pediococcus* species are the most common bacteria that are included in silage inoculants (Muck & Kung, 1997). The reason for using multiple species in some products is the opportunity of synergistic growth among bacterial species. Inoculated LAB can complement the epiphytic LAB present on the crop and facilitate the fermentation process (Muck & Kung, 1997). They have been reported to influence the rate and extent of silage fermentation. Typical ingredients found in inoculant may include enzymes, bacteria, moulds, micronutrients for micro-organisms or a mixtures of all these to influence forage respiration and fermentation rate (Parker, 1979).

However, the addition of LAB inoculants to herbage with low content of WSC (below 30 g/kg) has been shown to limit the effect upon silage fermentation (Seale, 1986). In contrast, Rooke (1990) demonstrated that an inoculant of LAB could improve silage fermentation even at a very low concentration of WSC (12.8 g/kg fresh grass). Haigh and Parker (1985) concluded that WSC content as low as 30 g/kg may be sufficient for a stable fermentation where an effective additive is added during ensiling. In many instances, a source of readily fermentable substrate for LAB is included with commercial bacterial inoculants. This combination has proved to be effective in securing more stable silage fermentation (Henderson, 1987).

Several studies (Kennedy *et al*., 1989, Schneider *et al*., 1995, Meeske & Basson, 1998, Okine, 2007) have used LAB inoculants on grasses, grass-legumes,

cereal crops and food by-products with mixed responses (positive, negative and no response) to treatments. In over 250 studies reviewed by Muck (1993), inoculation enhanced silage fermentation 75 % of the time with lucerne, 77 % of the time with grass silages, but only 40 % of the time with maize silages. The lower response cases with inoculated maize is expected as the pH in maize silage often drops to 4 within the first 48 hours of ensiling leaving very little room for improvement in rate of preservation (Meeske, 2005). Maize typically has high numbers of naturally-occuring epiphytic microorganisms with which inoculated bacteria must compete. Also, maize silage generally has high amounts of fermentable carbohydrates, allowing existing bacteria to generate a reasonably rapid pH decline.

Within recent years, inoculants and enzymes have become popular as a means of improving silage fermentation and nutritive value (Charmley, 2001). Commercially available inoculants not only vary in ingredients but in type of preparation (dried, liquid, freeze-dried) and packaging (bottles, vacuum packs and paper sacks). According to Whittenbury (1967, cited by Fish, 1991) the requirements of a quality silage micro-organism are as follows:

i)   it must be fast growing and able to compete with and dominate other micro-organisms in silage

ii)   it must be homofermentative

iii)   it must be acid tolerant down to a silage pH of 4.0

iv)   it must possess the ability to ferment glucose, fructose, sucrose, and preferably fructosans and pentosans

v)   it should have no action on organic acids

In addition, McCullough (1975) listed the following requirements for a cost effective quality inoculant:

i)   the cost of the additive must be less than the silage lost without the additive

ii)   addition of the additive must result in a more efficient fermentation than occurs naturally

iii)   the additive should produce a silage with a greater digestibility of energy and/or protein than untreated silage

There are mixed concerns regarding the effect of LAB inoculant on nutrient digestibility. Some suggest that inoculation usually has little or no effect on the fibre content of silages because most LAB contain little or no ability to degrade plant cell walls. According to McDonald (1981), the effects of inoculants on digestibility may be a consequence of improved nutrient preservation during the fermentation process and conservation of a greater proportion of digestible nutrients. Dry matter digestibility of inoculated silages was not affected in some studies (Kung *et al*., 1993, Rooke *et al*., 1988). The concentration of NDF was reported to be reduced by inoculation (Keady & Steen, 1994), which was due to partial hydrolysis of hemicelluloses (Muck & Kung, 1997). In contrast, Kung *et al*., (1987) and Rooke *et al*., (1988) did not observe a reduction in cell wall fractions from inoculated silage compared to the control.

In a review data up to the end of the 1980s, Spoelstra (1991) concluded that inoculation increased animal performance from silages by about 7 %. Muck (1993) also reached a similar conclusion, noting a 5 % improvement in milk production following inoculant use. Both reviewers concluded that a large part of the improvement in performance was due to an increase in digestibility, rather than an increase in intake. This was contrary to what was expected, since LAB are non-cellulytic (Charmley, 2001). The increase in digestibility may be a response to the

extensive fermentation of substrate to lactic acid in these silages. Under these conditions, acid hydrolysis of structural carbohydrate to soluble sugars will occur (Dewar *et al.*, 1963). The reduction in fibre concentration has been speculated to be responsible for increases in digestibility. On the other hand, Teller *et al.* (1993, cited by Charmely, 2001) have demonstrated that highly fermented silages are eaten more slowly than less fermented silages or hay. If these changes in eating behaviour influence ruminal retention time, then this could also explain why digestibility is increased by inoculant use. A possible direct, probiotic effect of silage inoculation on rumen fermentation has been proposed (Gordon, 1989, Weinberg *et al.*, 2007) to explain improved animal performance from inoculated silages, in the absence of changes in silage fermentation. Weinberg and Muck (1996) found animal performance effects with *L. plantarum* MTD1 inoculant appear to be independent on the effects of fermentation and on digestibility.

Weinberg *et al.* (2007) hypothesized that certain LAB strains interact with rumen micro-organisms to enhance rumen functionality and animal performance. These results strongly suggest that a silage inoculant `s effects on animal performance cannot be ascertained or surmised by the inoculant`s effectiveness on fermentation. Evidence is increasing to suggest that lactic acid in silage favours a glucogenic balance of VFA in the rumen (Martin *et al.*, 1994), which has led to a reduction in milk fat percent (Cushnahan & Mayne, 1995). If this proves to be a widespread phenomenon, inoculants may play a role in reducing milk fat.

One of the crucial roles for using microbial inoculants during ensiling of forages is that the populations of native LAB vary depending on the type of forage and the time of harvest. They are generally lower in alfalfa (10^5 cfu/g fresh material), and greater on perennial grasses (10^6 cfu/g fresh material), maize (10^7 cfu/g fresh

material) and sorghum (10^7 cfu/g fresh material) (Pahlow *et al*., 2003). It is therefore recommended that an inoculation rate of $10^5 - 10^6$ viable cells per gram crop is often sufficient for the inoculants LAB to overwhelm the epiphytic LAB and become the predominant population in the silage (Weinberg & Muck, 1996). Three major factors influencing the efficacy of silage inoculants include the nature of epiphytic LAB, the water-soluble carbohydrate content of the crop, and the characteristics of the bacterial strains included in the inoculant (Muck & Kung, 1997).

It has been reported that microbial inoculation reduced proteolysis during ensiling and resulted in improved efficiency of silage protein utilization and reduced N losses (Charmley, 2001). According to McDonald (1981), this effect arose as a result of pH reduction with inoculation which inhibits protein degradation in silages. It is known that proteolysis in silage increases with increasing pH in the range from pH 3-7 (Heron *et al*., 1989 cited by Driehuis *et al*., 2001). For effective reduction of proteolysis, the pH reduction must occur quickly. Although quick acidification to a pH below 4.0 reduces proteolysis, it is not completely inhibited (McDonald, 1981).

Several studies (Steen *et al.,* 1998, Okine, 2007) reported increased DMI with commercial inoculants with no obvious improvements in fermentation. Satter *et al.* (1988) have summarized 8 experiments involving various inoculant-treated silages made from high DM Lucerne and offered to lactating dairy cows. They concluded that whereas inoculant treatments improved silage fermentation characteristics, these usually did not result in measurable improvements in DMI or milk production. This was supported by the work of Keady and Steen (1996) who fed ruminants on inoculated silage and no improved animal performance was observed. In contrast, Sharp *et al.* (1994) reported LAB inoculated grass silages to be better preserved and resulted in greater voluntary intake in growing heifers. Similarly, Meeske and Basson

(1998) and Meeske *et al.* (1999) found lambs that were fed inoculated silage growing faster than those in the control silage diet. Interestingly, increases in animal performance are not always explained by improvements in fermentation characteristics. Several studies (Whittenberg *et al.*, 1983, Steen *et al.*, 1989, Kennedy *et al.*, 1989, Keady and Steen, 1994, McAllister *et al.*, 1995) have reported a lack of response in different types of silages (treated compared to untreated) to which LAB has been applied. Three factors determine whether an inoculant would be beneficial: the natural population on the chopped crop, the sugar content (WSC) in the crop and the strains of bacteria in the inoculant to be used.

In some studies, LAB inoculants on maize (Rust *et al.*, 1989) and barley (McAllister et al., 1995) did not affect animal weight gains and DM intake. More-over, Kennedy *et al.*, (1989) and Gwayumba (1997) observed a reduction in animal performance following being fed inoculated silage. This decrease in animal performance following inoculation may also be due to fermentation losses of the most readily degradable organic matter and cell wall fractions inadvertently increasing the relatively less degradable fractions in the silage.

Addition of microbial inoculants and beet pulp to wet brewers grains at ensiling was beneficial in promoting a more efficient fermentation (Schneider *et al.*, 1995). Adding LAB inoculant in a TMR silage increased the LA content and tended to decrease ruminal methane production while adding molasses did not increase LA and increased methane production (Cao *et al.* 2010).

2.3.1.1.2. Effects microbial LAB inoculants on aerobic stability of silage

Aerobic stability is a term that nutritionists have used to define the length of time that silage remains cool and does not spoil after it is exposed to air (McDonald, 1981). Aerobic stability of silage is especially important in intensive animal production worldwide because large operations often contract for and take delivery of silage sufficient for 2 to 4 days of feeding and store it unprotected and, hot weather can encourage rapid aerobic deterioration of such silage (Pitt *et al.*, 1991). The inability to remove sufficient quantities of silage from silos between feedings can result in prolonged exposure to air. An air ingress as small as 100 to 150 mg O_2/kg DM is adequate to make silage highly susceptible to aerobic deterioration (Woolford, 1990). Upon exposure to oxygen, conditions become favourable for proliferation of aerobic bacteria, yeasts and fungi (Moon, 1981). In most silages, yeasts have the ability to increase in numbers from $< 10^2$ to 10^{12} cfu/g DM by day 3 of aerobic exposure (Woolford, 1990). However, a high population of yeasts does not necessarily mean a silage will deteriorate (Nishino *et al.*, 2003), instead, quantity of lactate-utilizing yeasts decides whether a silage will deteriorate or not upon aerobic exposure (Woolford, 1990).

Thermophilic filamentous fungi are also found in deteriorating silage, however, their growth is generally lower and thus have a little effect on silage as feed (Fish, 1991). Regardless of the substrate utilized by these micro-organsisms, deterioration in forage crops is always accompanied by a loss of residual sugars and the evolution of ammonia and carbon dioxide (McDonald *et al.*, 1991). The latter can be directly equated to DM loss and its measurement can be used to monitor the progress of deterioration (Woolford, 1990). Furthermore the accumulation of lactic

acid could serve as a substrate for yeasts that degrade lactic acid into CO_2 and water, and generate heat, leading to nutrient losses (Rust *et al*., 1989).

Aerobic deterioration of silage is indicated by an increase in temperature and pH caused by metabolism of sugars and organic acids by yeasts and bacteria that assimilate lactic acid (McDonald *et al*., 1991). Furthermore, this deterioration of silage causes high DM losses and a risk of mycotoxin production in the feed, which are detrimental to animal health (Filya, 2003). Ironically, silages that have undergone a clostridial fermentation are very stable when exposed to air because they have high concentrations of VFA that are highly antifungal (Woolford, 1990).

Honig (1990) suggested that inoculation of silage with LAB might improve aerobic stability via competitive suppression of yeasts. However, in summary of studies conducted between 1990 and 1995, Muck and Kung (1997) reported that homolactic LAB inoculation of whole crop maize improved dry matter (DM) recovery and animal performance by 2 to 3 % and 3 to 5 % respectively. However, inoculants that contain mainly homofermentative LAB have often reduced the aerobic stability of silage because of insufficient production of volatile fatty acid (VFA) (Muck & Kung, 1997, Rust *et al*., 1989, Weinberg *et al*., 1993).

Furthermore, a review of studies published between 1990 and 1995 reported that inoculation had a similar level of success at improving fermentation characteristics, but noted that aerobic stability was not improved particularly when corn or small grains were evaluated (Bolsen *et al*., 1996, Muck & Kung, 1997). Inoculation with a homofermentative LAB inoculant probably reduced aerobic stability. Weinberg *et al*. (1993) hypothesized that high levels of residual WSC, combined with high lactic acid concentrations and a lack of sufficient concentrations of protective VFA in the silage inoculated with a homofermentative LAB were

associated with aerobic spoilage. In addition, inoculation with homofermentative LAB shifts the fermentation towards lactic acid rather than better inhibitors of yeasts such as acetic acid. A relationship between acetic acid and stability was proposed by Danner *et al*. (2003) who claimed that increasing acetic acid concentrations inhibit spoilage organisms, thereby promoting exponential increases in stability. Consequently the quest for LAB inoculants that would inhibit the growth of yeasts and enhance aerobic stability was initiated. Inoculants containing the heterofermentative species, *L. buchneri*, have been marketed mainly on their ability to improve the aerobic stability of silage (Weinberg & Muck, 1996, Ranjit & Kung, 2000).

Several subsequent studies have confirmed that *L. buchneri* application improves the aerobic stability of silages (Ranjit & Kung, 2000, Driehuis *et al*., 2001, Taylor *et al*., 2002, Nkosi *et al*., 2009b). The explanation for aerobic stability enhancing effect of *L. buchneri* is that, in silages inoculated with this organism, the concentration of acetic acid is increased which impair the activity of yeasts (Filya, 2003). According to previous research (Driehuis *et al*., 2001, Taylor *et al*., 2002, Nkosi *et al*., 2009b) inoculation with *L. buchneri* typically results in acetic acid concentrations ranging from 36 to 50 g/kg DM, suitable to control yeast during aerobic exposure of silage.

It is further reported that this inoculant is more effective in maize than alfalfa (*Medicago sativa*) or small grains because heterofermentative bacteria is less abundant in maize than alfalfa (Lin *et al*., 1992). Because of the lower levels of *L. buchneri* and other heterofermentative bacteria in maize, acetic acid is normally lower in maize than in alfalfa making maize more susceptible to aerobic stability problems.

When *L. buchneri* is used, it is recommended that a minimum of 45 – 60 days elapse before opening the silo in order to ensure good aerobic stability (Muck, 2008).

However, some limitations with the use of *L. buchneri* is that there are reports regarding losses of energy (1 – 2 %) and DM (5 – 10 %) in the silo and reduced intake due to high levels of acetic acid present in *L. buchneri* inoculated silage (Oude Elferink *et al*., 2001). However, feeding lactating cows and lambs silages treated with *L. buchneri* have shown that DMI is not reduced (Driehuis *et al*., 1999, Taylor *et al*., 2002, Nkosi *et al*., 2009b). The results reinforce the fact that the production of acetic acid via conversion of lactic acid to acetic acid by *L. buchneri* is different from the normal pathways of acetic acid production in silage (Oude Elferink *et al*., 2001). Furthermore the heterofermentative pathway of *L. buchneri* inoculants can cause greater silage pH and ammonia-N concentration (Neylon & Kung, 2003) and increased losses of WSC and DM during fermentation (Adesogan & Salawu, 2004). Moreover, some heterofermentative LAB such as *L. reuteri*, *L. crispatus* and *L. brevis* have been reported to produce ferulate esterases, which improve silage aerobic stability and increase digestibility and animal performance (Nsereko *et al*., 2008).

Although the fermentation efficiency of heterolactic bacteria is lower than homolactic bacteria (McDonald *et al*., 1991), any increase in dry matter losses during fermentation may be offset by improvements in the aerobic stability of the silage (Holzer *et al*., 2003). The acetic acid associated reduction in silage intake by cattle (Jones *et al*., 1980, Buchanan-Smith, 1990) differed from results of other studies (Driehuis *et al*., 1999, Taylor *et al*., 2002, Nkosi *et al*., 2009b). Consequently, improved stability through elevated acetic acid levels may be possible without a reduction in the intake of silage. Inclusion of propionic acid bacteria in inoculants

may also improve aerobic stability as propionate has also been shown to exhibit antifungal activity (Weinberg *et al*., 1995, Higginbotham *et al*., 1998).

The beneficial effects of homofermentative LAB on fermentation and retention of nutrients in silages, along with the ability of heterofermentative LAB to improve the aerobic stability of silage, has led to the development of inoculants containing of mixtures of these bacteria (Ranjit & Kung, 2000). These inoculants are called dual-purpose or combo inoculants and they improve the fermentation as well as the aerobic stability of silage as reported with ryegrass (Ashbell *et al.*, 2002, Filya, 2003) and wet bermudagrass silages (Adesogan *et al*., 2004). Combining *L. buchneri* with other LAB to obtain positive attributes when silages are exposed to air and active fermentation has been studied in cereal grain silages (Weinberg *et al*., 1999, Filya, 2003) and in grass silages (Adesogan *et al*., 2004).

Filya (2003) investigated the effects of *L. buchneri* and *L. plantarum*, alone or in combination, on the fermentation and aerobic stability of low dry matter corn and sorghum silages and observed higher levels of acetic acid in silages treated with *L. buchneri* alone and in combination with *L. plantarum* as compared to control silage or silage treated with *L. plantarum* alone. A subsequent study by Zahiroddini *et al*. (2006) silage treated with a combination of *L. buchneri* and *P. pentosaceus* had similar effects on acetic acid concentration with silage treated with a combination of *L. plantarum* and *E. faecium*. They suggested that *L. buchneri* was not able to compete with other silage micro-organisms when applied together with a homofermentative LAB. This was supported by Kleinschmit and Kung (2006) who reported that maize silage treated with *L. buchneri* 40788 and *Pediococcus pentosaceus* R1094 had normal fermentation characteristics, but the aerobic stability of silage was not consistently improved with this combination of organisms. Variation

in results using mixtures of *L. buchneri* and homofermentative LAB and others may reflect differences in the relative competitiveness of different strains of *L. buchneri* in varying ensiling environments.

2.3.1.2. Fermentation stimulants

Some forage crops may be low in WSC or may have lack of LAB which is responsible for the fermentation of the crop (McDonald, 1981), and silage additives / inoculants may be beneficial in this regard. To stimulate the fermentation process for the production of silage, a source of soluble carbohydrate such as whey has been used extensively as a silage additive (Thomas, 1978, Khattab *et al.,* 2000, Nkosi, 2003, Zobell *et al.,* 2004). Whey is a milk by-product resulting from cheese making and due to the addition of starter cultures in milk, may consist of large amounts of LAB as well as lactic acid, which result in a pH of 4.6 (Schroeder, 1999). Although whey can be beneficial in silage making, it is not recommended to be used with high moisture crops because it increased effluent production and may cause poor fermentation (Thomas, 1978, Zobell, *et al*., 2004). Consequently, many experts preferred using whey in a dried form (e.g. Dash *et al.,* 1974), of which the drying process is costly and may not be affordable to small scale farmers. Renewed interests of using liquid whey as a silage additive has evolved. In a study using liquid whey (Fazaeli *et al.,* 2003), higher lactic acid production was observed from whey treated silage compared to the control.

Another source of carbohydrate, sugarcane molasses, which is a by-product of the sugar cane industry that contains 650 g/kg DM soluble carbohydrates (Ashbell *et al.,* 1995, Meissner, 1999), has been used to improve the fermentation process (Bolsen

et al., 1996, Yunus *et al*., 2000, Van Niekerk *et al*., 2007, Nkosi *et al*., 2009a). Due to the viscosity of molasses, it is difficult to apply and should therefore be diluted preferably with a small volume of warm water to minimize seepage losses (Ashbell *et al*., 1995). With crops of low DM, a considerable proportion of molasses may be lost in the effluent during the first days of ensiling (Henderson, 1993), as reported by Ashbell (1992) when citrus peels of 140 – 210 g/kg DM was ensiled with molasses.

Furthermore, cell wall enzymes such as cellulases and hemicellulases, can be used as silage fermentation stimulants. Cellulase, hemicellulase and amylase enzymes have been widely tested as silage additives. These compounds have the potential to convert structural carbohydrates to soluble sugars which can be fermented by silage bacteria. Many experiments have shown that their use increases the level of fermentable substrate in silage, thus promoting extensive fermentation (Jakkolaa *et al*., 1991, Stokes, 1992). However, with relatively wet silages, there has not been an increase in digestibility and improvements in animal performance have been very small (Jakkolaa *et al*., 1991). This can be attributed to increased effluent losses, removing soluble compounds from the silage, since research with wilted silages has shown benefits in digestibility and on animal performance.

Application of fibrolytic enzymes, alone or in combination with bacterial inoculants, has been proposed as a means of directly improving fibre digestibility as well as increasing the availability of water soluble carbohydrates to serve as a substrate for LAB (McDonald *et al*., 1991). In a study by Zahiroddini *et al*. (2006) the inclusion of enzymes with inoculants did not seem to be effective either in decreasing the NDF content or increasing the WSC content of barley silage. Other researchers (Ranjit & Kung, 2000, Kung & Ranjit, 2001) have applied enzyme-containing inoculants onto barley silages with no effects on NDF and ADF concentrations.

Zahiroddini *et al.* (2004) have found higher concentrations of fibre in silages treated with enzyme-containing inoculant ensiled in mini-silos, but lower concentration of ADF in the same silages ensiled in large bag silos. They attributed this effect to the nature of ensiling environment.

2.3.1.3. Fermentation inhibitors

Mineral (sulphuric and hydrochloric) and organic (formic and lactic) acids give rise to an immediate low pH in the silo and create conditions that putrefactive micro-organisms cannot tolerate (Cole, 1992). The original use of mineral acids in silage preservation dates back to 1885 (Watson & Nash, 1960). Added acids are more effective than natural fermentation because acidification occurs within minutes of adding the additive. When relying on natural fermentation, acidification can take days or weeks (Charmley, 2001). Research conducted in Ireland (O`Kiely *et al.*, 1989) showed the use of sulphuric acid (at a concentration 45 % w/w) as a silage additive to have resulted in intakes equivalent to those of formic acid treated silages. Formic acid has been reported to reduce silage fermentation, and lowers the amount of acetic and butyric acids and the degree of proteolysis in the silo (Waldo, 1978 cited by Cole, 1992). Furthermore, increased DM intakes of growing cattle and dairy cows, coupled with improvements in animal performance have been associated with formic acid treated silages (Thomas & Thomas, 1985, Parker & Crawshaw, 1982), the improvements being greatest when the control silage is poorly preserved.

Chemical additives such as formic acid, sodium acetate, sodium chloride, etc. were used to improve the fermentation of ensiled high moisture by-products (Megias *et al.*, 1998, Kholif *et al.*, 2007). Application of formic acid results in a rapid

acidification of forage and partial inhibition of microbial growth (Woolford, 1984). Furthermore, experiments in laboratory and farm scale silos indicated that the addition of formic acid based preservatives at ensiling improved the fermentation pattern and aerobic stability of silage (Salawu *et al*., 2001, Filya & Sucu, 2007). Application of 4 ml/kg formic acid on wheat silages was reported to be effective in improving silage quality and aerobic stability, but did not affect organic matter digestibility (Filya & Sucu, 2007). Sterilants such as formaldehyde that inhibit the growth of microflora in general also restrict proteolysis in the silo. Problems of handling corrosive acids and poor intakes of the resultant silages have limited the use of chemical additives (Gwayumba, 1997). As a result, they have been replaced with biological additives such as inoculants (Weinberg & Muck, 1996).

