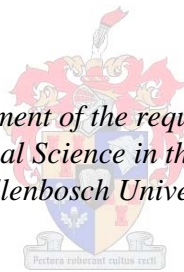


Maize silage based diets for feedlot finishing of Merino lambs

by
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Declaration

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Summary

The aim of the study was to determine the effect of increasing levels of Maize silage in finishing diets for Merino lambs on their feed intake, production performance, feed conversion ratio, digestibility and meat quality. Concerns exist regarding the intake of high moisture and fibre containing silage in sheep due to the physical fill effect thereof. To determine the efficiency of silage as feed ingredient for sheep, maize was cut at 27% dry matter (DM), compacted into 220 litre plastic drums, sealed and left to ferment for 60 days. The silage produced was analysed for fermentation end products and the nutritive value determined. The silage produced had an optimum pH, starch and water soluble carbohydrate (WSC) content. The crude protein (CP) content (112.2 g/kg DM) was higher than expected. Four diets containing, on a dry matter (DM) basis either, 0, 20, 50 or 70% maize silage was formulated on an iso-nutrient basis with exception of neutral detergent fibre (NDF). The aim was to establish the effect of increasing levels of silage on animal production with regard to dry matter intake, growth, digestibility and meat quality. Diets were formulated on an iso-nutrient basis to match the 70% silage diet and therefore had relatively low specifications due to the high inclusion of silage from the 70% silage diet. A growth study and an *in vivo* and *in vitro* digestibility study were conducted to determine the effect of the different diets on feedlot sheep production. Meat quality was also determined to establish whether the experimental diets had an effect on meat quality.

Forty lambs in a completely randomised block design, with four treatments, were used in a 60-day finishing study. The dry matter intake (DMI) of lambs decreased as silage inclusion increased above the 20% silage inclusion level. At the 20% inclusion rate, the feed intake of the animals was stimulated. This resulted in significant differences found between the cumulative intake of the low and the high silage diets. Feed conversion ratio (FCR) was poorer, however, for the control and 20% silage diets. The poor FCR most likely was related to the quality of the feed ingredients used in the formulation of the control feed and the concentrate in the 20% silage diet rather than the silage itself. Significant differences were also found in the dressing percentage of the slaughtered animals where the 20 and 50% silage diets had a higher dressing percentage than the control and 70% silage diets. It was concluded that silage can be successfully incorporated into sheep diets, especially at low levels where its inclusion stimulates intake.

Eight animals per group were used in an *in vivo* digestibility study to determine the apparent digestibility of the experimental diets. Feed, faeces and urine samples collected during the trial period were analysed for the respective nutrients. The 20% diet, even though having the best overall apparent digestibility, did not result in better production responses. Lambs on the 20% silage diet had the highest daily DM intake, which resulted in them having the highest energy intake. There were no differences in total energy excreted between the silage-based diets. This resulted in the 20% silage diet also having the best energy retention. Nitrogen retention was the highest for the control and 20% silage diets. This can be ascribed to the low quality of the concentrate part of the diet. The 20% silage diet, as previously stated, had the highest apparent DM and organic matter (OM) digestibility, while the control diet showed the lowest overall nutrient digestibility. The low nutrient digestibility of the control diet can be ascribed to the relatively poor quality ingredients used. There were no differences in the crude protein (CP) digestibility between the control and the 20% diet. Both proved to be higher than the CP digestibility of the 50 and 70% silage diet. As the neutral detergent insoluble nitrogen (NDIN) was higher for the 50 and 70%

diets, this observation was not surprising. Fibre content of the silage-based diets increased as the inclusion level of the silage increased, which resulted in a decrease in overall fibre digestion.

Three cannulated sheep were adapted on each experimental diet for two weeks before rumen fluid was collected for the *in vitro* digestibility study. No differences between the silage based diets were found for *in vitro* true digestibility (IVTD). The IVTD of the 20, 50 and 70% diets were higher, however, than the IVTD of the control diet confirming earlier observations on the choice of ingredients used in the control diet to formulate iso-nutrient diets. Degradability coefficients were determined for the DM and NDF fractions of the different experimental diets and fitted to the non-linear model; $p = a + b (1 - e^{-ct})$. The amount of DM that disappeared in a certain time (t) is represented by p . Constant a represents the fraction that was rapidly soluble, b represents the potential degradable fraction and c is the rate at which b was degraded. There were no differences between experimental diets for the rapidly soluble fraction. The silage-based diets had a higher potential degradable fraction (b) but did not differ in the degradability rate (c) from the control diet. Silage-based diets had higher overall effective degradability than the control but did not differ between one another. Constant a was not determined for NDF degradability since the NDF fraction did not have a rapidly soluble fraction. The control diet had the lowest potential degradable NDF fraction with the rate also being lower than the silage based diets. Effective NDF degradability was highest for the 50% silage diet.

Lambs used in the finishing study were slaughtered and meat samples taken for meat quality tests. The pH, colour, drip loss, cooking loss, shear force and fatty acid composition were determined on the *Longissimus dorsi* samples collected at Roelcor (Malmesbury, Western Cape, South Africa). Proximate analysis was also conducted on the meat samples. The experimental diets did not have a significant effect on the proximate chemical composition of the meat. Colour differences were found; however no clear pattern could be established. There were no differences in fatty acid composition. It can be concluded that up to 70% maize silage can be included in the finishing diets of Merino lambs with no adverse effects on the meat quality.

The study showed that 20% maize silage can be included in the finishing diets of Merino lambs without negatively affecting intake, production, digestibility or meat quality. Future research is needed to optimise the 20% silage diet, however, and to again look at the effect that it will have on animal production, including the effect thereof on total methane emissions.

Opsomming

Die doel van die studie was om te bepaal of mielie kuilvoer doeltreffend gebruik kan word as 'n komponent in die afronding van Merino lammers. Gedurende die proses is mielies gesny teen 27% droë materiaal (DM), en saamgepers in 220 liter plastiek dromme. Dit is toegelaat om te fermenteer vir 60 dae. Die kuilvoer wat daaruit geproduseer is, is geanaliseer vir fermentasie eindprodukte, en die voedingstofwaarde is bepaal. Vier diëte met onderskeidelik 0 (kontrole), 20, 50 en 70% kuilvoer is geformuleer op 'n iso-nutriëntbasis met die uitsondering van vesel (NDF). 'n Groeistudie, tesame met 'n *in vivo* en *in vitro* verteerbaarheidstudie is uitgevoer om die effek van die verskillende diëte op diere produksie te toets. Vleiskwaliteit toetse is ook gedoen om te kyk of die verskillende diëte 'n effek op vleiskwaliteit het.

Veertig lammers, in 'n ewekeurige blokontwerp, is gebruik in 'n 60 dae afrondingstudie. Dit is opgemerk dat die DM inname (DMI) afgeneem het soos die kuilvoer insluiting bo die 20% vlak toegeneem het. By die 20% insluitingskoers, is voerinnamte by die diere gestimuleer. Dit het veroorsaak dat beduidende verskille gevind is tussen die kumulatiewe inname van die lae en die hoë kuilvoer diëte. Die voeromsetkoers (VOK) was egter hoër vir die kontrole en 20% kuilvoer diëte. Beduidende verskille is ook gevind in die uitslagpersentasie van die diere, waar die 20% en 50% kuilvoer diëte 'n hoër uitslagpersentasie as die kontrole en 70% kuilvoer diëte gehad het.

Agt diere is per groep gebruik in 'n *in vivo* verteerbaarheidstudie om die skynbare verteerbaarheid van die eksperimentele diëte te toets. Voer, feses en urien monsters is gedurende die proefperiode ingesamel en geanaliseer. Die 20% kuilvoer dieet het die hoogste DM en organiese materiaal skynbare verteerbaarheid teenoor die kontrole diëte wat die laagste gehad het. Daar was geen verskille in die ru- proteïen (RP) verteerbaarheid van die kontrole en 20% kuilvoer diëte nie. Beide was hoër as die RP verteerbaarheid van die 50% en 70% kuilvoer diëte. Die veselinhoud van die kuilvoergebasseerde diëte het toegeneem soos die insluitingsvlak van die kuilvoer toegeneem het, wat 'n afname in veselvertering veroorsaak het. Lammers op die 20% kuilvoer diëte het die hoogste daaglikse DM inname gehad, wat die hoogste energie inname tot gevolg gehad het. Daar was geen verskille in die totale energie inname van die kuilvoergebasseerde diëte – dit het veroorsaak dat die 20% kuilvoer diëte ook die beste energie retensie gehad het. Stikstof retensie was die hoogste vir die kontrole en 20% kuilvoer diëte.

Drie gekannuleerde skape is vir twee weke op elke eksperimentele diëte aangepas voordat rumenvloeistof ingesamel is vir die *in vitro* verteerbaarheidstudie. Geen verskille is gevind vir die *in vitro* ware verteerbaarheid (IVWV) tussen die kuilvoergebasseerde diëte nie. Hulle was egter hoër as die IVWV van die kontrole diëte. Degradeerbaarheid koëffisiënte is bepaal vir die DM en NDF fraksies van die verskillende eksperimentele diëte en is gepas in die model $p = a + b(1 - e^{-ct})$. Die hoeveelheid DM wat verdwyn het binne 'n sekere tyd (t) word voorgestel deur p . Die konstante a verteenwoordig die fraksie wat vinnig oplosbaar is, b verteenwoordig die potensieel degradeerbare fraksie en c is die koers waarteen b gedegradeer is. Konstante a is nie bepaal vir die NDF degradeerbaarheid nie, aangesien die NDF fraksie nie 'n vinnig oplosbare fraksie gehad het nie. Daar was geen verskille in die vinnig oplosbare fraksie tussen eksperimentele diëte nie. Kuilvoergebasseerde diëte het 'n hoër potensieel degradeerbare fraksie gehad, maar daar was geen verskille in koers van degradering nie. Die kuilvoergebasseerde diëte het 'n hoër DM effektiewe degradeerbaarheid as die kontrole diëte. Effektiewe NDF degradeerbaarheid was die hoogste vir die 50% kuilvoer diëte.

Lammers in die studie gebruik is geslag en vleismonsters is geneem vir vleiskwaliteit toetse, insluitende pH, kleur, drupverlies, kookverlies en taatheid. Proksimale analise is ook uitgevoer op die vleismonsters. Die eksperimentele diëte het nie 'n beduidende effek op die proksimale chemiese samestelling van die vleis gehad nie. Kleur verskille is wel gevind, maar geen duidelike patroon kon vasgestel word nie. Daar was geen verskille in die vetsuur samestelling nie. Daar kan dus tot die gevolgtrekking gekom word dat mielie kuilvoer ingesluit kan word in die afrondingsdiëte van Merino lammers, tot by 70%, sonder enige negatiewe effekte op die vleiskwaliteit.

Daar is tot die gevolgtrekking gekom dat mielie kuilvoer suksesvol geïnkorporeer kan word in skaapdiëte, veral teen lae vlakke (20%) waar die gebruik nie net inname stimuleer nie, maar ook geen negatiewe effekte het op produksie en verteerbaarheid nie.

Dedication

I dedicate this thesis to my parents Hardie and Anne-Marié Beukes. Thank you for your love, support and encouragement over the years. Thank you for all the opportunities you gave me even when times got tough, for showing me what hard work really is, how to live life to the fullest and to respect the people around me. I will never be able to thank you enough.

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Notes

The language and style used in this thesis are in accordance with the requirements of the *South African Journal of Animal Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has been unavoidable.

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List of abbreviations

ADF	Acid detergent fibre
ADG	Average daily gain
ADIN	Acid detergent insoluble nitrogen
ADS	Acid detergent solution
a_w	Water activity
BC	Buffering capacity
$BW^{0.75}$	Metabolic body weight
CP	Crude protein
DE	Digestible energy
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
EUN	Endogenous urinary nitrogen
FCR	Feed conversion ratio
FME	Fermentable metabolisable energy
GE	Gross energy
IVTD	<i>In vitro</i> true digestibility
LAB	Lactic acid bacteria
MCP	Microbial crude protein
ME	Metabolisable energy
MFN	Metabolic faecal nitrogen
MJ	Mega joules
MUFA	Monounsaturated fatty acids
NDF	Neutral detergent fibre
NDIN	Neutral detergent insoluble nitrogen
NPN	Non protein nitrogen
n-3	Omega-3 fatty acids
n-6	Omega-6 fatty acids
OM	Organic matter
peNDF	Physical effective neutral detergent fibre
PUFA	Polyunsaturated fatty acids
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
SARA	Sub acute ruminal acidosis
SD	Standard deviation
SE	Standard error of the mean
SDMI	Silage dry matter intake
TDN	Total digestible nutrients
TMR	Total mixed ration
UDP	Undegradable protein
VFA	Volatile fatty acids
WSC	Water soluble carbohydrates

Chapter 1

General introduction

South Africa has approximately 8000 commercial and 5800 communal sheep farmers farming an estimated 28.8 million sheep (Department of Agriculture, Forestry and Fisheries, 2011). It is most extensive and concentrated in the more arid parts of the country, like the Northern Cape, Eastern Cape, Western Cape, Free State and Mpumalanga. Forage conservation plays an important role in animal production systems in South Africa to overcome the challenging annual dry season. Large parts of the country have a low and variable rainfall, with periodic droughts being the norm (Rouault, 2004). Furthermore, it is believed that livestock products will have to double in developing countries between 1993 and 2020 to meet the needs of the ever growing human population (Reddy *et al.*, 2003). The demand for meat has resulted in small stock farmers increasing their stocking rate of female animals and more producers therefore finish lambs in feedlots.

It is therefore of great importance for farmers to produce enough forage during the rainy season for preservation, not only to maximise production but also to have enough in reserve for seasonal droughts. There are two basic ways of forage preservation; hay making or the production of silage (McDonald *et al.*, 2002). Silage production is favoured in some instances since it is less weather dependent; fewer field and transportation losses occur during ensiling; and the silage is more palatable because it is cut at a younger growth stage, thus containing less structural carbohydrates (Blaser, 1964). The process of ensiling can be described as the controlled fermentation of a moisture crop containing between 50 and 82% moisture, in a bunker or silo to preserve it for later use (Dodds *et al.*, 1985; McDonald *et al.*, 2002). Ensiling follows two basic principles, the first of which is to obtain anaerobic conditions as quickly as possible to prevent aerobic spoilage. The second objective is to lower the pH to a level where unwanted micro-organisms, like clostridia and enterobacteria, will not grow (McDonald *et al.*, 1991; Muck, 2010). Silage is routinely used as a cost-effective feedstuff in dairy cattle nutrition, but it is not that commonly used in sheep production systems. European countries and Australia, however, make use of silage in their sheep enterprises to improve pasture utilisation; increase stocking rate; for use as a drought feed; and also for the finishing of lambs in a feedlot (Marley *et al.*, 2007; Stanley, 2003). The same principles can, theoretically, be applied to South Africa, not only to optimise pasture utilisation but also to optimise sheep production to meet the ever growing demand for animal protein. Sheep production systems in South Africa are traditionally extensive and sheep are more sensitive to silage quality than cattle, giving the impression that silage is not a practical or effective roughage source for sheep (Baumont *et al.*, 2000). Due to increasing feed prices and practices like no-till farming, where farmers remove stock from their fields in order to conserve ground cover as a means to counteract erosion, producers have to find ways to optimise their production systems. Using silage is proposed as one such option for the production of mutton.

Very little information is available in South Africa on the use of silage in sheep nutrition. The first objective was to determine the effect of silage on the dry matter intake (DMI) of the lambs and their growth response to the increasing levels of silage. Following this, the effect thereof on the *in vivo* and *in vitro* digestibility of the diets was determined. The third objective was to establish if silage

based diets, at different inclusion levels, will have any effects on meat quality. Work done in this study therefore lays the foundation for future research in optimising silage-based diets for the optimum production of feedlot lambs.

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Chapter 2

Literature review

Introduction

Intensive finishing of lambs by sheep farmers in South Africa takes place on an opportunistic basis mainly for economic reasons. Low feed prices or high demand for mutton makes it economically viable for the farmer to finish his/her own lambs before being slaughtered. South Africa has approximately 8000 commercial and 5800 communal sheep farmers farming an estimated 28.8 million sheep. These farms are mostly extensive and concentrated in the more arid parts of the country like the Northern Cape, Eastern Cape, Western Cape, Free State and Mpumalanga (Department of Agriculture, Forestry and Fisheries, 2011). More mutton is currently consumed in South Africa than is produced, with the amount of mutton consumed reaching a peak at 188 million kilograms in 2008, of which only 163 million kilograms were locally produced. Furthermore, sheep numbers have declined over the past decade, mainly as a result of predation and theft. This, together with the increase of the human population has increased the demand for meat, increasing the value thereof with the average gross production over the last ten years amounting to R 2 588 million (Department of Agriculture, Forestry and Fisheries, 2011). Intensive feeding of lambs has therefore become more popular, allowing farmers to increase their stocking rate without negatively affecting their pasture and also having better control over their flock. Many sheep breeds are used in feedlot production systems in South Africa. Meat and dual-purpose breeds like the Dorper, Dormer, Mutton merino and Dohne Merino are favoured above wool breeds such as the Merino, because of a better growth. The Merino, according to Campher *et al.* (1998), as cited in Van der Westhuizen (2010), accounts for almost half the sheep population in South Africa, however, and therefore also plays an important role in meat production. Being a later maturing breed, the Merino results in a higher dressing percentage with a lower fat content, making it more acceptable to the modern consumer.

The focus of the thesis was to determine whether maize silage can be used for the finishing of Merino lambs in a feedlot and, more specifically, to determine the optimum inclusion level. The literature review is therefore focused on the factors to be taken into account for the intensive feeding of mutton as well as the use of silage in ruminant diets, especially sheep.

Intensive feeding of sheep

Growth

Lawrie & Ledward (2006), define the growth of an animal as the increase in weight until the animal reaches a mature size. It follows a basic sigmoidal growth curve which can be divided into three phases, the first of which is a slow growth rate with time, after which the animal enters a phase of growing exponentially until it reaches mature size and the growth rate again declines (Lawrie & Ledward, 2006). Younger animals in the exponential growth phase therefore have a better feed conversion ratio than older sheep and are therefore the best to use in a feedlot. Maturity type as well as gender plays an important part in the selection of sheep to use in a feedlot. Early maturing breeds like the Dorper for example will grow faster than the late maturing Merino. The Dorper will therefore be ready for slaughter at a younger age but the Merino can be slaughtered at a higher live weight due to the fact that fat accumulation will take longer. Male animals will also perform better than female animals in a feedlot setup. The best feedlot lamb will vary depending on the

feed and prices and also on consumer demands. Later maturing breeds which can be slaughtered at a higher live weight without accumulating excessive fat is favoured in certain circumstances.

Dry matter intake

Dry matter intake (DMI), according to Jolly & Wallace (2006) will have the most profound effect on the growth of an animal. Factors that affect DMI include palatability, digestibility, rumen outflow rate, fibre content as well as the dry matter (DM) content of the feed (Jolly & Wallace, 2006; Manso *et al.*, 1998; McDonald *et al.*, 2002). It is important to try and predict the DMI of the animals to ensure optimum growth. It is important, furthermore, to predict the DMI to minimise wastage, since approximately 58% of the total cost of finishing lambs is feed related (Jolly & Wallace, 2006). Dry matter intake will vary with age and weight of lambs, but can generally be calculated as 3.8 – 4.2% of the live weight of the animal (NRC, 1985). Optimising the DMI of lambs in feedlot will therefore not only ensure optimum growth but also reduce wastage of expensive feed thereby increasing the profit margin.

Energy and protein requirements

The energy and protein requirements of animals will vary at different physiological stages. It is of utmost importance that these requirements are met to ensure optimum growth and development, especially those of a young growing animal. Energy requirements largely depend on the stage of maturity, growth rate, sex, and level of intake (NRC, 2007). The requirements is higher for animals in the exponential growth phase and can be calculated as follows (McDonald *et al.*, 2002):

- Castrates: $EV_g = 4.4 + 0.35W$
- Females: $EV_g = 2.1 + 0.45W$
- Males: $EV_g = 2.5 + 0.35W$

EV_g = energy value of live weight gain, MJ/kg

W = live weight gain, kg/day

The protein requirement of the growing animal is reliant on the energy density of the diet. It is generally accepted that 12 grams of crude protein is needed per MJ ME (Jolly & Wallace, 2006). At least 40% of the protein supplied must be in a rumen undegradable form to ensure that there is not a shortage in essential amino acids. The first limiting amino acids, being lysine and methionine, can also be supplied in a rumen protected form to ensure that requirements are met. The optimum ratio of lysine to methionine is 3:1 (McDonald *et al.*, 2002; NRC, 2007).

Fibre requirement

The fibre fraction of a diet can be defined nutritionally as the slowly digestible or indigestible fraction of a feed and is important in ruminant diets to ensure a healthy rumen environment (Mertens, 1997). It can be divided chemically into a neutral detergent (NDF) and an acid detergent fibre (ADF), both being inversely related to energy density (Kawas *et al.*, 1991). The NDF fraction is the slowly digestible fraction that consists of cellulose, hemicellulose and lignin. The ADF fraction, on the other, hand represents the indigestible contents such as crude lignin and cellulose (Smith, 2008). The NDF fraction of feeds is generally used to describe the fibre content of feeds since it isolates all the insoluble fibre contents and has generally been used in the formulation of ruminant diets (Kawas *et al.*, 1991). However it still is a chemical component and does not say much about the effectiveness of the fibre. The physical effectiveness of fibre (peNDF) is an

indication of the effect that the fibre has on chewing activity and subsequent rumen health (Mertens, 1997). Sheep have the ability to select a diet of optimum composition (Kyriazakis & Oldham, 2007). Supplying good quality roughage *ad libitum* will therefore supply their peNDF need for optimum rumination and rumen health.