2.3.1.4. Absorbents

One of the major setbacks in ensiling agro-industry by-products is their high moisture contents, which requires that the by-product be dehydrated or mixed with a dry source (absorbent) to improve compaction and ensiling (Khorvash *et al*., 2006). Absorbents can be added to silages, particularly those that are high in moisture content (e.g. by-products) or where acid additives are added to reduce the problems of pollution from effluent associated with these silages. They include materials such as barley, straw, bran, sugar beet pulp, hay, straws and poultry litter / manure. Nicholson *et al*. (1977) confirmed potato by-products to ensile satisfactory when mixed with dry roughage and observed positive results when fed to steers.

2.3.1.5. Nutrients

To sustain nutritional quality and enhance the fermentation process during ensiling, various additives (feedstuffs, nutrients, absorbents, etc.) have been used (Oude Elferink *et al*., 1999, Charmley, 2001). Urea is a common additive that provides both non-protein nitrogen (NPN) and the ammonia needed for optimal ruminal fermentation (Erfle *et al*., 1986, Leupp *et al*., 2006). Non-protein nitrogen sources (e.g. urea, anhydrous ammonia) not only increase the nutritive value, but also improve the aerobic stability of silage (Keller *et al*., 1994). The work of Leupp *et al*. (2006) concluded that the addition of urea to wet beet pulp at ensiling increased the DM content, enhanced fermentation environment, and increased nutrient quality. However, the use of NPN in high moisture (> 70 %) silages is often discouraged due to inability to achieve a low enough pH (4.0) to minimize the microbial activity that causes nutrient losses (Valadares *et al*., 1999). Nutrients such as ammonia, and minerals have also been used as additives during ensiling (McDonald, *et al*., 1991). Ferris and Mayne (1994) reported decreased VFA concentration with increasing levels of beet pulp ensiled with perennial ryegrass.

2.4. Effects of end-products of silage fermentation on intake

According to Charmley (2001) one of the major disadvantages associated with silage making is that the feeding value of silage is reduced relative to that of the original crop. However, silage research up to the present time has focussed on closing the gap between feeding value of the original crop and that of the resulting silage. Poorly preserved silages are consumed to a lesser extent than well preserved silages. Baker *et al*. (1991) produced two silages from the same sward grass, one was well ensiled using good silage techniques and an additive, whilst the second was made in a deliberate attempt to create a poor quality silage. When these were fed to dairy cattle,

considerably more of the well produced silage was eaten, the main difference between the two being their amine content.

Acetic acid from silages was negatively correlated with silage intake (Wilkins *et al.*, 1971) but inclusion of additional lactic acid or butyric acid reversed this effect (Buchanan-Smith, 1990). Subsequent studies with *L. buchneri* inoculation and reported improved silage intake with increased acetic acid concentration (Driehuis *et al.*, 2001, Taylor *et al.*, 2002, Nkosi *et al.*, 2009b). Accoring to Driehuis *et al.* (1999) reduced silage intake is reported only with poorly preserved silage.

Another fermentation end-product, butyric acid was first implicated as being responsible for reducing silage intake in 1963 (Charmley, 2001). Intake of silage by dairy cows declines as the concentrations of silage ammonia and butyric acid increase (Cushnahan *et al.*, 1995).

Low pH in silages is often associated with poor intake because low pH in rumen reduces cellulolytic activity and depress intake (Charmley, 2001). However, silage pH alone could not account for a significant part of feed intake (Kawamoto *et al.*, 2009), and its influence was indirect (Wilkins *et al.*, 1971). According to Rooke (1995) there is no relationship between silage pH and rumen pH, because silage is neutralized by saliva upon consumption (Charmley, 2001). Some studies (e.g. Newbold *et al.*, 1991) had reported that neutralization of silage with bicarbonate increased silage intake. Rooke (1995) also suggested that lactic acid may have a direct effect on palatability since a sour taste is associated with reduced palatability.

Ammonia-N in silage is predominantly a product of clostridial fermentation of amino acids, and has been associated with reduced silage intake (Steen *et al.*, 1998). It has been reported that silage with a high CP content and high solubility can result in high rumen ammonia concentration leading to a reduced silage intake (Charmley &

Veira, 1990). Under certain feeding situations, these conditions could lead to mild ammonia toxicosis which may reduce feed intake (Charmley, 2001).

Furthermore, various potential intake inhibitors with neuropharmacological effects, such as amines and histamine have been found in silage. These products are produced by protein degradation during silage fermentation, and are typically found in butyrate silage (Ohshima *et al.*, 1979).

2.5. Conclusions

It is apparent that ensiling forages with the use of silage additives or inoculants may improve the fermentation of the crop by restricting proteolysis and preserve more nutrients to be available to the animal. However, this homofermentative type of silage is prone to aerobic deterioration, and heterofermentative LAB inoculants (e.g. *L buchneri*) could be used to improve its aerobic stability. Chemical additives can result in better silage quality but are now gradually substituted with microbial inoculants. This is due to their corrosive nature to equipments and is dangerous to farmers. In reviewing inoculant studies, it is apparent that the effects of inoculants on animal performance are not consistently linked with effects on fermentation.

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CHAPTER 3

The effects of hay and poultry litter as absorbents and whey and molasses as silage additives on the quality of ensiled potato hash

3.1 Introduction

Feeding costs for livestock production represent between 60 and 80 % of the total costs (Henning, 1998). Cereals may have to be imported, requiring foreign exchange, and human consumptions has priority for the use of cereals (Briedenhann, 2008). In addition, there is a decline in the production of feeds worldwide (Leng, 2008) which makes feeds costly. It is therefore essential to reduce the cost of feeding by utilizing food by-products. Not only could the by-products be utilized as a source of nutrients for ruminants, but utilizing them to replace imported commercial feedstuffs could save energy in transportation, and possibly reduce environmental impact of burning them as wastes or burying them as landfill (Rogerson, 1993).

Potato hash (PH), a by-product that derives from the processing of snacks and chips, is produced at ± 50 tons per day and is currently dumped or given free to farmers (Vosloo, 2010, personal communication). One of the limitations for using potato by-products in animal nutrition is their high moisture content, which makes transportation costly (Charmley *et al*., 2006, Okine, 2007). Production of meal from potato by-products is technically feasible, but high drying and processing costs are economic deterrents (Charmley *et al*., 2006, Tawila *et al*., 2008). Consequently, some studies (Hoover *et al*., 1976, Nicholson *et al*., 1977) have shown that ensiling can be an efficient way for processing potato by-products and fed to animals. However this practice is very rare in South Africa because silage is mostly produced from maize, forage sorghum, lucerne, oats, barley and triticale, and tropical grasses (Meeske,

2000). Various studies (e.g. Kayouli & Lee, 1999, Cao *et al*., 2009) have shown that high moisture by-products can be preserved by ensiling when all essential principles for ensiling are followed. One of the major setbacks in ensiling potato by-products is its low dry matter (DM) and water-soluble carbohydrates (WSC) contents (Okine, 2007), which warrants the use of silage additives or absorbents to improve the fermentation process (Weinberg & Muck, 1996).

Materials with absorbent properties (e.g. chopped barley straw, sugar beet pulp, cereal grains) have been successfully added to various high moisture forages at ensiling to adjust DM content and improve nutritive value (Jones *et al*., 1990, Ferris & Mayne, 1994, Khorvash *et al*., 2006). However, these materials are not readily available to farmers in South Africa, and alternative materials that are accessible to the farmers are required. Therefore poultry litter (PL) and *Eragrostis curvula* hay which are easily accessible to farmers, were selected as absorbents for ensiling PH. Poultry litter has been shown to improve the crude protein (CP) content of maize silage (Fontenot *et al*., 1975) and sorghum silage (Al-Rokayan *et al*., 1998), and is often used by resource poor farmers as a CP supplement for their livestock. However, its use in animal nutrition is prohibited in South Africa and may need processing before being considered as an animal feed source (Act 36, 1947). Research has proved that ensiling improves the quality of PL by reducing pathogenic agents through fermentation (Al-Rokayan *et al.,* 1998, Knight *et al*., 1976).

In addition, other two by-products that have a stimulant effect on silage fermentation (i.e. whey and sugarcane molasses) were selected as additives for ensiling PH. Sugarcane molasses, a waste product of sugar production, has been widely used as a silage additive (Weinberg *et al*., 2008, Kwak *et al*., 2009, Nkosi *et al*., 2009). Whey from cheese production, contains large amounts of LAB and lactic

acid, and has also been used for silage making (Dash *et al*., 1974, Bautista-Trujillo *et al*., 2009, Zobell *et al*., 2004). Data on the nutritive value of PH, its ensiling with either molasses or liquid whey as additives, and PL and hay as absorbents is limited. Therefore the objectives of this study were to determine the nutritive value of PH, and the effects of additives and absorbents on fermentation and aerobic stability of PH silage under laboratory conditions.

3.2. Materials and Methods

3.2.1 Silage preparation and sampling

Potato hash (PH) was collected from Simba (336 Andre Greyvenstein road, Isando, Gauteng, South Africa), a local food producing factory in South Africa for chemical analysis and ensiling. Poultry litter (PL) and liquid whey were collected respectively from the Poultry and Dairy industry centres of ARC-API, Irene institute. Poultry litter was sun-dried and ground before silage making while liquid whey was screened for lactic acid bacteria (LAB) populations, and contained 6.95×10^5 LAB cfu/ml ($\pm$ 0.341 SEM) before ensiling. *Eragrostis curvula* hay was ground in a hammer mill through a 2.5 cm sieve before ensiling. Triplicate representative samples of PL, hay and PH were collected and analysed for chemical composition. Sugarcane molasses was obtained from a local feed supplier and was diluted with warm water (1 part molasses with 2 parts of water, 4 h before application), and sprayed over the mixture at a rate of 30 ml/kg fresh material (FM). Liquid whey was sprayed at 30 ml/kg FM to obtain at least $> 1.7 \times 10^4$ cfu/g FM. In order to add the same amount of water as with the treated silages, the control was sprayed with water at 30 ml/kg FM. Mixtures (as is basis) of PH with either hay (800 g PH/kg + 200 g hay/kg) or PL (800

g PH/kg + 200 g PL/kg) were produced and treated with: no additive (control), whey and molasses. The experiment was conducted in a 2 x 3 factorial design (i.e. 2 absorbents x 3 additives).

Mixtures were produced in a uniform manner with constant mixing to obtain homogenous mixtures and ensiled in hundred and eight (108) 1.5 litre anaerobic jars (J. Week, GmBHu. Co., Wehr-Oflingen, Germany) equipped with lids that enable gas release. There were 18 jars per treatment, and each jar was filled with approximately 850 g (wet weight) of the mixture. The mixtures were compacted with stamping sticks to obtain a packing density of 133.2 kg DM/m^3 per jar. The jars were stored at 24 - 28°C room temperature to follow the fermentation dynamics.

On day 0, triplicate samples of the mixtures were collected for subsequent chemical analysis. Three jars per treatment were opened on each of days 4, 10, 20, 40, 60 and 90 for determination of pH, DM, CP, ether extract (EE), metabolizable energy (ME), neutral detergent fibre (aNDF), acid detergent fibre (ADF), in vitro organic matter digestibility (IVOMD), in vitro dry matter digestibility (IVDMD), ash, WSC, lactic acid (LA), ammonia-N (NH$_3$-N) and volatile fatty acids (VFAs). Silages at 90 days were subjected to an aerobic stability test in three 2 l polyethylene terephtalate bottles for each treatment at room temperature (28°C), which lasted for 5 days following the procedure of Ashbell *et al.* (1991).

3.2.2 Chemical analysis

A 40 g silage sample from each jar was collected and mixed with 360 ml of distilled water in a stomacher bag, homogenized and left at 10^{0}C for 24 h (Suzuki & Lund, 1980). It was then homogenized for 4 min and filtered through a Whatman No. 4 filter paper (G.I.C. Scientific, Midrand, Gauteng, South Africa). The extract was

used for determination of pH, WSC, VFAs, LA and ammonia-N. The WSC were determined by the phenol-sulphuric acid method of Dubois *et al*. (1956) and LA was determined by the colorimetric method of Barker and Summerson (1941) as modified by Pryce (1969). The VFA were determined with a Varian 3300 FID Detector gas chromatograph (Varian Associates, Inc., Palo Alto, CA, USA) by the procedure of Suzuki and Lund (1980). Ammonia N was determined by distillation using a Buchi 342 apparatus and a Metrohm 655 Dosimat with a E526 titrator according to AOAC (ID 941.04, 1990). This method is based on the method of Pearson and Muslemuddin (1968) for determining volatile N.

The DM of pre-ensiled mixtures and that of silage was determined by drying the samples at 60^0C until a constant mass was achieved, and was corrected for loss of volatiles using the equation of Porter and Murray (2001). 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. The ADF was determined using a Fibertec System 1010 (FOSS Analytical AB, Sweden) by boiling samples in an acid solution followed by filtration (ID 973.18, AOAC, 1990), and aNDF was determined by using amylase and sodium sulphite (Van Soest *et al*., 1991). Separate samples were used for ADF and aNDF analysis and both included residual ash. Analyses for ash (ID 942.05) and EE (ID 920.39) were determined according to the procedures of AOAC (1990). The ME was determined by the gas production technique of Pienaar (1994), while IVOMD and IVDMD were determined according to Tilley and Terry (1963).

3.2.3 Statistical analysis

Differences between additives (control, whey and molasses) and absorbents (hay and PL) for fermentation characteristics and chemical composition were

analysed in a 2x3 factorial design by the analysis of variance (ANOVA) using Genstat (2000). Significant differences between the means were declared when *P* was <0.05 and the differences among the means were compared by the Fisher's protected least significance difference (LSD) test. Means for the fermentation characteristics and chemical composition were analysed for the effects of additives, absorbents and their interactions with the model:

$$Y_{ijk} = \mu + t_i + d_j + (td)_{ij} + \varepsilon_{ijk}$$

where: *Y* $_{ijk}$ is the individual observations of the i-th additive and the j-th absorbent and the k-th replicate, μ is the overall mean, t_i is the effect of the i-th additive, d_j is the effect of the j-th absorbent, *(td)* $_{ij}$ is the interaction between t and d, and ε_{ijk} is the residual error.

3.3 Results and Discussions

3.3.1 Chemical composition

3.3.1.1 Chemical composition of potato hash, hay and poultry litter

The 155 DM g/kg in PH (Table 3.1) was comparable to 170 g/kg in potato pulp reported by Okine (2007). This makes PH difficult to ensile satisfactorily and should be treated with a suitable additive or absorbent (McDonald *et al*., 2002, Wilkinson, 2005). Consequently, absorbents (hay and PL) were therefore used to improve its DM during ensiling. The results showed that PH contained 703 g starch/kg DM, which is higher than 177 g/kg DM obtained in potato pulp (Okine, 2007). This might be attributed to differences in processing methods between these by-products. Further, the 105 g CP/kg DM content in PH is in agreement with those

reported in other potato by-products (Onwubuemeli *et al.,* 1985, Hough *et al.*, 1993, Tawila *et al.,* 2008). These researchers further suggested that CF content in potato by-products could range between 16 to 175 g/kg. Accordingly that of the PH used in the current study was 59 g/kg DM.

Table 3.1 Chemical composition of potato hash, poultry litter and *E. curvula* hay (expressed on g/kg DM, unless stated otherwise)

Parameter	Potato hash	*Eragrostis curvula* hay	Poultry litter
DM g/kg	155±0.13	937±0.25	890±0.22
OM g/kg DM	957±0.05	961±0.42	750±0.06
CP g/kg DM	105±0.49	45±0.35	284±0.54
CF g/kg DM	59±0.07	408±0.24	146±0.07
aNDF g/kg DM	370±0.67	790±0.35	410±0.53
ADF g/kg DM	163±1.58	432±0.08	198±2.06
ADL g/kg DM	53.2±0.65	70.4±0.44	55.8±0.80
Ash g/kg DM	43.1±1.14	38.8±0.88	250±1.06
Urea g/kg DM	ND	ND	3.11±0.06
EE g/kg DM	110±0.06	32.5±0.09	ND
Starch g/kg DM	704±1.25	ND	ND
ME MJ/kg DM	11.4±0.57	5.52±0.06	ND
IVOMD (% DM)	82.5±1.59	36.6±0.14	ND
IVDMD %	81.6±0.24	37.04±0.33	ND

DM, dry matter; OM, organic matter; CP, crude protein; CF, crude fibre; aNDF, amylase treated neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; EE, ether extract; ME, metabolizable energy; IVOMD, in vitro organic matter digestibility; IVDMD, in vitro dry matter digestibility; ND, not determined

The fibre and ME values in PH were respectively higher and lower than those of grains such as maize, which is reported to have 117 g NDF/kg DM and 14.2 MJ ME/kg DM (McDonald *et al*., 2002). However, its ME (11.36 MJ/kg DM) was comparable to the 12.3 MJ ME/kg reported in oats (Meissner, 1999). The high energy, IVDMD and IVOMD values in PH suggest that it can replace some grains for energy in ruminant diets, as it was illustrated with other potato by-products in previous studies (Stanhope *et al*., 1980, Onwubuemeli *et al.,* 1985, Tawila *et al.,* 2008).

The DM content of 890 g/kg in PL in the present study revealed that it was well dried with acceptable moisture content according to the range of 120 and 250 g/kg proposed by Bagley *et al*., (1994, cited by Owen *et al*., 2008). However, the CP

content (283.5 g/kg DM) was higher than 200 g CP/kg DM reported by Owen *et al.* (2008). This might be attributed to differences in the type of bedding material, type of ration consumed by birds and methods of storing. Moreover, ash content of PL was higher than 158 g/kg reported by Migwi *et al.* (2000) and Ashbell *et al.* (1995), but was within the range of 150 – 260 g/kg reported by Bagley *et al.* (1994, cited by Owen *et al.*, 2008). The chemical composition of *E. curvula* hay is consistent to that reported by Snyman (1991) and Distel *et al.* (1994).

3.3.1.2 Chemical composition of pre-ensiled potato hash mixtures

The DM of a crop at ensiling has a strong influence on the rate and extent of the resulting fermentation, and a low DM content at ensiling with a low sugar content increases the chance of a clostridial fermentation (McDonald *et al.*, 2002). According to Wilkinson (2005) a DM content of 250 – 400 g/kg in forage is required for favourable fermentation. From Table 3.2 it is evident that the DM content for potato hash hay silage (PHHS) and potato hash poultry litter silage (PHPLS) were within the requirements for proper ensiling. The DM in PHHS is consistent to the 360 g DM/kg reported in a mixture of 770 g/kg potato wastes and 230 g hay/kg (as is basis) reported by Hough *et al.* (1993).

The PHPLS had higher CP and ash contents compared to PHHS mixtures, which could be attributed to higher CP and ash contents in the PL compared to hay (Table 3.1). However, the energy (ME) and IVOMD in PHHS mixtures were higher than that of PHPLS. The lower energy and IVOMD in PHPLS in comparison to PHHS could be attributed to the high ash content in PL, which seemed to have a more pronounced effect on the energy content in PHPLS than the higher fibre content in hay.

According to Kung and Shaver (2001) a forage with an ash content of > 150 g/kg DM or CP of > 230 g/kg DM will have a high pH during fermentation because of the higher buffering capacity associated with high ash and CP contents. On average, the PHPLS mixtures had a pH of 6.8 compared to 4.3 in PHHS at pre-ensiling (Table 3.2). The differences in ash or CP contents in PL and hay could contribute to these results. Mixing sudan fodder with PL at a 7:3 ratio resulted in a pH of 6.1 at pre-ensiling (Rasool *et al.*, 1998), which is comparable to that of PHPLS treatments.

Water-soluble carbohydrates are regarded as essential substrates for growth of LAB for proper fermentation and low levels may restrict LAB growth (McDonald *et al.*, 1991). According to Haigh (1990), a minimum concentration of 37 g/kg DM WSC in a herbage with a DM of 230 g/kg is crucial for a successful fermentation without the use of inoculant. The concentration of WSC in the PHHS mixture (control) at pre-ensiling was 18 g/kg DM and probably too low, for efficient fermentation. According to Huisden *et al.* (2009) forages with low WSC (< 50 g/kg) concentrations at ensiling are ideal candidates for examining the effect of molasses addition. From the results in Table 3.2 it is evident that the addition of whey and especially molasses, have increased the WSC concentration in the pre-ensiled mixtures to acceptable levels for favourable fermentation. This agrees with others (Migwi *et al.*, 2000, Kwak *et al.*, 2009, Nkosi *et al.*, 2009) who confirmed increased WSC concentration when molasses was added during ensiling. The reason for this improvement is that molasses contains about 650 g WSC/kg DM (Ashbell *et al.*, 1995, Meissner, 1999) which is higher than in whey and control treatments.

Table 3.2 Means (n=3) for chemical composition of pre-ensiled potato hash mixtures (g/kg DM unless stated otherwise)

Parameters	Absorbents	Additives			Significance (*P*)		
		Control	Whey	Molasses	Absorbents	Additives	Interaction
Dry matter g/kg	Hay	372.1	371.9	371.6	<0.001	0.094	<0.001
	Poultry litter	364	368	365			
Ash g/kg DM	Hay	44.2[b]	45.6[a]	46.9[a]	<0.001	0.001	<0.001
	Poultry litter	266[b]	270[a]	268[b]			
Crude Protein g/kg DM	Hay	79.2[b]	80.1[a]	81.7[a]	<0.001	0.003	<0.001
	Poultry litter	273[c]	275[b]	276[a]			
ME MJ/kg DM	Hay	11.9	12.4	12.4	<0.001	0.065	<0.001
	Poultry litter	9.4	9.5	9.5			
IVOMD %	Hay	71.8[b]	72.4[b]	74.2[a]	<0.001	0.018	<0.001
	Poultry litter	66.4[b]	67.9[b]	69.8[a]			
Ether extract g/kg DM	Hay	74.2[b]	79.9[a]	74.3[b]	<0.001	<0.001	<0.001
	Poultry litter	33.9[b]	37.5[a]	32.6[b]			
aNDF g/kg DM	Hay	451	450	451	<0.001	0.071	0.013
	Poultry litter	335	331	334			
ADF g/kg DM	Hay	283.0	282.6	282.9	<0.001	<0.055	0.041
	Poultry litter	181	180.5	181			
pH	Hay	4.3	4.3	4.2	<0.001	0.082	<0.001
	Poultry litter	6.9	6.7	6.8			
WSC g/kg DM	Hay	18.1[c]	34.6[b]	47.6[a]	<0.001	<0.001	<0.001
	Poultry litter	33.8[c]	35.8[b]	49.7[a]			

[a,b,c] Means of the same row with different superscript differ (*P*<0.05)

DM, dry matter; CP, crude protein; ME, metabolizable energy; IVOMD, in vitro organic matter digestibility; ADF, acid detergent fibre; aNDF, amylase treated neutral detergent fibre; WSC, water soluble carbohydrate

3.3.1.3 Chemical composition of potato hash silage

Data on the effects of absorbent on chemical composition of the PH silages is shown in Table 3.3. An absorbent x additive interaction (P<0.05) occurred for all the chemical composition parameters, indicating that the effect of additives differ within a particular absorbent. The DM in PHHS was higher (P<0.01) than that of PHPLS which is due to the high DM content of hay compared to PL. In accordance with the pre-ensiled material, the contents of ash and CP were higher (P<0.01) in PHPLS compared to PHHS. This could be attributed to the high ash and CP in PL compared to hay. These findings agreed with others (Migwi *et al.*, 2000, Ashbell *et al.*, 1995) who reported increased ash and CP contents in silages that contained PL. Due to the higher fibre content of hay compared to PL, the addition of hay increased (P<0.01) the fibre content of PH silage. Furthermore, higher (P<0.01) IVOMD in PHHS compared to PHPLS occurred, which indicates a substantial better potential feeding value of PHHS for ruminants (Migwi *et al.*, 2000). This is inconsistent with Hadjipanayiotou (1994) who reported reduced digestibility of tomato pulp silage with PL addition. Furthermore, PHPLS had lower GE and EE compared to PHHS. The lower GE in the PHPLS was probably due to the higher ash and lower EE in PL.