The use of silage for finishing of feedlot lambs

Silage is widely used as a cost-effective source of roughage in dairy cattle nutrition in South Africa. It is not that commonly used in sheep production systems, however. European countries and Australia, on the other hand, make use of silage in their sheep enterprises to improve pasture utilisation and increase stocking rate, as well as a drought feed and also for the finishing of lambs in a feedlot (Marley *et al.*, 2007; Stanley, 2003). The same principles can, theoretically, be applied to South Africa; not only to optimise pasture utilisation, but also to optimise sheep production to meet the ever growing demand for animal protein. Sheep production systems in South Africa are traditionally extensive and sheep are more sensitive to silage quality than cattle, which gives the impression that silage is not a practical or effective source of roughage for sheep (Baumont *et al.*, 2000). As a result of increasing feed prices and practices like conservation farming, where farmers move away from grazing to protect ground cover to retain more water and stop erosion. Farmers have to find ways to optimise their production systems. Using silage is proposed as one such option. There are a few concerns regarding the use of silage for the finishing of feedlot lambs, however. The first limiting factor is a low ME content and a lack of fermentable metabolisable energy (FME), which will result in a sub optimum environment for rumen micro-organisms and therefore a reduction in fibre utilisation (Chumpawadee *et al.*, 2006). Another concern, due to excessive fermentation, is the high level of non protein nitrogen (NPN) resulting in low undegradable protein (UDP) content. Management-related factors, like excess acidity, excess water, chop length and contaminants can all reduce the quality of the silage and therefore of animal production. It is critical, for the effective use of silage, to know the quality of the silage and to optimise the diets accordingly to maintain optimum animal production (Wilkinson, 2005).

It is therefore important, for the effective use of silage for mutton production, to understand the processes of ensiling as well as the management-related factors affecting silage quality. Silages are mostly used in total mixed rations (TMR) and it is thus important to also determine the nutritive value to ensure that animals receive a balanced diet.

Voluntary intake

Factors such as fermentation end products, DM and length of cut all have an effect on ruminant intake, more so for sheep than cattle, with the average voluntary intake being 27% lower when silage is fed compared to fresh herbage (Forbes, 2007). According to Forbes (2007), is it largely a result of the fermentation end products due to the fact that the depression of voluntary intake is significantly lower when partially fermented silages are fed. There is much controversy around the fermentation end products that depress intake, but it seems to be ammonia and the volatile fatty acid (VFA) butyrate (>1%) that are the main intake depressing factors (Forbes, 2007; Oba & Allen, 2003; Allen *et al.*, 2009; Charmley, 2001; Kung & Stokes, 2001). It is therefore of great importance to produce good quality silage, as close as possible to the original crop, to ensure optimum dry matter intakes by sheep. Dry matter content, on the other hand, is directly correlated to voluntary feed intake. The higher the DM the less bulky the silage and therefore more can be eaten before rumen fill is reached (Kokkonen *et al.*, 2000). This is only true to a certain point; intake of poor quality roughages will decline as the DM is increased. Chop length also has an effect on voluntary

food intake. The ideal chop length for optimum packing of the silo is 8 to 12 mm (Meeske, 2011). This will help with the fermentation process due to the fact that less air will be trapped in the silo, reaching the anaerobic phase quicker. Chopping length will also have an effect on ruminant intake. A chopping length of less than 10 mm can result in an increased DM intake of up to 25% in sheep, when compared to silage cut at an average length of 75 mm (Wilkinson, 2005). There is risk in feeding silage with a shorter chop length, however, especially when large amounts of concentrates are included before the animals have had time to adapt. The shorter chop length will decrease the peNDF, thereby reducing the time spent ruminating (Stone, 2004). Less rumination in turn results in less saliva reaching the rumen, and therefore less bicarbonate secretion to buffer the decreasing pH in the rumen (Mertens, 1997). The rumen pH can drop to below the critical 5.6 mark in such cases and result in sub acute ruminal acidosis (SARA) (Krause & Oetzel, 2005). Signs of acidosis include reduced DMI, laminitis and diarrhoea (Kleen *et al.*, 2003). Supplying roughage *ad libitum* or adding sodium bicarbonate will counteract SARA (Krause & Oetzel, 2006).

Supplementing silage for optimum animal production

The main concerns when feeding high moisture silage to ruminants is the low UDP, high ammonia and relatively low FME fraction as a result of excessive fermentation (Givens & Rulquin, 2004; Stanley, 2003; Wilkinson, 2005). The protein content of fresh grass can be anywhere between 75 and 90% of the total nitrogen. The remaining nitrogen is in the form of free amino acids, peptides and compounds containing nitrogen. This changes drastically during the ensiling process (Givens & Rulquin, 2004). Plant proteases rapidly break down plant proteins to ammonia and amino acids increase the soluble NPN fraction of the feed. This, together with a relatively low FME due to the use of water soluble carbohydrates (WSC) by bacteria during fermentation, lowers the efficiency of NPN use by rumen microorganisms (McDonald *et al.*, 2002). Excess ammonia is carried to the liver by the portal blood where it is converted to urea. Some of the urea is recycled, but most of it is lost via excretion in the urine (Chanjula *et al.*, 2004). The low UDP fraction is also a concern. It is commonly known that high producing dairy cattle or fast growing ruminants cannot reach their genetic potential only relying on microbial protein (MCP). They also have to be fed a fraction of protein that cannot be broken down by the rumen micro-organisms and will pass to the duodenum, thereby providing essential amino acids (EAA). This UDP fraction must not duplicate the amino acids already provided by MCP but rather uplift the amino acid deficiency resulting from feeding only rumen degradable protein (RDP). When optimising silage-based diets, it is therefore necessary to take these concerns into consideration. It is important to supplement silage with cereal grains to supply additional energy and UDP sources like toasted soybean meal to optimise animal production (Stanley, 2003). Other feed ingredients can successfully be used include:

- Maize gluten meal
- Groundnut meal
- Brewers' grains
- Canola oilcake
- Rumen protected amino acids

The optimum ratio of RDP to FME for the growth of rumen micro-organisms is 10:1 (Wilkinson, 2005). Attaining this ratio will not be an issue for high-energy silages like maize or whole grain

cereals that underwent optimum fermentation (Wilkinson, 2005). On the other hand, silages with lower energy content, like grass and legume silages, should ideally be mixed with high-energy silages or supplemented to obtain the optimum RDP: FME ratio.

Effect of diet on meat quality

Factors effecting meat quality of ruminants can be divided into animal related factors and environmental factors. The animal factors are directly related to the animal and include the age, breed and sex. Environmental factors, on the other hand, are the factors that are not animal related, like diet, weather and processing of the meat (Priolo *et al.*, 2001). Diet has a considerable effect on meat quality and thus on the consumable product. This is especially true in an intensive system where animals are of the same sex, breed and age. It is thus important to understand the effect that diet has on the meat to ensure the production of a high-quality product according to consumer demands.

Fat and long chain fatty acids have an effect on the taste, nutritional value and storage stability of meat and therefore play an important role in the acceptability of meat to the consumer (Webb & O'Neill, 2008; Webb & Casey, 1995). Research has shown that ruminants raised on different production systems will vary in acceptance by the consumer (Kerth *et al.*, 2007); the main contributing factor to this is meat flavour resulting from the differences in the fatty acid profile. Fatty acids of animal origin typically have more than 12 carbon atoms and are referred to as long-chain fatty acids. They can be classified as saturated (SFA), monounsaturated (MUFA) or polyunsaturated fatty acids (PUFA) according to their carbon bonds (Webb & O'Neill, 2008). Omega-3 (n-3) and Omega-6 (n-6) fatty acids are polyunsaturated fatty acids and are also regarded as dietary essential fatty acids. These fatty acids have to be included in the diet of monogastric animals (including humans) and not only play an important role in the immune response, but also act as carriers for fat soluble vitamins (Hwang, 1990; Webb & O'Neill, 2008). The optimum ratio of PUFA: SFA for meat is no less than 0.4 and less than four for n-6/n-3. A feed that will change the concentration of the flavour precursors will have an effect on the flavour and therefore on the consumable product (Duckett & Kuber, 2001; Melton, 1990). Pasture-finished ruminants will have a higher concentration of n-3 PUFA and ruminants finished on concentrate diet will have a higher n-6 PUFA concentration (Enser *et al.*, 1998). This is because grass has a higher precursor fatty acid for α -linolenic acid (18:3) whereas concentrate diets have a higher precursor fatty acid for linoleic acid (18:2) (Marmer *et al.*, 1984). Finishing studies with beef cattle, like the one done by O'Sullivan *et al.* (2002), showed that maize silage resulted in a significant lower 18:3 content than grass silage and will therefore have an higher n-6/n-3 ratio. This will have an effect on consumer acceptability due to the fact that meat with a higher n-3 content, therefore lower n-6/n-3 content is favoured by the consumer. Stanley (2003), however, stated that feeding high silage diets will have no adverse effect on meat quality. Meat quality attributes like tenderness and colour may be affected because diets containing large amounts of silage will most likely have a lower energy density. Sheep will therefore take longer to reach optimum weight for slaughter, which may affect tenderness and the colour of the meat (Muir *et al.*, 1998). It remains important, however, to test the effect of silage-based diets on meat quality, even though Stanley (2003) has stated that no differences can be expected. The reason for this is that it is generally accepted that animals finished on pasture, which is roughage-based like silage, will be less acceptable to the consumer than concentrate-finished animals.

It is important, therefore, when determining whether silage can effectively be used for the finishing of Merino lambs, to study the effect that it will have on the growth, digestibility and meat quality of the lambs. The first step, however, is to produce good quality silage. For this, one must have understanding of the principles of ensiling, the type of fermentation taking place in the silo, and the crop characteristics affecting silage quality.

Principles of ensilage

The main aim of ensiling a crop is to ensure that its nutrient quality when fed to the animals is as close as possible to the chemical composition when the crop was first cut. Ensiling therefore is a method of preserving crops with certain characteristics, as discussed later on. Anaerobic conditions have to be reached as fast as possible to inhibit oxidation. The first factor playing a role in reaching anaerobic conditions is bunker density; the optimum for maize and lucerne is 750 and 600 kg/m³ respectively (Meeske, 2011). A higher bunker density will lead to better preservation due to the fact that more air will be forced out of the bunker and the circulation of air during filling, storing and feed-out will also be inhibited (Muck, 2000). A factor contributing to bunker density and therefore to anaerobic conditions is the moisture content of the crop. Low moisture content will increase the porosity of the silo, thereby allowing more air into the bunker. The optimum moisture content differs between crops, but all fall between 50 and 82% (Table 2.1) (Dodds *et al.*, 1985; McDonald *et al.*, 2002). Another factor affecting bunker density is the chop length; a shorter chop length means better compaction and less air trapped in the bunker (Wilkinson, 2005). The optimum chop length can vary from 8 – 12 mm, depending on the crop (Dodds *et al.*, 1985; Meeske, 2011). Crop-related factors affecting ensiling will be discussed later on.

Table 2.1 Estimated percentage of moisture for major forage crops for silage production (Dodds, 1985)

Crop	Est % moisture
Grass crops	
Maize	70 - 65
Small grains	75 - 70
Sorghum	70
Perennial grasses	75 - 70
Legumes	
Lucerne	82 - 70
Sweet clover	82 - 75

The bunker must be sealed properly to ensure no re-entering and circulation of air. According to Sprague (1974), as cited in Woolford (1990), 90% of the oxygen will be removed by respiratory enzymes together with aerobic bacteria naturally present on the crop after just 15 min in an adequately sealed bunker, with less than 0.5% remaining after 30 min (McDonald *et al.*, 1991; Woolford, 1990; Dodds, 1985a). Failing to seal the bunker properly will result in excessive growth of unwanted aerobic bacteria. These aerobic bacteria will use up the more readily available carbohydrates and produce heat, carbon dioxide and water (Dodds, 1985; McDonald *et al.*, 2002). This then results in dry matter (DM) losses, lower protein digestibility, growth of yeasts and moulds and an overall reduction of forage quality (Dodds, 1985).

The second principle of ensiling, after the anaerobic conditions, is to lower the pH to inhibit the growth of unwanted micro-organisms like clostridia and enterobacteria. Clostridia is an anaerobe bacterium naturally present on the crop that produces butyric acid and is responsible for the

degradation of amino acids, thus lowering the quality of the silage and reducing the intake by ruminants. Enterobacteria will ferment sugars to produce secondary products responsible for protein degradation, but it also produces acetic acid responsible for inhibiting the growth of yeasts and moulds (Castenada & Birch, 2011; McDonald *et al.*, 1991). The most effective way to inhibit the growth of unwanted bacteria is to reduce the pH to a level where they cannot grow and multiply. This can be done by optimising the conditions for lactic acid production by lactic acid bacteria (LAB). Lactic acid bacteria, like clostridia and enterobacteria are naturally present on the crop and ferment soluble carbohydrates to mainly lactic acid. The production of lactic acid will lower the pH to a level where the unwanted micro-organism cannot survive, thereby preserving the crop (Castenada & Birch, 2011; Holzer *et al.*, 2003). A pH of four is generally accepted as sufficient for inhibiting the growth of unwanted micro-organisms such as clostridia and enterobacteria (McDonald *et al.*, 1991; McDonald *et al.*, 2002; Meeske, 2011; Wilkinson, 2005).

Silage fermentation

Aerobic phase

There are two main phases after the silo is packed and sealed. The first is the aerobic phase; during this phase air (oxygen) is still trapped in the silo (Kung, 2001b). Plant respiration will thus take place removing the oxygen and generating CO₂ and heat as can be seen here:



Enzymes, like amylases and hemicellulases released from plant cells when the crop was cut, will start to break down amylose and hemicellulose and increase the level of water soluble carbohydrates (WSC) in the plant material (Bolsen, 1995). Plant proteases are still active, resulting in the breakdown of proteins to amino acids, peptides, amides and NH₃. Excessive temperatures (above 42° to 44°C) resulting in the Maillard reaction can be reached if the silo is not adequately sealed. Free amino acids and sugars are formed into polymers, lowering the digestibility, not only of the protein but also of the fibre (Bolsen, 1995; McDonald *et al.*, 2002). Unwanted microorganisms like moulds, yeasts and enterobacteria will compete for the WSC needed by the LAB for the reduction of the pH. This will result in a higher end pH, thus anaerobic bacteria like clostridia will be able to grow and multiply. For optimum fermentation to take place, the crop must be cut at the right length and optimum DM or wilted until the optimum DM is reached. The silo/bunker must be filled rapidly and compacted to the right density and sealed off properly (Kung, 2001b).

Anaerobic phase

The anaerobic phase or fermentation will start as soon as the oxygen is depleted. Lactic acid bacteria together with unwanted micro-organism like clostridia and enterobacteria multiply under these anaerobic conditions due to a high pH and the availability of WSC. Proteolytic enzymes are also active during the onset of this phase, resulting in the breakdown of plant proteins to soluble non protein nitrogen (NPN). The pH of the ensiled material has to be lowered to inhibit the growth of these unwanted bacteria and to stop the breakdown of plant proteins. This is done by the production of lactic acid. Lactic acid bacteria is therefore the most important micro-organism during the ensiling process (Bolsen, 1995; McDonald *et al.*, 1991; McDonald & Greenhalgh, 2002; Wilkinson, 2005). Water soluble carbohydrates released due to the breakdown of the intact plant cells under the anaerobic conditions will be fermented. Fermentation end products will consist

mainly out of lactic acid with some acetic acid, carbon dioxide and ethanol. Lactic acid, being the strongest acid, rapidly lowers the pH, thereby inactivating the proteolytic enzymes and inhibiting the growth of enterobacteria and clostridia (Bolsen, 1995).

Silage additives

The main aim of adding additives to silage is to optimise the environment for the growth of Lactic acid bacteria (LAB), thereby ensuring that LAB will dominate fermentation. Early additives used, like molasses, served as a relatively cheap source of water soluble carbohydrates. Mineral acids, on the other hand, were used to reduce the pH as quickly as possible to 3.5 in the belief that it would stop plant enzyme and microbial activity (McDonald *et al.*, 1991). The focus today is still to control fermentation, thereby reducing DM losses, but also to improve the nutritional value of the silage. There are five main categories which silage additives can be grouped by:

- Fermentation stimulants
- Fermentation inhibitors
- Aerobic deterioration inhibitors
- Nutrients
- Absorbents

Fermentation stimulants are the most studied of all the additives currently under investigation. They are readily available to farmers and on-farm application is easy and safe (Elferink *et al.*, 2012). Fermentation stimulants can be divided into carbohydrate sources and bacterial cultures. Carbohydrate sources include molasses, cereals and pulps like beet and citrus (McDonald *et al.*, 1991). These carbohydrate sources serve as an energy source for the LAB, thereby optimising their growing environment and resulting in optimum fermentation when other management issues are resolved (Nishino & Uchida, 1999; McDonald *et al.*, 1991). Bacterial cultures such as LAB, also known as inoculants, are widely used to improve the quality of the silage and are added to crops during harvesting or filling of the silo. Lactic acid bacteria, as previously mentioned, will produce lactic acid which, in turn, will lower the pH of the ensiled material (Muck, 1996). The reduction in pH will stop the breakdown of plant proteins by inactivating the proteolytic enzymes. It will also inhibit the growth of unwanted micro-organisms such as clostridia and enterobacteria, thereby preserving the ensiled plant material. Lactic acid bacteria can be divided into six genera, each having its own species, which can further be grouped into homo- or heterofermentative (Table 2.2) (Bolsen, 1995).

Table 2.2 Classification of lactic acid bacteria typically found in silage; according to genus, species and glucose fermentation (McDonald *et al.*, 1991)

Genus	Species	Glucose fermentation
<i>Lactobacillus</i>	<i>acidophilus</i>	Homofermentative
	<i>casei</i>	
	<i>cornyiformis</i>	
	<i>curvatus</i>	
	<i>plantarum</i>	
	<i>salivarius</i>	
	<i>brevis</i>	Heterofermentative
	<i>buchneri</i>	
	<i>fermentum</i>	
	<i>viridescens</i>	
<i>Pediococcus</i>	<i>acidilactici</i>	Homofermentative
	<i>cerevisae</i>	
	<i>pentosaceus</i>	
<i>Enterococcus</i>	<i>faecalis</i>	Homofermentative
	<i>faecium</i>	
<i>Lactococcus</i>	<i>lactis</i>	Homofermentative
<i>Streptococcus</i>	<i>bovis</i>	Homofermentative
<i>Leuconostoc</i>	<i>meseteroides</i>	

Homofermentative LAB (Table 2.2) ferments six-carbon sugars to produce lactic acid (Table 2.3) and has been used widely over the last few decades to enhance silage quality since it is a strong acid that will quickly lower the pH (Driehuis *et al.*, 1997). Heterofermentative bacteria, on the other hand, produce lactic acid together with acetic acid, ethanol and carbon dioxide (Table 2.3). This will result in DM losses due to a loss in carbon, but will also enhance the aerobic stability due to the acetic acid production (Muck, 2010).

Table 2.3 End products of fermentation with their DM and energy recovery percentages (Bolsen, 1995)

Fermentation	End products	Substrate recovery, %	
		Dry matter	Energy
<i>Homofermentative</i>			
1 glucose →	2 lactic acid	100	97
<i>Heterofermentative</i>			
1 glucose →	1 lactic acid	76	97
	1 ethanol		
	1 CO ₂		
3 fructose →	1 lactic acid	95	98
	2 mannitol		
	1 acetic acid		
	1 CO ₂		

Homofermentative inoculants will have the best DM and energy recoveries if aerobic stability is maintained, and is theoretically the best inoculant to use (Muck, 2004). However, there has been a lot of controversy regarding the effectiveness thereof. The reasons for this vary from not applying the inoculant correctly to some of the inoculants being inactive. Other factors playing a role is the type of crop ensiled. Crops like maize and small grains have natural homofermentative fermentations, thus adding an inoculant will not have a significant effect, but adding an heterofermentative inoculant can increase aerobic stability (Muck, 2010; Kristensen *et al.*, 2010). Grasses and lucerne, on the other hand, do not have natural homofermentative fermentations, thus adding the inoculant will have a greater effect, but aerobic stability can still be a problem. Although lactic acid is more effective than acetic acid in preserving the ensiled material, it does not contribute to inhibiting the growth of yeasts and moulds after the silo is opened (Muck, 2010). The best inoculant to use will depend on the specific need. If silos can be properly sealed during feed out and the primary objective is to preserve the ensiled crop, it will be best to use a homofermentative inoculant. A lot of farms have open bunkers sealed with plastic or wrapped bales, however. Under these circumstances it will be best to use a heterofermentative inoculant to enhance aerobic stability (Weinberg *et al.*, 1999). Combinations of *Lactobacillus buchneri* and heterofermentative LAB are also available (Table 2.4). A good DM recovery will thus be reached due to initial lactic acid production by the homofermentative strain, after which the *L. buchneri* will ferment some of the lactic acid to acetic, thereby enhancing aerobic stability (Filya, 2003b; Muck, 2010; Kung, 2001a; Driehuis *et al.*, 2001).

Table 2.4 Silage characteristics after exposure to air for five days, treated with a homo- and heterofermentative LAB or a combination of the two, Filya (2003a) as cited by Muck (2010)

Forage	Treatment	pH	CO ₂ production,%	Yeast, log cfu/g	Moulds, log cfu/g
			DM	DM	DM
Wheat	Untreated	4.9	2.94	6.8	3.5
	<i>L. buchneri</i>	3.9	0.46	< 2.0	< 2.0
	<i>L. plantarum</i>	5.3	3.73	8.1	3.1
	Both	4.1	0.68	2.2	< 2.0
Sorghum	Untreated	6.4	3.16	7.6	3.7
	<i>L. buchneri</i>	4.3	0.54	2.0	< 2.0
	<i>L. plantarum</i>	6.4	4.53	8.4	3.0
	Both	4.6	0.88	2.6	< 2.0
Maize	Untreated	6.1	2.55	6.5	3.3
	<i>L. buchneri</i>	4.2	0.41	< 2.0	< 2.0
	<i>L. plantarum</i>	5.8	0.76	7.7	2.8
	Both	4.8	0.7	2.0	< 2.0

Fermentation inhibitors like acids and formaldehyde was one of the first additives used in the development of modern silage production. The initial aim was to stop all microbial activity by lowering the pH to lower than 3 by adding a whole array of acids, like mineral acids, formic-, acetic- and lactic acid (Henderson, 1993). This lowered the palatability of the silage, however, and resulted in reduced intake by ruminants (McDonald *et al.*, 1991). Adding acids to the crop was also dangerous for the operator and corrosive to equipment. Fermentation inhibitors are still used today; not to stop all microbial activity, but rather to optimise the environment for LAB by reducing the initial pH to a level where it favours the growth of LAB.

Aerobic deterioration inhibitors are used to reduce DM losses during the feed-out phase. They include heterofermentative inoculants, as previously mentioned, and propionic acid, which will inhibit the growth of yeasts and moulds as soon as the silage is exposed to air (McDonald *et al.*, 1991).

The additives classified under fermentation stimulants as carbohydrate sources can also be grouped as nutrients due to the fact that they improve the nutritive value of the silage, which will result in better utilisation by the ruminant (McDonald *et al.*, 1991). There also are nutrients such as nitrogenous compounds and minerals that can be added to increase the nutritive value of the silage, but without enhancing fermentation characteristics. Urea, for example, can be added to a crop with a low nitrogen content, like maize; this will increase the nitrogen content but also the buffering capacity (discussed later on), which will result in a higher end pH. The same is true when

minerals are added to a crop before ensiling, which will result in less stable silage with a higher risk of clostridial growth and is therefore not recommended. Fermentation stimulants like molasses should therefore be ensiled together with minerals and urea to counteract the increase in buffering capacity (McDonald *et al.*, 1991). It will be best to add protein-rich food and minerals as a premix to the silage after fermentation to lower the risk of unstable fermentation and low quality silage.

Ensiling high moisture crops result in effluent production, especially when grasses are ensiled without being wilted (Haigh, 1994). Absorbents, like straw, are used to lower effluent production, resulting in less pollution and loss of nutrients (McDonald *et al.*, 1991).

Crop characteristics affecting silage quality

Silage quality does not only depend on good management practices such as optimum time of cut, wilting and bunker management, but also on plant factors which play a role in the quality of the silage produced. Although most crops can be ensiled, only some have the required characteristics to produce good quality silage. A silage crop must have a high dry matter yield per hectare and an optimum level of water soluble carbohydrates (WSC) to sustain the growth of LAB. A low buffering capacity (BC) will ensure a low end pH, high digestibility and optimum water content (McDonald *et al.*, 1991; Wilkinson, 2005).

Water soluble carbohydrates (WSC) consist of mono- and disaccharides such as glucose, fructose, sucrose and fructans. The glucose and fructose are present as free sugars and serve as a substrate for the LAB and therefore are the most important WSC. The WSC content of whole crop maize during ensiling can be up to 120 g WSC/kg DM and can go down to 20 g WSC/kg DM during the fermentation process (Wilkinson, 2005; Knicky, 2005). Fructans and sucrose will also be used by the LAB as an energy source, but it must first undergo acid hydrolysis. Acid hydrolysis will break down sucrose to its mono-saccharide building blocks, glucose and fructose, which are more readily available for the LAB. Fructans, on the other hand, is made up from fructose residues that are bound by 2,1- and 2,6-linkages; the complexity of the branches will determine how easily it is broken down to fructose and therefore the availability thereof for LAB (Knicky, 2005). Crops with a high WSC fraction are therefore more suitable for silage production. It has to be noted, however, that stage of maturity will again play a role regarding to the WSC content of the crop. This is especially the case when ensiling whole-crop grains such as oats and maize. The WSC fraction of these crops consists largely of glucose and fructose during the early growth stages. As the crops mature, the WSC fraction will decline while the fibre fraction increases. It is therefore important to cut the crop between the milk and early dough stage when the WSC fraction is at its highest (Filya, 2003a).

The buffering capacity (BC) is the ability of a crop to resist a drop in pH to 4.0 (Knicky, 2005). Weak salts of organic acids like succinate, malate and citrate and other anions like nitrate and sulphate have the biggest effect on the BC of a crop. Although BC will reduce as the crop matures and increase with N fertiliser, it is mainly affected by crop species (Wilkinson, 2005; Knicky, 2005). Crops like lucerne that have a high mineral content, will therefore have a higher BC and will be harder to ensile than maize or oats.

Crop DM, as previously mentioned, plays an important role in the fermentation process during ensiling because of the influence it has on the micro-organisms in the ensiled plant material. Water activity (a_w) is the term used to describe the amount of water available for the growth of the micro-organisms. Micro-organisms react differently to changing water activities with clostridia being more

sensitive to lower a_w than LAB. It is thus possible to control the microbial growth by altering the crop DM, which is negatively correlated to a_w (Knicky, 2005).

Analysing silage for quality

Forage, preserved as silage, can make up to 90% of the ruminant diet (Charmley, 2001). It is therefore important to determine the quality of the silage before feeding it to ruminants. On farm quality testing is not easy to do and therefore most farmers rely on physical attributes like smell, feel and taste to give them an idea of the quality silage produced (Table 2.5) (Wilkinson, 2005). Silage however has to be analysed before it can be used in ruminant nutrition. The analysis of most importance to the farmers is the crude protein (CP) content and the total digestible nutrients (TDN). This however does not reflect the quality of the silage and type of fermentation due to the fact that the major factors contributing to this are time of cut and the specific type of crop ensiled. Silage quality can be described as the feeding value of the silage and is not affected by the digestibility of the silage only, but also the type of fermentation that occurs during ensiling (Huhtanen *et al.*, 2002). It is therefore important, when testing for silage quality, not to look at crop related factors only, but also at factors related to the type of fermentation that occurred (Table 3.6). These factors play a role in animal production and it is therefore best to analyse silage for all the factors affecting quality to ensure that the animals receive a balanced diet. In modern analysis of silages, therefore, factors such as pH, CP, NDF, WSC, starch content and organic acid content and profile have to be determined.

Table 2.5 On-farm assessment of silage quality as adopted from Wilkenson (2005)

Assessment	Possible indications
Colour	
Yellow	Low protein/secondary fermentation if at base of silo and wet
Dark green	High protein if also leafy
Brown	Overheated/protein damage – Maillard reaction
Grey/white	Bad aerobic stability – mouldy
Texture	
Wet	Low DM with risk of secondary fermentation
Slimy	Secondary fermentation
Dry	High DM
Leafy	High energy and protein
Stemmy	Low energy and protein
Rough/Abrasive	Low intake if also stemmy
Soft	High intake if also leafy
Sticky	Residual WSC
Smell/taste	
Sweet	Lactic acid – good fermentation
Vinegar	Acetic acid – mixed fermentation
Fruity	Yeast activity – mixed fermentation
Vomit	Butyric acid – secondary fermentation
Sharp	Excess acidity – pH too low
pH	pH between 3.7-4.5 – good fermentation

Table 2.6 Crop and fermentation-related factors affecting silage quality (Charmley, 2001)

Crop related factors	Fermentation related factors
Crude protein (CP)	pH
Acid detergent fibre (ADF)	Protein solubility
Neutral detergent fibre (NDF)	Organic acid content and profile
Total digestible nutrients (TDN)	Water soluble carbohydrates (WSC)
	Ammonia

Moisture, energy and protein

Moisture content is the first analysis done to characterise the fermentation that has taken place and therefore the quality of the silage. It is determined by drying an as-fed sample in an oven and then calculating the difference. The optimum moisture content is critical for effective packing of the silo to exclude air as fast as possible and for the effective growth of LAB (McDonald *et al.*, 2002; Knicky 2005). High moisture content (above 75%) can prolong fermentation, which, in turn, will lower the energy content, increase the risk of secondary fermentation and lead to excessive break down of plant proteins, thereby increasing the non-protein-N (NPN) fraction. Excess air, on the other hand, will be trapped in the silo if the moisture content is too low, which will lead to secondary fermentation resulting in DM losses. Plant maturity also plays an important role in silage quality and can be determined by analysing the silage for ADF and NDF. In lucerne and grass silage, these values are an indication of WSC available for the rumen micro-organisms. A crop like lucerne ensiled with high ADF and NDF values will therefore deplete all available WSC during fermentation, leaving no reserves to maintain optimum rumen function. Maize fibre values, on the other hand, are not a good indication of available WSC, due to high starch content (Wilkinson, 2005).

The main contributing factor to the energy content and digestibility value of silage is the extent to which the fibre is bound to lignin. Lignin, as previously stated, gives plants their structural strength and increases as the plant matures. The older the plant, the more lignin accumulates and the less digestible it becomes, which will reduce the energy value thereof (Danley & Vetter, 1973). It is more accurate to determine the digestibility of a feedstuff by an *in vivo* study, even though there is a relationship between digestibility and lignin content. The energy value can then be estimated from the *in vivo* digestibility studies and normally ranges between 9 and 12 MJ ME/kg DM. Fermentable metabolisable energy (FME) is the ME potentially available for the rumen micro-organisms. The ratio of FME:ME for optimum rumen micro-organism growth is 3:4. A lower ratio is an indication of extensive fermentation and it is advised to add either starch or sugar to the diet to ensure optimal rumen function and therefore optimum utilisation of rumen degradable protein (RDP) (Wilkinson, 2005). Water soluble carbohydrates (WSC) present in the silage is also an indication of the effect that the silage will have on the rumen environment. It serves as an energy source for the rumen micro-organisms, having the same effect as the FME. Extensive fermentation will deplete the WSC in the crop and also reduce the FME. It is accepted as a general rule of thumb that silages with a high WSC content has higher nutritional quality. The content can vary from 20 to 120 g WSC/kg DM (Wilkinson, 2005; Knicky, 2005).

The CP content of silage will vary considerably depending on the crop ensiled, the fertiliser used and the climate. Mature whole-crop wheat and maize contains 10 g CP/kg DM and 8.9 g CP/kg DM respectively, with lucerne silage being in the extent of 200 g CP/kg (NRC, 2007). Maturity will also play a role, since the younger, leafy crop will have a higher CP value than the more mature crop containing proportionally more stem material. Silage protein mainly serve as rumen degradable protein (RDP) since a lot of the plant proteins are broken down during fermentation to non-protein nitrogen, like ammonia nitrogen, and is therefore readily available to rumen micro-organisms (Papadopoulos & Mckersie, 1983). This results in only 10% of the initial plant protein reaching the abomasum for digestion and therefore silage mainly serves as a source of RDP in ruminant diets (Wilkinson, 2005). Another fraction of the protein can be bound to ADF as a result of excessive heating due to secondary fermentation. This fraction is unavailable to ruminants and is measured by determining the acid detergent insoluble nitrogen (ADIN). Neutral detergent insoluble nitrogen (NDIN), although some can be digested, is also negatively correlated to the nutritive value of silage. Silages with a higher ADIN and NDIN value will have a lower overall digestibility (Van Soest & Mason, 1991).

Silage intake by ruminants is complex due to the fact that it is affected by the fermentation quality, digestibility and nitrogen factors. Huhtanen *et al.* (2002) formulated an equation to determine an index for silage DM intake (SDMI):

$$\text{SDMI Index} = 100 + 0.151 \times (D\text{-value} - 690) - 0.000531 \times (\text{TA}^2 - 6400) - 4.7650 [\text{Ln}(\text{Ammonia N}) - \text{Ln}(50)]$$

- *D*-value = silage digestibility (g/kg DM)
- TA = total acid (g/kg DM)
- Ammonia N expressed as g/kg of the total N

Grass silage with high digestibility and fermentation quality can have a potential intake index value of more than 100.

Silage pH

One of the on-farm methods to estimate whether optimum fermentation has taken place is to measure the pH of the silage. This can be done by mixing a sample in distilled water for one to two minutes and measuring the effluent either by a pH probe or pH-sensitive paper that will change colour according to the acidity (Seglar, 2003). Normal pH values range between 3.7 and 4.5, depending on certain crop characteristics. Maize silage that is well preserved will have a pH range of 3.7-4.5 whereas Lucerne silage, with a higher BC, will fall in the range of 4.2-5 (Seglar, 2003; Kung, 2001a). A low pH, as previously mentioned, is an indication of good LAB fermentation that resulted in the optimum production of lactic acid to inhibit the growth of unwanted micro-organisms such as clostridia and enterobacteria. Bad fermentation will therefore result in a high end pH of up to 7.5. This can be due a whole array of factors such as lack of WSC, growth of clostridia and enterobacteria, high a_w or even cold conditions (Kung & Stokes, 2001). A high pH (Table 2.8) due to clostridia will result in reduced intakes by ruminants due to butyrate production (Kung, 2001b). It was found that a low silage pH will reduce intake. This is not due to the pH of the silage affecting the rumen pH thereby reducing the cellulolytic activity, however, but rather to the organic acids affecting the palatability of the silage. Silage pH however is affected by organic acids. These

organic acids, namely lactic, acetic, propionic and butyric acid also have to be analysed to fully understand the type of fermentation that took place. Lactic acid is the most important organic acid regarding silage quality since it is the most abundant, contributing 65 to 70% to the total acids. It is also stronger than the VFA's and therefore has the greatest effect on the silage pH (Kung, 2001b; Seglar, 2003). It can range anywhere from 3-8% for lucerne silage and 4-6% for maize silage on a DM basis. High moisture maize on the other hand can have lactic acid concentrations ranging between 1-3% (Kung & Stokes, 2001; Seglar, 2003). Acetic, propionic and butyric acids are the VFAs contributing to the decline in pH during fermentation, giving silage its characteristic smell and contributing to the aerobic stability of silage during the feed-out phase. The VFA that has the biggest impact on aerobic stability is acetic acid and is found at concentrations up to 3% (Filya, 2003b; Kung, 2001a; Muck, 2010; Danner *et al.*, 2003). A high level of acetic acid is not ideal since the production thereof will result in a loss of carbon, thereby resulting in DM losses. The ratio of lactic acid to acetic acid is an important factor to take into consideration since lactic acid can be fermented to acetic acid (Filya, 2003a). A lactic acid to acetic acid ratio of 3:1 indicates that optimum fermentation took place (Kung & Stokes, 2001). Propionic acid levels in well-fermented silage will be less than 1%, with butyric acid being undetectable (<0.1%). High butyric acid levels, resulting in a rancid smell, are an indication of secondary fermentations that will result in lower energy levels, reduced DM intakes, and also DM losses. It is also an indication that extensive protein degradation had taken place, which would increase the fraction of soluble protein. There seems to be controversy around the effects that these acids have on ruminant DMI, with butyric acid being the first acid identified to reduce ruminant DMI. A lot of studies was done to determine the effect of the other VFAs and lactic acid on the DMI, but little correlation was found and results were inconsistent (Charmley, 2001). Ethanol is another factor contributing to the acidity of silage and is a result of yeast activity that can metabolise lactic acid, resulting in a high end pH. The amount of ethanol produced depends on the crop ensiled. In maize, silage the optimum level is 1-3% with legume and grass silage being <1.5% (Kung & Stokes, 2001; Seglar, 2003).

Ammonia concentration is also an important fermentation factor contributing to silage quality; it is expressed as a percentage of the nitrogen content of the silage. Elevated levels of ammonia indicate that extensive protein breakdown occurred, either due to a high end pH, which resulted in proteolytic plant enzymes not being deactivated, or due to clostridial fermentation which, again, will also result in butyric acid production. Lucerne and grass silage with a higher CP level will have an ammonia content of up to 15% whereas maize with lower CP will contain less than 10% CP (Kung & Stokes 2001; Seglar, 2003). Cushnahan *et al.* (1995) found that an increase in ammonia will result in reduced DM intakes in dairy cattle (Charmley, 2001).

With new technologies, such as the use of inoculants, and better management practices, the gap between intake of freshly cut forages and those preserved as forages has slowly been closed. Studies now suggest that there is little difference in DM intake of good quality silage and that of the forage. The effect of the different fermentation end products are summarised in Table 2.7 (Kung, 2001b; Charmley, 2001). Table 2.8 gives an indication of the optimum DM and fermentation end products expected for different silages.

Table 2.7 Fermentation end products and their effect on silage quality (Kung, 2001b)

Item	Positive or Negative	Actions
pH	Positive	Low pH inhibits unwanted bacterial activity
Lactic acid	Positive	Inhibits bacterial activity by lowering the pH
Acetic acid	Positive & Negative	Associated with undesirable fermentations. Inhibits yeasts responsible for aerobic spoilage
Butyric acid	Negative	Associated with protein degradation, toxin formation, and large losses of DM and energy
Ethanol	Negative	Indicator of undesirable yeast fermentation and high DM losses
Ammonia	Negative	High levels indicate excessive protein breakdown
Acid detergent insoluble nitrogen (ADIN)	Negative	High levels indicate heat-damaged protein and low energy content.

Table 2.8 Amounts of common fermentation end products in various silages (Kung & Stokes, 2001)

	Lucerne silage (low moisture)	Lucerne silage (low moisture)	Grass silage	Maize silage	High moisture maize silage
DM %	30 – 35	45 – 55	25 – 35	35 – 40	75
pH	4.3 – 4.5	4.7 – 5.0	4.3 – 4.7	3.7 – 4.2	4.0 – 4.5
Lactic acid %	7 – 8	2 – 4	6 – 10	4 – 7	0.5 – 2.0
Acetic acid %	2 – 3	0.5 – 2.0	1 – 3	1 - 3	< 0.5
Propionic acid %	< 0.5	< 0.1	< 0.1	< 0.1	< 0.1
Butyric acid %	< 0.5	0	0.5 – 1.0	0	0
Ethanol %	0.5 – 1.0	0.5	0.5 – 1.0	1 – 3	0.2 – 2.0
Ammonia-N %	10 – 15	< 12	8 – 12	5 – 7	< 10

It is important, when determining whether maize silage can be used effectively as an ingredient in the finishing of sheep, to look at the effect it will have on animal growth, digestibility and also meat quality. The first step, however, would be to analyse silage for quality and nutritive value to optimally formulate a balanced diet.

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Chapter 3

General materials and methods

Abstract

This study aimed to evaluate the effectiveness of corn silage as a feed ingredient in feedlot sheep diets. The quality of silage is of utmost importance to sustain good animal production. In this chapter, the general materials and methods are discussed as a first part in addressing the aim of the study. The experimental design and the compilation of the experimental diets will be discussed in length here and be referred back to in the subsequent chapters

Procedures used in this study were all approved by the Research Ethics Committee: Animal Care and Use, University of Stellenbosch (Ref: 11LV_VAN01).

Animals and experimental design

Forty, three month old, Merino lambs (26.6 kg \pm 0.63) were sourced from Ouplaas in the Bredasdorp district in the Southern Cape of South Africa and transported to Welgevallen Experimental Farm, Stellenbosch, South Africa where the study was conducted. On arrival at Welgevallen, lambs were tagged, dosed with 3ml/10kg live weight *Levicon* (Bayer, Isando, Gauteng, South Africa) for internal parasites and vaccinated with *Multivax P* (1 ml, Inervet, Kempton Park, Gauteng, South Africa) against pulpy kidney and pasteurilla. Lambs also received a 35 ml dose of *Lacticon-s* (Afrivet, Megastarter Biotech, Centurion, Gauteng, South Africa) to assist with their adaption to the experimental diets and were divided into four groups of equal initial weight. Each group of lambs was randomly assigned to one of the four treatments; control (containing no silage), 20% silage, 50% silage or 70% silage on a DM basis. Lambs were kept and fed indoors, each lamb housed separately in 1.8m x 1.2m pens. Individual intakes were determined during the adaptation period. Feeding troughs were cleaned every morning to maximise intake and prevent mould from forming on the silage. Feed was weighed, hand mixed and given at 07:00 in the morning and again at 16:00 in the afternoon (Table 3.1). *Ad libitum* fresh drinking water was available at all time. All refusals were bagged daily and weighed once a week. Lambs were weighed once a week, before being fed, to determine average daily gain (ADG) and feed conversion ratio (FCR).

Table 3.1 Example of Maize silage to concentrate ratio for lambs receiving 2kg As Is daily of the experimental diets containing 0 (control), 20, 50 or 70 % maize silage on a DM basis

	Control	20%	50%	70%
Maize silage, kg	0.0	0.9	1.5	1.8
Concentrate, kg	2.0	1.1	0.5	0.2
Total, kg	2.0	2.0	2.0	2.0

Physical and chemical composition of dietary treatments

Diets consisted of a control containing no silage, diets with 20, 50, and 70% maize silage (on a DM basis) in the ration. All the diets were formulated on an iso-nutrient basis, except for fibre (NDF) (Table 3.1).