Table 3.3 Effects of absorbents and additives on the chemical composition of potato hash silage after 90 days of ensiling (n = 3)

Parameters	Absorbents	Additives			Significance (P)		
		Control	Whey	Molasses	Absorbents	Additives	Interaction
Dry matter g/kg	Hay	331.7	329.9	335.6	<0.001	<0.001	<0.001
	Poultry litter	308.1[c]	340.0[a]	321.4[b]			
Ash g/kg DM	Hay	53.9[b]	62.7[a]	65.2[a]	<0.001	<0.001	<0.001
	Poultry litter	279[b]	288[b]	297[a]			
Crude Protein g/kg DM	Hay	89.8	92.9	95.3	<0.001	<0.001	<0.001
	Poultry litter	122.8[b]	140.3[a]	139.7[a]			
Gross energy MJ/kg DM	Hay	17.6	18.2	17.8	<0.001	0.004	<0.001
	Poultry litter	13.6[b]	14.9[a]	13.9[b]			
aNDF g/kg DM	Hay	507[a]	453[b]	469[b]	<0.001	<0.001	0.013
	Poultry litter	378[a]	336[b]	327[b]			
ADF g/kg DM	Hay	474[a]	428[b]	422[b]	<0.001	<0.001	0.041
	Poultry litter	346[a]	321[b]	317[b]			
Ether extract g/kg DM	Hay	77.4[b]	82.4[a]	73.9[b]	<0.001	<0.001	<0.001
	Poultry litter	22.4[b]	35.1[a]	20.8[b]			
IVOMD %	Hay	58.4[c]	62.3[b]	69.7[a]	<0.001	0.018	<0.001
	Poultry litter	51.7[c]	56.9[b]	61.9[a]			

[a,b,c] Means of the same row with different superscript differ ($P<0.05$)

aNDF, amylase treated neutral detergent fibre; ADF, acid detergent fibre; IVOMD, in vitro organic matter digestibility

According to Table 3.3, molasses and whey addition increased ($P<0.05$) the ash content of the silage. This could be attributed to the 132 g/kg DM of ash in molasses (Xande *et al*., 2010) and up to 80 g/kg DM of ash in whey (Ben Salem & Fraj, 2007). Consistent to the results of the present study, some researchers (Mahala & Khalifa, 2007) reported an increased ash content of molasses containing sorghum silage. However, the work of Dash *et al*. (1974) and Bautista-Trujillo *et al*. (2009) did not confirm increased ash content of silage with whey addition, which is not in agreement with our study.

There are conflicting reports regarding the effects of molasses on CP content in silage. Some researchers (Migwi *et al*., 2000, Baytok *et al.*, 2005, Mahala & Khalifa, 2007) reported increased, while others (Van Niekerk *et al.*, 2007, Kwak *et al*., 2009) reported no effect or even decreased (Moore & Kennedy, 1994) CP contents in silage with molasses. The effect of additives on CP in PHHS was not significant. However, whey and molasses in PHPLS increased ($P<0.05$) CP compared to the control. These results supported those of Migwi *et al.* (2000), Baytok *et al*. (2005). The increased CP in PHPLS with whey in the present study was assumed to have been caused by 106 - 130 CP g/kg DM in whey (Weinberg, 2004, Formigoni *et al*., 2006, Ben Salem & Fraj, 2007). However, the increase in CP with molasses is difficult to explain because molasses is reported to contain low levels (44 CP g/kg DM) of CP (Xande *et al.,* 2010).

The effect of additives on the gross energy content of PHHS were not significant (P>0.05). However, whey in PHPLS increased ($P<0.05$) gross energy content compared to the molasses treatment. According to Dolz and De Blas (1992), dietary energy increases with an increase in EE of the diet. This could explain the increase in gross energy of the whey treated PHPLS since it contained a higher

(P<0.05) EE content compared to the other treatments (Table 3.2). Moreover, whey is reported to contain 20 – 80 g/kg DM of EE (Nasi *et al*., 1995, Rapetti *et al*., 1995, Formigoni *et al*., 2006) while no EE was detected in molasses (Xande *et al*., 2010).

High dietary fibre indicates low levels of cell solubles and hence low digestibility (McDonald *et al*., 2002). The fibre content (ADF and aNDF) in the present study were reduced (P<0.05) with molasses and whey, which agrees with Baytok *et al*. (2005) and Bautista-Trujillo *et al.* (2009) in molasses treated silages, and Fazaeli *et al.* (2003) in whey treated silage. This decrease in fibre may have resulted from increased cell wall digestion due to improved silage fermentation caused by these additives (Bautista-Trujillo *et al*., 2009). Furthermore, improved (P<0.05) IVOMD occurred with whey and molasses addition compared to the control. It is assumed that the reduced IVOMD in the control could be related to its high fibre content which led to an increase in indigestible materials (McDonald *et al*., 1991). This agreed with Jeon *et al.* (2003) who reported a reduced digestibility in forest by-product silage that contained high fibre. The improved IVOMD with whey addition could also be attributed to the high concentration (734 g/kg) of readily fermentable carbohydrate (lactose) in whey (Formigoni *et al*., 2006), and agreed with previous observations (Dash *et al*., 1974, Khorvash *et al*., 2006) when whey or lactose was added. More-over, improved IVOMD with molasses addition could be attributed to the added sugars that have not been completely fermented during ensiling (Khorvash *et al*., 2006).

3.3.2 Fermentation

3.3.2.1 Effects of absorbents on silage fermentation

The influence of absorbents on the fermentation characteristics of PH silage is set out in Table 3.4 and Figures 3.1 to 3.3. According to Table 3.4 absorbent x additive interactions occurred for all the fermentation characteristics. From Table 3.4 and Figure 3.1, it is apparent that the addition of PL to PH at ensiling increased silage pH. This may be attributed to the high nitrogen and mineral contents in PL that prevented pH reduction, owing to its high buffering capacity (Ashbell *et al.,* 1995). This is in agreement with Saylor and Long (1972, cited by AI-Rokayan *et al.,* 1998) who achieved a pH of 5.8 from ensiled mixture of ground orchard grass and PL. This increase in pH promoted a clostridial type of fermentation as indicated by its elevated butyric acid and ammonia-N concentrations that reduced silage quality (McDonald *et al.*, 1991, Woolford, 1984). In contrast, ensiling a mixture of pineapple wastes and PL (8:2 as is basis) for 60 days resulted in pH of 3.75 (Nguyeni Thi Hong Nhnan *et al.*, 2009), which is lower than that of PHPLS. This might be attributed to the lower pH (3.97) in the fresh pineapple wastes and a pH of 4.14 in the PL they used. However, the pH in PHHS (Table 4) was also lower than the 4.01 obtained in silage mixture of 770 g/kg potato waste and 230 g/kg hay (Hough *et al*., 1993).

Table 3.4 Effects of absorbents and additives on the fermentation characteristics of potato hash silage after 90 days of ensiling and aerobic stability (CO_2 g/kg DM) after 5 days of aerobic exposure (n = 3)

Parameters	Absorbents	Additives			Significance (P)			CV %
		Control	Whey	Molasses	Absorbents	Additives	Interaction	
pH	Hay	3.6	3.5	3.5	<0.001	0.002	<0.001	2.7
	Poultry litter	6.8[a]	6.4[a]	5.5[b]				
WSC g/kg DM	Hay	13.3[a]	10.2[b]	9.6[b]	<0.001	<0.001	<0.001	9.2
	Poultry litter	7.0[a]	6.2[b]	5.6[b]				
Lactic acid g/kg DM	Hay	48.7[b]	51.2[b]	59.9[a]	0.004	<0.001	<0.001	9.4
	Poultry litter	1.18[b]	2.47[b]	34.6[a]				
Acetic acid g/kg DM	Hay	15.3[a]	13.4[b]	11.7[c]	0.041	<0.001	<0.001	11.6
	Poultry litter	21.1[a]	18.9[b]	18.3[b]				
Propionic acid g/kg DM	Hay	2.8	2.1	1.9	0.061	0.059	0.082	12.3
	Poultry litter	2.4	1.9	1.4				
Butyric acid g/kg DM	Hay	0.43[a]	0.16[b]	0.10[c]	<0.001	0.020	0.031	13.4
	Poultry litter	5.3[a]	4.5[a]	3.2[b]				
NH_3-N g/kg TN	Hay	13	12	11	<0.001	<0.001	0.002	11.9
	Poultry litter	68.5[a]	57.1[a]	25.2[b]				
CO_2 g/kg DM	Hay	7.6	7.3	7.1	<0.001	0.301	<0.001	
	Poultry litter	24.9	24.4	25.6				

[a,b,c] Means of the same row with different superscript differ (P<0.05)

WSC, water soluble carbohydrates; NH_3-N, ammonia nitrogen; CO_2, carbon dioxide

control
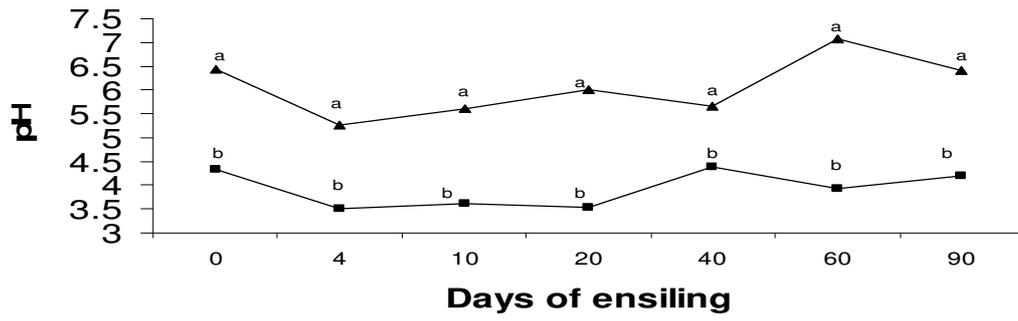
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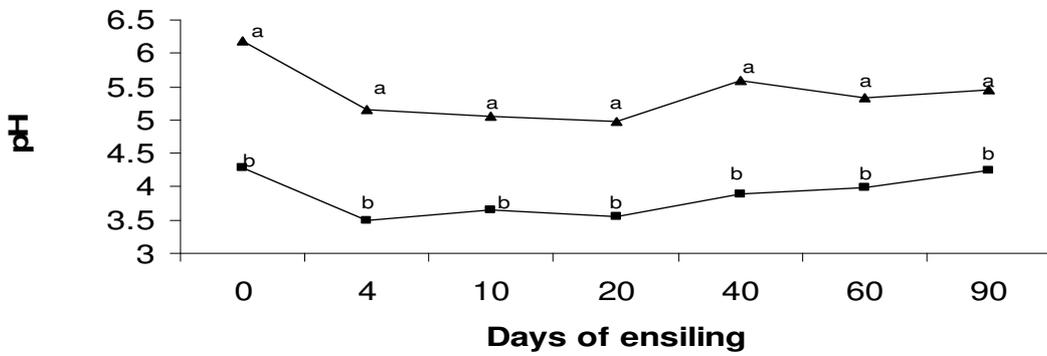
molasses
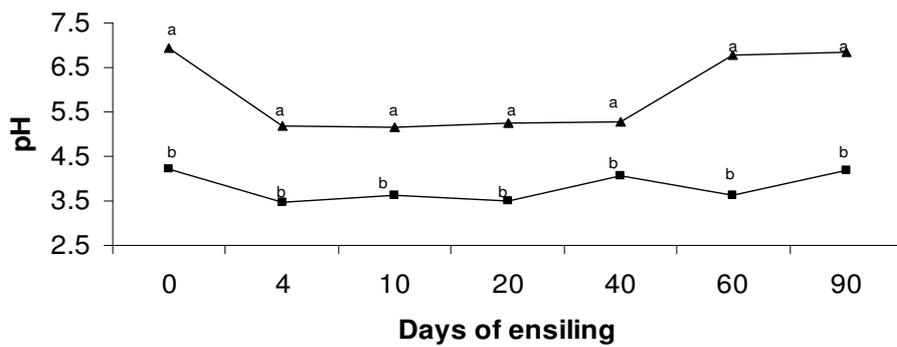
[a,b] Means of the same days with different superscripts differ (*P*<0.05)

Figure 3.1 Effects of absorbents (hay (■) and poultry litter (▲)) on the pH of potato hash silage

The high pH in the PHPLS increased and decreased the concentration of butyric acid and LA respectively compared to PHHS (Table 3.4; Figures 3.2 and 3.3), indications of a clostridial type of fermentation in the silage. In silages made from tropical grasses, a pH of 4.2 has been reported as the maximum to consider silage to be well-preserved (McDonald *et al*., 2002). However, PHHS was well preserved compared to PHPLS in terms of recommended pH. Ensiling a mixture of cashew apple waste with PL (80:20) for 30 days resulted in 43.8 g/kg DM LA concentration and a pH of 4.6 (La Van Kinh *et al*., 1997), which was not achieved in the PHPLS of the present study.

It has been reported that higher acetic acid concentration in silage is a result of inefficient silage fermentation or prolonged fermentation due to high buffering capacity of legume silage (McDonald *et al*., 1991). As a guideline, silages of less than 250 g DM/kg are expected to contain more than 30 g/kg DM of acetic acid (Kung & Shaver, 2001). Although PHPLS had a higher DM compared to PHHS, it contained higher acetic acid concentration than PHHS, which can be attributed to the higher buffering capacity of PL that prevented faster reduction rate in pH (Ashbell *et al*., 1995). According to McDonald *et al*. (1991) a high concentration of acetic acid *vs* LA in silage is an indication of poorly fermented silage. It is clear that the concentrations of acetic acid were higher than that of LA in the PHPLS (Table 3.4). This could be due to its high pH that might have inhibited the activity of LAB which should have produced more LA.

control
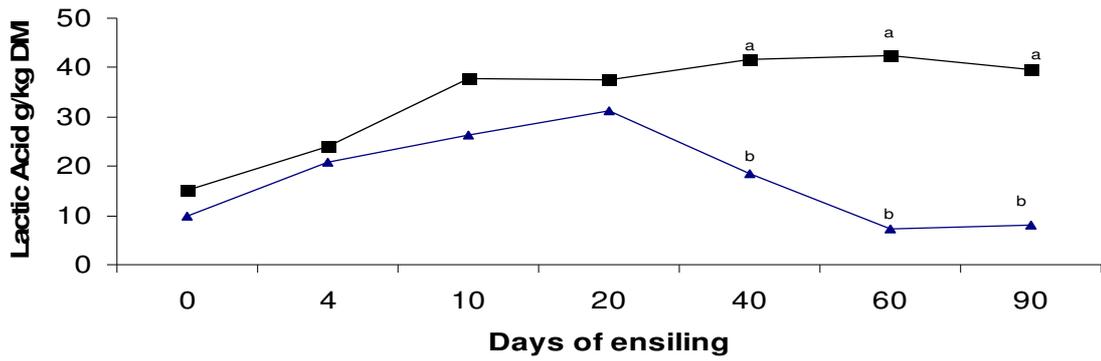
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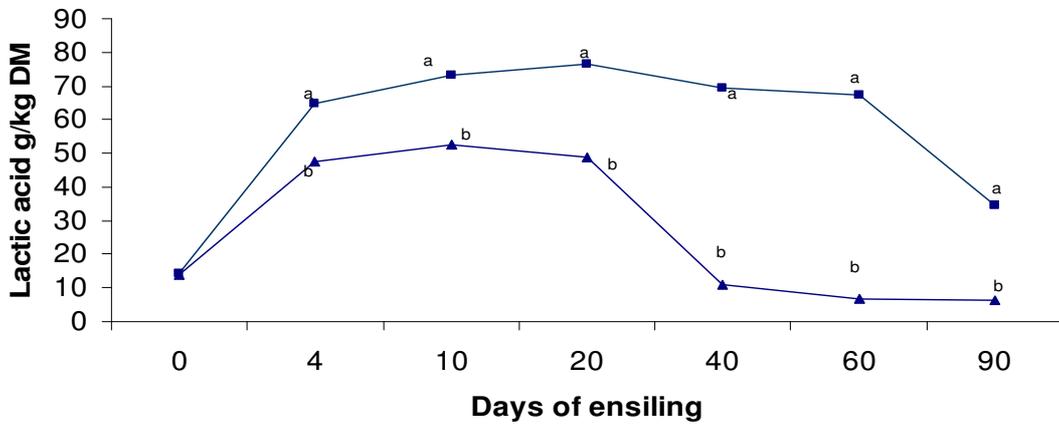
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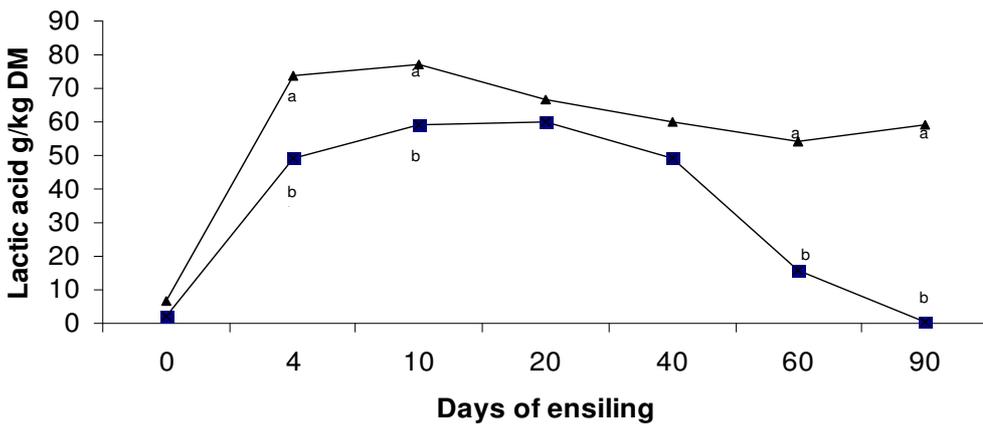
[a,b] Means of the same days with different superscripts differ (*P*<0.05)

Figure 3.2 Effects of absorbents (hay (■) and poultry litter (▲)) on the lactic acid concentration of potato hash silage

control
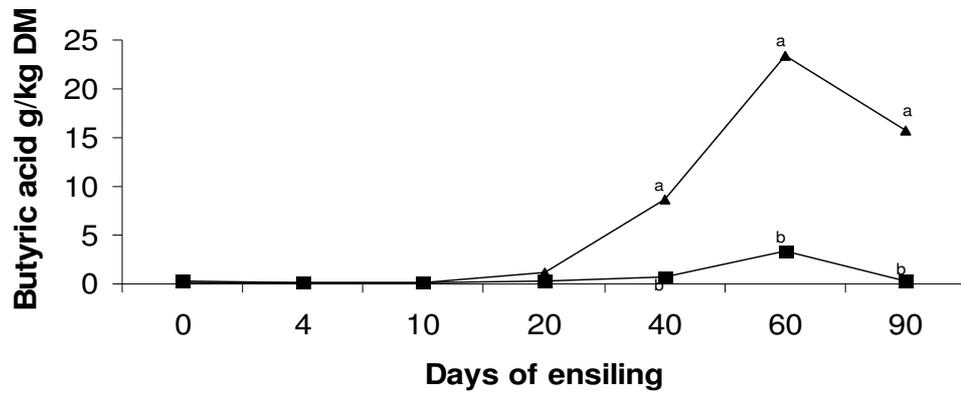
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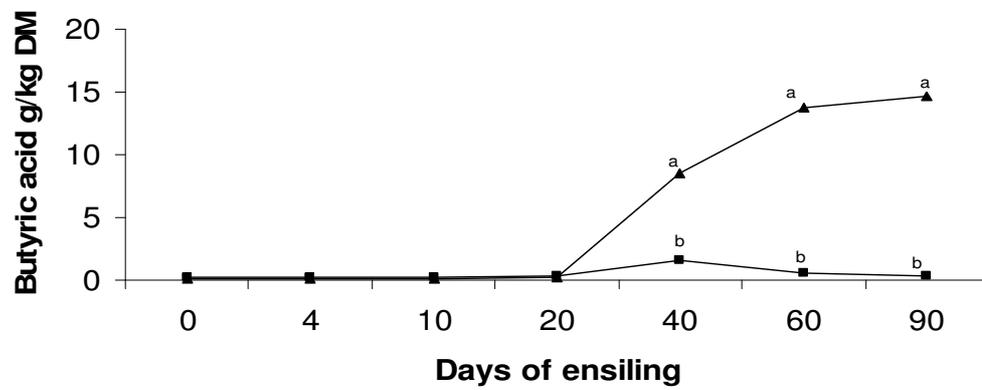
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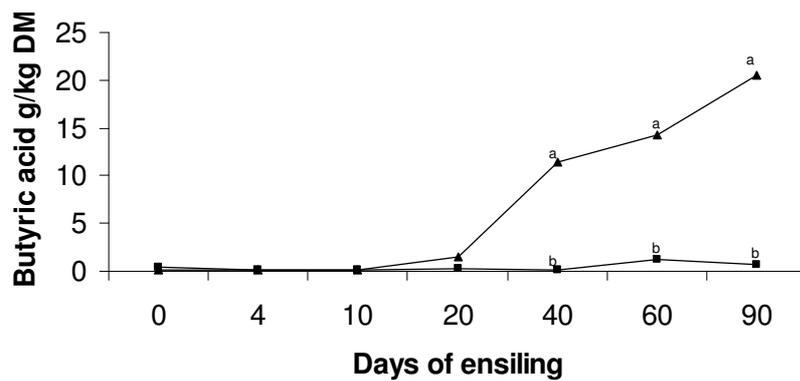
[a,b] Means of the same days with different superscripts differ (*P*<0.05)

Figure 3.3. Effects of absorbents (hay (■) and poultry litter (▲)) on the butyric acid concentration of potato hash silage

The rate of pH decline is important in determining the extent of proteolysis. If the rate of pH decline is slow, more protein will be hydrolysed (McDonald *et al.*, 2002). Thus the higher pH in PHPLS during ensiling increased ammonia-N (Table 3.4), which could have arisen from proteolysis or by the conversion of other nitrogenous constituents into ammonia (Harmon *et al.*, 1975). This could be due to its high pH that might have inhibited the activity of LAB which should have produced more LA.