Whole crop maize was harvested at a DM of 27% with a commercial silage harvester on the farm Faircape near Durbanville in the Western Cape of South Africa. The untreated crop was compacted by hand into 220 L plastic drums and sealed with plastic bags within six hours after harvest. Drums were transported to the Welgevallen Experimental Farm in Stellenbosch, where they were further sealed by putting sand bags on their lids. It was left to ferment for 60 days before sampling five random drums with a silage drill (1.5 m stainless steel pipe with a sharpened edge) to obtain representative samples for proximal analysis. Silage samples were taken, after a 60 day fermentation period, at the same depth in each drum (40 cm). Raw materials for the concentrate part of the diets were mixed and pelleted at Mariendal Experimental Farm, Stellenbosch, South Africa. The control feed was mixed by Tanqua Feeds, Riversonderend, South Africa, to supply the same nutrient concentrations as the other experimental diets, with the exception of fibre (NDF)

Table 3.2 Physical and chemical composition of four diets fed to the Merino lambs

DM Basis, g/kg	Maize silage inclusion level			
Physical composition, g/kg	Control	20%	50%	70%
Maize silage (pH 3.62)	0	200	500	700
Yellow Maize	545	513	303	144
Lucerne meal	150	0	0	0
Cottonseed oil cake	60	100	103	104
Molasses (meal)	88	50	40	0
Salt	5	5	5	5
Sodium bicarbonate	20	20	20	20
Ammonium chloride	5	5	5	5
Limestone	18	18	18	18
MCP	3	3	3	3
Urea	5	5	2	0
min/vit	1	1	1	1
Oat hay	100	80	0	0
Total, g/kg	1000	1000	1000	1000
Chemical composition, DM				
DM g/kg	909.2	778.8	357.5	281.8
Organic matter g/kg	900.1	938.6	937.5	933.8
Ether extract g/kg	19.2	32.8	31.2	36.8
Crude Protein g/kg	165.2	159	154.5	153.3
Acid detergent fibre g/kg	204.1	121.6	184.6	204.1
Neutral detergent fibre g/kg	421.7	242.0	316.7	341.8
ME (MJ/kg)	10.3	10.6	10.3	10.0

Analytical methodologies for feed, faeces and meat sample analysis

Moisture

AOAC (2002) official method 934.01: Determination of moisture content

Porcelain crucibles were washed in hot soapy water and dried in an oven for two hours at 100°C before being placed in a desiccator for 30 minutes to cool down. The weight of the crucibles was recorded, the scale tared and 2.5 g meat/ 2 g feed sample accurately weighed into the crucible. The crucible with the meat/ feed sample was placed in an oven at 100°C for 24h after which it was

again placed in a desiccator and left to cool down for 30 minutes before being weighed. Percentage moisture was calculated as follows:

$\% \text{ Moisture} = [(\text{Dry crucible mass} + \text{Sample mass} - \text{Mass of dry sample in crucible}) / (\text{Sample mass})] \times 100\%$

Ash

AOAC (2002) official method 942.05: Determination of ash content

A 2g moist-free sample was weighed into a porcelain crucible with a known mass and placed in a furnace at 500°C for six hours. It was then allowed to cool for two hours before being placed in a desiccator for 30 minutes before being weighed back. The percentage ash was calculated as follows:

$\% \text{ Ash} = [(\text{Mass of crucible and ash} - \text{Empty dry crucible}) / \text{Sample weight}] \times 100$

$\% \text{ Organic matter} = 100 - \% \text{ Ash}$

Crude protein

AOAC (2002) official method 968.06: Determination of Nitrogen by the Dumas method

Apparatus: LECO FP-528, Nitrogen Determinator (Leco Corporation; St. Joseph, USA)

Accessories: 502-186 Tin foil cups

Sample weight: 0.1000 g

Furnace temperature: 850°C

Protein factor: 6.25

Calibration of the Leco

1. Check that the filter is clean;
2. Change crucible if full (350 analysis/ crucible);
3. Make sure all three gas bottles are open;
4. Open gas lines by selecting "ON";
5. Run three to six blank analyses;
6. EDTA is used for the calibration of the Leco for samples such as meat where more than 20% protein is expected (% Nitrogen range: 9.45 – 9.60). For samples with a protein content of less than 20%, Alfalfa is used for the calibration (% Nitrogen range: 3.96 – 4.04).

The samples used for the analysis of Nitrogen were ground and fat and moisture were removed. The foil cup was placed on the scale and zeroed before a 0.1000 g sample was weighed into the cup. The cup was then folded in the shape of a teardrop and the final weight recorded. Samples

were placed in the Leco and analysed for nitrogen content. Crude protein was calculated as follows:

$$\% \text{ Crude protein} = \% \text{ Nitrogen} \times 6.25$$

Ether extract for feed and faeces samples

AOAC (2002) official method 920.39: Determination of fat in feed samples

Apparatus: Tecator Soxtec System HT 1043 Extraction unit

Reagents: Diethyl ether

Method:

1. Clean aluminium cups (soxhlet flasks) were prepared by putting them in a drying oven at 100°C for two hours, after which they were cooled in a desiccator for 30 minutes and accurately weighed to the fourth decimal;
2. Two grams of a dry sample were then accurately weighed into thimbles and covered with a small piece of defatted cotton wool to keep the sample in place during extraction;
3. Approximately 50 ml diethyl ether was added to aluminium cups;
4. The water supply for the condensation was turned on together with the oil bath and cooling system;
5. The extraction thimble containing the sample was placed in the extractor as soon as the ready light started to flash, after which the suction tube handle was pulled down, sealing the joints of the extraction apparatus;
6. Thimbles were dropped into the ether and left to boil for 15 minutes;
7. Thimbles were lifted after boiling and left for 30 minutes;
8. Taps were closed for 15 minutes to capture ether;
9. Aluminium cups were removed and placed in drying oven for at least two hours or till all the ether had evaporated;
10. Aluminium cups were then placed in desiccator and left to cool down for 30 minutes before being weighed.

Percentage fat was calculated as follows:

$$\% \text{ Fat} = \frac{[(\text{Mass of soxhlet cup} + \text{fat}) - (\text{Mass of soxhlet cup})] / \text{Mass of sample}}{1} \times 100$$

Ether extract of meat samples

The ether extraction for the meat samples was done as described by Lee *et al.* (1996).

Reagents: Chloroform, methanol and sodium chloride (5 g/ 1L distilled H₂O)

Method:

1. Clean glass beakers were placed in drying oven for two hours to dry, after which they were placed in a desiccator for 30 minutes to cool down;
2. Beakers were weighed, zeroed and a 5g meat sample was accurately weighed into the beaker;
3. Chloroform/ methanol 1:2 is used for samples with less than 5% fat whereas a 2:1 ratio is used for meat samples with more than 5% fat;
4. Fifty millilitres of chloroform/ methanol (2:1) was added and mixed with a Bamix blender for one minute;
5. Contents of the beaker were filtered through a Whatman no. 1 filter paper and 20 ml 0.5% NaCl was added, after it was mixed;
6. It was left to stand for 30 minutes before bottom layer was tapped into a 100 ml Erlenmeyer flask, after which 5 ml was pipetted into fat beaker with known mass and placed in a heated sand bath for at 45 min;
7. Fat beakers were placed in a desiccator for 30 minutes to cool down and accurately weighed.

The percentage fat was calculated as follows:

$$\text{Fat \%} = [(\text{Fat beaker} + \text{Fat}) - (\text{Fat beaker}) / \text{Sample mass}] \times (\text{Chloroform volume}^A / 5) \times 100$$

$$A - \text{Chloroform/ methanol (2:1)} = 50 \times 2/3 = 33 \text{ ml}$$

$$A - \text{Chloroform/ methanol (1:2)} = 50 \times 1/3 = 16.7 \text{ ml}$$

Neutral detergent fibre (NDF)

Neutral detergent fibre was determined by the method described by Van Soest *et al.* (1991) with the help of an ANKOM 220 Fibre Analyser (ANKOM Technologies, Fairport, NY, USA).

The neutral detergent solution was prepared by adding 30 g sodium lauryl sulphate, 18.61 g Ethylenediaminetetraacetic Disodium Salt (Dihydrate), 6.81 g sodium tetraborate decahydrate, 4.56 g sodium phosphate dibasic (anhydrous) and 10 ml triethylene glycol to distilled water and filling to volume (1 L).

Procedure:

1. The F57 Filter Bags were weighed (W_1) and the scale zeroed;
2. An air-dried sample milled through a 1 mm screen was directly weighed ($0.5\text{g} \pm 0.05 \text{ g}$) into the filter bags (W_2); a blank bag (C_1) was also included to determine a correction factor for bag weight;
3. Bags were sealed with a heat sealer (0.5 cm from the open edge) and flicked to spread the sample uniformly;

4. Three bags per tray (eight trays) were stacked on the centre post, each level rotating 120 degrees, and placed in the ANKOM fibre analyser with a weight on top to keep the samples submerged;
5. For the processing of 24 bags, 1.9 – 2 L of NDS is used together with 20 g sodium sulphite and 4 ml heat-stable alpha-amylase;
6. *Agitate* and *Heat* were turned on and the lid closed after confirming that the Bag Suspender was agitating properly;
7. It was left to boil for 75 minutes, after which *Agitate* and *Heat* were turned *OFF* and the drain valve opened to release the pressure before opening the lid;
8. The drain valve was closed after the solution was exhausted and 2 L hot water (90-100°C), together with 4 ml alpha-amylase, added;
9. The lid was closed, but not sealed, with *Agitate* on for three to five minutes;
10. Steps 8 and 9 were repeated three times with alpha-amylase only added twice;
11. The bags were removed after the final rinse and the water pressed out gently;
12. Bags were placed in a beaker with acetone and left to soak for three minutes before being removed, gently blotted dry and left so that acetone could evaporate;
13. Bags were then dried in the oven at 105°C for two hours before being placed in a desiccator to cool down before being weighed (W_3).

The percentage NDF was calculated as follows:

$$\text{aNDF (As-is basis)} = [(W_3 - (W_1 \times C_1)) \times 100] / W_2$$

$$\text{aNDF (DM basis)} = [(W_3 - (W_1 \times C_1)) \times 100] / (W_2 \times \text{DM})$$

Acid detergent fibre (ADF)

Acid detergent fibre was determined by the method described by Goering & Van Soest (1970) with the help of an ANKOM 220 Fibre Analyser (ANKOM Technologies, Fairport, NY).

Acid detergent solution (ADS) was prepared by adding 20 g N-Cetyl-N,N,N-Trimethyl Ammonium Bromide (CTAB) to 1 L of standardised H_2SO_4 . The standardised H_2SO_4 was prepared by measuring 28 ml of 98% H_2SO_4 and filling to volume (1 L) with distilled water. Procedures followed for ADF determination were the same as for the determination of NDF, with the following differences:

- No sodium sulphite or heat-stable alpha amylase was used during step five and 1.9 – 2 L of ADS was added instead of NDS;
- The solution was left to boil for 60 minutes during step seven, instead of 75 minutes.

Percentage ADF was calculated as follows:

$$\text{ADF (As-is basis)} = [(W_3 - (W_1 \times C_1)) \times 100] / W_2$$

$$\text{ADF (DM basis)} = [(W_3 - (W_1 \times C_1)) \times 100] / (W_2 \times \text{DM})$$

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Chapter 4

Chemical composition and quality of maize silage prepared for inclusion in sheep feed

Abstract

Although maize silage is not commonly used in South African sheep production systems; it is, however, used effectively in Europe and Australia. The aim of this study was to determine the chemical composition of maize silage to establish whether it could be used effectively in the diets of Merino lambs. Whole-crop maize was harvested at 270 g/kg DM, packed into 220 L plastic drums and left to ferment for 60 days. The resulting silage was analysed for nutrient content as well as fermentation end products to determine if it could be used effectively as an ingredient in ruminant diets. Silage quality is characterised according to its DM, CP, pH, WSC and starch content. Dry matter (270 g/kg DM) content was lower and crude protein (CP) higher (112.2 g/kg) than generally accepted for maize silage. The pH (3.62) was low enough to inhibit the growth of unwanted bacteria, mainly as a result of the lactic acid content (4.6%) of the silage. The silage contained an optimum amount of water soluble carbohydrates (WSC) (3.4%) and starch (27.6%). It had an acetic acid content of 5.4% and a combined propionic and butyric acid content of less than one percent. An ammonia nitrogen content of 10.6% also indicated that no excessive protein degradation had taken place. It was therefore concluded that the silage produced was of good overall quality and could therefore be included in the diet of Merino lambs. The silage was subsequently included in sheep feedlot diets as described in Chapter 6.

Key words: fermentation end products; nutrient content

Introduction

Forage conservation plays an important role in animal production systems in South Africa. Large parts of the country have a low and variable rainfall with periodic droughts being the norm (Rouault, 2004). Furthermore, it is believed that developing countries will have to double livestock products between 1993 and 2020 to meet the needs of an ever growing human population (Reddy *et al.*, 2003). It is therefore of great importance for farmers to produce enough forage during the rainy season, not only to maximise production but also to have enough in reserve in case of a drought. There are two basic ways of forage conservation: hay making and the production of silage (McDonald *et al.*, 2002). Silage production is favoured in some instances because it is less weather dependent and because less field and transportation losses occur during ensiling, while the silage is more palatable due to the fact that it is cut at a younger growth stage, thus containing less structural carbohydrates (Blaser, 1964). The process of ensiling can be described as the controlled fermentation of a high moisture crop (containing between 50 and 82% moisture) in a bunker or silo to preserve it for later use (Dodds *et al.*, 1985; McDonald *et al.*, 2002). Ensiling has two basic objectives, the first of which is to obtain anaerobic conditions as quickly as possible to prevent aerobic spoilage. The second objective is to lower the pH to a level where unwanted micro-organisms, like clostridia and enterobacteria, will not grow (McDonald *et al.*, 1991; Muck, 2010). Forage preserved as silage can make up to 90% of the ruminant diet (Charmley, 2001). It is therefore important to determine the quality of the silage before feeding it to ruminants. On-farm quality testing is not easy to do and many farmers therefore rely on physical attributes like smell, feel and taste to give them an idea of the quality of the silage produced (Wilkinson, 2005). Silage

has to be analysed before it can be used in ruminant nutrition, however. The analysis of most importance to farmers is the crude protein (CP) content and the total digestible nutrients (TDN). This, however, does not say much about the quality of the silage or the type of fermentation that occurred. Major factors influencing silage quality are type and number of lactic acid bacteria on the crop before ensiling, WSC content of the crop, chopping, compaction and sealing of the bunker. Analysis should therefore also include pH, WSC, lactic acid, starch and organic acid content and profile. Silage quality can be described as the feeding value of the silage and is not affected by the digestibility of the silage only, but also by the type of fermentation that occurred during ensiling (Huhtanen *et al.*, 2002).

When testing for silage quality, it is therefore important not to look at crop-related factors only, but also at factors related to the type of fermentation that occurred. To ensure that the animals receive a balanced diet, it is best to analyse silage for all the factors affecting the quality, which in turn will affect animal production.

Materials and methods

Whole-crop maize was harvested at a DM of 27%, with a commercial silage harvester (12 mm chop length) on the farm Faircape (Durbanville, Western Cape, South Africa). The untreated crop was compacted into 220-litre plastic drums and sealed within six hours. Drums were transported to the Welgevallen Experimental Farm in Stellenbosch (Western Cape, South Africa) where it was sealed further by placing sand bags on top of the plastic, covering the maize silage, to prevent entering of air. Drums were left to ferment for 60 days before samples were taken for chemical analysis. Samples taken were analysed for DM, ether extract, nitrogen content, ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and for gross energy (GE). A separate sample, also taken 60 days after ensiling, was vacuum-packed and frozen for later lactic acid, volatile fatty acid (VFA), water soluble carbohydrate (WSC) and ammonia-N analysis. The pH measurements were taken during feed out as the individual drums were opened. See Chapter 3 for general materials and methods. One reading was taken immediately the drum was opened and a second reading was taken when the drum was emptied.

Proximate analysis

Silage samples were weighed, dried at 60°C for 96 h, air-equilibrated and weighed again. As described in Chapter 3 (General materials and methods), the samples were milled through a 1.5-mm screen (Scientes RSA, Hammer mill, Ser No 372) and analysed according to the AOAC International (2002) official methods of analysis (17th edition), for ether extract (EE), moisture and ash content. All N analysis was performed according to the combustion method (Method 992.15) with a Leco FP-428 N and protein Analyser (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI, USA). Acid detergent fibre (ADF) and NDF were determined with an ANKOM²²⁰ Fibre Analyser (ANKOM Technologies, Fairport, NY, USA) and the gross energy by adiabatic oxygen bomb calorimetry (Animal Production Laboratory, Elsenburg, Stellenbosch, South Africa). Please see Chapter 3 for the complete methodologies.

Neutral detergent insoluble nitrogen

Neutral detergent fibre analysis was done on samples by the method described by Van Soest *et al.* (1991) with the help of an ANKOM 220 Fibre Analyser (ANKOM Technologies, Fairport, NY, USA). See Chapter 3 (General materials and methods) for the complete procedure. The samples were

then dried in an oven at 60°C for 96 h after NDF analysis and analysed for nitrogen content as described in Chapter 3. Neutral detergent insoluble nitrogen was expressed as gN/kg DM.

Maize silage pH

Two pH measurements per drum were taken during feed out, the first when the drum was opened and the second measurement on the last silage in the drum. The top layer was discarded before the first sample was taken. An electronic pH meter (pH meter number: 285146, Wissenschaftlich, Weilheim, Germany) was used for measuring the pH. The pH meter was calibrated before each measurement, according to the method described by the manufacturers (WTW Wissenschaftlich-Technische Werkstätten, 82362, Weilheim, Germany). The first sample that was measured was taken when the drum was opened and the second 48h after opening. A 40g representative silage sample was mixed with 40 ml distilled water, left to soak and the pH measured. The pH meter was calibrated before each measurement was taken.

Lactic acid determination

Lactic acid of a representative maize silage sample was determined according to the colorimetric method described by Pryce (1969). A 50g frozen silage sample was diluted with 250 ml distilled water and shaken by hand for three minutes, after which it was stored at 5°C for 24 hours. The mixture was shaken again for three minutes during the cooling period on two separate occasions. The diluent was filtrated through Whatman no. 4 paper after the 24-hour cooling down period to remove all the plant material and then the supernatant was stored in a refrigerator before being sent to Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Gauteng, South Africa) for lactic acid analysis.

Starch

Starch analysis was done according to the (A) starch gelatinisation and hydrolysis method modified from Holm *et al.* (1986) and (B) glucose analysis method modified from Karkalas (1985).

A:

Samples were prepared by drying them in an oven at 60°C for 96 h, after which they were milled through a 1-mm screen.

Reagent:

- 0.1M sodium acetate buffer with a pH of 4.5

Enzyme:

- Heat stable α amylase, Sigma Aldrich no. A3306
- Amyloglucosidase, Sigma Aldrich no. A1602

Equipment:

- Water baths at 93°C and 60°C
- Funnels
- Erlenmeyer flasks

- Volumetric flasks, 50 ml and 100 ml
- Glass wool
- Aluminium foil

A 0.2g sample was accurately weighed, recorded and placed in an Erlenmeyer flask. Then 20 ml distilled water was added and stirred with a magnetic stir bar. Heat stable α amylase (100 μ l) was added and it was stirred again. The 93°C water bath was prepared and the Erlenmeyer flask was covered with foil before it was submerged in the water bath for one hour. The flask was left to cool down for 15 min before its contents was filtered through glass wool-plugged funnels into volumetric flasks (100 ml) and filled to volume. Two millilitre samples were transferred to a 50-ml volumetric flask after which 8 ml of 0.1M sodium acetate buffer and 50 μ l Amyloglucosidase were added and swirled to mix. The solution was placed in the water bath (60°C) for 30 min and stirred every 10 min. Flasks were made to volume pending assay for glucose.

B:

Reagents:

- Glucose oxidase peroxidase reagent (GOP)
- Glucose standard solutions
- Distilled water

Reagents were prepared as described by Lingnau (2011).

Equipment:

- Vortex
- Volumetric flasks (100 ml)
- Screw cap test tubes
- Water bath 40°C
- Spectrophotometer ($\lambda = 505$ nm)

The sample prepared in A as well as 1-ml sample of each of the glucose standard solutions were transferred to test tubes after which 5 ml GOP reagent was added and vortexed. The tubes were closed and placed in a 40°C water bath for 45 minutes before being removed and placed in a dark room to cool down. A sample blank was used to zero the spectrophotometer for the sample and the standard blank for the stock standard. The solution, after it cooled down, was transferred to the spectrophotometer vials and the absorbance measured at $\lambda = 505$ nm.

The following formula was used for the calculation of glucose in the standard solution (Lingnau, 2011):

$$\text{Glucose } \mu\text{g/ml} = [(\text{glucose stock solution, } \mu\text{g/ml}) \times V_a] / V_s$$

Where:

V_a = aliquot volume of stock solution

V_s = final dilution volume

A graph was constructed with the glucose concentration of the standard solution ($\mu\text{g/ml}$) on the y axis and absorbance values ($\lambda = 505 \text{ nm}$) of the standard solutions on the x axis. The regression was calculated as:

$$y = mx + c$$

This took the following form:

$$\text{Glucose } \mu\text{g/ml} = \text{value of slope} \times \text{absorbance} + \text{value of intercept}$$

By using the regression equation, the glucose ($\mu\text{g/ml}$) concentration of each sample was calculated and multiplied by 0.9 to determine the starch percentage.