3.3.2.2. Effects of additives on silage fermentation

A silage with a pH that falls within a range of 3.8 – 4.2 is considered well preserved (McDonald *et al.*, 1973). After 90 days of ensiling (Table 3.4) the pH in PHHS was reduced to less than 3.6 while that of PHPLS was still high (> 5), indicatives of poor fermentation in the latter. However, it is clear from Table 3.4 and Figure 3.4 that additives did not influence the pH in the PHHS at days 60 and 90. This is consistent with Bautista-Trujillo *et al.* (2009) who reported that whey and molasses addition to maize at ensiling did not influence the pH. In contrast several researchers reported reduced silage pH with the addition of whey (Zobell *et al.*, 2004) and molasses (Baytok *et al.*, 2005, Kwak *et al.*, 2009). However, the effect of additives on pH were significant (P<0.01) in the PHPLS.

PHHS (Potato hash hay silage)
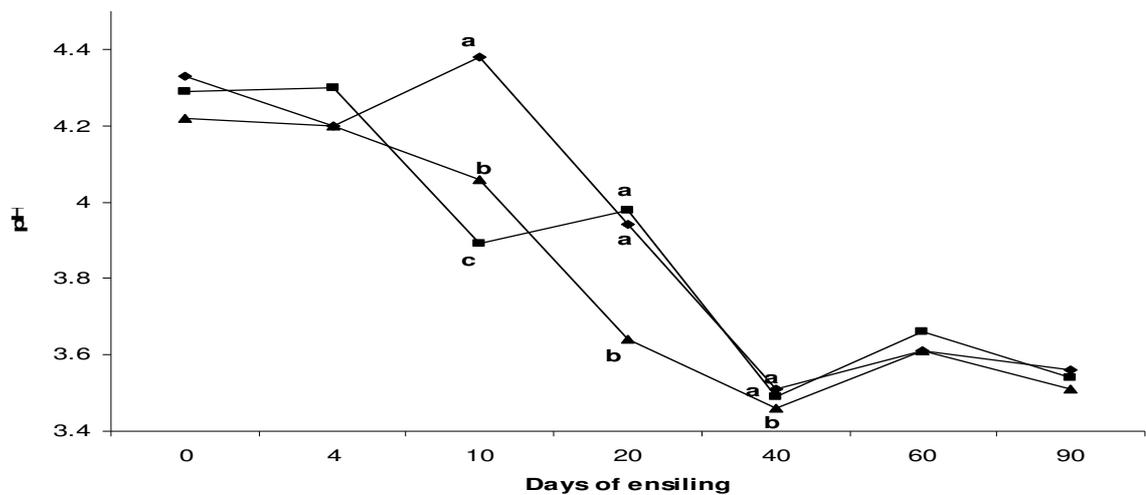
Means of the same days with different superscripts differ (*P*<0.05)

PHPLS (Potato hash poultry litter silage)
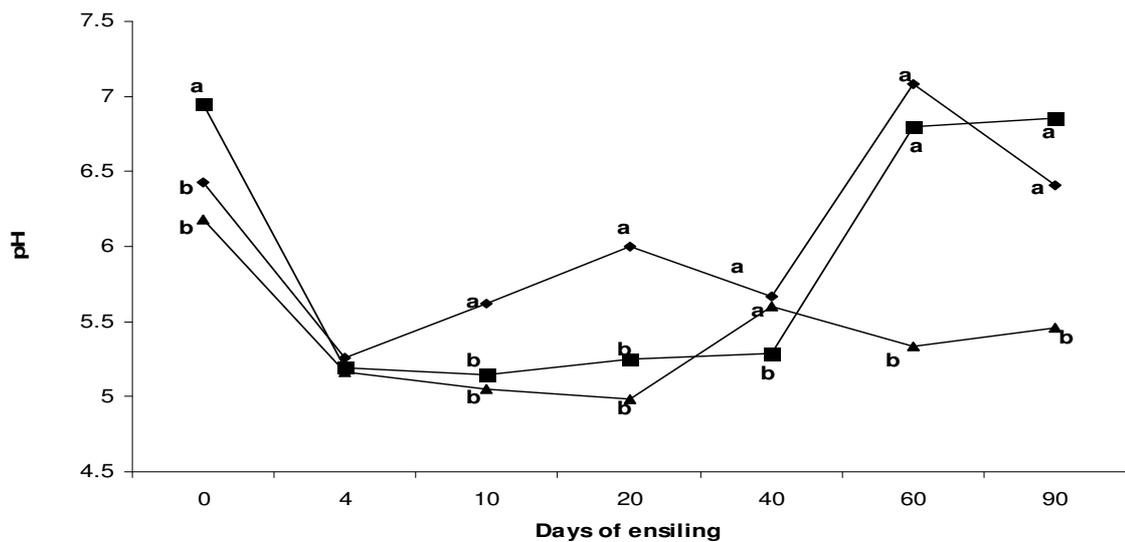
Means of the same days with different superscripts differ (*P*<0.05)

Figure 3.4 Effects of treatments (control ♦, whey ■ and molasses ▲) on pH of potato hash silage

Molasses addition reduced (*P*<0.05) the pH. Accordingly, molasses further resulted in a reduced ammonia-N and butyric acid as well as increased LA concentration in the PHPLS (Table 3.4). This is consistent with others (Baytok *et al.*, 2005, Bautista-Trujillo *et al.,* 2009, Kwak *et al.*, 2009) who reported reduced pH with

molasses addition. Although a pH of < 3.6 occurred in PHHS and could depress silage intake by ruminants, studies from Shaver *et al*. (1985) and Moloney and O`Kiely (1999) did not report a depressed intake in steers fed grass silage that contained low pH (3.5). However, if intake of this silage becomes a problem, the addition of sodium bicarbonate to neutralize silage acidity can be a solution.

High quality silage is likely to be achieved when LA is the predominant acid produced, as it is the most efficient fermentation acid and reduces silage pH more efficiently than other fermentation products (McDonald *et al*., 2002). According to De Brouwer *et al*. (1991), a good silage is characterised by 80 – 120 g/kg DM of LA concentrations. The LA concentrations of PHHS (Table 3.4) of the present study were lower than the proposed 80 g/kg DM. These low LA concentrations are an indication that the WSC concentrations at pre-ensiling were not sufficient enough to promote efficient fermentation (Yang *et al*., 2006). As expected, molasses increased (*P*<0.01) LA concentration compared to the other treatments in both PHHS and PHPLS (Table 3.4 and Figure 3.5), supporting other researchers (Van Niekerk *et al*., 2007, Weinberg *et al*., 2008, Kwak *et al*., 2009, Nkosi *et al*., 2009). This might be attributed to the rapid degradation of WSC by LAB to produce LA thereby reducing silage pH (McDonald *et al*., 1991).

It seems from the results in Table 3.4 that acetic acid concentrations were reduced by whey and molasses addition. Accordingly there are some reports that confirmed reduced acetic acid in silage with molasses and whey additions (Baytok *et al*., 2005, Van Niekerk *et al*., 2007, Kwak *et al*., 2009). In contrast, Nkosi (2003) reported increased acetic acid in citrus and mango leaves silages treated with whey at

ensiling. This is because the silage in the latter study was not well preserved as indicated by its higher concentration of butyric acid.

Butyric acid is an indication of loss of energy in silage (McDonald *et al.*, 1991) and less than 0.1 g/kg DM is normally found in well preserved silage (Kung & Shaver, 2001). It has been reported that molasses addition to a high moisture forage at ensiling may result in a heterofermentative type (butyric acid production) of fermentation (Woolford, 1984). It was therefore expected that molasses and whey addition to the PH mixtures at ensiling will result in a heterofermentation of the silage. In contrast, whey and molasses in PHHS reduced ($P<0.05$) butyric acid concentration compared to the control. However, only PHHS containing molasses had less than 0.1 g butyric acid/kg DM, indicative of well preserved silage. This supported others (Baytok *et al.*, 2005, Van Niekerk *et al.*, 2007, Nkosi *et al.*, 2009) who confirmed reduced butyric acid concentration with molasses addition.

Ammonia-N in silage reflects the degree of protein degradation, and extensive proteolysis adversely affects the availability of N to ruminants (Wilkinson, 2005). Well-preserved silages should contain less than 100 g NH_3-N/kg TN (McDonald, *et al.*, 2002). It has been reported that biological additives reduced proteolysis during ensiling and resulted in improved efficiency of silage protein utilization and reduced N losses (Charmley, 2001). It is evident from Table 3.4 that the additives did not ($P>0.05$) influenced the concentration of ammonia-N in the PHHS. However, molasses in PHPLS reduced ($P<0.05$) ammonia-N concentrations compared to other treatments. These findings corroborated the work of Migwi *et al.* (2000) and Kwak *et al.* (2009). In contrast, Baytok *et al.* (2005) obtained higher ammonia-N in molasses treated silage compared to the control, citing high moisture content of forage and the level of molasses used.

PHHS (Potato hash hay silage)
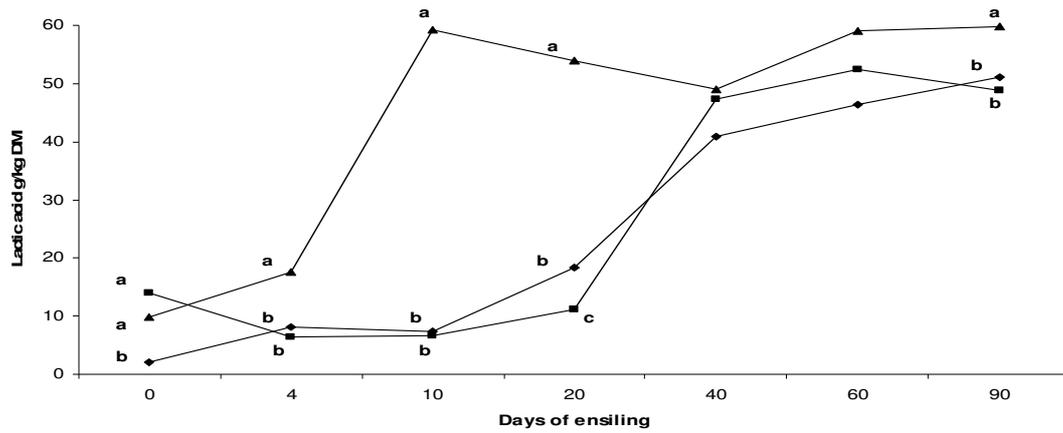
a,b,c Means of the same days with different superscripts differ ($P<0.05$)

PHPLS (Potato hash poultry litter silage)
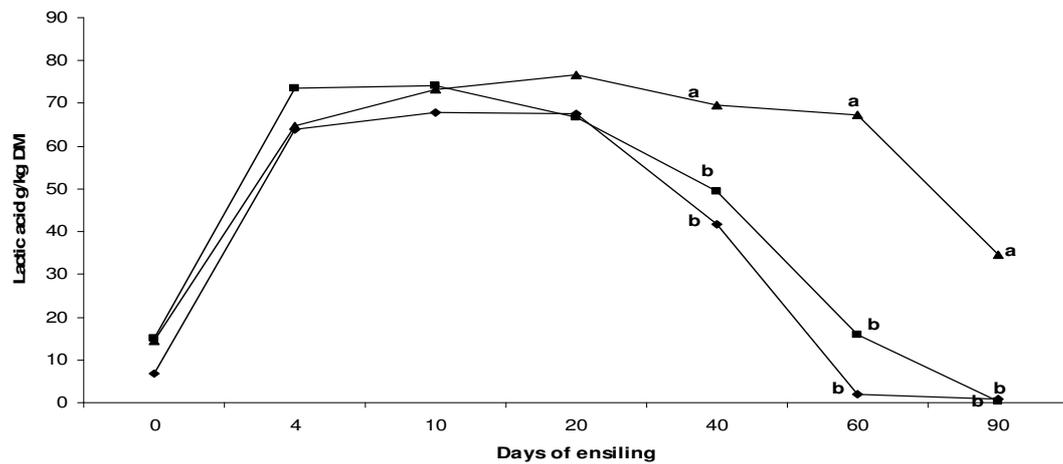
a,b Means of the same days with different superscripts differ ($P<0.05$)

Figure 3.5 Effects of treatments (control ♦, whey ■ and molasses ▲) on the lactic acid concentrations of potato hash silage
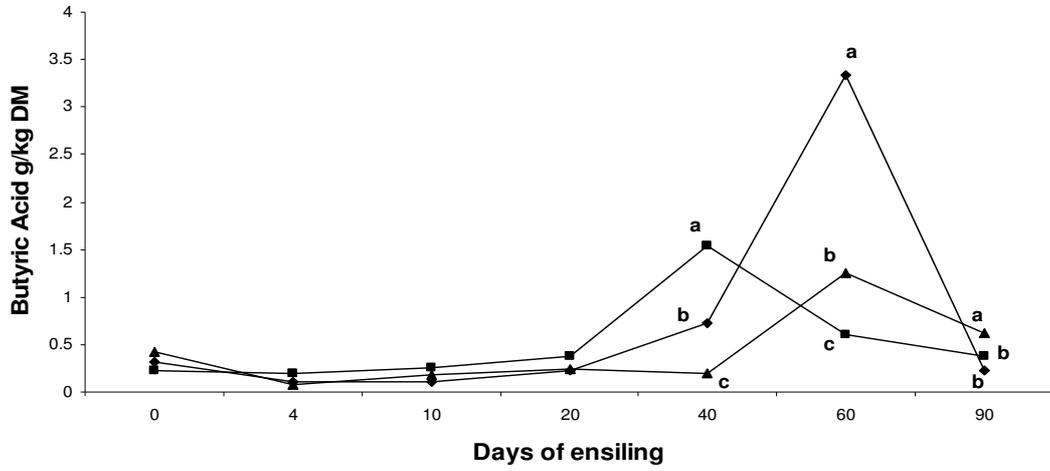
PHHS (Potato hash hay silage)

a,b,c Means of the same days with different superscripts differ (*P*<0.05)

PHPLS (Potato hash poultry litter silage)
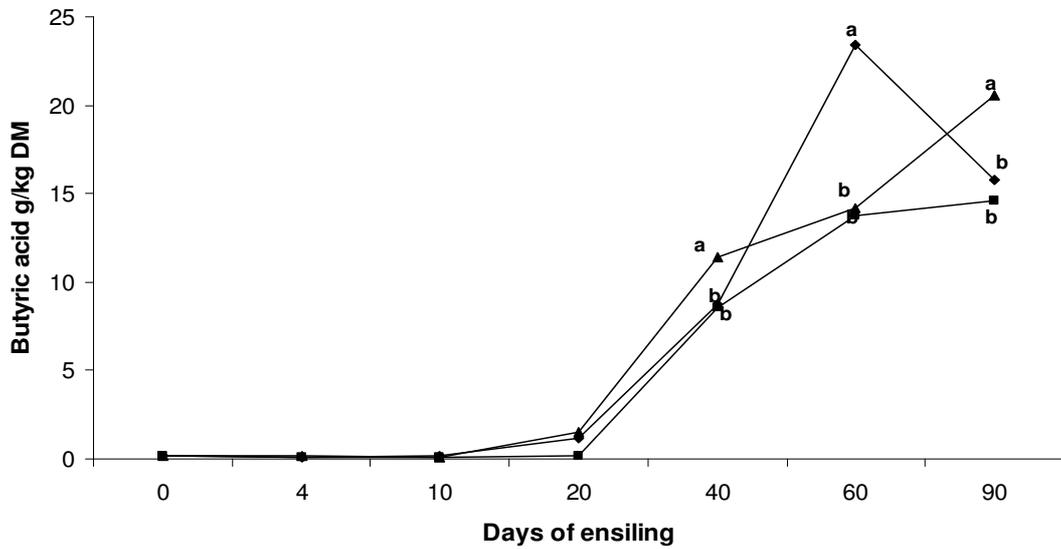
a,b, Means of the same days with different superscripts differ (*P*<0.05)

Figure 3.6 Effects of treatments (control ♦, whey ■ and molasses ▲) on the butyric acid concentration of potato hash silage

3.3.3 Aerobic stability of silage

3.3.3.1 Effect of absorbents on aerobic stability

A higher CO_2 production in silage indicates the activity of yeasts and moulds, which cause a rise in temperature and deteriorate the quality of silage (Ashbell *et al.*, 1991). According to O`Kiely (1989) badly preserved silages are usually more stable under anaerobic conditions than well preserved silages. From the results in Table 3.4 it is clear that PHPLS was badly preserved and also had higher CO_2 production and therefore lower aerobic stability than PHHS. This is despite of its higher acetic acid concentration, which is known to have a positive effect on silage aerobic stability (Danner *et al.*, 2003).

3.3.3.2. Effect of additives on aerobic stability

It has been established that improved aerobic stability of silages is associated with higher concentrations of acetic acid compared to untreated silages (Weinberg & Muck, 1996, Driehuis *et al.*, 1999, Danner *et al.*, 2003, Nkosi *et al.*, 2009). According to Weissbach (1996) a concentration of more than 3 g/kg DM of acetic acid is enough to stabilize silage during aerobic exposure. Acetic acid concentrations in silages in the present study were higher than 3 g/kg DM, indications that they were stable when exposed to air. Accordingly it seems from the CO_2 results that the addition of whey and molasses to PH silage did not influence aerobic stability. It has been reported that whey addition to grass silage (Negron, 2006) and bermudagrass (Umana *et al.*, 1991) reduced the aerobic stability of silage as indicated by an increased temperature. In

contrast, Nkosi (2003) reported improved aerobic stability in citrus and mango silages treated with whey.

3.4. Conclusions

The high ME, IVDMD and IVOMD in PH suggest that it can be used as an energy feed source. Ensiling PH with ground hay compared to PL as an absorbent, resulted in a better quality silage as indicated by improved fermentation characteristics and chemical composition. Although the addition of PL significantly increased the CP and ash contents in PH silage, fermentation characteristics were influenced detrimentally as indicated by higher pH, butyric acid, acetic acid and lower LA concentrations. Furthermore, the aerobic stability of PH silage containing PL was poor and could influence animal performance negatively. Furthermore it seems that whey and molasses were effective in improving the IVOMD and fermentation characteristics of PH. However the best results regarding energy (IVOMD), LA, acetic acid and butyric acid concentrations were obtained with molasses addition. Further studies are needed to elucidate the impact of the silage on animal performance.

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CHAPTER 4

Effects of whey and sugarcane molasses as silage additives on potato hash silage quality and growth performance by lambs

4.1 Introduction

A major constraint to a profitable livestock production under resource poor farmers in the Gauteng Province (South Africa) is the scarcity of feed supply throughout the year (GADS, 2006). This province is experiencing a rapid growing human population coupled with high demand for housing which limit land suitable for fodder cultivation. The utilization of less traditional feeds such as by-products combined with roughage sources may provide farmers with a variety of feeding options. By-product feeds are produced by a number of food processing industries, and such resources may impact traditional ruminant feeding practices by reducing the amount of concentrate fed to ruminants, providing feeding options when there is a scarcity of feed and reduce feed cost. Potato hash, a by-product from the production of snacks and chips, is available in large amounts in the Gauteng Province and is not widely used in livestock feeding. If it is not consumed, it often gets mouldy and sour, and is unlikely to be used as animal feed. Because of problems associated with the fresh form of potato hash, drying and ensiling are two common methods of preserving high moisture by-products. However, the drying process is costly and may not be affordable to the resource poor farmers.

Interest in conserving by-products by ensiling is steadily increasing largely due to the increase in their use as animal feed (Megias *et al.*, 1998, Kayouli & Lee, 1999, Bakshi *et al.*, 2006, Kholif *et al.*, 2007). Properly ensiled silage from high moisture by-products can replace costly feeds such as maize silage in ruminant diets

(e.g. Itavo *et al*., 2000, Lallo *et al*., 2003, Pirmohammadi *et al*. 2006). However, ensiling of potato hash requires substantial amounts of fermentable sugars to produce sufficient lactic acid which lower the pH and stabilize the product (McDonald, 1981, Wilkinson, 2005). Generally, potato by-products contain relatively low concentrations of water soluble carbohydrates (WSC) and lactic acid bacteria (LAB) (O`Kiely *et al*., 2002, Okine, 2007) due to damages to LAB during food processing of the crops (Moon, 1981). Thus it requires silage additives to improve the fermentation during ensiling. Some food by-products are successfully ensiled with chemical additives with subsequent improvement in fermentation quality and digestibility of the silages (Megias *et al*., 1998, Kholif *et al*., 2007).

However, chemical additives have their own limitations as they are corrosive to the equipment used and can be dangerous to handle. Due to this, biological additives are often preferred (Gwayumba, 1997). Biological additives also have their own limitation. They are costly to the farmer and their effectiveness can be less reliable, since it is based on the activity of living organisms (Weinberg & Muck, 1996). Alternatively, food waste materials such as whey (Nkosi, 2003, Zobell *et al.*, 2004, Bautista-Trujillo *et al*., 2009) and sugarcane molasses (Bolsen *et al*., 1996, Yunus *et al*., 2000, Van Niekerk *et al*., 2007, Nkosi *et al*., 2009) can be used as silage additives to ensile high moisture potato hash. The present study compared the fermentation characteristics of potato hash silage produced with or without additives (whey and molasses) with that of maize silage, and its subsequent effect on digestibility, feed intake and performance of lambs fed on the silage diet.

4.2 Materials and Methods

4.2.1. Silage fermentation

Potato hash was collected from Simba (PTY) LTD (Andre Greyvensteyn Avenue, Isando, South Africa), a food producing factory in the Gauteng Province and brought to the Agricultural Research Council -Irene Institute (longitude 28^0 13`S : latitude $25^0$ 55`E, altitude 1524 m) South Africa for chemical analysis, silage making and lamb feeding experiment. To prevent effluent loss during ensiling, potato hash silage was produced by mixing 800 g/kg potato hash (850 g moisture, 105 g CP/ kg DM, 43 g ash/kg DM, 370 g aNDF/kg DM, 163 g ADF/kg DM, 110 g EE/kg DM) with 200 g/kg *Eragrostis curvula* hay (60 g moisture, 45 g CP/kg DM, 39 g ash/kg DM, 789 g aNDF/kg DM, 432 g ADF/kg DM, 33 g EE/kg DM) and treated with: untreated (no additive), whey or molasses. Where molasses was used, it was diluted with warm water at a ratio of 1:2 (4 h before application), and was sprayed over the material at a application rate of 30 l per ton fresh material (FM). Whey was screened for lactic acid bacteria (LAB) populations and had LAB population of 6.95×10^5 LAB cfu/ml ($\pm$ 0.341 SEM) before ensiling. Whey was sprayed at 30 l per ton fresh materials to obtain at least $> 1.7 \times 10^4$ cfu/g FM. Maize silage, a fourth silage treatment was produced by chopping whole crop maize (Senkuil, Sensako, Brits, South Africa) (360 g DM/kg, pH of 5.7 and 43 g WSC/kg DM) with a Feraboli 945 precision silage chopper (Fondata Nel, Cremona, Italy) to obtain a 5 mm chop length, and was ensiled in 210 l drums without an additive. In order to compensate water that was added to the treated silage, both the maize and untreated potato hash were sprayed with 30 l of distilled water over a ton of fresh material to keep them in the same level of moisture as with the treated silages. The materials were ensiled in 210 l drums that were lined with plastic bags and were closed with a rubber lid to prevent

damages to the bags by rodents. After 90 days of ensiling, the drums were opened and samples were collected and analysed for chemical composition and fermentation characteristics following standard procedures.

4.2.2. Lamb growth study

Experimental diets that contained either potato hash silages or maize silage were formulated as shown in Table 4.1. The 4 dietary treatments were: a) maize silage (MS), b) untreated potato hash silage (UPHS), c) whey treated potato hash silage (WPHS) and d) molasses treated potato hash silage (MPHS). Samples of the diets were collected fortnightly and analysed for chemical composition. The diets were fed *ad libitum* to 32 South African Dorper lambs (23.5 ± 0.873 kg live weight) housed in individual metabolic crates (2.2 m^2) in an insulated well-ventilated barn. The lambs were allocated in a complete randomized design on the basis of live weight to the four diets, resulting in 8 lambs per treatment. Lambs were ear tagged, and treated against internal and external parasites before the commencement of the trial. Feed intake was measured daily while live weight was measured at the start of the trial and on weekly intervals until the end of the trial. A 14 day dietary adaptation period was offered and the trial lasted for 63 days.

4.2.3. Digestion study

A 7 day faecal collection period was conducted a week after the growth study. Lambs were fitted with leather harnesses and canvass bags attached to the back of each lamb 3 days before the digestion trial started. Daily feed intake and faeces were collected. Faeces accumulated for the 7 day period were pooled per lamb and sub-samples were collected for laboratory analyses. Feaces samples were frozen at - 20˚C.

4.2.4. Potato hash silage intake and digestibility study

This study was done to evaluate the effects of feeding potato hash silage to ruminants without supplementation on feed intake and digestion. Potato hash silages (UPHS, WPHS and MPHS) with the fermentation and chemical compositions that are shown in Table 4.2 were fed to 9 mature South African Dorper sheep (43.5 kg ± 0.214 live weight) in a 3 x 3 Latin square experimental design for 30 d. The sheep were randomly assigned to treatment in the first period (10 d, i.e. 5 d adaptation and 5 d faecal collection periods). There were 3 sheep per treatment per period. Sheep were fitted with leather harnesses and canvass bags attached to the back of each sheep 3 days before the digestion trial started. Daily feed intake and faecal outputs were recorded. Faeces accumulated for the 5 d period were pooled per sheep and sub-samples were collected for the determination for chemical analyses and saved frozen.

Table 4.1 Composition of experimental diets (%, as is basis)

	Treatments			
Ingredient %	UPHS	MPHS	WPHS	MS
Maize meal	52	54	51	44
Wheat bran	10	10	10	10
Molasses meal	10	10	10	10
Silage	20	18	19	20
Hay (*E. curvula*)	0	0	0	8
Cotton oil cake	5	5	7	5
Feedlime	1.4	1.4	1.4	1
Ammonium sulphate	0.5	0.5	0.5	0.5
Urea	0.6	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Mineral premix[a]	0.06	0.06	0.06	0.06

UPHS; untreated potato hash silage, MPHS; molasses potato hash silage, WPHS; whey potato hash silage, MS; maize silage

[a] DM basis: vitamin A 8 mg, vitamin D3 1.6 mg, vitamin E 20 mg, selenium 10 mg/kg, potassium 215 mg/kg, iron 50 mg/kg, cobalt 20 mg/kg, zinc 50 mg/kg, manganese 1600 mg/kg, copper 300 mg/kg, iodine 70 mg/kg, calcium 220 mg/kg, phosphorus 280 mg/kg, sulphur 30 g/kg, salt 950 g/kg.

4.2.5. Chemical analysis

A 40 g silage sample from each drum was collected and mixed with 360 ml of distilled water in a stomacher bag, homogenized and left at 10^0C for 24 h (Suzuki & 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 used for determination of pH, water soluble carbohydrates (WSC), volatile fatty acids (VFAs), lactic acid (LA) and ammonia-N (NH_3-N). The WSC were determined by the phenol-sulphuric acid method according to Dubois *et al*. (1956) and lactic acid was determined by the colorimetric method of Barker and Summerson (1941) as modified by Pryce (1969). The VFAs were determined with a Varian 3300 FID Detector gas chromatograph (Varian Associates, Inc., Palo Alto, CA, USA) by the procedure of Suzuki and Lund (1980). Ammonia N was determined by distillation using a Buchi 342 apparatus and a Metrohm 655 Dosimat with a E526 titrator according to AOAC (1990). This method is based on the method of Pearson and Muslemuddin (1968) for determining volatile N.

The dry matter (DM) of silage, diets and faeces was determined by drying the samples at 60^{0}C in a force air oven until a constant mass was achieved, following the procedure of AOAC (1990). After drying, the samples were ground through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) for chemical analyses. Following the procedures of Van Soest *et al.* (1991), the neutral detergent fibre (aNDF) concentration was determined using amylase (Sigma-Aldrich Co. LTD., Gillingham, UK, no. A-1278) and 2-ethoxyethanol, and the acid detergent fibre (ADF) concentration was determined using the Fibretec System equipment (Tecator LTD., Thornbury, Bristol, UK). Residual ash content was taken into account for both aNDF and ADF results. Crude protein (CP), ash and ether extract (EE) were determined according to the procedure of AOAC (1990), while metabolizable energy (ME) was determined by using the gas production technique of Pienaar (1994).

4.2.6 Statistical analysis

Data for the fermentation and chemical composition of the silage and diets was subjected to ANOVA for randomized complete design, while that of growth and digestibility studies were subjected to a completely randomized design (CRD) using Genstat (2000). Where P value is significant (P<0.05), statistical differences between the means were declared using the Fisher's protected least significance difference (LSD) using the model: $Y_{ijk} = \mu + t_i + d_j + (td)_{ij} + \varepsilon_{ijk}$

where: Y_{ijk} is the individual observations of the i-th treatment and the j-th day and the k-th replicate, μ is the overall mean, t_i is the effect of the i-th treatment, d_j is the effect of the j-th day, $(td)_{ij}$ is the interaction between t and d, and ε_{ijk} is the residual error.

Data on potato hash silage intake and digestibility was analyzed in a 3 x 3 Latin Square Design using the model: $Y_{ijk} = \mu + p_i + s_j + t_k + \varepsilon_{ijk}$

where: Y_{ijk} is the individual observations of the i-th row (period), the j-th column (sheep) and the k-th treatment, μ is the general effect, P_i is the effect of the i-th row, S_j is the effect of the j-th column, t_k is the effect of the k-th treatment, and ε_{ijk} is the random variation or experimental error.

4.3. Results and Discussions

4.3.1 Silage fermentation

Data on the chemical composition and fermentation characteristics of the silages is presented in Table 4.2. After 90 days of ensiling, the DM of MS was higher (*P*<0.05) than the potato hash silages due to low DM content (250 g/kg) of the latter at pre-ensiling. Water-soluble carbohydrates are regarded as essential substrates for the growth of LAB for proper fermentation, and low levels may restrict LAB growth (McDonald *et al*., 1991). Haigh and Parker (1985) suggested that a concentration of more than 30 g/kg DM of WSC in a herbage is critical for a successful fermentation. The concentration of WSC in the potato hash mixture at pre-ensiling was 22 g/kg DM, indicating that it was not enough for efficient fermentation. This justified the addition of whey and molasses to improve the fermentation process.

The pH of potato hash silages after 90 days of ensiling was reduced to 4.5 for the UPHS and 4.2 for the MPHS and WPHS. However, the pH of UPHS was not low enough for efficient preservation, because it should be 4.20 or 4.35 at a DM content of 200 and 250 g/kg (Weissbach, 1968) respectively. According to McDonald *et al*.

(2002) silage with a pH range of 3.8 to 4.2 is considered well preserved and the UPHS did not achieve this target. Moreover, good quality silage is characterized by 30 – 140 g/kg lactic acid concentration (Zobell *et al.*, 2004) and UPHS had lactic acid concentration (26 g/kg DM) lower than this level. Maize silage had the lowest ($P<0.05$) pH, acetic acid, butyric acid, propionic acid and ammonia-N, and highest lactic acid ($P<0.05$) concentration compared to the potato hash silages. It has been reported that silage from maize can be produced without the use of additives due to the fact that maize has a low buffering capacity and has enough sugar for efficient fermentation (McDonald, 1981, Meeske, 2005). This might be the reasons for its better fermentation quality compared to the potato hash silages.

The fermentation characteristics of MS recorded in the present study corresponds well with that of maize silage of 300 g/kg to 400 g/kg DM content reported by Kung and Shaver (2001). This study further revealed that whey and molasses addition increased ($P<0.05$) the concentration of lactic acid, reduced silage pH and the concentrations of butyric acid and ammonia-N compared to the UPHS, indications of well-preserved silages (McDonald *et al.*, 1991). This result agrees well with previous work that reported higher lactic acid concentrations, lower pH and ammonia-N content when molasses (Bolsen *et al.*, 1996, Yunus *et al.*, 2000) and whey (Bautista-Trujillo *et al.*, 2009, Zobell *et al.*, 2004) were added to a forage at ensiling compared to the control. Moreover, whey and molasses addition reduced ($P<0.05$) the fibre content of the silage as compared to MS and UPHS. This could be attributed to partial hydrolysis of hemicelluloses in the treated silages (Muck & Kung, 1997). Our result agreed with Fazaeli *et al.* (2003) and Guney *et al.* (2007) who reported a decrease in fibre content for liquid whey treated straw silage and for molasses treated sorghum silage, respectively.

Table 4.2 Chemical composition and fermentation characteristics of pre-ensiled potato hash, potato hash silage and maize silage after 90 days of ensiling (n = 7)

	Pre-ensiled potato hash mixture	Ensiled Treatments				
		UPHS	MPHS	WPHS	MS	SEM
DM g/kg	250	232[b]	236[b]	230[c]	320[a]	0.530
Ash g/kg DM	59.4	60.4[a]	55.0[b]	61.1[a]	53.7[c]	1.81
CP g/kg DM	89.1	72.3[c]	82.5[b]	86.0[a]	86.0[a]	0.003
CF g/kg DM	259	334[b]	303[c]	275[d]	340[a]	0.4
EE g/kg DM	73	45[a]	37[b]	34[c]	23[d]	0.283
ME MJ/kg DM	10.0	7.8[c]	9.6[b]	9.7[b]	11.8[a]	0.05
pH	4.8	4.5[a]	4.2[b]	4.2[b]	3.9[c]	0.01
WSC g/kg DM	22	13[c]	17[b]	15[bc]	36[a]	0.27
LA g/kg DM		26.1[d]	47.5[b]	42.5[c]	77.6[a]	0.18
AA g/kg DM		28.5[a]	21.0[c]	23.5[b]	3.7[d]	0.26
PA g/kg DM		6.3[a]	6.7[a]	5.2[b]	0.1[c]	0.88
BA g/kg DM		0.91[a]	0.42[c]	0.52[b]	0.01[d]	0.03
NH$_3$-N as %TN		9.8[a]	7.5[b]	7.5[b]	5.1[c]	0.09

[a,b,c] Means with different letters in a row differ significantly (P<0.05)
UPHS; untreated potato hash silage, MPHS; molasses potato hash silage, WPHS; whey potato hash silage, MS; maize silage, LA; lactic acid, WSC; water-soluble carbohydrates, AA; acetic acid, PA; propionic acid, BA; butyric acid, TN; total nitrogen.

Ammonia-N in silage reflects the degree of protein degradation (Wilkinson, 2005), and well-preserved silages contain less than 100 g NH$_3$-N/kg TN (McDonald *et al*., 2002). The silages in the present study had ammonia-N concentrations of less than 100 g NH$_3$-N/kg TN. However, treating potato hash silage at ensiling with either whey or molasses further reduced (*P*<0.05) the ammonia-N concentration compared to the UPHS, confirming the work of other researchers (Yunus *et al*., 2000, Bautista-Trujillo *et al*., 2009, Nkosi *et al*., 2009) who found reduced NH$_3$-N production. This can be explained by the fact that whey and molasses reduced the pH resulting in a decreased production of NH$_3$-N in the silage compared to the UPHS. The higher concentration of NH$_3$-N in UPHS led to a decrease in the CP content of the silage compared to the other silages.

Higher (*P*<0.05) concentration of butyric acid occurred in the UPHS, leading to a reduced energy content of the silage compared to the other silages. It is well established that adding molasses and whey reduced the concentration of butyric acid

in silage (Bautista-Trujillo *et al*., 2009, Nkosi *et al*., 2009). A concentration of < 0.1 g/kg DM butyric acid is typical found in well preserved silage (Kung & Shaver, 2001) and only the MS had the acceptable butyric acid concentration compared to the potato hash silage. Butyric acid is associated with a clostridial type of fermentation and usually associated with high moisture silages (McDonald, 1981) and MS had higher DM content compared to the potato hash silages. The ME content in the MPHS, WPHS and MS is within the range of 9.6 – 12.2 ME MJ/kg DM typically reported for silages (Wilkinson, 2005). The reduction of ME in the UPHS might be attributed to the high butyric acid content, which is an indication for the loss of energy in the silage (McDonald, 1981).

Table 4.3 Chemical composition of experimental diets formulated with either potato hash silages or maize silage and fed to lambs (n = 7)

	Treatments				
	UPHS	**MPHS**	**WPHS**	**MS**	**SEM**
DM g/kg	774[b]	781[a]	775[b]	784[a]	0.53
Ash g/kg DM	73.8[a]	68.2[b]	67.6[b]	63.4[c]	1.59
CP g/kg DM	134[b]	141[a]	137[ab]	141[a]	1.03
ME MJ/kg DM	11.8	11.9	11.8	12.0	0.69
ADF g/kg DM	132[b]	89[d]	103[c]	181[a]	0.37
aNDF g/kg DM	309[b]	222[d]	253[c]	348[a]	1.17
EE g/kg DM	45.1[a]	35.3[c]	37.6[b]	35.8[bc]	0.57
IVOMD %	71[d]	76[b]	75[c]	77[a]	0.02

[a,b,c] Means with different letters in a row differ significantly ($P<0.05$)

UPHS; untreated potato hash silage, MPHS; molasses potato hash silage, WPHS; whey potato hash silage, MS; maize silage, DM; dry matter, CP; crude protein, ME; metabolizable energy, ADF; acid detergent fibre, aNDF; amylase treated neautral detergent fibre, EE; ether extract, IVOMD; in vitro organic matter digestibility

4.3.2 Lamb growth study

According to Wilkinson (2005) silages may contain 72 – 89 g/kg DM of CP (McDonald, 1981) and 9.6 – 12.2 ME MJ/kg DM and require supplementation to achieve a daily gain of more than 150 g/d in lamb commercial operations (Marley *et al*., 2007). Feeding lambs on silage alone generally leads to either loss of live weight or limited daily gains (Fitzgerald, 1986), and Speijers *et al*. (2005) obtained daily gains of 45 g/d from lambs that were fed Lucerne silage supplemented with 250 g sugarbeet pellets. Data on the chemical composition of the silages (Table 4.2) showed that they were low in DM, CP and energy contents. Therefore diets that contained < 200 g/kg silage (either potato hash or maize silages) were formulated (Table 4.1) to improve the nutritive value of the silage diets. The chemical composition of the diets (Table 4.3) shows that the diets had similar (*P*>0.05) energy content, but had different (*P*<0.05) contents of DM, CP, fibre, EE and IVOMD. Maize silage had higher (*P*<0.05) fibre fractions while the fibre fractions for WPHS and MPHS was low.

Data on the growth performance and nutrient digestibility in lambs fed the experimental diets is shown in Table 4.4. Lambs fed the MS and MPHS diets had higher (*P*<0.05) DMI, ADG and final live weights compared to the other diets. This might be attributed to higher DM content in the two silages which is known to improve DMI and growth rates in ruminants (Mustafa *et al*., 2008). In addition, the MS and MPHS had lower butyric acid content compared to the other silages. Increased concentration of butyric acid in silage is associated with poorly fermented silages and usually results in depressed intake by ruminants (McDonald *et al*., 2002). Moreover, feeding sheep on maize silage is known to result in a positive effect on feed intake (Provenza, 1995). It has been reported that finishing lambs on a diet that

contained 180 g/kg apple pomace silage depressed DMI compared to a dried apple pomace diet (Karami *et al.,* 1996 cited by Taasoli & Kafilzadeh, 2008). In contrast, Jetana *et al.* (2009) did not report a depressed DMI when a diet containing 200 g/kg pineapple wastes silage was fed to buffaloes. The present study recorded DMI of 1056, 1099, 1188 and 1250 g/d for the UPHS, WPHS, MPHS and the MS diets respectively. Taasoli and Kafilzadeh (2008) recorded DMI of 938 g/d in lambs fed a diet that contained 300 g/kg apple pomace silage, which is lower than those of the present study.

Table 4.4 Mean intake (g/d) and apparent digestibility co-efficiency (g/kg DM) of experimental diets fed to lambs (n = 8)

| | Treatments | | | | |
	UPHS	**MPHS**	**WPHS**	**MS**	**SEM**
DMI g/d	1099[b]	1188[a]	1056[b]	1250[a]	68.3
ADG g/d	192[d]	250[a]	205[c]	218[b]	0.2
FCR kg/kg	5.7[a]	4.8[c]	5.2[b]	5.7[a]	0.19
IBW kg	23.8	23.2	23.7	23.3	0.87
FBW kg	35.9[b]	38.8[a]	36.7[b]	39.6[a]	1.88
Digestibility co-efficiency (g/kg DM)					
DMD	628[b]	725[a]	702[a]	712[a]	37.6
OMD	652[b]	739[a]	710[a]	742[a]	38.1
CPD	532[b]	641[a]	535[b]	640[a]	19.7
EED	587[a]	566[b]	520[b]	556[b]	10.2
ADFD	457[b]	436[c]	425[c]	548[a]	14.0
aNDFD	676[b]	662[b]	688[b]	748[a]	27.4

[a,b,c] Means with different letters in a row differ significantly (*P*<0.05)
UPHS; untreated potato hash silage, MPHS; molasses potato hash silage, WPHS; whey potato hash silage, MS; maize silage, SEM; standard error of means, ADI, average daily intake, ADG; average daily gains, FCR; feed conversion rate, IBW, initial body weight, FBW; final body weight, DMD; dry matter digestibility, OMD; organic matter digestibility, CPD; crude protein digestibility, EED; ether extract digestibility, ADFD; acid detergent fibre digestibility, aNDFD; amylase treated neautral detergent fibre digestibility

The present study recorded daily gains of 192, 205, 218 and 250 g/d for UPHS, WPHS, MS and MPHS respectively. Rams fed on a halophytic silage without supplementation recorded gains of 162 g/d (Abdul-Aziz *et al.*, 2001) which is lower than those of the present study. However, the work of Taasoli and Kafilzadeh (2008) recorded daily gains of 199.8 g/d which are comparable to those of the present study.

Meeske and Basson (1998) recorded daily gains of 255 g/d in lambs fed a ration that contained 600 g maize silage/kg DM of diet, which is comparable to the MS diet of the present study. However, Bosman *et al*. (2000) obtained a growth rate of 154 g/d in lambs fed a diet that contained 700 g/kg maize silage which is lower than that of MS diet in the present study. Higher (P<0.05) final weights were obtained in the MS and MPHS.

Lambs with higher FCR require longer finishing periods and a FCR of < 5 indicates a relatively good feeding management with the diet (Bosman *et al*., 2000). Better (P<0.05) FCR was obtained with MPHS and WPHS (4.8 and 5.2) compared to 5.7 from the other diets. Dietary addition of 300 g/kg apple pomace silage resulted in a FCR of 4.69 in lambs (Taasoli & Kafilzadeh, 2008), comparable to the MPHS in the present study. Higher FCR (9 – 12) were recorded in Omani lambs fed ration that contained 60 % forage (Mahgoub *et al*., 2000). Other workers reported lower values (5.7 to 4.1) but these were obtained with rations of higher concentrate inclusion rate (Archimede *et al*., 2007, Pineda *et al*., 1998) which are in agreement with the results of the present study.

4.3.3 Digestibility study

The apparent digestibility of DM and OM was similar (*P*>0.05) for the MS, MPHS and WPHS diets, but higher (*P*<0.05) than that of UPHS. The MS and MPHS diets had higher (*P*<0.05) digestibility of CP compared to the other diets which could led to improved growth performance in lambs compared to those fed on other diets.

4.3.4 Silage intake preferences and digestibility

It is well established that feed intake is more likely to be lower when ruminants are fed solely on silage, and poor animal performance can be expected (Fitzgerald, 1986). The sheep recorded DMI of < 700 g/d which warranted the need for supplementation to achieve a better lamb performance. Higher lamb performance can be achieved if the DM of silage is > 300 g/kg (Phipps & Wilkinson, 1985), and the silages in the present had DM content of < 250 DM g/kg (Table 4.2).

Data on the intake and digestibility of potato hash silages (UPHS, MPHS and WPHS) by sheep is shown in Table 4.5. The results show that there were differences ($P<0.05$) in the intake of silages by sheep, which could be attributed to variations in the chemical composition and fermentation end products of the silages (Wilkinson *et al.*, 1971, Steen *et al.*, 1998, Kriszan & Randby, 2007). Higher ($P<0.05$) intake of DM, OM, CP and fibre (ADF and aNDF) were obtained in the MPHS and WPHS compared to the UPHS. This supported the work of other researchers who reported that whey addition (Khattab *et al.*, 2000) and molasses (Baytok *et al.*, 2005) to forage at ensiling improved silage intake compared to the control.

Moreover, the voluntary intake of silage has been found to correlate positively with CP concentration and negatively with ammonia-N concentration (Kriszan & Randby, 2007, Wilkins *et al.*, 1971). Higher CP contents in final silages are required for adequate intakes, and any reduction in the CP content during the fermentation of forage may adversely impact intake (Wilkinson, 2005). The WPHS and MPHS silages had lower ($P<0.05$) concentrations of ammonia-N and butyric acid, and had higher ($P<0.05$) CP content compared to the UPHS, and were most preferred by the sheep compared to the UPHS. However, the CP content of the silages (Table 4.2) was lower

than 140 g CP/kg DM required for growing lambs (NRC, 2007), which resulted in

lower CP intake (Table 4.5) for sheep fed the silage. This warrants the need for CP

supplementation in these silages to achieve better lamb performance.

Table 4.5 Mean values for the feed intake (g/d) and digestibility of potato hash silages by lambs (n = 3)

Intake	Treatments			
	UPHS	**MPHS**	**WPHS**	**SEM**
DMI	619[b]	681[a]	631[ab]	44.0
OMI	609[b]	658[a]	684[a]	41.9
CPI	45.19[b]	56.52[a]	54.27[a]	6.60
EEI	27.89[a]	25.20[b]	21.45[c]	1.442
ADFI	197.5[b]	209.1[a]	202.3[ab]	11.03
aNDFI	283.9[b]	305.6[a]	288.7[ab]	13.31
Digestibility co efficiency (g/kg DM)				
DMD	493[b]	593[a]	581[a]	35.9
OMD	489[b]	605[a]	595[a]	26.5
CPD	404[b]	597[a]	653[a]	43.2
EED	700[c]	771[b]	863[a]	22.6
ADFD	401[b]	571[a]	605[a]	55.3
aNDFD	430[b]	588[a]	628[a]	52.6

[a,b,c,] Means with different letters in a row differ significantly ($P<0.05$)
UPHS; untreated potato hash silage, MPHS; molasses potato hash silage, WPHS; whey potato hash silage, SEM; standard error of means, DMI; dry matter intake, OMI; organic matter intake, CPI; crude protein intake, EEI; ether extract intake, ADFI; acid detergent fibre intake, aNDFI; amylase treated detergent fibre intake, DM; dry matter, OM; organic matter, CP; crude protein, EE; ether extract, ADF; acid detergent fibre, aNDF; amylase treated neutral detergent fibre.