Water soluble carbohydrates

A 40 g representative maize silage sample was sent to Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Gauteng, South Africa) for water soluble carbohydrate determination. The method used for determining the WSC content was based on the phenol-sulphuric acid method by Dubois *et al.* (1956) where a 40g frozen silage sample was diluted with 360 ml distilled water and homogenized for 4 minutes with a bamix homogenizer, after which it was filtrated through a Whatman no 1 filter paper. The supernatant was diluted to 1:10 by pipetting 1 ml into a test tube with 9 ml distilled water (solution A). Solution A was further diluted to 1:1000 by pipetting 1 ml into another test tube with 9 ml distilled water (solution B). Two test tubes were prepared for the spectrophotometer. The first had 1 ml of distilled water and served as a blank. The second was prepared by adding 0.15 ml phenol (80%) to 1 ml of solution B and vortexed for 10 seconds, after which 5 ml sulphuric acid 98% (H_2SO_4) was added to the solution and it was again vortexed for 10 seconds. Samples were analysed by the spectrophotometer and WSC determined after 30 minutes, referring to a standard curve constructed for the specific sugar (glucose) under examination. Water soluble carbohydrates were calculated as follow:

$$\% \text{ WSC} = \frac{(\text{Absorption} - y \text{ intercept}) / x}{\% \text{ DM}} * \text{dilution factor}$$

Volatile fatty acids

Volatile fatty acids were determined by using a Gas Chromatograph (Varian 3300 FID Detector Gas Chromatograph, Varian Associates, Inc. 1985, United States of America, Column: CP Wax 58 (FFP) CB Cat no. 7654 25 m, 0.53 mm, 2.0 μm) at Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Gauteng, South Africa). Determination of VFA was done as described by Suzuki and Lund (1980). A 50g representative maize silage sample was diluted in 200 ml distilled water and shaken on a horizontal shaker for 6 hours at 180 rpm. Plant material was removed by filtering the mixture through four layers of cheesecloth. The supernatant was centrifuged for 20 minutes at 4500 rpm in a cooled chamber, after which it was filtrated through a Cameo 30 (0.45 μm) filter. A 1 μl sample was injected into the gas chromatograph with the standard being repeatedly injected until consecutive results were comparable. The GC conditions for the VFA determination were as follow:

- Initial column temperature – 50°C
- Initial column temperature – 50°C
- Initial column hold time – 2°C
- Temp program column – Yes
- Program 1 final column temp – 190°C
- Program 1 column rate in °/min – 15
- Program 1 column hold time – 5 min
- Temp program column – No
- End time – 16.33 min
- Injector temperature – 250°C
- Detector temperature – 260°C
- FID A initial attenuation – 2
- FID A initial range – 11
- FID A autozero on – yes
- Program 1 FID A time in min – tune
- Program 1 FID attenuation – INF
- Add next FID A program – No
- Initial relays 1
- Time program relays – No
- Method complete, end time – 16.33 min

Ammonia nitrogen

Ammonia nitrogen content was determined according to the method of Pearson and Muslemuddin (1968) by the Central Analytical Facilities (University of Stellenbosch, Western Cape, South Africa). A 50g representative sample of maize silage was homogenized with a bamix homogenizer in a 250 ml 0.1 N H₂SO₄ solution for three minutes and filtered through a Whatman no. 4 filter paper. The filtrate was used to determine the ammonia nitrogen by distillation, using a Buchi 342 apparatus (Labortechnik AG, Flawil, Switzerland) and a MetröhM 655 Dosimat with an E526 titrator (MetröhM AG, Ionenstrasse, Herisau, Switzerland).

Results and discussion

One of the first analyses done to determine the quality of the silage and to give an idea of the type of fermentation that took place is the moisture content of the ensiled material. The optimum moisture content of harvested crop is critical for effective packing of the silo to exclude air as fast as possible and for the effective growth of LAB (McDonald *et al.*, 2002; Knicky 2005). A high moisture content can prolong fermentation, which, in turn, will lower the energy content, introduce the risk of secondary fermentation, and lead to excessive break-down of plant proteins, thereby increasing the non-protein-N (NPN) fraction. Excess air, on the other hand, will be trapped in the silo if the moisture content is too low, which will lead to secondary fermentation resulting in DM

losses. The optimum DM for maize at harvesting for silage can range from 30 to 45% with high-moisture maize being as low as 25%. Maize produced during this trial had a DM of 27% (Table 5.1). This is an indication that the crop was cut at the right age and moisture content for the production of optimum silage.

The CP content of silage will vary considerably depending on the crop ensiled, fertilizer use and climate. Maize silage will have a CP content of around 80 g/kg DM whereas, the CP content of lucerne silage can be in the range of 180 g/kg (NRC, 2007). Maturity will also play a role since younger leafy crops will have a higher CP value than more mature crops containing relatively more indigestible or poorly digestible stem material. Albeit low, silage protein mainly serves as a rumen degradable protein (RDP) source due to the fact that a lot of the plant proteins are broken down during fermentation and are readily available to rumen micro-organisms (Papadopoulos & Mckersie, 1983). Maize silage produced during this study had higher crude protein content, 112.2 g/kg DM, than expected and could be the result of fertilizer given to the crop. It is however most likely a result of the whole-crop maize being cut at a younger growth stage. Generally, maize silage contains 80 g/kg DM CP (NRC, 2007).

Analysis for fermentation end products is necessary to determine the type of fermentation that took place, which, in turn, will give an indication of the quality of the silage produced. Regarding silage quality, lactic acid is the most important of the organic acids produced during fermentation. It is the most abundant acid (75% of the total acids contained in silage) and also stronger than the volatile fatty acids (VFA) and therefore has the greatest effect on silage pH (Kung, 2001b; Seglar, 2003). It can range between 4-6% on a DM basis with high moisture maize ranging between 1-3% (Seglar, 2003). The lactic acid content of the maize silage (4.6%) fell within the range and resulted in an optimum pH (3.62) being reached. A low pH is an indication of good LAB fermentation that resulted in optimum production of lactic acid to inhibit the growth of unwanted micro-organisms such as clostridia and enterobacteria. Bad fermentation will result in a high end pH of up to 7.5. The pH of the maize silage produced for this study therefore is an indication that optimum fermentation took place, resulting in a low end pH and thereby inhibiting the growth of unwanted micro-organisms like clostridia and enterobacteria. Normal pH values range between 3.7 and 4.5, depending on certain crop characteristics. Silage that is well preserved will have a pH range of 3.7-4.5, whereas lucerne silage, with a higher buffering capacity (BC), will fall in the range of 4.2-5.0 (Kung, 2001a; Seglar, 2003).

Water soluble carbohydrates (WSC) present in the silage is an indication of the effect that the silage will have on the rumen environment. It serves as an energy source for the rumen micro-organisms. Extensive fermentation will deplete the WSC because it also serves as an energy source for the LAB. It is accepted as a general rule of thumb that silages with a high WSC content has a higher nutritional quality. The WSC content of silages can vary from 20 – 120 g WSC/kg DM, depending on the type of fermentation that has taken place (Knicky, 2005; Wilkinson, 2005). Excessive fermentation will result in more WSC being depleted and thus in lower WSC content after ensiling. The WSC of the maize silage (22 g WSC/kg DM) produced in the current study fell within the range generally accepted, although on the low side. The starch content of the silage not only serves as an energy source for the animal, but also for the rumen microorganism and therefore in the production of microbial protein for the ruminant. According to Weiss and Firkins (2007), maize silage ought to have a starch content of 30%; this accords with the 27.6% starch content of the maize silage in the current study.

Acetic, propionic and butyric acids are the VFAs contributing to the decline in pH during fermentation, which gives silage its characteristic smell and contributes to the aerobic stability of silage during the feed-out phase (Kung, 2001b). The VFA that has the biggest impact on aerobic stability is acetic acid, which is found in concentrations of up to 3% (Danner & Holzer, 2003; Filya, 2003b; Kung, 2001a; Muck, 2010). A high level of acetic acid is not ideal because the production thereof will result in a loss of carbon, thereby resulting in DM losses. The ratio of lactic acid to acetic acid is an important factor to take into consideration since lactic acid can be fermented to acetic acid (Filya, 2003a). A lactic acid to acetic acid ratio of 3:1 indicates that optimum fermentation took place (Kung & Stokes, 2001). This, however, was not the case for the silage produced in this study. The acetic acid concentration of the silage was substantially higher (5.4%) than expected, resulting in a lactic acid to acetic acid ratio of 1:1 (Table 4.1). Aerobic stability would have increased, but a loss in DM could be expected together with possible reduction in intake (Forbes, 2007). The excessive fermentation, resulting in the high acetic concentration, may be a reason for the low WSC content of the silage.

Propionic acid in well-fermented silage will be less than 1%, with butyric acid being undetectable (< 0.1%). High butyric acid levels resulting in a rancid smell are an indication of secondary fermentations that result in lower energy levels, reduced DM intake and also DM losses. It is also an indication that extensive protein degradation has taken place, which will increase the fraction of soluble protein. Extensive protein degradation and secondary fermentations did not take place during the ensiling of the maize. Propionic and butyric acid concentrations were 0.1 and 0% respectively for the maize silage in this study.

Ammonia concentration is another fermentation factor contributing to silage quality; it is expressed as a percentage of the CP content of the silage. Elevated levels of ammonia indicate that extensive protein breakdown has occurred, either due to a high end pH, which can result in proteolytic plant enzymes not being deactivated, or due to clostridial fermentation which will also result in butyric acid production. Lucerne and grass silage with a higher CP level will have an ammonia content of up to 15%, whereas maize, with lower CP, will contain less than 10% ammonia (Kung & Stokes 2001; Seglar, 2003). Cushnahan *et al.* (1995) found that an increase in ammonia will result in reduced DM intakes in dairy cattle (Charmley, 2001). The ammonia-N content (10.6%) expressed as a percentage of the CP content of the silage was at the upper limits of what can be expected for maize silage.

Table 4.1 Chemical composition of maize silage (MS1) produced under irrigation in the Durbanville area of the Western Cape of South Africa compared to normal chemical composition of maize silage (MS2)

	MS1	MS2
Nutritional		
DM g/kg	270.0	250.0-450.0
CP g/kg	112.2	80
Fermentation		
pH	3.6	3.7-4.5
Starch g/kg	276.0	300.0
WSC g/kg	22.0	20.0-120.0
Lactic Acid %	4.6	1.0-6.0
Acetic acid %	5.4	3.0
Propionic acid %	0.1	< 1.0
Butyric acid %	0.0	< 0.1
Ammonia-N %	10.6	< 10.0
Lactic: Acetic acid	1:1	3:1

Conclusion

The main concerns regarding the quality of the maize silage was the high moisture content which could lead to reduced DMI, especially for sheep. Another concern with the maize silage produced for this study was the high acetic content which indicated that unnecessary dry matter losses occurred. However, it is evident from the data obtained from this study that the silage produced was of a good overall quality, not only regarding the nutritive value thereof but also the fermentation end products. The maize silage had a good pH, indicating that it was well preserved, which resulted in it having an optimum starch and CP content. The reason for higher CP levels is most likely due the fact that that the maize was cut at a young age. The maize silage contained normal levels of starch, WSC and lactic acid.

It can be concluded that the silage was of good enough quality to be effectively used as an ingredient in ruminant diets.

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Chapter 5

The effect of increasing levels of maize silage diets on the production performance of feedlot Merino lambs

Abstract

The aim of the study was to determine the effect of increasing levels of maize silage in the diets of Merino feedlot lambs on the feed intake, production performance and feed efficiency ratio. One of the concerns regarding the use of silage as the main ingredient of a sheep feed is its high moisture and NDF content and relatively poor protein value. Forty lambs, in a four-way completely randomised block design, were used in a 60-day finishing study. The diets consisted of a control with no silage and three diets containing, on a DM basis, 20, 50 or 70% maize silage. With the exception of fibre (NDF and ADF), all diets were formulated on an iso-nutrient base. It was observed that the dry matter intake (DMI) decreased as silage inclusion increased above the 20% level. At 20% inclusion rate, the feed intake of the animals was stimulated. This resulted in significant differences between the cumulative intake of the low and the high silage diets. The feed conversion ratio (FCR) was higher, however, for the control and 20% silage diets. Significant differences were also found in the dressing percentage of the slaughtered animals where the 20 and 50% silage diets had a higher dressing percentage than the control and 70% silage diets. It was concluded that silage can be successfully incorporated into sheep diets, especially at low levels where its use stimulates intake.

Key words: dressing percentage; dry matter intake; feed conversion ratio

Introduction

In South Africa silage is widely used as cost-effective feed ingredient in TMR systems for dairy cattle nutrition, but it is not that commonly used in sheep production systems. European countries and Australia, on the other hand, make use of silage in their sheep enterprises to improve pasture utilisation, increase stocking rate, for use as a drought feed and also for the finishing of lambs in a feedlot (Marley *et al.*, 2007; Stanley, 2003). The same principles can, theoretically, be applied to South Africa, not only to optimise pasture utilisation but also to optimise sheep production to meet the ever-growing demand for animal protein. Sheep production systems in South Africa traditionally are extensive and sheep are more sensitive to silage quality than cattle (Baumont *et al.*, 2000), giving the impression that silage is not a practical or effective roughage source for sheep. Due to increasing feed prices and practices like conservation farming, however, farmers have to find ways to optimise their production systems. Using silage is proposed as one such option.

A few factors that will affect the performance of lambs receiving silage diets have to be taken into consideration, however. First of these is the dry matter (DM) content, which is directly correlated to voluntary feed intake. Silage with higher DM content should result in a higher DMI by sheep (Kokkonen *et al.*, 2000). Supplementing deficiencies of silage diets also needs to be taken into account. Plant proteins, for instance, will be broken down to ammonia nitrogen during the ensiling process, resulting in a higher amount of rumen degradable protein (Papadopoulos & Mckersie, 1983). It is therefore necessary to add rumen bypass protein such as, for example, soybean or canola meal (Wilkinson, 2005), or other available oil cakes with adequate UDP levels. To improve

the nitrogen retention, it is important to supplement silage based diets with fermentable carbohydrate sources such as cereal grains. This will provide the energy needed by the rumen microorganisms to utilise the degradable nitrogen entering the rumen (Stanley, 2003). Care has to be taken, however, when supplementing grain since it will lower the rumen pH to unsatisfactorily levels, resulting in acidosis (Slyter, 1976).

Plant material that is ensiled will have an effect on animal performance. Legume silages, according to Fraser *et al.* (2002) as cited in Stanley (2003), will have better production responses and voluntary intakes than silages produced from cereal grains and grass. Increasing the yield by cutting the plant material at a later stage will lower the digestibility due to an increase in structural carbohydrates (Van Soest, 1978). A study conducted by Fitzgerald (1987) on sheep found that as the digestibility of perennial ryegrass silage decreased so did the voluntary intake. Fitzgerald (1987) also found that the best gain/ha is obtained when grass is ensiled at ear emergence. This is not exactly the same for maize, however, since the filling of the grain will compensate for increase of structural carbohydrates. It is found that the optimum time to ensile maize will be at half milk line or 35% DM

the trial when the animals were slaughtered and calculated as carcass weight (with kidneys intact) as percentage of live weight. A more indepth discussion on carcass quality and other meat characteristics are given in Chapter 8.

For the complete experimental design and analytical methodologies, please see Chapter 3. The information in Table 5.1, containing the experimental diets, is repeated in this chapter for the sake of convenience.

Table 5.1 Physical and chemical composition of four diets fed to the Merino Lambs

DM Basis, g/kg Physical composition, g/kg	Control	Maize silage inclusion level		
		20%	50%	70%
Maize silage (pH 3.62)	0	200	500	700
Yellow Maize	545	513	303	144
Lucerne meal	150	0	0	0
Cottonseed oil cake	60	100	103	104
Molasses (meal)	88	50	40	0
Salt	5	5	5	5
Sodium bicarbonate	20	20	20	20
Ammonium chloride	5	5	5	5
Limestone	18	18	18	18
MCP	3	3	3	3
Urea	5	5	2	0
min/vit	1	1	1	1
Oat hay	100	80	0	0
Total, g/kg	1000	1000	1000	1000
Chemical composition, DM basis				
Dry matter g/kg	909.2	778.8	357.5	281.8
Organic matter g/kg	900.1	938.6	937.5	933.8
Ether extract g/kg	19.2	32.8	31.2	36.8
Crude Protein g/kg	165.2	159.0	154.5	153.3
Acid detergent fibre g/kg	204.1	121.6	184.6	204.1
Neutral detergent fibre g/kg	421.7	242.0	316.7	341.8
ME (MJ/kg)	10.3	10.6	10.3	10.0

Rumen characteristics: rumen pH, volatile fatty acid composition (VFA) of rumen fluid and rumen wall histology

A separate study was conducted to determine the effect that silage-based diets had on the pH of the rumen. Three fistulised sheep were used to determine the rumen pH profile of the control, 50% maize silage and 100% maize silage diets in a two x three cross-over design study. Sheep were dewormed and dosed with *Lacticon-S* (Megastarter Biotech, Centurion, Gauteng, South Africa) to help them adapt to the experimental diets. Rumen fluid, after a two-week adaptation period, were taken every hour for 24 hours and the pH measured (pH meter number: 285146, Wissenschaftlich, Weilheim, Germany) to determine the effect that the specific feed had on the rumen pH. Rumen fluid samples were taken with a syringe and the pH meter was calibrated before each measurement. The process was repeated for the 50% and 100% maize silage groups. One of the sheep did not adapt to the 100% maize silage, however, and had to be taken out of the study,

therefore, no statistical analysis was done and results are presented as is to give an indication of the treatment effects of rumen pH and VFA composition.

Rumen fluid samples were taken at four-hour intervals from 07:00 am to 01:00 am the next morning to determine the effect that the control and 100% maize silage diets had on the VFA composition. The results that are presented can therefore only give an indication of the treatment effects. The rumen fluid samples were frozen (minus 10°C) pending analysis. Samples were prepared according to the modified method proposed by Siegfried *et al.* (1984). A calcium hydroxide solution (CHS) and a cupric sulphate solution (CSR) were added to the rumen fluid sample to deproteinise as well as remove sugars. The solution that remained consisted of fermentation products such as short-chain alcohols, non-volatile fatty acids as well as VFAs. Reagents were prepared as follows:

- CHS – Ca(OH)₂ (52.9 g) was accurately weighed into a 250-ml Erlenmeyer flask and a stir bar added. Ultra pure water (200 ml) was added to the flask, after which the flask was inverted a few times to suspend the solution. The resultant slurry was placed on a stir plate during pipetting to ensure a homogenous solution.
- CSR – CuSO₄·5H₂O (50 g) was again accurately weighed into a volumetric flask (500 ml). Ultra pure water (400 ml) was added to dissolve the CuSO₄·5H₂O. Crotonic acid (2 g) was added to the solution, then mixed and the flask filled to volume with distilled water.

The procedure used was as follows:

A rumen fluid sample (1.5 ml) was transferred into a 1.7 ml centrifuge tube and centrifuged at 12000 x g for ten minutes. The tube was taken out of the centrifuge and 600 µl was transferred to a clean 1.7 ml centrifuge tube. CHS (600 µl) and CSR (300 µl) were added to the tube before being capped, vortexed and frozen at minus 10°C.

Tubes were thawed, centrifuged for ten minutes and 1000 µl of the supernatant was transferred to a new 1.7 ml centrifuge tube. The new tubes already contained concentrated H₂SO₄ (28 µl). Tubes were again capped and frozen. For the next, step the tubes had to be thawed and frozen again for a final time. The tubes were again centrifuged for ten minutes after they had been thawed for the final time and supernatant was transferred to HPLC vials.

Vials were taken to the Central Analytical Facilities (Stellenbosch, Western Cape, South Africa) for VFA analysis according to the HPLC method.

The HPLC solvent was prepared as follows:

Three point five litres ultra pure water, 1.66 ml concentrated H₂SO₄ and 0.40 g ethylenediaminetetraacetic acid (EDTA) was added to a 4 L Erlenmeyer flask. The Erlenmeyer flask was heated for 2 min and then it was left to cool overnight whilst being stirred to completely dissolve EDTA. The flask was filled to volume with ultra pure water and the solution was vacuum filtered through a 0.2 µm membrane before being transferred to the HPLC reservoir bottle. The apparatus used was a Focus GC Model (Thermo Finnigan, Austin, Texas, USA) with the following conditions:

- Internal standard – Crotonic acid
- Column – BPX21, 30 m x 0.25 mm ID, 0.25 µm film
- Initial temperature – 60°C, 5 min
- Rate 1 – 7°C/min
- Final temperature – 160°C
- Detector – FID, 260°C
- Injector – 220°C
- Split flow – 20:80
- Split ratio – 80
- Carrier gas – Hydrogen, 1 ml/min
- Injector volume – 1 µl
- Injection volume – 1 µl
- Run time – 35 min

Eighty millilitres of ultra pure water was added to a 100 ml Erlenmeyer flask, the VFAs added and the flask filled to volume. Volatile fatty acids, their volumes and time to peak are given in Table 5.2.

Table 5.2 Volatile fatty acid standard solution made up in 100 ml ultra pure water together with the time (min) when peaks appeared

	Volume of VFA (µl)	Time to peak (min)
Acetic acid	57.3	13.8
Propionic acid	74.6	16.5
Iso-butyric acid	92.8	18.8
Butyric acid	91.8	20.5
Iso-valeric acid	54.5	24.0
Valeric acid	108.4	29.5
Concentrated sulphuric acid	41.5	–

Rumen wall samples were also taken, at day of slaughter, to determine the histological effects the experimental diets had on the rumen. Two-by-three-centimetre rumen wall samples were taken in the left cranial ventral sac at approximately 2.5 centimetres right of the reticulo rumen fold. The method used for collecting rumen wall samples was adapted from Greenwood *et al.* (1997). The samples were preserved in formaldehyde (4%) solution and processed (embedded in wax, mounted and H&E staining) by the Department of Agriculture, WC, Veterinary services, Stellenbosch, South Africa.

Measurements were done with an Olympus CH30 Microscope (Nikon Digital sight, 4 x magnification, no: 0271225, Wirsam Scientific, Cape Town) with a Zeiss lens (West Germany). The total magnification was 40x when the 10x ocular magnification is included. Pictures for measuring the papillae parameters were taken with a Nikon DS-Fi1 camera (0.99 µm/px, RGB 8 bit: 2555 x 4982, Achromat 4 x (uncalibrated), Tokyo, Japan). Three slides were produced per lamb; the length and width of two of the longest and shortest papillae of each slide were measured in µm. A total of six long and six short papillae per lamb were therefore measured. Measurements of the submucosa were also taken on the left and right side of the papillae (Sub1 and Sub 2), as well as of the thickness of the rumen wall (RW) (Figure 5.1).

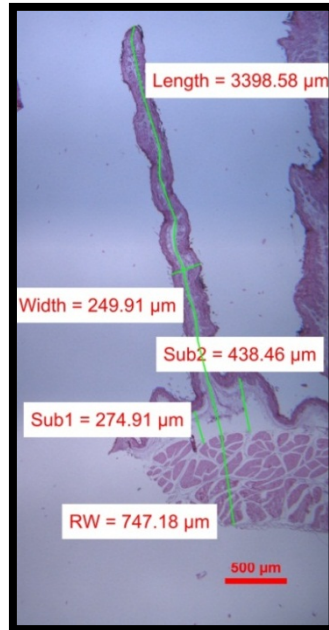


Figure 5.1 Method for measuring rumen parameters in µm:
 Sub1 = Submucosa on the left side of papilla;
 Sub2 = Submucosa on the right side of papillae;
 RW = Rumen wall thickness;
 Length = Papillae length;
 Width = Papillae width.