Forage fibre (ADF and aNDF) content has been regarded as an important

factor in the regulation of forage intake (Meissner *et al.*, 1989, Van Soest *et al.*, 1991).

The UPHS had higher ($P<0.05$) fibre contents than the other silages, which might be

one of the reasons for its lower DM, OM and CP intakes. Whey and molasses addition

reduced ($P<0.05$) the fibre content of the silage as compared to UPHS, which could

be attributed to partial hydrolysis of hemicelluloses in the treated silages (Muck &

Kung, 1997). This agreed with Fazaeli *et al.* (2003) who reported a decrease in fibre

content in liquid whey treated straw silage, and Guney *et al.* (2007) in molasses

treated sorghum silage compared to the control. Both studies recorded improved digestibility of DM and OM compared to the control. Moreover, Khattab *et al.* (2000) reported improved nutrient digestibility from whey treated banana waste silage compared to the control which is in agreement with the present study. In contrast, Zobell *et al.* (2006) did not observe improvements in the digestibility of DM when liquid whey was added to wheat straw and wheat middlings at ensiling compared to the control. The lower digestibility of DM, OM and higher concentrations of ammonia-N and EE in the UPHS might have contributed to the low preference for this silage. Moore *et al.* (1986) reported a depressed digestibility of fibre when a diet containing higher fat was fed to steers.

4.4 Conclusions

It can be concluded that whey and molasses addition improved the fermentation characteristics of potato hash silage. Improved lamb performance was obtained with MPHS followed by MS diets, suggesting that MPHS can replace MS in finishing diets for lambs, containing 20 % silage on as is basis. Furthermore, molasses addition to potato hash at ensiling improved its acceptability and nutrient digestibility in lambs compared to the control.

Feeding potato hash silage without supplementation may lead to poor animal performance due to a lower DMI and CPI of lambs. Therefore silage without supplementation is recommended to ruminants for body maintenance, especially during periods of feed scarcity.

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CHAPTER 5

Effects of homofermentative and heterofermentative bacterial silage inoculants on potato hash silage fermentation and nutrient digestibility in rams

5.1. Introduction

Potato hash (PH), a by-product of the potato food producing industry, is available in South Africa as an animal feed. It contains 150 g dry matter (DM)/kg, 700 g starch/kg DM, 11.2 MJ metabolizable energy (ME)/kg DM, 105 g crude protein (CP) /kg DM (see Table 3.1, Chapter 3). An estimated amount of 50 t per day is produced in South Africa. However, if it is not consumed in a short period of time by animals, it gets mouldy and becomes useless as animal feed. The production of meal from potato waste products is technically feasible, but high drying and processing costs are economic deterrents (Charmley *et al*., 2006, Tawila *et al*., 2008). Ensiling can be considered as an efficient way of preserving high moisture by-products if all essential principles of ensiling are followed (Kayouli & Lee, 1999, Cao *et al*., 2009). For proper ensiling, a material must have high concentrations of water soluble carbohydrates (WSC), low buffering capacity, a DM content of 250 to 400 g/kg and adequate lactic acid bacteria (LAB) prior to ensiling (Wilkinson, 2005). Potatoes, although mainly starch, contain some soluble sugars and, when mixed with legumes and other crops, have produced satisfactory silage when ensiled (McDonald *et al*. 1991). However, potato by-products may contain relatively low DM, WSC and LAB (O`Kiely *et al*., 2002, Okine, 2007) due to processing (Moon, 1981). Consequently, silage additives are used to improve the concentrations of WSC and LAB prior to ensiling (McDonald *et al*., 1991).

Potato by-products have been ensiled with or without homofermentative LAB inoculants in some studies (Okine *et al*., 2005, Okine, 2007, Oshita *et al*., 2007). The results revealed an increase in temperature of the silage when silage was exposed to air. Research has shown that heterofermentative LAB inoculants improve aerobic stability of silage through high production of acetic acid and that this subsequently improves animal performance (Driehuis *et al.*, 2001, Ranjit *et al*., 2002). Research on the use of heterofermentative LAB during the ensiling of potato hash is limited. The aim of this study was to investigate the effects of ensiling potato hash with LAB inoculants *Lalsil fresh LB* (heterofermentative) and *bonsilage forte* (homofermentative) on silage fermentation and digestibility in rams.

5.2. Materials and Methods

5.2.1. Fermentation study

Potato hash was collected from Simba (336 Andre Greyvenstein road, Isando, Gauteng, South Africa), a local food producing factory in South Africa for nutrient analysis, ensiling and a digestibility study with rams. Mixtures of 700 g PH/kg and 300 g wheat bran/kg (330 g DM/kg, 101 g CP/kg DM, 570 g aNDF/kg DM, 326 g ADF/kg DM) were produced to achieve at least 350 g/kg DM. A heterofermentative LAB inoculant, *Lalsil Fresh LB* (*Lactobacillus buchneri* NCIMB 40788, Lallemand SAS, Cedex, France) was applied at a rate of 2 l per t of freshly mixed potato hash (5 g of inoculant was dissolved in 2 l water 4 h before application) to obtain at least 6×10^5 cfu LAB/g fresh material. A homofermentative LAB inoculant, *Bonsilage Forte* (Schaumann, Agri Austria GmbH, Brunn am Gebirge, Austria) contains strains of *Lactobacillus paracasei* (DSM 16245), *Lactobacillus lactis* (NCIMB 30160) and *Pediococcus acidilactici* (DSM 16243). The inoculants were applied at a rate of 2 l per

t of freshly mixed potato hash (5 g of inoculant was dissolved in 2 l water 4 h before application) to provide at least 2.5 x 10^5 cfu/g of fresh material. In order to ensure the same amount of moisture as in the treated potato hash, the control silage was sprayed with 2 l of water per t of fresh material. Treatments were control, *bonsilage forte* (BF) and *Lalsil Fresh LB* (LFLB). Application rates of the inoculants were in accordance with the level of LAB in the inoculants as specified by the manufacturers. The treatments were compacted in 210 l drums (4 drums/treatment) which were lined with a double layer of polyethylene, equipped with clamps and weighted down with heavy bricks. The treatments were compacted (822 ± 33.5 kg/m^3) by trampling and the drums were individually sealed after expelling air and stored at 22 to 25^{0}C. After 3 months of ensiling, drums were opened and triplicate samples were collected before diet formulation and analyzed for DM, pH, lactic acid (LA), volatile fatty acids (VFA) and ammonia N (NH_3-N). Further, aerobic stability was determined by exposing silage to air for 5 d (30°C) and CO_2 production was determined as described by Ashbell *et al.* (1991).

5.2.2. Digestibility study

Potato hash silage was mixed with soybean meal (891 g DM/kg, 942 g organic matter (OM)/kg DM, 462 g CP/kg DM, 53 g ether extract (EE)/kg DM, 14 MJ ME/kg DM and 79 g neutral detergent fibre (aNDF)/kg DM) at a ratio of 9:1 (as fed basis). Samples of the diets were collected weekly and analysed for DM, OM, CP, gross energy (GE), EE and fibre (ADF and aNDF). Diets were fed individually *ad libitum* to 15 South African Mutton Merino rams (37.2 ± 2.21 kg live weight) with five replicate rams per diet. Rams had *ad libitum* access to fresh water and feed intake was measured daily. Rams were adapted to the experimental diets and metabolism crates for 14 days,

followed by 7 day urine and faeces collection. Rams were fitted with leather harnesses and canvass bags attached to the back of each ram three days before the digestion trial started. Urine was collected with a funnel fitted under the cages using 10 l buckets that contained 100 ml of 100 g sulphuric acid/kg solution. Daily outputs of faeces and urine were recorded, subsampled and kept frozen. Faeces and urine that were accumulated for the 7 d period were pooled and subsamples were collected for laboratory analyses.

5.2.3. *Chemical analysis*

A 40 g silage sample from each drum was collected and mixed with 360 ml of distilled water in a stomacher bag, homogenized and left at 10^0C for 24 h (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 used for determination of pH, WSC, VFAs, LA and ammonia-N. The WSC were determined by the phenol-sulphuric acid method of Dubois et al. (1956) and lactic acid was determined by the colorimetric method of Barker and Summerson (1941) as modified by Pryce (1969). The VFAs were determined with a Varian 3300 FID Detector gas chromatograph (Varian Associates, Inc., Palo Alto, CA, USA) by the procedure of Suzuki and Lund (1980). Ammonia N was determined by distillation using a Buchi 342 apparatus and a Metrohm 655 Dosimat with a E526 titrator according to AOAC (ID 941.04, 1990). This method is based on the method of Pearson and Muslemuddin (1968) for determining volatile N.

The DM of silage was determined by drying the samples at 60^0C to a constant mass, and was corrected for loss of volatiles using the equation of Porter and Murray (2001). After drying, samples were ground through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) for chemical analyses.

The ADF was determined using a Fibertec System 1010 (FOSS Analytical AB, Sweden) by boiling samples in an acid solution followed by filtration (ID 973.18, AOAC, 1990), and aNDF was determined by using amylase and sodium sulphite (Van Soest et al., 1991). Separate samples were used for ADF and aNDF analysis and both included residual ash. Crude protein (ID 968.06), OM (ID 942.05) and EE (ID 963.15) were determined according to the procedures of AOAC (1990), while the GE was determined with bomb calorimetry. Analysis of N in the feeds, faeces and urine samples was done the by Kjedahl method following the procedure of AOAC (ID 984.13, 1995).

5.2.4. Statistical analysis

Data on effects of treatments on fermentation, chemical composition and aerobic stability of silage were analysed in a completely randomized design by ANOVA using Genstat (2000). Differences among treatment means were compared with Fisher`s least significant difference (LSD) and significance was declared at the 0.05 % probability level. Data was fitted to the model: $Y_{ij} = \mu + t_i + \varepsilon_{ij}$ where: Y_{ij} is the individual observations of the i-th treatment and the j-th replicate, μ is the general effect, t_i is the effect of the i-th treatment and ε_{ij} is the random variation or residual error.

Effects of treatments (inoculants) on nutrient digestibility in rams were analysed with the model: $Y_{ij} = \mu + t_i + \beta_j + \varepsilon_{ij}$ where: Y_{ij} is the individual observations of the i-th treatment and the j-th replicate, μ is the general effect, t_i is the effect of the i-th treatment, β_j is the effect of the j-th replicate, ε_{ij} is the random variation or experimental error.

5.3. Results and Discussions

5.3.1 Fermentation

The main objective of using LAB inoculants when making silage is to obtain a lactic acid type fermentation that results in well preserved silage. It is generally believed that microbial inoculation of silage has positive effects on fermentation by decreasing pH and butyric acid while increasing the concentration of lactic acid (Muck, 1996). Data on the fermentation characteristics, aerobic stability and nutritive value of pre-ensiled and ensiled potato hash is shown in Table 5.1. The pH of the silages after 90 days of ensiling was reduced to 4.6 or less, which is considered acceptable for silages with a DM content of 350 g/kg (Weissbach, 1968, Cherney *et al*., 2006). The inoculants significantly (P<0.05) increased production of lactic acid and decreased silage pH as well as concentrations of butyric acid and ammonia-N compared to control. This confirmed results from previous studies (Meeske & Basson, 1998, Aksu *et al*. 2004) that LAB increased lactic acid concentrations and reduced pH in maize silage. However, higher (P<0.05) lactic acid and reduced acetic acid, butyric acid and ammonia-N occurred with BF compared to LFLB. This supports other studies that confirmed improved fermentation quality of silage with a homofermentative inoculants compared with heterofermentative inoculants (McDonald *et al*., 2002, Sucu & Filya, 2006).

Water-soluble carbohydrates are regarded as essential substrates for the growth of LAB for proper fermentation (McDonald *et al*., 1991), and low levels may restrict LAB growth. Haigh and Parker (1985) suggested that a concentration of more than 30 g/kg DM of WSC in herbage is critical for successful fermentation. The concentration of WSC in the potato hash mixture pre-ensiling was 78 g/kg DM, indicative of sufficient WSC for efficient fermentation. Residual WSC was lower

(P<0.05) in the inoculated silages compared to the control after 90 days of ensiling, indicating that WSC was better utilized by LAB in the inoculated silages.

Table 5.1 Effects of homo-fermentative and hetero-fermentative bacterial inoculation on the chemical composition, fermentation characteristics, aerobic stability and nutritional composition of potato hash silage after 90 days of ensiling (n = 3)

	Pre-ensiled	Treatments			SEM
		Cont	BF	LFLB	
Chemical composition of silage					
DM, g/kg	413	408[a]	394[b]	392[b]	16.2
OM, g/kg DM		945	953	951	20.7
CP, g/kg DM		136[b]	145[a]	143[a]	9.2
EE, g/kg DM		46.8[c]	71.8[a]	64.8[b]	25.34
ADF, g/kg DM		203[a]	160[b]	176[b]	42.6
aNDF, g/kg DM		471[a]	430[b]	430[b]	40.8
Fermentation characteristics					
pH	6.9	4.6[a]	3.9[b]	3.9[b]	0.07
WSC, g/kg DM	78	37[a]	27[b]	27[b]	9.9
LA, g/kg DM		64.6[c]	78.9[a]	70.8[b]	1.89
AA, g/kg DM		2.4[b]	2.2[c]	2.5[a]	0.28
PA, g/kg DM		0.03[a]	0.02[b]	0.03[a]	0.014
BA, g/kg DM		0.05[a]	0.02[c]	0.04[b]	0.031
NH_3 –N, g/kg TN		34[a]	24[b]	24[b]	9.8
Aerobic stability					
CO_2, g/kg DM		4.0[b]	5.6[a]	1.7[c]	0.26
Nutritional composition of diets					
DM, g/kg		451	437	441	15.3
OM, g/kg DM		946	948	941	8.5
CP, g/kg DM		164[b]	171[a]	169[a]	6.6
GE MJ/kg DM		16.4	16.4	16.9	0.63
EE, g/kg DM		52.6[c]	75.9[a]	64.1[b]	11.83
ADF, g/kg DM		226[a]	182[b]	191[b]	35.3
aNDF, g/kg DM		489[a]	440[b]	445[b]	44.4

[a-c] Means with different letters in a row differ (*P*<0.05)
DM, dry matter; WSC, water-soluble carbohydrates; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; NH_3-N, ammonia N, TN, total nitrogen
Treatments: Cont, control; BF, bonsilage forte; LFLB, lalsil fresh LB

Ammonia-N in silage reflects the degree of protein degradation, and extensive proteolysis adversely affects utilization of N by ruminants (Wilkinson, 2005). Well preserved silages should contain less than 100 g NH_3-N/kg TN (McDonald *et al*., 2002), and the potato hash silages had ammonia N concentrations of less than 100 g NH_3-N/kg TN. However, inoculating the potato hash at ensiling with either BF or LFLB reduced the ammonia N concentration compared to control, supporting findings of other researchers (Gordon, 1989, Rooke *et al*., 1988, Anderson *et al*., 1989). This may be because BF and LFLB inoculants had a positive effect on pH reduction resulting in a decreased production of NH_3-N in the silage. McDonald *et al*. (1991) indicated that a rapid decline in pH is desirable to reduce the amount of protein degradation in the silo. The inoculants in the present study reduced butyric acid and ammonia N concentrations compared to the control, which agrees with results of Aksu *et al*. (2004). Concentrations of butyric acid from all the silages were < 0.1 g/kg DM, which is indicative of well preserved silages (Kung & Shaver, 2001).

It has been reported that inoculation of silage with a homofermentative LAB inoculant often reduces the aerobic stability of silage (Muck & Kung, 1997, Sucu & Filya, 2006), due to lower production of anti-fungal compounds, giving silage little protection against aerobic spoilage (McDonald *et al*. 1991). However, it has been shown that the aerobic stability of silage can be improved by use of heterofermentative inoculants (Driehuis *et al*., 2001, Ranjit *et al*., 2002). The results in the present study clearly indicate that the LAB inoculants had different effects on the aerobic stability of the potato hash silage. Inoculating potato hash silage with BF resulted in a higher CO_2 production and thus lower aerobic stability compared to the other silages, which is in

agreement with Ashbell *et al.* (1991). Similarly, Sucu and Filya (2006) obtained higher (16.9 g/kg DM) production of CO_2 in maize silage that was inoculated with a homofermentative inoculant compared to 7.5 g/kg DM of CO_2 in the control maize silage. Inoculating potato hash diet with LFLB at ensiling increased the concentration of acetic acid and lowered CO_2 production. This supports research of others (Driehuis *et al.*, 1999, Ranjit *et al.*, 2002, Nkosi *et al.*, 2009a, Nkosi *et al.*, 2009b) who reported increased acetic acid concentration, and improved aerobic stability of silage when a heterofermentative LAB inoculant was used.

5.3.2 Apparent digestibility of diets

Although an equal amount of 100 g/kg soybean meal was included in all treatments, the diets that contained the inoculated potato hash silage had higher CP and lower fibre contents (ADF and aNDF) compared to the diet that contained the control silage (Table 5.1). This might be due to the differences in the fermentation quality of the silages since lower ammonia N was obtained in the inoculated silages compared to the control, an indication that proteolysis was restricted by inoculation. This is in consistent with Gordon (1989), Rooke *et al.* (1988) and Anderson *et al.* (1989).

A review by Huhtanen *et al.* (2003) suggested that the fermentation quality of silages has a major effect on feed intake, nutrient utilization and milk production in ruminants. Similarly, Rooke *et al.* (1988) and Meeske (2000) reported increased DM intake in silages treated with LAB inoculants compared to the control. Data on the effect of inoculation on intake, nutrient digestibility and N utilization by sheep is shown in Table 5.2. The DM intake in the present study did not differ amongst treatments, which

agrees with other researchers (Higginbotham *et al.*, 1998, Keady & Steen, 1994) who reported a lack of improvement on DM intake when an inoculated silage was fed compared to untreated. In a number of studies, a negative relationship between silage intake and the concentrations of acetic acids have been reported (Wilkins *et al*., 1971, Steen *et al*., 1998). The present study does not support those findings, since DM intake was not affected by the concentration of acetic acid. However, it supports other researchers (Driehuis *et al*., 1999, Ranjit *et al*., 2002, Krizsan *et al*., 2006, Nkosi *et al*., 2009b) who reported no reduction in DM intake when *L. buchneri* inoculated silage was fed to ruminants.

5.2. Effects of homo-fermentative and hetero-fermentative bacterial inoculation to potato hash silage on intake (g/kg DM), apparent nutrient digestibility (g/kg) and N utilization by sheep (n = 5)

	Treatments			SEM
	Cont	BF	LFLB	
Intake, g/kg DM				
DMI	1236	1353	1283	119.2
OMI	1170	1266	1217	97.4
CPI	198[b]	220[a]	213[a]	8.4
GEI MJ/d	19.5[b]	21.7[a]	21.0[a]	2.76
Apparent nutrient digestibility, g/kg				
DM	672	656	678	0.211
OM	690	698	704	0.151
CP	609[c]	675[a]	668[b]	0.073
GE	632[b]	691[a]	664[a]	0.271
aNDF	391[c]	487[a]	435[b]	0.543
EE	845[b]	870[a]	835[b]	0.312
DE MJ/kgDM	11.6[b]	14.6[a]	15.0[a]	3.40
N utilization				
NI, g/kg DM	31.7[b]	35.2[a]	34.1[a]	3.54
Faecal N g/d	17.2[a]	14.4[b]	15.5[b]	1.24
N urine g/d	2.2	2.5	1.7	1.37
TN excretion g/d	19.4[a]	16.9[b]	17.2[b]	1.57
N retention g/d	12.3[b]	18.3[a]	16.9[a]	4.02
N retention as a % of intake	38.8[c]	52.1[a]	50.4[b]	0.18

[a-c] Means with different letters in a row differ (*P*<0.05)
DMI, dry matter intake; OMI, organic matter intake; CPI, crude protein intake; GEI, gross energy intake; DM, dry matter; OM, organic matter; CP, crude protein; aNDF, neutral detergent fibre; EE, ether extract; DE, digestible energy; NI, nitrogen intake; TN, total N. Treatments: Cont, control; BF, bonsilage forte; LFLB, lalsil fresh LB

That the DM digestibility of the diets was not affected by the inoculants, agrees with Okine *et al*. (2005) in potato pulp silages. However, the digestibility of CP, GE, aNDF and EE was improved in the diets that contained the inoculated silage. These findings support other researchers (*e.g*., Gordon 1989, Aksu *et al*., 2004) who reported improved nutrient digestibility from inoculated silages, which may be a consequence of improved nutrient preservation during the fermentation process and conservation of a higher proportion of digestible nutrients (McDonald *et al*., 1991). According to Muck and Kung (1997), improved digestibility of NDF might be attributed to partial acid hydrolysis of hemicellulose. In contrast, Wittenberg *et al*. (1983) found that corn silage inoculated with *L. plantarum* and *S. faecium* did not influence nutrient digestibility, due to a lack of improvement of the fermentation characteristics of the inoculated silages compared to control. The DE (14 MJ/kg DM) in the BF and LFLB inoculated silages is comparable to the 13 MJ/kg DM reported in potato pulp silage (Okine *et al*., 2005), an indication that the inoculated potato hash silages could be used as an energy source for ruminants.

Inoculation of potato hash silage with either BF or LFLB improved N intake and retention (g/day or as a proportion of intake) compared to control. However, the N retention (as a proportion of intake) was higher (P<0.05) in lambs fed the BF inoculated silage compared to those fed on the LFLB inoculated silage. This could be attributed to improved digestibility of CP in the BF compared to the LFLB.

Total N excretion was highest in sheep fed the control diet, a reflection of lower efficiency of N utilization. According to McDonald *et al.* (1991), improved CP digestibility in inoculated silages could be related to the higher N retention as it might have improved microbial N synthesis in the rumen. The increase in N intake could be

attributed to better digestibility of CP which resulted in increase N absorption (Okine, 2007), showing more efficient N use when BF and LFLB inoculated silages were used in sheep diets. In contrast, Wittenberg *et al*. (1983) did not observe improvement in N retention for lambs fed inoculated silages, which contrasts with the results of the present study.

5.4 Conclusions

Results show that both heterofermentative and homofermentative bacterial inoculants have a beneficial effect on fermentation and nutritive quality of potato hash silage. This was manifested by a higher lactic acid production and lower pH, as well as lower butyric acid and ammonia N concentrations. Accordingly, the apparent digestibility of CP, EE and GE as well as N retention, was improved with both bacterial inoculants. However, silage fermentation, apparent digestibility of CP and fibre, and N retention were improved more with BF inoculation in comparison with LFLB. However, BF inoculation reduced aerobic stability of silage while it was enhanced by LFLB inoculation.

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CHAPTER 6

Effects of ensiling a totally mixed potato hash ration with or without a heterofermentative bacterial inoculant on silage fermentation and lamb growth performance

6.1. Introduction

The rapid human population growth in South Africa has been accompanied by simultaneous increase in the demand of land for settlement development, which resulted in a limited area for fodder cultivation (NDA, 2006, Provincial Fact Sheet, 1997). There is also a growing pressure to improve livestock production which has greatly boosted the demand for conventional feed resources, leading to an increase in the imports of feed ingredients from foreign countries. In addition to this, fluctuations in the price of cereal grains and high demands of cereal for human consumptions resulted in a demand for alternative, traceable and low cost feed resources for livestock (Neelakantan & Singh, 1998). In pursuit of sustainable and economically viable livestock systems, many farmers worldwide are under increasing pressure to maximize the use of available agro-industry by-products based diets for their livestock (Marley *et al*., 2007, Mirzaei-Aghsaghali & Maheri-Sis, 2008).

Potato hash (PH), a mixture of potato skins, starch, fats and yellow maize obtained after the production of snacks, is one of the agro-industrial by-products that are available in appreciable quantities in South Africa, and its chemical composition is given in Table 3.1, Chapter 3. Despite the fact that potato by-products can be processed (e.g. drying) and fed to animals (Charmley *et al*., 2006, Okine, 2007, Tawila *et al*., 2008), PH

is usually fed as fresh to animals by farmers. However, this by-product is produced in high volumes, particularly during the peak periods and if it is not consumed in a short period of time by animals, it gets mouldy and becomes useless for animal feeding.

Because of the high moisture content in fresh PH, it is more advantageous to formulate a total mixed ration (TMR) that contains PH before ensiling. This technique helps to omit the time of mixing before feeding, minimize the risk of effluent production and avoids self selection of feeds by animals (Nishino *et al*., 2003, Wang & Nishino, 2008). Potato hash silage can be a major portion of the forage in ruminant diets, and can also spoil when exposed to air. The study in Chapter 5 confirmed that the aerobic stability of potato hash silage can be improved with a heterofermentative LAB inoculant. Work that evaluated the effect of a heterofermentative LAB inoculant on a TMR silage quality and animal growth performance is limited. The objective of the present study was therefore to evaluate the effects of ensiling a totally mixed potato hash ration with or without *Lalsil Fresh Lactobacillus buchneri (*LB*)* on silage fermentation quality and lamb growth performance.

6.2. Materials and Methods

6.2.1. Silage fermentation

Potato hash (PH) was collected from Simba (336 Andre Greyvenstein road, Isando, Gauteng, South Africa), a local food producing factory in South Africa for nutrient analysis, ensiling and in vivo growth and nutrient digestibility studies using lambs. Totally mixed rations (TMRs) that contained 804 g PH/kg were formulated (Table 6.1) and ensiled with or without a LAB inoculant. The inoculant, *Lactobacillus buchneri*

NCIMB 40788 (*Lalsil Fresh LB*, Lallemand Animal Nutrition, BP 59, Cedex, France), a heterofermentative LAB was mixed in water (1 g in 200 ml) and sprayed over 100 kg of TMR to obtain at least 3×10^5 CFU/g in the material as determined by the manufacturer. In order to achieve the same amount of moisture as in the treated TMR, the untreated TMR (U-TMR) was sprayed with 200 ml sterilized water on a 100 kg TMR. The TMRs were ensiled in 1.5 L anaerobic glass jars (J. Weck, GmBHu. Co., Wehr-Oflingen, Germany) equipped with lids to enable gas release. Each jar was filled with approximately 850 g (wet weight) of TMR without a headspace, and a packing density of 566.7 kg FM/m^3 was obtained. Treatments were: U-TMR silage and *Lalsil Fresh LB* treated TMR (LB-TMR) silage and were produced in a uniform manner with constant mixing. A total of forty two jars were filled (21 jars per treatment), and they were stored at a temperature of $24^0C – 28^0C$ to follow fermentation dynamics.

Three samples per treatment were collected before ensiling and three jars were opened on each of days 3, 7, 10, 21, 45, 60 and 90 of ensiling to determine pH, dry matter (DM), in vitro organic matter digestibility (IVOMD), crude protein (CP), ether extract (EE), metabolizable energy (ME), neutral detergent fibre (aNDF), acid detergent fibre (ADF), ash, water-soluble carbohydrates (WSCs), lactic acid (LA), ammonia-N (NH_3-N) and volatile fatty acids (VFAs). After opening the jars at day 90, samples of the silages were subjected to an aerobic stability test for 5 days in three 2-litre polyethylene terephtalate bottles for each treatment at room temperature (28^0C) according to Ashbell *et al.* (1991). After aerobic exposure (day 95), pH, LA, acetic acid and CO_2 production were measured. Furthermore, enumeration of yeasts was done from the freshly collected

samples (day 90) and after aerobic exposure (day 95) following the procedure of IDF (1990).

6.2.2. Lamb growth and digestibility studies

The U-TMR and LB-TMR were formulated as in the laboratory study and ensiled in 210 L drums. There LB (10 g of inoculant dissolved in 2 L water 4 h before application) was applied at a rate of 2 L per t of fresh TMR. An additional TMR that contained 801 g/kg whole crop maize (Senkuil, Sensako, Brits, South Africa) of 320 g DM/kg of a theoretical 5 mm chop length, was produced (Table 6.1) and used as a reference treatment. In order to ensure the same amount of moisture, a ton of the maize TMR (M-TMR) and U-TMR was sprayed with 2 L of sterilized water. The treatments (M-TMR, U-TMR and LB-TMR) were ensiled by compacting in 210 L drums (9 drums / treatment) lined with a plastic bag, and closed with a rubber lid to prevent damages to the bags by rodents. A packing density of 966 ± 42.1 kg/m^3 was obtained and the drums were stored at 22 to 25^{0}C. After 3 months of ensiling, a drum of each treatment was opened and sampled weekly for the determination of fermentation characteristics and chemical compositions.

The diets were fed *ad libitum* to 24 South African Dorper lambs (20 $\pm$ 0.152 kg live weight) housed in individual metabolic crates (2.2 m^2) in an insulated well-ventilated barn. The metabolic crates were designed to allow total collection of urine. The lambs were allocated in a complete randomized design on the basis of live weight to the three diets, and this resulted into 8 lambs per diet. Lambs had free access to water and were fed once daily and feed intake was measured. The amount of feed offered was always 100

g/kg higher than the previous intake to ensure *ad libitum* intake. Prior to the start of the trial, lambs were identified with ear tags, and treated against internal parasites using valbazen® (Pfizer South Africa, 85 Bute Lane, Sandton, Gauteng Province, South Africa), and external parasites using deadline (Bayer PTY Limited, 27 Wrench road, Isando, Gauteng Province, South Africa). Furthermore, they were weighed on full stomach before the start, and continued weekly until the end of the study. A 14 d dietary adaptation period was allowed and the trial lasted for 63 d.

A 5 d total collection of faeces and urine was conducted a week after the growth study. Lambs were fitted with leather harnesses and canvass bags that were attached to the back of each lamb 3 d before the collection. Urine was collected with a funnel fitted under the cages using 10 L buckets that contained 100 ml of 100 g sulphuric acid/kg solution. The urine was measured daily and 150 ml was frozen and stored for N analysis. Faeces and urine collected during the 5 d period were pooled and sub-sampled for laboratory analyses.

Table 6.1 Feed ingredients used for formulating totally mixed rations (TMRs) either with potato hash or maize silage and their nutritive values

Ingredient g/kg	Potato hash TMR[a]	Maize TMR
Maize meal	61	105.4
Soybean oil cake	39.2	54.1
Molasses meal	4.9	31
Potato hash	804	0
Maize	0	801.3
Feedlime	3.4	4.8
Eragrostis curvula hay	85	0
Salt	1.8	2.4
Vitamin premix[b]	0.6	0.9
Nutritional values (g/kg DM, unless stated otherwise)		
DM	340	330
CP	124.8	126.1
EE	53.5	33.0
ADF	232.2	335.8
aNDF	348.6	425.1
TDN g/kg	0.707	0.696
ME MJ/kg DM	11.4	10.5
Ca	5.1	7.2
P	2.8	3.2

[a] was formulated and ensiled with or without LB,
[b] DM basis: vitamin A 8 mg, vitamin D3 1.6 mg, vitamin E 20 mg, selenium 10 mg/kg, potassium 215 mg/kg, iron 50 mg/kg, cobalt 20 mg/kg, zinc 50 mg/kg, manganese 1600 mg/kg, copper 300 mg/kg, iodine 70 mg/kg, calcium 220 mg/kg, phosphorus 280 mg/kg, sulphur 30 g/kg, salt 950 g/kg.
DM; dry matter, CP; crude protein, EE; ether extract, ADF; acid detergent fibre, aNDF; amylase treated neutral detergent fibre, TDN; total digestible nutrient, ME; metabolizable energy, Ca; calcium, P; phosphorus

6.2.3 Chemical analysis

A 40 g sample of ensiled TMR was collected from each jar and mixed with 360 ml of distilled water in a stomacher bag, homogenized and left at 10^0C for 24 h (Suzuki & Lund, 1980, Speijers *et al*., 2003). It was then homogenized for 4 min and filtered through a Whatman No. 4 filter paper (G.I.C. Scientific, Midrand, South Africa) and the extract was used for determination of pH, WSC, LA, VFAs and ammonia-N. The WSC were determined by the phenol-sulphuric acid method of Dubois *et al*. (1956) and lactic acid was determined by the colorimetric method of Pryce (1969). The VFAs were determined with a Varian 3300 FID Detector gas chromatograph (Varian Associates, Inc., Palo Alto, CA, USA) by the procedure of Suzuki and Lund (1980). Ammonia-N was

determined by distillation using a Buchi 342 apparatus and a Metrohm 655 Dosimat with a E526 titrator according to AOAC (ID 941.04, 1990).

The DM of silage was determined by drying the samples at 60^0C to a constant mass, and was corrected for loss of volatiles using the equation of Porter and Murray (2001). After drying, the samples were ground through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) for chemical analyses. Following the procedures of Van Soest *et al*. (1991), the neutral detergent fibre (aNDF) concentration was determined using heat stable α-amylase (Sigma-Aldrich Co. LTD., Gillingham, UK, no. A-1278) with sodium sulfite, and the acid detergent fibre (ADF) concentration was determined using the 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 procedure of AOAC (1990). The ME was determined from the digestible energy (DE) values by multiplying with 0.08 (Blaxter & Clapperton, 1965) and IVOMD was determined by the method of Tilley and Terry (1963). Analysis of N in the feeds, faeces and urine samples was done the by Kjeldahl method following AOAC (ID 984.13, 1990).

6.2.4. Statistical analysis

Data on effects of treatments on fermentation, chemical composition and aerobic stability of silage was analysed (three replicates for each treatment at each time of ensiling) in a completely randomized design by ANOVA using Genstat (2000). Fisher`s least square means were used to test significant differences among treatments, and

significance was declared at *P*<0.05 probability level. Data on the fermentation characteristics and chemical composition of silage produced in small silos were analysed for the effects of treatments, day and treatment x day interaction with the model:

$$Y_{ijk} = \mu + t_i + d_j + (td)_{ij} + \varepsilon_{ijk}$$

where: Y_{ijk} is the individual observations of the i-th treatment and the j-th day and the k-th replicate, μ is the overall mean, t_i is the effect of the i-th treatment, d_j is the effect of the j-th day, $(td)_{ij}$ is the interaction between t and d, and ε_{ijk} is the residual error.

The effects of treatments on the aerobic stability (CO_2 production and yeast enumeration) of the silages at day 95 were analysed using the afore-mentioned model, but effects of day and treatment x day interaction were removed from the model.

Contrasts were constructed to evaluate effects of treatments (inoculants) on growth and nutrient digestibility in lambs, and single degree of freedom orthogonal comparisons were U-TMR vs LB-TMR + M-TMR. The data was fitted with the model: $Y_{ij} = \mu + t_i + \beta_j + \varepsilon_{ij}$ where: Y_{ij} is the individual observations of the i-th treatment and the j-th replicate, μ is the general effect, t_i is the effect of the i-th treatment, β_j is the effect of the j-th replicate (8 lambs/treatment), ε_{ij} is the random variation or experimental error.

6.3 Results and Discussions

6.3.1 Silage fermentation

The DM content of a crop at ensiling has a strong influence on the rate and extent of the resulting fermentation. A low DM content at ensiling, with a low sugar content increases the chance of a clostridial fermentation and subsequent poor acceptance of the silage by the animals (Fraser *et al*., 2000). For proper ensiling, a material must have high

concentrations of water soluble carbohydrates (WSC), low buffering capacity, a DM content of 250 to 400 g/kg and adequate lactic acid bacteria (LAB) prior to ensiling (Wilkinson, 2005). The DM in the present study was 373 g DM/kg and the WSC concentration was 30.1g/kg DM at pre-ensiling, which is critical for a successful fermentation (Haigh, 1990).

The TMRs had an initial pH of 6.5 (Figure 6.1). However, a rapid decline in pH between days 0 and 3 of ensiling occurred, namely 4.0 and 4.7 in the LB-TMR and the U-TMR silages respectively. After 90 day, the pH of the silages was reduced to 4.1 or less, characteristics of a well preserved silage (De Brouwer *et al*., 1991). Moreover, inoculation of LB to the TMR silage at ensiling increased the concentration of LA (Figure 6.2) which resulted in a more rapid drop in pH compared to U-TMR silage. It was assumed that low levels of WSC (30.1 g/kg DM) in the PH TMR at ensiling restricted the growth of LAB, preventing a faster drop in pH in U-TMR silage. Accordingly, Meeske *et al*. (1999) reported that the pH of *Digitaria eriantha* silage was still at 5.28 after 2 days of ensiling due to lack of WSC. The slower rate of pH drop allowed more time for the growth of clostridia, which can be inhibited by a pH of below 5 (Jonsson, 1991, cited by Meeske *et al*., 1999). Okine (2007) recorded a pH of 3.60 from potato pulp silage treated with a homofermentative LAB inoculant, which is comparable to the 3.9 in LB-TMR silage in the current study.

Inoculation PH TMR with LB at ensiling reduced ($P < 0.05$) ammonia-N, butyric acid, and increased the concentrations of WSC, LA, acetic acid and CP compared to U-TMR silage (Table 6.2). This agrees with others (Mari *et al*., 2009, Nkosi *et al*., 2009) who confirmed increased LA concentrations and reduced pH in LAB inoculated maize

silage compared to control. The LA concentration in silages of the present study was > 80 g/kg DM recommended in a well preserved silage (De Brouwer *et al*., 1991).

Ammonia-N in silage reflects the degree of protein degradation (Wilkinson, 2005) and well-preserved silages should contain less than 100 g NH_3-N/kg TN (McDonald *et al*., 2002). The concentration of ammonia-N in our study is less than 83 g NH_3-N/kg TN, indicative of well preserved silages. The effects of LAB inoculants on ammonia-N reduction in silage compared to untreated silage have been reported (Rooke *et al*., 1988, Gordon, 1989, Anderson *et al*., 1989). Inoculation of LB to TMR silage restricted ($P<0.05$) proteolysis as indicated by its higher CP and low ammonia-N contents compared to U-TMR silage (Table 6.2). In contrast, inoculating maize (< 270 g/kg DM) with *L. buchneri* at ensiling was reported to increase concentration of ammonia-N due to relatively higher pH due to high metabolic activity of the bacterium in the silage (Driehuis *et al.*, 2001). However, the DM content in LB-TMR silage was 353.9 g/kg, hence the concentration of ammonia-N was not increased by the inoculant while a rapid decrease in pH occurred.

**Table 6.2 Chemical composition and fermentation characteristics of pre-ensiled totally mixed rations (TMR) and ensiled TMR
with or without *LB* after 90 days of ensiling (g/kg DM, unless otherwise stated)**

| | Pre-ensiled TMR (n = 3) | | | Silage (n = 21) | | | | | | |
| | | | Treatments | | | | | *P* | | |
	TMR	SD	U-TMR	LB-TMR	LSD	SEM	Trt	day	Trt x day
Chemical composition									
DM g/kg	373	2.5	300.3[b]	353.9[a]	1.85	0.61	0.001	0.001	0.001
CP g/kg DM	133.85	3.6	107.2[b]	138.5[a]	0.601	0.198	0.001	0.009	0.022
Ash g/kg DM	81	2.6	88.2[a]	83.6[b]	3.32	1.09	0.013	0.010	0.008
ME MJ/kg DM	11.53	0.08	10.19	10.74	0.190	0.063	0.061	0.001	0.001
IVOMD g/kg DM	730	0.76	691[b]	728[a]	0.88	0.29	0.001	0.001	0.001
EE g/kg DM	34.1	0.86	46.3[a]	34.0[b]	1.39	0.46	0.001	0.001	0.007
ADF g/kg DM	234.05	7.50	209.4[a]	149.9[b]	0.591	0.195	0.003	0.005	0.082
aNDF g/kg DM	342.7	3.54	326.8[a]	208.4[b]	1.353	0.223	0.001	0.001	0.152
Ca g/kg DM	6.6	0.11	6.4	6.3	0.58	0.19	0.057	0.088	0.064
P g/kg DM	2.45	0.04	1.98	2.61	0.060	0.020	0.081	0.001	0.001
Fermentation characteristics									
pH	6.37	0.103	4.1[a]	3.9[b]	0.046	0.015	0.004	0.001	0.001
WSC g/kg DM	30.1	0.26	13.0[b]	15.9[a]	0.30	0.100	0.001	0.001	0.001
LA g/kg DM			80.3[b]	95.7[a]	0.596	0.196	0.003	0.005	0.014
AA g/kg DM			23.9[b]	45.6[a]	0.085	0.265	0.001	0.001	0.021
PA g/kg DM			2.6[b]	6.5[a]	0.119	0.039	0.001	0.001	0.001
BA g/kg DM			9.9[a]	5.3[b]	0.355	0.117	0.001	0.001	0.001
NH$_3$-N g/kg TN			83.6[a]	60.8[b]	1.45	0.48	0.001	0.001	0.001

[a,b]Means with different letters in a row differ (*P*<0.05)

SD, standard deviation; SEM, Standard Error of Means; LSD, Least Significant Difference; EE, ether extract; IVOMD, in vitro organic matter digestibility; CP, crude protein; ca; Calcium; P, phosphorus; WSC, water-soluble carbohydrates; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; Treatments: U-TMR; untreated totally mixed ration, LB-TMR; Lactobacillus buchneri treated totally mixed ration, Trt, treatment; Trt x day, treatment and day interactions

Higher concentration of acetic acid is an indicator of less than desirable silage fermentation (McDonald *et al.*, 1991). However, it is well established that inoculating silage with a heterofermentative LAB improved aerobic stability of silage compared to untreated silages (Taylor *et al.*, 2002, Kung *et al.*, 2003, Nkosi *et al.*, 2009). It is evident from the current study that levels of acetic acid were higher in the LB-TMR silage especially 45 days after ensiling (Figure 6.3). It is reported that a concentration of acetic acid that ranges from 36 to 50 g/kg DM is suitable to control yeasts during aerobic exposure of silage (Driehuis *et al.*, 2001, Taylor *et al.*, 2002). The concentration of acetic acid was 46 g/kg DM in the LB-TMR, which is enough to reduce yeasts. It is further reported that LA is converted into acetic acid with *L.buchneri* inoculation, and higher acetic acid compared to LA can be obtained (Oude Elferink *et al.*, 2001, Filya, 2003). However, the acetic acid concentrations in silages in this study were generally low compared to the LA concentration (Table 6.2), indicative of good preseved silage (McDonald *et al.*, 1991). This agrees with Mari *et al.* (2009) who reported increased LA and acetic acid in corn silage inoculated with *L. buchneri 40788*.

Microbial inoculation usually has little or no effect on the fibre content of silages because most LAB contain little or no ability to degrade plant cell walls (McDonald *et al.*, 1991). Inoculation of LB to TMR silage reduced (*P*<0.05) concentration of ADF and aNDF compared to U-TMR, which may be due to partial acid hydrolysis of hemicellulose (Muck & Kung, 1997). This is consistent with Keady and Steen (1994) but contrasting the findings of Kung *et al.* (1987) and Rooke *et al.* (1988) who reported no reduction in fibre concentration of silage with LAB inoculation. Moreover, LB-TMR silage had higher (*P*<0.05) levels of IVOMD (728 g/kg) compared to 691 g/kg in U-TMR silage, which might be attributed to higher

losses of organic matter (OM) in U-TMR silage during fermentation. Meeske and Basson (1998) reported higher IVOMD in LAB inoculated maize silage compared to the control, which is in agreement with the findings of our study. In contrast, others (*e.g.*, Black *et al.*, 1980, Meeske *et al.*, 1999) could not confirm improved IVOMD with inoculation compared to untreated silages.
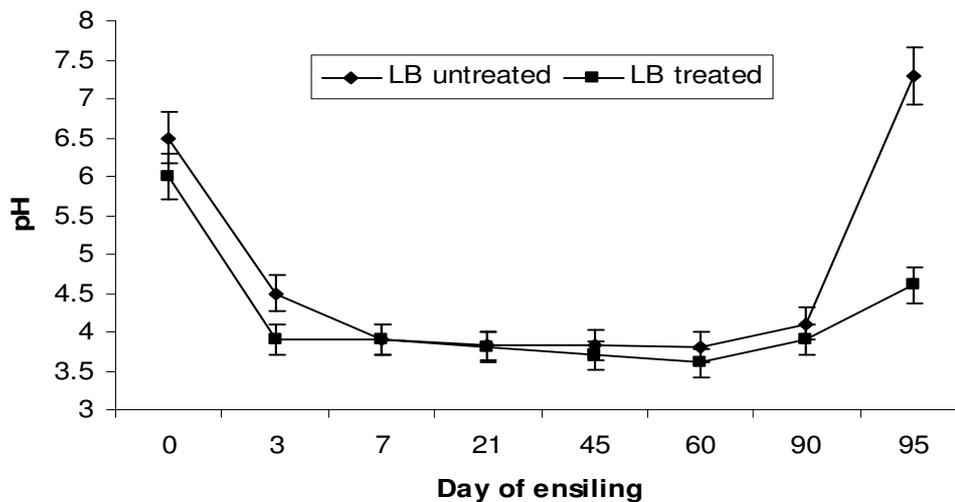
Figure 6.1 Effects of LB treatment on pH and aerobic exposure (from day 90 – 95) of total mixed ration silage

When exposed to air for five days (Table 6.3), the LB-TMR silage had lower ($P<0.05$) CO_2 production (2.2 g/kg DM) compared to 5.5 g/kg DM in U-TMR silage. This supports previous work (Nkosi *et al.*, 2009) when LB inoculated maize silage had lower CO_2 production compared to the control. According to the evidence that silages of higher LA concentrations or those with more residual sugar contents are less stable when exposed to air (Weinberg *et al.* 1993), it was anticipated that the TMR silages would deteriorate fast. The opposite result observed in our study is difficult to explain but low numbers of yeasts in silages may be associated with enhanced stability (Nishino *et al.*, 2003).