Statistical analysis

The effect of silage-based diets on production efficiency of Merino lambs was analysed by using PROC GLM and PROC ANOVA of Statistica (Data analysis software system, Statsoft Inc. 2011, version 10.0). The Least Square Means were calculated for all effects tested and significance was declared at $P \leq 0.05$.

As described earlier, no statistical responses were determined for pH or VFA samples and only descriptive results are presented.

Results and discussion

One of the concerns regarding the use of silage as the sole feed for small ruminants is its high moisture content. Wilkens *et al.* (1971) (as cited in Dulphy & Van Os, 1996), stated that wilting silage will increase DMI due to the lowered moisture content. Duckworth & Shirlaw (1958) came to the same conclusion and found that feed with a DM of less than 28-24% DM will reduce intake. Silage is therefore seen as a high rumen fill feedstuff that results in lower dry matter intakes by sheep. Weekly intake data (As is) obtained from this study (Figure 5.2) are in agreement with the work previously done by other researchers. Lambs receiving the 50 and 70% silage diets constantly had to increase their intake to meet their nutrient demand. This was only possible when the lambs grew bigger in body size as time went on (Figure 5.2) and therefore had a bigger rumen capacity. This was not the case for the lambs receiving diets containing less silage or the control diet. They were able to match their nutrient demand with their intake and did not have to adapt their intake as drastically as the lambs on the higher silage diets. From Figure 5.2, this point is demonstrated clearly where As Is intake of the 50 and 70% silage diets continuously increased

during the 60-d study period. Their DMI therefore also increased concurrently while the DMI of the 20% silage treatment reached a plateau after approximately three weeks on the diet (Figure 5.3).

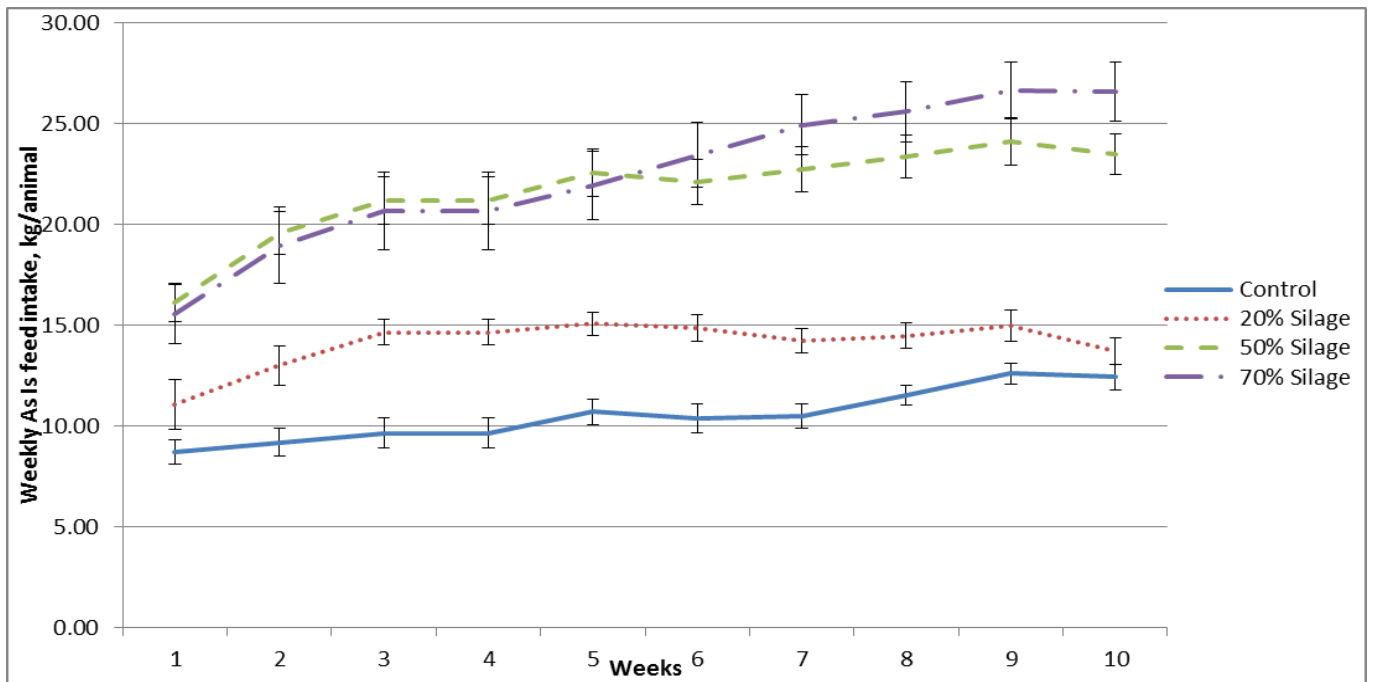


Figure 5.2 Weekly As Is intake (kg/animal) of sheep fed a control diet or an experimental diet containing 20, 50 or 70% maize silage (error bars represent the SEM)

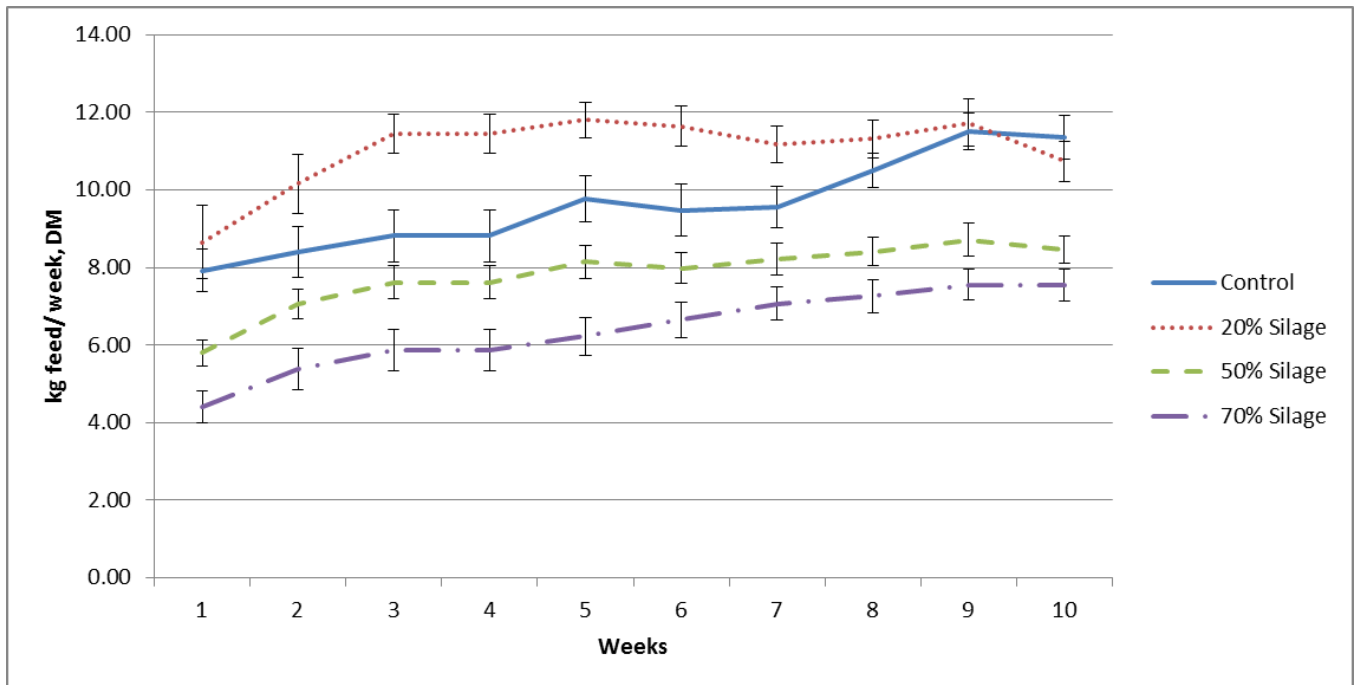


Figure 5.3 Weekly DMI (kg/animal) of sheep fed a control diet or an experimental diet containing 20, 50 or 70% maize silage (error bars represent the SEM)

The lambs on the 50 and 70% silage diets experienced a physical restriction due to the high moisture content and were not able to reach the same DMI over the 60-day study period as the lambs on the control and 20% silage diets (Figure 5.2). These animals constantly increased their

intake as they grew bigger physically, but still were not able to reach the same intake as the sheep on the control or 20% silage diets, as indicated by the lower cumulative DMI in Table 5.3. Furthermore, the silage treatment groups were observed to consume their feed at a higher initial rate than the control group, indicating that silage inclusion had a positive palatability effect in the diet. In Figure 5.2, it can be seen that the weekly feed intake for the 20% silage treatment group was the highest ($P < 0.05$), supporting the palatability effect of the silage. It was expected that the NDF content of the diets will also have an effect on DMI. This was however not the case. The control diet having the highest NDF content (421.7 g/kg) as well as the highest daily NDF intake (0.57 kg/day DM) had the highest daily intake, together with the 20% silage diet. The 20% diet on the other hand had the lowest NDF content (242 g/kg) and a daily NDF intake of 0.38 kg/day (DM). These findings therefore confirm that the moisture content had the most profound effect on DMI.

Optimum growth has to be achieved if silage-based diets are to be effective in finishing diets of lambs. The production parameters obtained from this study is summarised in Table 6.3. Work done by Stanley (2003) showed that good quality, highly digestible legume silage can be used for the finishing of second-cross lambs. Live weight gains of 150 g/d were observed when only legume silage was used and up to 300 g/d gains were observed when replaced with 70% grain. Meeske & Basson (1998) in a separate study found average daily gains (ADGs) of 255 g when silage was fed at 60%, combined with a concentrate, to South African Mutton Merinos.

Table 5.3 Mean (\pm SE) growth parameters of Merino lambs fed four diets differing in silage content

	Maize silage inclusion level				P-Value
	Control	20%	50%	70%	
Initial body weight (kg)	26.3 \pm 1.11	27.5 \pm 1.21	27.0 \pm 1.61	25.7 \pm 1.37	0.804
Final body weight (kg)	40.7 \pm 1.31	40.3 \pm 1.28	42.2 \pm 1.95	38.2 \pm 1.78	0.381
Body weight gain (kg)	14.4 \pm 0.81	12.8 \pm 0.81	15.3 \pm 1.10	12.5 \pm 0.89	0.123
Cumulative feed intake (kg DM)	87.3 ^a \pm 3.82	98.7 ^a \pm 3.25	70.4 ^b \pm 3.03	58.0 ^b \pm 3.87	< 0.001
Daily feed intake (kg DM)	1.4 ^a \pm 0.06	1.6 ^a \pm 0.05	1.1 ^b \pm 0.05	0.9 ^b \pm 0.06	< 0.001
ADG (kg/day)	0.2 \pm 0.01	0.2 \pm 0.01	0.2 \pm 0.02	0.2 \pm 0.01	0.123
FCR	6.1 ^a \pm 0.25	7.9 ^b \pm 0.40	4.7 ^c \pm 0.27	4.7 ^c \pm 0.19	< 0.001
Cold carcass weight (kg)	17.1 \pm 0.77	18.4 \pm 0.82	19.9 \pm 1.02	17.1 \pm 0.99	0.123
Dressing percentage (%)	42.0 ^b \pm 0.75	45.6 ^a \pm 1.28	47.0 ^a \pm 0.68	44.6 ^{ab} \pm 0.84	0.004

^{a,b,c} Row means with different superscripts differed significantly at $P \leq 0.05$

As expected, there were no significant differences ($P > 0.05$) in initial and final body weight due to the fact that animals were randomly assigned to treatments at the beginning of the trail and fed to approximately 40 kg before being slaughtered. The lack in differences between treatments for the final body weight or body weight gain therefore indicates that the animals' performance was remarkably similar regardless of the use of silage in their diet, or not. This is not surprising, as the diets were formulated to be similar in terms of the nutrients supplied. Average daily gains were somewhat lower than reported by Meeske & Basson (1998). The reason for this could be that Meeske & Basson used South African Mutton Merinos, a breed selected for its capability to produce meat. They further implanted lambs with a growth stimulant, Ralgro®, to obtain better average daily gains and therefore observed better feed conversion ratios (FCRs).

The most important findings in our study were related to feed intake and FCR effects of silage inclusion in sheep diets. Feed conversion ratios differed significantly between the groups in the current study. The 50 and 70% silage diets resulted in the best FCR and differed significantly ($P < 0.01$) from the control and the 20% silage diet. Of the four dietary treatments, the 20% silage diet had the highest FCR (7.91 ± 0.401), which by all standards, is not optimum for the production of sheep in a feedlot. When looking at the higher DM intake, it was expected that the 20% silage diet would not only have a higher ADG than the other silage diets but also a better FCR, since animals reached the same final body weights. This, however, was not the case. The FCR increased as the amount of concentrate in the diet increased, indicating that the concentrate added to the silage was of a low quality. This was the case indeed, as the concentrate portion of the diets consisted predominantly of yellow maize, cottonseed oil cake, molasses meal and oat hay (Table 5.1). To formulate the diets on an iso-nutrient basis matching the specifications of silage (with the main variable in the diet being silage), relatively low specification feedstuffs were used for the concentrate portion of the diet. The lower silage diets containing more concentrate therefore had a higher FCR. This, however, was not the only factor influencing the FCR. It is also clear, from the DMI data (Figure 5.3), that sheep on the 50 and 70% diets could not reach the same intakes as the lambs fed the control or 20% silage diets, indicating a physical restriction on DMI. Hicks *et al.* (1990), working on feedlot steers and heifers, found that restricting the DMI of the animals will improve their FCR. This can be ascribed to better diet digestibility because of a longer retention time and a reduction in refusals when DM feed intake is limited. Daily DM refusals for the control ($0.19 \text{ kg} \pm 0.014$) and 20% silage ($0.25 \text{ kg} \pm 0.012$) diets were also higher than the 50 ($0.08 \text{ kg} \pm 0.007$) and 70% ($0.05 \text{ kg} \pm 0.005$) silage diets ($P < 0.001$). It is therefore also possible that less nutrients were taken in, resulting in a lower FCR.

Significant differences ($P = 0.04$) were found between the dressing percentages of the dietary groups. Lambs receiving the 20 and 50% silage diets had a higher ($45.6\% \pm 1.28$ and $47.0\% \pm 0.68$ respectively) dressing percentage than lambs on the control diet ($42.0\% \pm 0.75$). This was related to the diets having higher ME values, even though diets were formulated on an iso-nitrogenous basis. Note that the metabolisable energy (ME) content (Table 5.1) was determined by an *in vivo* digestibility study.

Only three fistulated animals were available for the measurements of the rumen pH. One of the animals, as previously stated, also refused the 100% silage diet and had to be removed from the study. Results obtained must therefore be interpreted with care since only summative statistics can be reported. The data obtained showed that the rumen profile followed the same trend as seen in dairy cows receiving maize silage diets as the sole feed (Figure 5.4) (Beauchemin & Yang, 2005). The pH started out high and fell directly after feeding before it slowly started to increase again. Another dip in pH was seen after the second feeding, after which it returned to the initial high pH during the night. Rumen pH was not determined with data loggers as rumen fluid samples had to be removed hourly. The strict anaerobic conditions of the rumen therefore were slightly disrupted during the 24-h collection period, offering a possible explanation for the observed lower rumen pH at the second 07:00 AM reading. It was interesting to find that the 100% silage diet never resulted in a pH drop below the critical pH of 5.5, even though the average pH of the maize silage itself was 3.62. This can be ascribed to the fact that there was no concentrate in the diet and that the 100% silage stimulated rumination. More bicarbonate was therefore secreted with salivation to buffer the rumen, inhibiting a sharp drop in pH (Kaufmann, 1976).

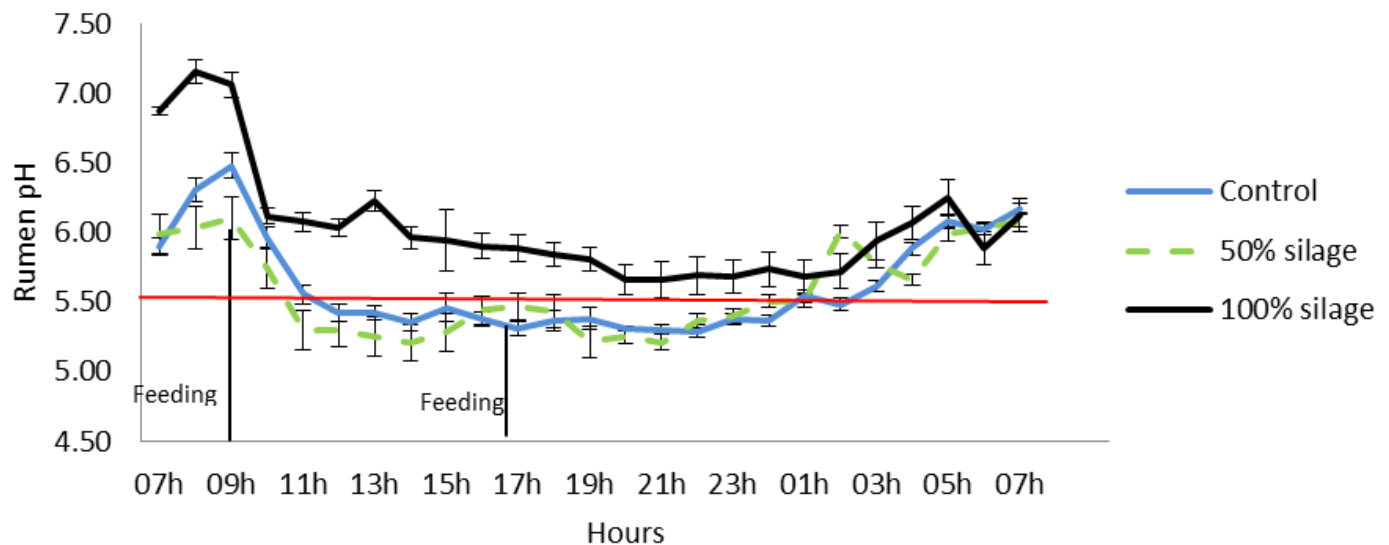


Figure 5.4 Effect of Control, 50 and 100% maize silage diets on rumen pH (error bars represent the SEM)

The mean VFA content of the rumen fluid samples taken at six-hour intervals are given in Table 5.4. The data, due to a lack of experimental animals, are not statistically viable and only give an indication of what can be expected. The total concentrations of VFAs, according to McDonald *et al.* (2002), can vary from 70 – 150 mmol/litre depending on the diet the animal receives. It is generally accepted that diets high in fibre, like the maize silage, will have acetic acid proportions of up to 70%. Diets comprising mostly of concentrates, however, will have a lower acetic acid proportion but a higher proportion of propionic acid (McDonald *et al.*, 2002). Rumen fluid obtained from the control group had a total VFA content of 119.5 mmol/litre and the silage group had a total VFA content of 80.3 mmol/litre (McDonald *et al.*, 2002). The molar proportions of acetate, propionate and butyrate for the control diet were 0.48, 0.22 and 0.16 respectively. The 100% maize silage diet had molar proportions of 0.45, 0.19 and 0.15 for acetate, propionate and butyrate.

Table 5.4 Mean (\pm SD) volatile fatty acids in the rumen fluid of sheep receiving a control diet containing 0% maize silage and sheep receiving 100% maize silage

Volatile fatty acid (m moles/ litre)	Time	Control	100% Maize silage
Acetic acid	07:00	53.9 \pm 5.69	29.3 \pm 4.19
	13:00	48.8 \pm 5.19	36.3 \pm 0.34
	19:00	66.7 \pm 0.44	40.8 \pm 0.81
	01:00	62.5 \pm 0.90	38.3 \pm 1.11
Propionic acid	07:00	22.7 \pm 1.45	10.6 \pm 1.58
	13:00	20.9 \pm 0.49	16.9 \pm 0.76
	19:00	31.7 \pm 3.95	17.1 \pm 0.72
	01:00	31.4 \pm 2.81	15.3 \pm 0.04
Isobutyric acid	07:00	4.7 \pm 0.12	4.0 \pm 0.03
	13:00	4.1 \pm 0.22	4.0 \pm 0.01
	19:00	4.2 \pm 0.08	4.1 \pm 0.02
	01:00	4.3 \pm 0.06	4.1 \pm 0.03
Butyric acid	07:00	16.9 \pm 3.13	9.5 \pm 1.11
	13:00	14.3 \pm 2.58	12.1 \pm 0.12
	19:00	18.7 \pm 2.14	13.8 \pm 0.37
	01:00	17.2 \pm 1.64	13.2 \pm 0.51
Isovaleric acid	07:00	9.5 \pm 0.23	8.0 \pm 0.18
	13:00	8.4 \pm 0.21	8.1 \pm 0.08
	19:00	8.6 \pm 0.11	8.0 \pm 0.02
	01:00	9.0 \pm 0.07	8.0 \pm 0.00
Valeric acid	07:00	5.2 \pm 0.25	4.3 \pm 0.13
	13:00	4.9 \pm 0.19	4.8 \pm 0.04
	19:00	5.7 \pm 0.31	4.8 \pm 0.04
	01:00	5.6 \pm 0.03	4.7 \pm 0.04

Rumen development, health and subsequent efficiency largely depend on the type of diet the animal receives. It is known that starch-rich concentrate diets will facilitate the development of rumen papillae (Suárez *et al.*, 2006). This, according to Sander *et al.* (1959), as cited in Baldwin *et al.* (2004), is due to the higher butyrate and propionate proportions produced by fermentation of concentrates. Roughage, on the other hand, is necessary for rumen muscularisation and also helps to maintain the integrity of the epithelium (Norouzzian *et al.*, 2011). It is therefore necessary to obtain the optimum ratio of concentrate: roughage not only to stimulate the development of the rumen but also to ensure rumen health and thereby optimum production of the animal. Measurements of papillae and rumen wall thickness are summarised in Table 5.5.

Table 5.5 Mean (\pm SE) for the shortest and longest papillae and rumen wall thickness measured from Merino lambs receiving experimental diets containing 0 (control), 20, 50 and 70% maize silage

Parameter (μm)		Maize silage inclusion level				P- Value
		Control Diet 1	20% Diet 2	50% Diet 3	70% Diet 4	
Shortest papillae	Width	444.2 \pm 29.10	463.0 \pm 33.80	430.1 \pm 29.57	475.2 \pm 28.06	0.729
	Length	1463.1 \pm 154.63	1986.2 \pm 217.10	1881.7 \pm 114.26	1665.9 \pm 148.85	0.135
Longest papillae	Width	395.7 \pm 29.52	440.2 \pm 28.58	431.4 \pm 36.55	457.7 \pm 23.88	0.529
	Length	4388.8 \pm 345.83	5443.4 \pm 354.28	4393.1 \pm 305.21	4906.6 \pm 186.10	0.067
Submucosa1		204.8 \pm 23.71	192.2 \pm 22.18	173.1 \pm 17.37	185.1 \pm 21.63	0.763
Submucosa2		216.7 \pm 24.58	192.4 \pm 19.55	183.9 \pm 20.03	191.1 \pm 27.54	0.769
Rumen wall thickness		1090.5 \pm 99.10	1113.0 \pm 87.69	956.4 \pm 65.35	1133.2 \pm 925.57	0.319

No differences were observed for papillae length, rumen wall thickness or submucosa thickness between dietary groups. A study conducted by Le Roux (2011) investigated the effect that different creep feeds had on rumen development in lambs. The length and width of the short papillae in the current study correlate with the results found by Le Roux (2011) for the group of lambs on pasture. For the longer papillae, however, these correlate with the lambs receiving the creep feeds (4027.3 – 4483.0 μm). The rumen wall thickness in the current study is thinner than the lambs on pasture from Le Roux's (2011) study indicating that the concentrate in the experimental silage diets had an effect on the rumen wall thickness. There would most likely have been significant differences in rumen papillae if the diets were not formulated on an iso-nitrogenous basis (Table 5.1). The reason why there were no differences in rumen wall thickness between the control and silage diets most likely concerns the high fibre content of the control diet. Results would have been different if the diets were optimised for energy and protein content. Research indicates that diets formulated with higher energy concentrations will stimulate the development of rumen papillary (Suárez *et al.*, 2006).

Conclusion

It is evident from the data that were obtained that diets containing more than 50% silage will restrict the DMI of Merino lambs. The lambs will constantly adapt their intake as they physically grow bigger but will still not reach the same DM intakes as those on the control and lower silage diets. The lower DMI of the diets containing more than 50% silage is mainly due to the high moisture content. Lambs on the control and 20% silage diets therefore had higher cumulative feed intakes than those on the higher silage diets. The FCR, however, was higher for the control and 20% silage diet. The poor FCR has most likely more to do with the low quality of the control feed and the concentrate portion of the 20% silage diet than the silage itself. It is also known that restricting intake will result in a better FCR due to better diet digestibility and less feed refusals. Even though the feed intake was not deliberately restricted the 50 and 70% diets did in effect restrict the intake due to the low DM content, resulting in a better FCR. The silage diets had no negative effect on rumen parameters. Effects of the 0, 50 and 100% diets on rumen pH profile have to be interpreted with care, since too few fistulated animals were available to distinguish data statistically. The diets had the same effect on the rumen pH as seen in dairy cows, except in the case of the 100% silage diet.

The current research has laid the groundwork for further research. The next step will be to optimise the 20 and 50% diets to meet higher nutrient demands for lamb growth and to compare it with a proven efficient feedlot finishing diet.

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Chapter 6

The digestibility of silage-based diets fed to Merino lambs

Abstract

Digestibility and rumen degradability of four diets containing 0, 20, 50 and 70% maize silage were compared using an *in vivo* and *in vitro* digestibility study. Eight lambs per group were fitted with faecal collection harnesses and urine funnels for quantitative collection. Feed, faeces and urine collected during the seven-day period were analysed and the apparent nutrient digestibility calculated. The 20% silage diet presented the highest dry matter (DM) and organic matter (OM) digestibility. No differences were found between NDF digestibilities of the silage-based diets, it was higher, however, than with the control diet. The control diet presented the lowest overall *in vivo* apparent digestibility. Dry matter intake was the highest for the 20% silage diet and therefore resulted in the highest energy intake and the best energy retention. The 50 and 70% silage diet presented the lowest N retention while the 20% silage diet had the highest N retention. Results obtained from the *in vitro* digestibility study correlated with those found in the *in vivo* study regarding the DM and neutral detergent fibre (NDF) digestibility. The control diet presented lower DM and NDF degradability than the silage-based diets. *In vitro* true digestibility (IVTD) values did not differ between the silage based diets, but was higher than the IVTD of the control diet.

Key words: *in vitro* digestibility; *in vivo* digestibility; rumen degradability

Introduction

Silage is widely used in South Africa as a cost-effective source of roughage in dairy cattle nutrition. It is not that commonly used in sheep production systems, however. European countries and Australia, on the other hand, make use of silage in their sheep enterprises to improve pasture utilisation, increase stocking rate, for use as a drought feed and also for the finishing of lambs in a feedlot (Marley *et al.*, 2007; Stanley, 2003). The same principles can, theoretically, be applied to South Africa; not only to optimise pasture utilisation, but also to optimise sheep production to meet the ever-growing demand for animal protein. Sheep production systems in South Africa traditionally are extensive and sheep are more sensitive to the physical form of silage. For example, the chop length and quality of the silage affects sheep production more than production in cattle (Aerts *et al.*, 1984; Baumont *et al.*, 2000), giving the impression that silage is not a practical or effective source of roughage for sheep. Due to increasing feed prices and practices like conservation farming, however, farmers have to find ways to optimise their production systems. Using silage is proposed as one such option.

Factors that may affect the digestibility and therefore efficiency of silage based-diets for the production of sheep need to be taken into account, however. First of these is the physical form of silage-based diets. Silage can either be supplied *ad libitum*, with a restricted amount of concentrate given to lambs, or the concentrate can be mixed into the silage and given together as a total mixed ration (TMR). The form thereof, however, will vary from that of a pelleted diet. It is generally accepted that a pelleted diet will result in a higher intake than roughage-based diets (Forbes, 2007). The higher intake is associated with a lower retention time resulting in lower digestibility of nutrients (Forbes, 2007). Another factor that needs to

be taken into account is the particle length or physical effective fibre (peNDF) content of a diet. A lower peNDF fraction will result in less time spent ruminating (Stone, 2004). This, in turn, will result in less saliva reaching the rumen and therefore less bicarbonate to buffer the rumen (Allen, 1997; Mertens, 1997). The rumen pH will drop to below the critical 5.6 mark, resulting in subacute ruminal acidosis (SARA) (Krause & Oetzel, 2005). Physical effective fibre plays an important role in rumen health and function and therefore in the digestibility of nutrients. Yang & Beauchemin (2005) furthermore stated that peNDF is positively associated with nutrient digestibility when the diet has a low peNDF fraction.

The aim of the current study was to determine the optimum inclusion level of maize silage and its effect on the nutritive value of the diet. There is not a lot of data available in South Africa regarding the use of silage-based diets for the production of lambs. It is therefore important to determine the effect that maize silage inclusion will have on the nutritive value of the diet.

Materials and methods

Animals and sampling

Forty Merino lambs were sourced from Ouplaas in Bredasdorp in the Southern Cape of South Africa and transported to Welgevallen experimental farm, Stellenbosch, South Africa where the study was conducted. Ten animals per treatment were fed one of four dietary treatments containing 20, 50 or 70% (on a DM basis) silage or a control diet in an effort to determine the effect of silage on the digestibility of DM, NDF and CP of such lambs. Feed was prepared fresh daily and fed at 07:00 am and again at 05:00 pm. For the complete experimental design and analytical methodologies, please see Chapter 3. Table 6.1, containing the experimental diets, is repeated in this chapter for the sake of convenience.

Table 6.1 Physical and chemical composition of four diets containing 0, 20, 50 and 70% maize silage fed to Merino lambs

Physical composition DM Basis, g/kg	Maize silage inclusion level			
	Control	20%	50%	70%
Maize silage (pH 3.62)	0	200	500	700
Yellow Maize	545	513	303	144
Lucerne meal	150	0	0	0
Cottonseed oil cake	60	100	103	104
Molasses (meal)	88	50	40	0
Salt	5	5	5	5
Sodium bicarbonate	20	20	20	20
Ammonium chloride	5	5	5	5
Limestone	18	18	18	18
MCP	3	3	3	3
Urea	5	5	2	0
min/vit	1	1	1	1
Oat hay	100	80	0	0
Total, g/kg	1000	1000	1000	1000
Chemical composition, DM				
Dry matter g/kg	909.2	778.8	357.5	281.8
Organic matter g/kg	900.1	938.6	937.5	933.8
Ether extract g/kg	19.2	32.8	31.2	36.8
Crude Protein g/kg	165.2	159.0	154.5	153.3
Acid detergent fibre g/kg	204.1	121.6	184.6	204.1
Neutral detergent fibre g/kg	421.7	242.0	316.7	341.8
ME (MJ/kg)	10.3	10.6	10.3	10.0

In vivo digestibility study

The *in vivo* study was done, as described by McDonald *et al.* (2002), to determine the digestibility of the nutrients of the experimental diets. Eight lambs per group were fitted with faecal collection harnesses and urine funnels for the quantitative collection of faeces and urine. The faeces and feed samples were collected at 07:00 in the morning. Faeces were weighed and a representative sample (10%) was bagged and frozen for later analysis. A representative sample (5%) of the urine was also frozen after the total volume was

measured. Excess faeces and urine was disposed of. It is of importance to state that all digestible values are *apparent* digestibility coefficients and not true digestibility coefficients.

The faeces collected during the digestibility trial were weighed, dried at 60°C for 96 h, air-equilibrated and weighed again to determine its DM content. Dried feed and faeces samples were milled through a 1.5mm screen (Scientes RSA, Hammer mill, Ser No 372) and analysed, according to the AOAC International (2002) official methods of analysis (17th edition) for ether extract (EE), moisture and ash content. All N analyses were performed according to the combustion method (Method 992.15) with a Leco FP-428 N and protein analyser (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI, USA). Urine samples were dried in an oven at 100°C to determine the dry matter (DM) and also analysed for nitrogen content. ADF and NDF were determined with an ANKOM²²⁰ Fibre Analyser (ANKOM Technologies, Fairport, NY, USA) according to the procedures described by the manufacturer. Gross energy of the feed, faeces and urine samples was determined by adiabatic oxygen bomb calorimetry (Animal Production Laboratory, Elsenburg, Stellenbosch, South Africa). Methane gas production (MJ/day) was calculated as 8% of the total gross energy intake as described by McDonald *et al.* (2002). Nitrogen retention was determined by calculating the difference between the N-intake and N-excreted and expressed as gN/metabolic live weight ($W^{0.75}$). Nitrogen excreted was corrected for metabolic faecal N (MFN) as well as endogenous urinary N (EUN) (McDonald *et al.*, 1988).

MFN (g) = 5 g N/kg DM intake

EUN (g) = 0.18 g N/kg $BW^{0.75}$ /day

N retention (g N/kg $BW^{0.75}$ /day) = $[N_{\text{intake}} - (N_{\text{faeces}} - \text{MFN}) - (N_{\text{urine}} - \text{EUN})] / BW^{0.75} / \text{days}$

***In vitro* digestibility study**

The *in vitro* true digestibility of the experimental diets was determined with the help of an ANKOM® Daisy^{II} *in vitro* fermentation system (ANKOM Technologies, Fairport, NY) according to the protocol as proposed by the manufacturer. The buffer solution used, however, was modified and was based on the buffer system of Goering & Van Soest (1970).

The complete buffer solution comprised:

- Deionised water – 500 ml
- Rumen buffer solution – 250 ml
- Macro mineral solution – 250 ml
- Resazurin (0.2 %, w/v) – 2 ml
- Micromineral solution – 0.12 ml
- Trypticase – 1.25 g
- Reducing agent – 53 ml

Rumen buffer solution – A 2.0 L volumetric flask was filled to half with deionised water. To this, 8.0 g NH_4HCO_3 and 70.0 g NaHCO_3 were added before the flask was filled to volume and the medium dissolved.

Macro mineral solution – 11.4 g Na_2HPO_4 , 12.4 g KH_2PO_4 and 1.17 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were added to a 2 L volumetric flask and filled to volume with deionised water before being dissolved.

Micromineral solution – Fifty millilitres of deionised water was added to a 100-ml volumetric flask, after which 13.2 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 10.0 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.0 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and 8.0 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were added. The flask was filled to volume.

Cysteine sulphide reducing agent – 312 mg Cysteine hydrochloride, 20 ml 1 N NaOH and 312 mg $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ were added to 48 ml deionised water.

Rumen inoculum was collected from cannulated sheep on the experimental farm Welgevallen (Stellenbosch, South Africa). Sheep had been adapted for two weeks on the experimental diets prior to collection of rumen inoculum. Rumen contents were filtered through a double layer of cheesecloth into a heated (39°C) thermo flask. The flasks were filled to the brim and capped to ensure anaerobic conditions and the thermo flasks containing the rumen inoculum were immediately transported to the *in vitro* laboratory (Department of Animal Sciences, Stellenbosch, South Africa). Inoculum was poured into a preheated blender and the surface purged with CO_2 to maintain anaerobic conditions. After the microbes had been separated from the rumen material by the blender, the rumen inoculum (400 ml per jar) was poured into Daisy^{II} incubator jars whilst being purged with CO_2 for 30 seconds. To this, 1600 ml pre-heated (39°C) and reduced buffer were added.

Experimental diets were dried in an oven for 48 h at 60°C before being milled through a 1 mm screen using a hammer mill (Scientes RSA, Hammer mill, Ser No 372). Samples were then sieved through a 106 µm screen using a horizontal shaker to remove small particles. Filter bags (F57) were soaked in acetone and dried in an oven at 100°C for 24 hours before being weighed. The weight of the bags was recorded before a 0.5g ± 0.05 sample was weighed into each bag. Instead of only determining *in vitro* true digestibility dry matter (DM), neutral detergent fibre (NDF) and protein digested were also determined. Six bags per experimental diet for the determination of NDF digested were taken out of the Daisy^{II} incubator at 0, 3, 6, 12, 24, 36, 48 and 72 h. Bags were washed with cold water to stop microbial activity and frozen pending NDF analysis. Neutral detergent fibre analysis was done with the help of an ANKOM 220 Fibre Analyser (ANKOM Technologies, Fairport, NY); see Chapter 3 for the complete procedure. The experiment was repeated to determine the protein degradation. A total of 96 bags per experimental diet were weighed, heat sealed and evenly spread out in Daisy^{II} incubator jars (24 bags per jar, 4 jars). Blank bags were also weighed, heat sealed and included to determine a correction factor.

In vitro true digestibility was done on the 48h incubation bags. Bags underwent the same NDF procedure as previously mentioned and the IVTD was calculated as follows:

$$\% \text{IVTD} = 100 - [((\text{NDF}_v - (W_1 \times C_1)) \times 100) / W_2]$$

W_1 = Bag tare weight

W_2 = Sample weight (DM)

NDF_v = Final bag weight after NDF

C_1 = Blank bag correction

Dry matter and NDF disappearance data were fitted to the nonlinear model, as described by Ørskov & McDonald (1979):

$$p = a + b(1 - e^{-ct})$$

- p = amount of DM or NDF that disappeared in time t (hours)
- a = rapidly soluble fraction
- b = potential degradable fraction
- c = rate of degradation for time t

Statistical analysis

The effect that different silage inclusion levels had on diet digestibility as analysed by using PROC GLM and PROC ANOVA of Statistica (Data analysis software system, Statsoft Inc. 2011, version 10.0). The Least Square Means were calculated for all effects tested and significance was declared at $P \leq 0.05$.

Results and discussion

In vivo digestibility study

The *in vivo* digestibility results for the control, 20, 50 and 70% silage diets are first given separately in Tables 6.2, 6.3, 6.4 and 6.5 respectively. The results are then compared and presented in Table 6.6. The total nutrients consumed and excreted were used, together with proximate values, to determine the nutrient digestibility as described by McDonald *et al.*, (2002). The digestibility coefficients of the different diets were also determined and are included in the tables. Table 6.6 presents a summary of the *in vivo* digestibility study and provides the chemical composition of the feed and the faeces, as well as the apparent digestibility of the four diets in comparison with one another.

Significant differences ($P < 0.001$) were found between the apparent DM digestibilities of the four experimental diets. Digestibility decreased as the ADF and NDF fraction of the experimental diets increased as can be seen in Table 6.6. The 20% silage diet had the lowest ADF and NDF fractions and therefore the highest DM digestibility ($82.49\% \pm 0.010$). The control diet, on the other hand, had the highest ADF and NDF fractions, which resulted in the lowest ($61.93\% \pm 0.017$) DM digestibility. There were no differences in DM digestibility between the 50 and 70% silage diets. A study by Moran *et al.* (1988) on sheep receiving maize silage-based diets had a DM digestibility of 72.2%, which falls in the same range (71 – 74%) as results obtained in the current study for the 50 and 70% silage diets.

Crude protein digestibility was higher for the control ($77.96\% \pm 0.005$) and 20% silage ($80.96\% \pm 0.012$) diets and differed significantly from the 50 ($68.26\% \pm 0.013$) and 70% ($67.20\% \pm 0.013$) diets ($P \leq 0.001$). It was again observed that the CP digestibility decreased as the silage fraction, and therefore the ADF and NDF fraction, increased. The CP digestibility of the control diet was higher than that of the 50 and 70% silage diets, even though it had the highest ADF and NDF fraction. This could be because the neutral detergent insoluble nitrogen (NDIN) content of the control diet (6.5 g/kg DM) was lower than in the silage-based diets. As the silage content of the diet increased, so did the NDIN fraction (Table 6.6).

Both ADF and NDF digestibility differed significantly, as expected, due to the increasing amounts of silage in the diets ($P \leq 0.001$). Acid detergent ($21.78\% \pm 0.043$) and neutral detergent ($21.80\% \pm 0.040$) fibre digestion were the lowest for the control diet. This likely

was due to the higher passage rate of the pelleted control diet (Rodrigue & Allen, 1960; Udén, 1988). Significant differences were also found between the digestibilities of fat ($P \leq 0.001$), as can be seen in Table 6.6.