Table 6.3 Aerobic stability of ensiled total mixed potato hash rations treated with or without LB (n = 3)

	Anaerobic Treatments			Aerobic Treatments		
	U-TMR	LB-TMR	SEM	U-TMR	LB-TMR	SEM
DM g/kg	300.0[b]	353.9[a]	0.61	298.4[b]	351.2[a]	6.05
pH	4.1[a]	3.9[b]	0.015	7.3[a]	4.6[b]	0.154
Yeast $\log_{10}$	4.30	3.25	0.190	6.59[a]	4.67[b]	0.025
CO_2 g/kg DM				5.52[a]	2.16[b]	0.267

[a,b] Means with different letters in row differ significantly ($P<0.05$)
U-TMR; untreated totally mixed ration, LB-TMR; Lactobacillus buchneri treated totally mixed ration

Yeasts are known to initiate aerobic deterioration of silage and evidence has shown that silages of more than 10^5 cfu/g yeasts are prone to deteriorate on air exposure (McDonald *et al*., 1991). In contrast, Nishino et al. (2004) reported improved aerobic stability in ensiled TMR due to high concentrations of undissociated acids irrespective of $> 10^6$ cfu/g yeasts population. The results of the present study confirm lower ($P<0.05$) yeast numbers in LB-TMR silage compared to U-TMR silage (Table 6.3). This agrees with others (Ranjit *et al*., 2002, Taylor *et al*., 2002) who reported decreased numbers of yeasts in *L. buchneri* inoculated maize silage. The explanation for the aerobic stability enhancing effect of *L. buchneri* is that, in silages inoculated with this organism, the concentration of acetic acid is increased which impair the activity of yeasts (Danner *et al*., 2003, Filya, 2003). Moreover, a slow rate in the decrease of acetic and LA concentrations and slow increase in pH occurred in LB-TMR silage (Figures 6.1 - 3) compared to U-TMR silage, indicative of improved aerobic stability.
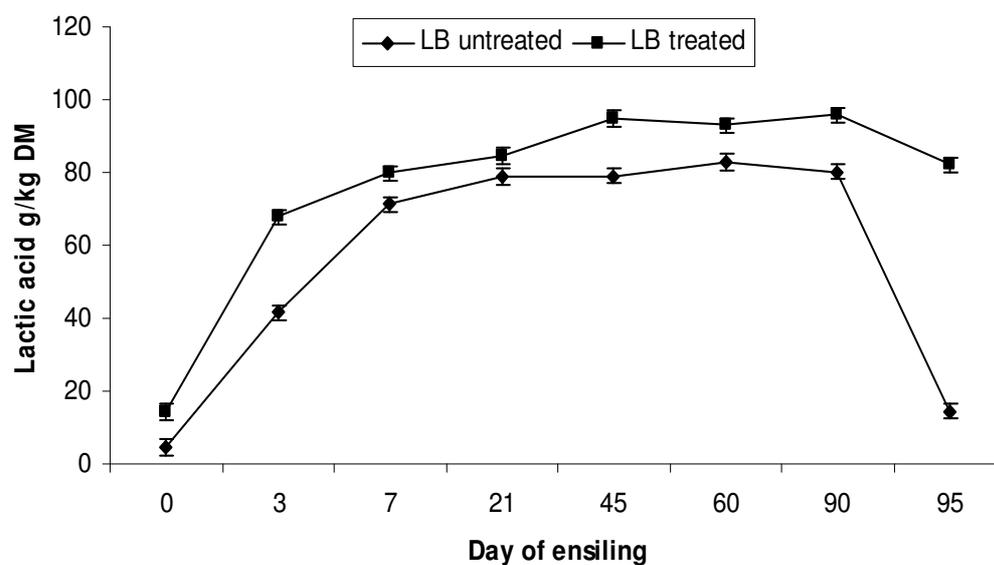
Figure 6.2 Effects of LB treatment on the lactic acid concentration and aerobic exposure (from day 90 – 95) of total mixed ration silage
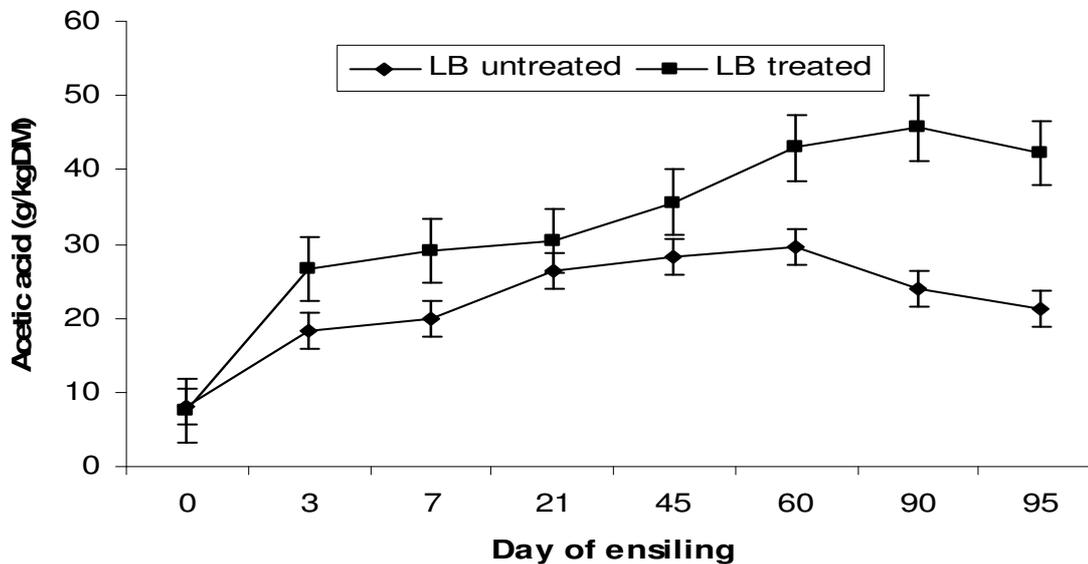

Figure 6.3 Effects of LB treatment on the acetic acid concentration and aerobic exposure (from day 90 – 95) of total mixed ration silage

6.3.2. Lamb growth and digestibility studies

The fermentation quality of silages has a major effect on feed intake, nutrient utilization and milk production in ruminants (Huhtanen *et al*., 2003). The silages used for growth and digestibility studies (Table 6.4) were characterized by LA content that ranged from 92 to 113 g/kg DM, trace quantities of butyric acid (2 - 5 g/kg DM) and ammonia-N concentrations (13 - 78 g/kg TN), indicatives of well fermented silages (McDonald *et al.,*1991).

Lambs fed on LB-TMR silage had higher *(P<0.05)* DM intake and ADG, which led to higher *(P<0.05)* final body weights compared to those fed the other silages (Table 6.5). This might be attributed to higher DM and improved fibre digestibility in LB-TMR silage, which are known to improve DM intake and growth rates in ruminants (Mustafa *et al*., 2008). Okine (2007) recorded daily DM intakes of 1070 and 1160 g/d in lambs fed potato pulp silage treated with different inoculants, which are lower than that of LB-TMR silage. This improvement in feed intake from the LB-TMR silage agrees with Rooke *et al*. (1988), Anderson *et al*. (1989) and Meeske and Basson (1998) who reported increased voluntary DM intake in LAB inoculated silages compared to control. Contrary to these findings, some researchers (Keady & Steen, 1994, Levital *et al*., 2009) could not report any improvement on DM intake in ruminants fed inoculated silage, while others (Kennedy *et al*., 1989) reported a reduced animal performance following feeding of inoculated silage. High level of butyric acid is an indication for loss of feed energy content in the silage (Seven & Cerci, 2003), and poor intake of silage by the animals was reported (Anderson *et al*., 1989). This might be one of the reasons for reduced intake in lambs fed U-TMR silage since it had considerably higher butyric acid content than the other silages.

Table 6.4 Chemical composition and fermentation characteristics of silages ensiled in 210 l drums for 3 months (n = 12)

	Treatments				Contrasts (*P*)		
	M-TMR	U-TMR	LB-TMR	SEM	1	2	3
Chemical composition							
DM g/kg	330.6[b]	340.3[b]	360.1[a]	0.38	0.001	0.001	0.001
Ash g/kg DM	57.3[c]	80.9[a]	74.9[b]	0.22	0.001	0.001	0.001
CP g/kg DM	128.2[b]	124.5[b]	133.2[a]	0.89	0.001	0.001	0.006
ADF g/kg DM	335.2[a]	211.7[b]	148.7[c]	0.65	0.001	0.001	0.001
aNDF g/kg DM	425.4[a]	326.8[b]	208.4[c]	0.59	0.001	0.001	0.001
EE g/kg DM	31.8[b]	42.9[a]	35.1[b]	0.28	0.001	0.001	0.001
IVOMD g/kg DM	787[a]	724[b]	782[a]	0.015	0.001	0.001	0.544
ME MJ/kg DM	12.6[a]	11.9[b]	12.2[a]	0.04	0.001	0.001	0.101
P g/kg DM	1.5[b]	1.5[b]	2.1[a]	0.05	0.001	0.001	0.059
Ca g/kg DM	3.8[b]	7.2[a]	6.8[a]	0.118	0.001	0.001	0.086
Fermentation characteristics							
pH	3.6	3.8	3.6	0.03	0.215	0.051	1.000
WSC g/kg DM	7.95[a]	4.39[c]	6.27[b]	0.007	0.001	0.001	0.001
Lactic acid g/kg DM	113.5[a]	92.8[c]	97.9[b]	0.247	0.001	0.001	0.001
Acetic acid g/kg DM	2.16[c]	6.06[b]	15.24[a]	0.022	0.001	0.001	0.001
Propionic acid g/kg DM	0.49[c]	0.96[b]	1.76[a]	0.011	0.001	0.001	0.008
Butyric acid g/kg DM	2.62[b]	5.34[a]	3.11[b]	0.015	0.001	0.001	0.002
Ammonia-N g/kg TN	13.1[c]	78.4[a]	42.3[b]	0.12	0.001	0.001	0.001

[a,b,c] Means with different letters in a row differ (*P*<0.05)
DM, dry matter; CP, crude protein; ADF, acid detergent fibre; aNDF, amylase treated neutral detergent fibre; EE, ether extract; IVOMD, in vitro organic matter digestibility; ME, metabolizable energy; ca; Calcium; P, phosphorus; WSC, water-soluble carbohydrate; NT, total nitrogen
Treatments: M-TMR, maize totally mixed ration; U-TMR; untreated totally mixed ration, LB-TMR; Lactobacillus buchneri treated totally mixed ration
Contrasts: 1 = treatments; 2 = U-TMR vs M-TMR + LB-TMR; 3 = M-TMR vs LB-TMR

Since higher concentrations of acetic acids were produced in the LB-TMR silage compared to the other silages, a depressed DM intake was expected because sheep may consume less of an acetic acid silage due to its taste and odour (Buchanan-Smith, 1990). In a number of studies (Wilkins *et al*., 1971, Steen *et al*., 1998) negative relationships between silage intake and the concentrations of acetic acids have been reported. However, Driehuis *et al*. (2001) and Krizsan *et al*. (2006) did not observed any negative effect of *L. buchneri* inoculated silage on feed intake, despite having higher acetic acid concentrations. Furthermore, our previous study (Nkosi *et al*., 2009) reported higher intake in lambs fed LFLB inoculated maize silage that contained 73 g/kg DM acetic acid. Driehuis *et al*. (2001) argued that the depression of intake in

silages may be explained by the fact that poor quality silages often contain high concentrations of acetic acid, and no studies showing intake depression with well-preserved silages. This statement agrees with our study because LB-TMR silage was well-fermented as indicated by lower pH and higher LA concentrations.

Table 6.5 Effects of silage on the intake, growth performance and apparent nutrient digestibility co-efficient (g/kg) (n = 8)

| | Treatments | | | | Contrasts (*P*) | | |
	M-TMR	U-TMR	LB-TMR	SEM	1	2	3
IBW kg	21.3	21.4	21.7	0.17	0.942	0.902	0.750
FBW kg	36.6[b]	32.8[c]	42.5[a]	0.24	0.001	0.001	0.001
DMI g/lamb/d	929[b]	723[c]	1318[a]	5.04	0.001	0.001	0.001
OMI g/lamb/d	836[b]	651[c]	1186[a]	7.47	0.001	0.001	0.001
ADG g/d	190[b]	162[c]	282[a]	1.39	0.001	0.001	0.001
FCR kg/kg	4.9	4.5	4.6	0.06	0.158	0.793	0.059
Digestibility co-efficient (g/kg, unless stated otherwise)							
DM	0.73[b]	0.73[b]	0.78[a]	0.13	0.001	0.001	0.001
OM	0.75[b]	0.76[b]	0.80[a]	0.22	0.001	0.001	0.001
ADF	0.67[b]	0.67[b]	0.77[a]	0.29	0.001	0.001	0.001
aNDF	0.65[b]	0.66[b]	0.76[a]	0.39	0.001	0.001	0.001
Digestible energy MJ/kg	13.2[a]	11.7[b]	13.6[a]	1.33	0.001	0.001	0.001

[a,b,c] Means with different letters in row differ (*P*<0.05)
IBW, initial body weight; FBW, final body weight; DMI, dry matter intake; OMI, organic matter intake; ADG, average daily gain; FCR, feed conversion ratio
Treatments: M-TMR, maize totally mixed ration; U-TMR; untreated totally mixed ration, LB-TMR; Lactobacillus buchneri treated totally mixed ration
Contrasts: 1 = treatments; 2 = U-TMR vs M-TMR + LB-TMR; 3 = M-TMR vs LB-TMR

Feeding sheep on maize silage has been reported to result in a positive effect on feed intake (Provenza, 1995). However, our results showed that lambs fed on M-TMR silage were outperformed by those fed LB-TMR silage. This could be explained by lower DM and reduced fibre digestibility in M-TMR silage compared to the LB-TMR silage. This supported others (Hoover *et al*., 1976, Nicholson *et al*., 1977) who reported that potato based silage diets outperformed maize silage when fed to ruminants.

A target growth rate for finishing lambs under commercial conditions is > 150 g/d (Marley *et al*., 2007, Savage *et al*., 2008) and feeding lambs on silage without supplementation resulted in growth rate of 50 g/d because of nutrient imbalances in

silage (Speijers *et al*., 2005). Our results recorded growth rates of > 160 g/d in lambs without the use of additional concentrate, highlighting the potential of using these diets to achieve a more sustainable and nutrient-efficient lamb-finishing system. However, average daily gains in lambs fed LB-TMR silage was higher than those fed the other silages, supporting our previous work (Nkosi *et al*., 2009) which reported higher ADG in lambs fed inoculated maize silage. Ranjit *et al*. (2002) reported DM intake of 935 g/d and ADG of 140 g/d in sheep fed on *L. buchneri 40788* inoculated maize silage, which is lower than that of LB-TMR silage but comparable to M-TMR silage.

The FCR recorded in the present study was higher than those reported by Seven and Cerci (2006) for lambs fed silage produced from a mixture of whole barley and vetch, and the 3.8 g/g reported in lambs fed a high concentrate diet (Haddad & Husein, 2004). Nevertheless, the FCR in the present study is less than 5, indicative of a relative good feed efficiency ratio. Some workers obtained a lower FCR (5.7 to 4.1) with rations containing a higher concentrate inclusion rates (Pineda *et al.,* 1998, Archimede *et al.,* 2007).

According to Cushnahan *et al*. (1995) silage treated with LAB inoculants appeared to be more digestible than untreated silages, seemingly due to the ingestion of fermentation end-products that modify the rumen fermentation. Inoculating TMR with LB improved ($P<0.05$) the apparent digestibility of DM, fibre and gross energy compared to the other silages (Table 6.5). These findings support other researchers (*e.g*., Gordon 1989, Aksu *et al*., 2004) who reported improved nutrient digestibility from inoculated silages. This may be a consequence of improved nutrient preservation during the fermentation process and conservation of a higher proportion of digestible

nutrients (McDonald *et al*., 1991). According to Muck and Kung (1997), improved digestibility of NDF might be attributed to partial acid hydrolysis of hemicellulose. In contrast, Wittenberg *et al*. (1983) found that corn silage inoculated with *L. plantarum* and *S. faecium* did not influence nutrient digestibility, due to a lack of improvement of the fermentation characteristics of the inoculated silages compared to the control.

Lambs fed the LB-TMR silage had higher ($P<0.05$) N intake and retention (g/day or as a proportion of intake) compared to those in the other silages (Table 6.6). The higher N intake in LB-TMR silage is largely a reflection of its higher DM intake and N content in the silage, which is consistent with Keery *et al*. (1991) and Okine *et al*. (2005). The digestibility of N was higher ($P<0.05$) in lambs fed LB-TMR silage compared to the other silages. This could be related to the higher N retention that may have improved microbial N synthesis within the rumen (McDonald *et al*., 1991, Macedo *et al*., 2007). Hoover *et al*. (1976) confirmed that potato silage is equivalent or superior to corn silage in feed intake and the retention of N and energy, which supports the results of the present study.

Table 6.6 Effects of silage diets on N intake (g/kg DM), excretion and retention in lambs (n = 5)

	Treatments				Contrasts (*P*)		
	M-TMR	U-TMR	LB-TMR	SEM	1	2	3
NI g N /d	8.4[b]	7.8[c]	14.7[a]	0.084	0.001	0.001	0.001
Faecal N g/d	3.89[b]	3.44[c]	4.73[a]	0.054	0.001	0.001	0.001
N urine g/d	2.3[a]	2.3[a]	1.9[b]	0.061	0.001	0.001	0.001
TN excretion g/d	6.2[b]	5.7[b]	6.6[a]	0.09	0.001	0.001	0.001
N retention g/d	2.2[b]	2.1[b]	8.1[a]	0.05	0.001	0.001	0.001
N retention (% NI)	26.2[b]	26.9[b]	55.1[a]	0.13	0.001	0.001	0.001
Digest N (as % of NI)	53.6[b]	54.9[b]	67.6[a]	0.178	0.001	0.001	0.001
Faecal output g DM/d	145.8[b]	124.3[c]	187.1[a]	0.44	0.001	0.001	0.001
Urine output ml/d	623	534	489	85.1	0.125	0.057	0.099

[a,b,c] Means with different letters in row differ (*P*<0.05)
Treatments: M-TMR, maize totally mixed ration; U-TMR; untreated totally mixed ration, LB-TMR; Lactobacillus buchneri treated totally mixed ration
NI; nitrogen intake, TN; total nitrogen, SEM; standard error of means
Contrasts: 1 = treatments; 2 = U-TMR vs M-TMR + LB-TMR; 3 = M-TMR vs LB-TMR

The increase in N intake with LB-TMR silage is allied to the better digestibility of N, resulted in an increase in absorbed N, showing a more efficient N use when LB-TMR silage is used in sheep diets. This finding agrees with Okine *et al*. (2005) and Grenet and Demarquilly (1982, cited by Fraser *et al*., 2000), but inconsistent with Aibibula *et al*. (2004, cited by Okine *et al*, 2005) who reported no improvement in N retention in sheep fed inoculated potato pulp silage compared to those fed untreated silage.

6. 4 Conclusions

It seems from the results of the present study that LB is effective in producing a better quality PH TMR silage, as indicated by improved fermentation, aerobic stability, lamb growth performance and the digestibility of the TMR PH silage. This study further supports the concept that *Lactobacillus buchneri 40788* improves the aerobic stability of silages. Although the resulting silage was higher in acetic acid, when fed to lambs, there were no deleterious effects on feed intake and nutrient digestibility. Animal performance was improved when well preserved high moisture PH TMR silage replaced maize silage in growth diets for lambs.

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CHAPTER 7

General Conclusions and Recommendations

The present study showed that potato hash (PH) is a by-product that is rich in some nutrients (e.g. starch and energy) that may be beneficial to animal feeding. This by-product is used by livestock farmers around the Gauteng Province for livestock feeding without using the correct feeding strategies. Consequently, poor animal performance occurred. Due to the fact that there was a lack of data pertaining the use of PH as a livestock feed, and it was in the interest of the present study to develop methods that can be feasible to be adopted by farmers when feeding this by-product.

One of the major drawbacks for the use of PH in animal nutrition is its low DM (150 g DM/kg) and CP (105 g CP/kg DM) contents. However, the present study showed that this can be corrected by mixing with other feed supplements to supply enough nutrients that are required for optimal ruminant production. The availability of PH is not consistent throughout the year, and may need to be preserved for future use. Due to the fact that the production of meal from high moisture by-products needs machinery facilities and may not be affordable to the farmers, ensiling was chosen as the cheapest method to be adopted by the farmers. The present study showed that PH can be ensiled successfully with the addition of absorbents (hay, poultry litter, wheat bran), additives (whey and molasses) and microbial inoculants (Lalsil Fresh LB and bonsilage forte). The present study further showed that although PH contains high levels of starch (700 g starch/kg DM), it needs the addition of whey or molasses to supplement its sugar content for efficient fermentation.

Potato hash silages were produced in 210 L drums, which can be bought at R400.00 each and the silage can be produced manually. This will help to solve the problem of labour costs and the digging of pits on farm for silage production. The study showed that the addition of whey and molasses during the ensiling of PH managed to reduce the silage pH, increase lactic acid concentration and reduce ammonia-N and butyric acid. However, poor quality silage was produced with the addition of poultry litter, and was not tested for livestock growth performance. Further work to improve silage production with mixtures of PH and poultry litter is warranted.

It is generally known that forages such as maize, wheat, sorghum, etc are typically used for the production of silage in South Africa. This practice is generally not attainable to the smallholder farmers. Some reasons being that i) some farmers who are residing in proximity with the high industrialized areas or urban centres may lack with available land for crop production and ii) forages such as maize are primarily produced for human consumption and the production of silage from maize may be an expensive commodity. As a result, the present study compared silage produced from PH to that of maize on the growth performance of animals. The results of the present study showed that PH silage that was treated with molasses can replace maize silage at 20 % inclusion levels in the diet of lambs. However, feeding PH silage without supplementation resulted in poor feed intake and poor nutrient digestibility. The silage cannot be fed to growing lambs or high producing animals, but can be fed for body maintenance. Furthermore, it is feasible to produce a total mixed ration silage that contained 800 gPH /kg and inoculated with Lalsil Fresh LB to improve both the fermentation indices and animal performance. The silage can replace maize silage without negative impacts on animal performance.

Based on the findings of the present study, it is economically feasible to produce silage using PH and feed to animals. This is because there were no health problems occurred when feeding PH silage to lambs, and animal performance was comparable to that of maize silage. It should however, be noted that the production of silage requires good management skills since poor silage production may lead to poor feed quality, which will adversely affect the animals to be fed. Farmers who are currently feeding PH to livestock must be taught on the technology of silage production from potato hash. This will capacitate them to produce silage independently at their farms. Furthermore, the use of PH in the form of silage in animal nutrition will reduce environmental pollution that may occur due to dumping of PH. This will be a better way of recycling the wastes back to human nutrition through animal feeding.

Rations prepared using PH silages proved to be practical and farmers can use them in their lamb fattening operations. Ensiling a TMR that contained PH is the best way to improve the utilization of the by-product as a feed resource. However, more comprehensive research is needed to evaluate the effect of the proposed silages especially when other ruminants are used. The following conclusions and recommendations can be drawn out of the present study:

1. Ensiling PH requires the use of absorbents (e.g. wheat bran, hay, etc) to adjust its DM content, and silage additives/inoculants to improve its fermentation quality.

2. Quality of treated potato hash silage was good as indicated by the research results.

3. *Lalsil Fresh LB* and bonsilage forte inoculants improved the fermentation quality of potato hash silage, but the latter reduced its aerobic stability.

4. Nutrient digestibility of PH silage was improved with whey, molasses, b*onsilage forte* and *Lalsil Fresh LB* treatments.

5. Potato hash silage should be supplemented with other feed sources to improve animal performance.

6. Ensiled TMR that contained 800 g PH/kg (as fed basis) and inoculated with Lalsil Fresh LB improved lamb performance compared to that contained whole crop maize.