Table 6.2 Mean (\pm SD) *in vivo* digestibility (DM basis) parameters for Merino lambs receiving the Control diet

Control	Dry matter	Organic matter	Crude protein	Acid detergent fibre	Neutral detergent fibre	Ether extract
Feed, g/kg		900.1	164.3	204.1	364.6	19.2
Faeces, g/kg		898.6 \pm 0.94	96.0 \pm 0.88	418.1 \pm 1.73	747.6 \pm 1.68	7.8 \pm 0.13
Nutrients (g/week):						
Consumed	8869.3 \pm 1142.01	7983.2 \pm 1027.92	1457.2 \pm 187.63	1810.6 \pm 233.13	3233.4 \pm 416.34	170.7 \pm 21.98
Excreted	3379.2 \pm 679.80	3036.4 \pm 613.91	324.3 \pm 57.54	1412.8 \pm 304.80	2526.2 \pm 536.40	26.3 \pm 6.38
Digested	5490.0 \pm 805.40	4946.8 \pm 732.30	1132.9 \pm 135.51	397.8 \pm 242.81	707.3 \pm 401.53	144.4 \pm 19.57
Digestibility coefficients	0.6 \pm 0.05	0.6 \pm 0.05	0.9 \pm 0.01	0.2 \pm 0.12	0.2 \pm 0.11	0.9 \pm 0.03

Table 6.3 Mean (\pm SD) *in vivo* digestibility (DM basis) parameters for Merino lambs receiving the 20% Silage diet

20% silage	Dry matter	Organic matter	Crude protein	Acid detergent fibre	Neutral detergent fibre	Ether extract
Feed, g/kg		938.6	159.0	121.6	241.9	32.8
Faeces, g/kg		896.9 \pm 1.26	175.6 \pm 1.04	358.8 \pm 2.14	602.9 \pm 3.72	12.6 \pm 0.20
Nutrients (g/week):						
Consumed	11403.1 \pm 1517.48	10703.0 \pm 1424.31	1813.1 \pm 241.28	1386.5 \pm 184.51	2758.5 \pm 366.39	374.6 \pm 49.85
Excreted	2032.5 \pm 356.21	1822.9 \pm 331.37	356.9 \pm 73.78	729.2 \pm 115.77	1225.4 \pm 233.10	25.7 \pm 5.95
Digested	9370.6 \pm 1406.72	8880.1 \pm 1319.74	1456.2 \pm 208.02	657.3 \pm 201.20	1533.1 \pm 400.93	348.9 \pm 47.42
Digestibility coefficients	0.8 \pm 0.03	0.83 \pm 0.03	0.8 \pm 0.03	0.5 \pm 0.10	0.6 \pm 0.09	0.9 \pm 0.01

Table 6.4 Mean (\pm SD) *in vivo* digestibility (DM basis) parameters of Merino lambs receiving the 50% Silage diet

50% silage	Dry matter	Organic matter	Crude protein	Acid detergent fibre	Neutral detergent fibre	Ether extract
Feed, g/kg		937.5	155.5	157.2	316.7	31.2
Faeces, g/kg		875.4 \pm 1.32	189.1 \pm 1.71	347.1 \pm 2.99	582.1 \pm 3.62	12.3 \pm 0.17
Nutrients (g/week):						
Consumed	7094.6 \pm 1282.81	6651.2 \pm 1202.63	1102.9 \pm 199.42	1115.0 \pm 201.61	2247.0 \pm 406.3	221.2 \pm 40.0
Excreted	1848.6 \pm 330.07	1618.4 \pm 301.78	349.5 \pm 50.89	641.6 \pm 150.83	1076.1 \pm 239.63	22.8 \pm 5.02
Digested	5246.0 \pm 1003.74	5032.8 \pm 946.10	753.4 \pm 161.58	473.4 \pm 95.37	1170.9 \pm 244.38	198.4 \pm 36.05
Digestibility coefficients	0.7 \pm 0.02	0.8 \pm 0.03	0.7 \pm 0.04	0.4 \pm 0.07	0.5 \pm 0.06	0.9 \pm 0.01

Table 6.5 Mean (\pm SD) *in vivo* digestibility (DM basis) parameters of Merino lambs receiving the 70% silage diet

70% silage	Dry matter	Organic matter	Crude protein	Acid detergent fibre	Neutral detergent fibre	Ether extract
Feed, g/kg		933.8	153.3	184.5	341.8	36.8
Faeces, g/kg		889.1 \pm 1.59	174.7 \pm 0.96	388.9 \pm 0.62	613.9 \pm 3.19	15.9 \pm 0.31
Nutrients (g/week):						
Consumed	6900.1 \pm 971.08	6443.3 \pm 907.46	1057.6 \pm 148.96	1273.3 \pm 179.33	2358.7 \pm 332.19	253.7 \pm 35.73
Excreted	1997.2 \pm 410.27	1775.6 \pm 379.49	348.9 \pm 61.32	776.6 \pm 158.52	1226.0 \pm 298.49	31.8 \pm 5.40
Digested	4902.9 \pm 713.97	4667.7 \pm 661.63	708.8 \pm 110.80	496.7 \pm 115.66	1132.7 \pm 218.02	222.0 \pm 35.39
Digestibility coefficients	0.7 \pm 0.04	0.7 \pm 0.04	0.7 \pm 0.04	0.4 \pm 0.08	0.5 \pm 0.08	0.9 \pm 0.03

Table 6.6 Results of the *in vivo* digestibility trial for four diets containing 0, 20, 50 or 70% maize silage fed to Merino lambs during a seven day period

Feed chemical composition (g/kg)	Maize silage inclusion level			
	Control	20%	50%	70%
Dry matter	956.2	898.1	878.7	870.3
Organic matter	900.1	938.6	937.5	933.8
Crude protein	165.2	159	154.5	153.3
Acid detergent fibre	204.1	121.6	184.6	204.1
Neutral detergent fibre	421.7	242	316.7	341.8
Ether extract	19.2	32.8	31.2	36.8
NDIN ¹ (g/kg DM)	6.5	11.3	23.6	29.1

Faecal chemical composition (g/kg)	Control	Maize silage inclusion level			P-value
		20%	50%	70%	
Dry matter	932.8 ± 0.41	926.2 ± 0.44	928.1 ± 0.46	922.8 ± 0.41	0.434
Organic matter	898.5 ^a ± 0.33	893.1 ^{ab} ± 0.44	875.4 ^b ± 0.47	889.1 ^{ab} ± 0.56	0.009
Crude protein	89.5 ^b ± 0.28	160.7 ^a ± 0.35	175.4 ^a ± 0.56	161.5 ^a ± 0.30	< 0.001
Acid detergent fibre	415.5 ^a ± 0.69	359.0 ^{bc} ± 0.92	344.4 ^b ± 1.06	385.2 ^{ac} ± 0.33	< 0.001
Neutral detergent fibre	742.9 ^a ± 0.84	598.4 ^b ± 1.54	577.6 ^b ± 1.31	608.5 ^b ± 1.44	< 0.001
Ether extract	7.7 ^b ± 0.05	14.9 ^a ± 0.24	12.2 ^{ab} ± 0.05	15.7 ^a ± 0.11	0.001

Apparent digestibility of the chemical component (%)	Control	Maize silage inclusion level			P-value
		20%	50%	70%	
Dry matter	61.9 ^c ± 0.02	82.5 ^a ± 0.01	73.9 ^b ± 0.01	71.1 ^b ± 0.01	< 0.001
Organic matter	62.0 ^c ± 0.02	83.2 ^a ± 0.01	75.6 ^b ± 0.01	72.5 ^b ± 0.01	< 0.001
Crude protein	78.0 ^a ± 0.01	81.0 ^a ± 0.01	68.3 ^b ± 0.01	67.2 ^b ± 0.01	< 0.001
Acid detergent fibre	21.8 ^b ± 0.04	47.9 ^a ± 0.03	42.4 ^a ± 0.02	39.2 ^a ± 0.03	< 0.001
Neutral detergent fibre	21.8 ^b ± 0.04	56.1 ^a ± 0.03	51.9 ^a ± 0.02	48.1 ^a ± 0.03	< 0.001
Ether extract	84.6 ^c ± 0.01	92.6 ^a ± 0.01	89.7 ^{ab} ± 0.01	87.6 ^{bc} ± 0.01	< 0.001

^{a,b,c} LS Means with a different superscript within a row were significantly different ($P \leq 0.05$)

¹Neutral detergent insoluble nitrogen

The mean energy metabolism of the Merino lambs receiving the experimental diets is presented in Table 6.7. Lambs receiving the 20% silage diet had the highest daily DM intake (1.61 kg ± 0.077), which resulted in them also having the highest daily energy intake (28.90 MJ ME ± 1.374). Increased intake of the 20% silage over the control diet can be ascribed to the positive effect that the silage had on the palatability of the diet (Chapter 5). Diets consisting of more than 50% silage had a negative effect on DM intake due to the high moisture and fibre content resulting in a lower daily energy intake, which is in agreement with findings of Duckworth and Shirlaw (1958). The higher intake of the 20% silage diet also resulted in higher methane production, which was calculated at 8% of the total energy intake (McDonald *et al.*, 2002). This, however, does not mean that silage-based diets will result in higher methane emissions. The reason here is mainly that it is a calculated value and does not take the different raw materials of the control diet into account. A study conducted by Moss *et al.* (1995) on sheep receiving a combination of concentrate and grass silage found that methane production will decline if the silage fraction is increased.

It is generally accepted that a higher intake will increase the passage rate and result in lower digestibility of nutrients and therefore greater energy loss via methane, urine and faeces (Campling & Freer, 1966). This was not the case in the current study. The control diet had a higher total energy excreted value (10.38 MJ/day \pm 0.665) than the 20% silage diet (7.78 MJ/day \pm 0.435) even though it had a lower DM intake ($P < 0.001$). This could be because silage, in general, has a higher physical effective NDF (peNDF) content, depending on chopping length, and therefore a higher retention time. However, peNDF was not determined in this study and cannot be reported. Yang and Beauchemin (2005) found that increasing the peNDF of maize silage-based diets for dairy cows will improve the digestibility of the diet. They furthermore stated that peNDF is positively associated with nutrient digestibility when the level of peNDF in the diet is low (8.9%). Results of the current study seem to agree with their findings. The lower silage diet had the highest DM and OM digestibility (Table 6.6). The increase in silage of the 50 and 70%, therefore resulting in higher peNDF, led to reduced DM and OM digestibility. The higher DM and GE intake of the 20% silage diet, together with lower total energy excreted, resulted in the diet having the highest energy retention (21.12 MJ/day \pm 1.083) and metabolisable energy (13.10 MJ/kg \pm 0.175).

Table 6.7 Mean (\pm SE) energy metabolism of Merino lambs fed a control diet or one of three diets containing 20, 50 or 70% maize silage

	Maize silage inclusion level				P-Value
	Control	20%	50%	70%	
Dry matter intake (kg/day)	1.3 ^b \pm 0.06	1.6 ^a \pm 0.08	1.0 ^c \pm 0.07	1.0 ^c \pm 0.05	< 0.001
Gross energy (MJ/kg)	17.0	17.9	17.8	17.1	
Energy intake (MJ/day)	21.1 ^b \pm 0.98	28.9 ^a \pm 1.37	18.0 ^{bc} \pm 1.15	16.9 ^c \pm 0.84	< 0.001
Faecal excretion DM (kg/day)	0.5 ^a \pm 0.03	0.3 ^b \pm 0.02	0.3 ^b \pm 0.02	0.3 ^b \pm 0.02	< 0.001
Faecal energy content (MJ/kg)	17.3 \pm 0.06	18.0 \pm 0.17	17.6 \pm 0.11	18.0 \pm 0.40	0.101
Faecal energy (MJ/day)	8.4 ^a \pm 0.59	5.0 ^b \pm 0.35	4.6 ^b \pm 0.29	5.1 ^b \pm 0.33	< 0.001
Urine (kg/day)	0.0 ^b \pm 0.01	0.0 ^{ab} \pm 0.00	0.1 ^a \pm 0.01	0.1 ^a \pm 0.00	0.022
Urinary energy content (MJ GE/kg)	11.3 \pm 1.22	9.4 \pm 1.31	10.1 \pm 1.83	10.8 \pm 1.25	0.781
Urinary energy (MJ/day)	0.3 \pm 0.05	0.4 \pm 0.08	0.5 \pm 0.19	0.5 \pm 0.26	0.128
Methane gas production (MJ/day)	1.7 ^b \pm 0.08	2.3 ^a \pm 0.11	1.4 ^{bc} \pm 0.09	1.4 ^c \pm 0.07	< 0.001
Total energy excreted (MJ/day)	10.4 ^a \pm 0.67	7.8 ^b \pm 0.44	6.6 ^b \pm 0.43	7.3 ^b \pm 0.46	< 0.001
Energy retention (MJ/day)	11.1 ^b \pm 0.64	21.1 ^a \pm 1.08	11.4 ^b \pm 0.84	9.7 ^b \pm 0.57	< 0.001
ME (MJ/kg)	8.8 ^c \pm 0.31	13.1 ^a \pm 0.18	11.3 ^b \pm 0.26	9.8 ^c \pm 0.29	< 0.001

^{a,b,c} LS Means with a different superscript within a row are significantly different ($P \leq 0.05$)

*Methane gas production was calculated as 8% of the total gross energy intake

The nitrogen (N) balance determined by the *in vivo* digestibility study for lambs receiving the experimental diets is given in Table 6.8. Significant differences, as previously mentioned, were found between DM intakes of experimental groups ($P < 0.001$). Faecal excretion was higher for the lambs receiving the control diet (Table 6.7), most likely due to the higher passage rate which, in turn, lowered the apparent digestibility thereof (Table 6.6).

Total daily N intake was higher (41.01 g/day \pm 0.002) for the lambs receiving the 20% silage diet due to the higher DMI. No differences were found for N out as urine ($P = 0.783$) or N out as faeces ($P = 0.838$). The higher N intake of the 20% silage diet together with the fact that the same amount of N was excreted as the other diets resulted in a higher (1.79 gN/kg BW^{0.75}/day \pm 0.149) N retention for the 20% silage diet ($P < 0.001$).

Table 6.8 Mean (\pm SE) nitrogen balance of Merino lambs fed a control diet or one of three different diets containing 20, 50 or 70% maize silage

	Maize silage inclusion level				P-Value
	Control Diet 1	20% Diet 2	50% Diet 3	70% Diet 4	
Dry matter intake (kg/day)	1.3 ^b \pm 0.06	1.6 ^a \pm 0.08	1.0 ^c \pm 0.07	1.0 ^c \pm 0.05	< 0.001
Feed N content (gN/kg)	0.03	0.03	0.03	0.02	
Total N intake (kg/day)	0.03 ^b \pm 0.00	0.04 ^a \pm 0.00	0.03 ^c \pm 0.00	0.02 ^c \pm 0.00	< 0.001
Total N intake (gN/kg BW ^{0.75} /day)	2.4 ^b \pm 0.18	3.0 ^a \pm 0.15	1.8 ^c \pm 0.11	1.8 ^c \pm 0.06	< 0.001
Total faecal excretion DM (g/day)	482.8 ^a \pm 0.03	280.5 ^b \pm 0.02	264.1 ^b \pm 0.02	285.3 ^b \pm 0.02	< 0.001
Faecal N content (gN/kg)	0.02 ^b \pm 0.05	0.03 ^a \pm 0.06	0.03 ^a \pm 0.10	0.03 ^a \pm 0.05	< 0.001
Faecal N lost (g/day)	7.4 \pm 0.00	7.8 \pm 0.00	7.9 \pm 0.00	7.9 \pm 0.00	0.839
Total urinary excretion (g/day)	28.5 ^b \pm 0.01	41.5 ^a \pm 0.00	46.5 ^a \pm 0.01	46.4 ^a \pm 0.00	0.022
Urinary N content (gN/kg)	0.3 ^a \pm 1.52	0.2 ^{ab} \pm 2.88	0.2 ^b \pm 1.38	0.2 ^b \pm 0.74	0.005
Urinary N lost (g/day)	7.2 \pm 0.00	8.4 \pm 0.00	8.5 \pm 0.00	7.6 \pm 0.00	0.783
MFN ¹ (g/day)	0.006 ^b	0.008 ^a	0.005 ^c	0.005 ^c	< 0.001
EUN ² (g/day)	0.002	0.002	0.002	0.002	0.651
N out Faeces (g/day)	7.4 \pm 0.47	7.8 \pm 0.60	7.9 \pm 0.50	7.9 \pm 0.50	0.838
N out Urine (g/day)	7.2 \pm 1.04	8.4 \pm 1.37	8.5 \pm 0.96	7.6 \pm 0.71	0.783
N retention (gN/kg BW ^{0.75} /day)	1.3 ^a \pm 0.12	1.8 ^a \pm 0.15	0.6 ^b \pm 0.16	0.7 ^b \pm 0.06	< 0.001

^{a,b,c}LS Means with a different superscript within a row are significantly different ($P < 0.05$)

¹Metabolic faecal nitrogen

²Endogenous urinary nitrogen

***In vitro* digestibility study**

The term *in vitro* digestibility refers to the rumen degradable fraction of the feed and is therefore interchangeable with the term degradability. Rumen degradability of feed can either be determined with *in sacco* or *in vitro* studies. The *in sacco* method is the most effective way of determining the degradability of feedstuffs in the rumen. It is not favoured, however, due to the cost, as well as animal welfare implications (Mohammed & Chaundhy, 2008). For this study, an *in vitro* degradability study was therefore used as an indication of the degradability of feedstuffs.

Results for *in vitro* true digestibility (IVTD) are presented in Table 6.9. There were no differences between the silage treatments, but degradability was lower for the control diet ($P < 0.001$) supporting earlier findings that the Control diet's feed ingredients were of low

quality. These results correlated with those found in the *in vivo* study and, as expected, was somewhat lower as it is documented that *in vitro* studies slightly under predict true degradability (McDonald *et al.*, 2002).

Table 6.9 Mean (\pm SE) 48h *in vitro* true digestibility of four experimental diets containing 0 (control), 20, 50 and 70% maize silage

	Maize silage inclusion level				P-Value
	Control	20%	50%	70%	
IVTD, % ¹	48.3 ^b \pm 1.84	65.1 ^a \pm 1.90	63.9 ^a \pm 1.87	63.0 ^a \pm 2.08	< 0.001

^{a,b} LS Means with a different superscript in the same row differ significantly ($P < 0.05$)

¹ *In vitro* true digestibility

Degradability kinetic coefficients for the DM and NDF fractions for the different experimental diets are given in Table 6.10. Constants were determined according to the model described by Ørskov & McDonald (1979); $p = a + b(1 - e^{-ct})$. The amount of DM that disappeared in a certain time (t) is represented by p . Constant a represented the fraction that is rapidly soluble, b represents the potential degradable fraction and c is the rate at which b was degraded. Constant a was not determined for NDF degradability since the NDF fraction did not have a rapidly soluble fraction.

No differences between the rapidly soluble fractions of the four experimental diets for DM degradability were recorded. The potential degradable fraction between the treatments did however differ ($P < 0.001$). Silage-based diets, according to the model, had a higher potential degradable fraction than the control diet containing no silage. However, the rate at which the potential degradable fraction was degraded did not differ ($P = 0.831$). As a result of the higher potential degradable fraction of the silage-based diets, the effective degradability was higher than the control diet ($P < 0.001$). Differences in the potential degradable fraction were also found for NDF degradability. The control diet again had a lower potential degradable fraction than the silage-based diets ($P < 0.001$). The rate at which the potential degradable fraction disappeared was also lower for the control diet containing no silage ($P < 0.001$). Effective degradability of neutral detergent fibre was the highest for the 50% silage diet (62.59% \pm 1.055) and the lowest for the control (34.48% \pm 1.029) and the 70% silage (35.48% \pm 1.029) diet ($P < 0.001$). This could be because the control and 70% silage diets had a higher overall NDF fraction (Table 6.6), resulting in lower effective degradability.

Table 6.10 Mean (\pm SE) *in vitro* degradability kinetic coefficients of experimental diets containing 0 (control), 20, 50 or 70% maize silage

	Maize silage inclusion level				P-Value
	Control	20%	50%	70%	
	Diet 1	Diet 2	Diet 3	Diet 4	
DMD					
a ¹	5.2 \pm 1.24	1.3 \pm 0.65	2.4 \pm 0.95	5.9 \pm 1.95	0.062
b ²	48.6 ^b \pm 1.54	69.7 ^a \pm 1.84	67.5 ^a \pm 2.83	65.7 ^a \pm 3.19	< 0.001
c ³	0.1 \pm 0.01	0.1 \pm 0.01	0.1 \pm 0.01	0.1 \pm 0.01	0.831
Effective degradability	30.7 ^b \pm 0.87	38.4 ^a \pm 0.55	38.8 ^a \pm 0.65	38.0 ^a \pm 0.77	< 0.001
NDF					
b ²	67.5 ^b \pm 1.55	81.3 ^a \pm 2.13	84.2 ^a \pm 0.68	83.5 ^a \pm 1.48	< 0.001
c ³	0.06 ^c \pm 0.01	0.13 ^{ab} \pm 0.01	0.2 ^a \pm 0.01	0.10 ^b \pm 0.01	< 0.001
Effective degradability	35.5 ^c \pm 1.03	58.1 ^b \pm 0.54	62.6 ^a \pm 1.06	35.5 ^c \pm 1.03	< 0.001

^{a,b,c}LS Means with a different superscript within a row are significantly different ($P < 0.05$)

Constants determined according to the model described by Ørskov & McDonald (1979) were $p = a + b(1 - e^{-ct})$

¹a = rapidly soluble fraction

²b = potential degradable fraction

³c = rate at which b is degraded in the rumen

The estimated DM and NDF disappearance as predicted by the model are illustrated in Figures 6.1 and 6.2, respectively. From the graphs, and in accordance with Table 6.10, the *In vitro* dry matter disappearance was higher for the silage-based diets than for the control diet. *In vitro* NDF disappearance followed the same basic trend with the control diet recorded lower than the silage-based diets. The rate at which the NDF in the silage-based diets disappeared differed, however. Diets containing more than 20% silage disappeared at a slower rate than the 20% silage diet. After 48 h the same percentage of NDF disappearance took place in all diets containing silage.

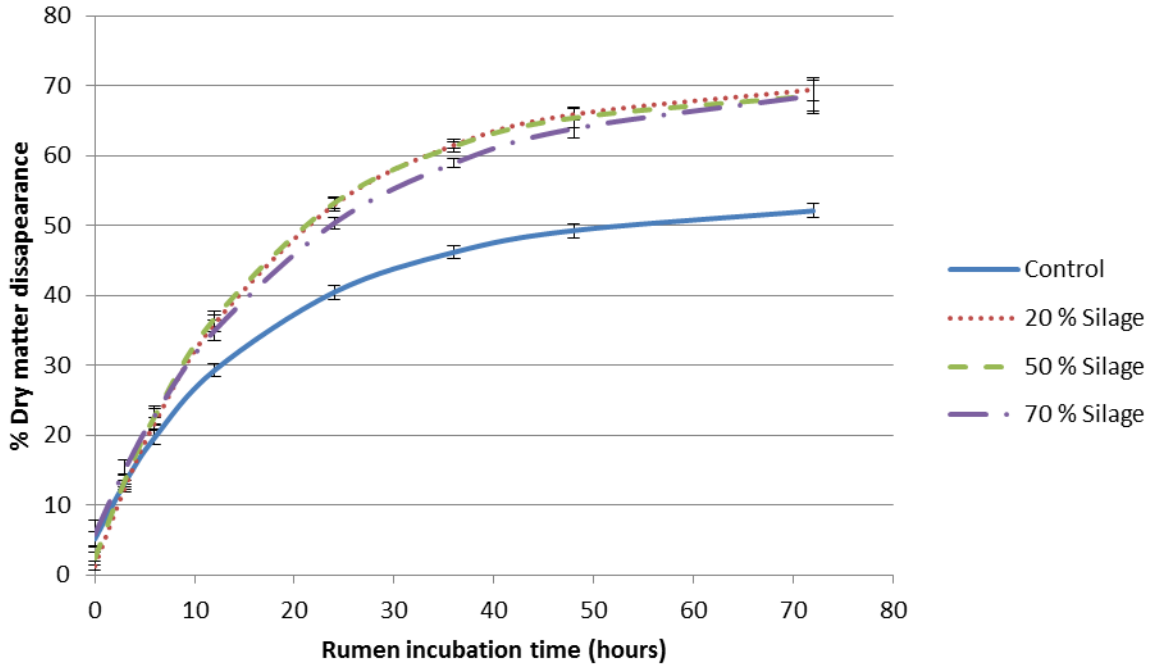


Figure 6.1 *In vitro* dry matter disappearance of experimental diets containing 0, 20, 50 and 70% maize silage

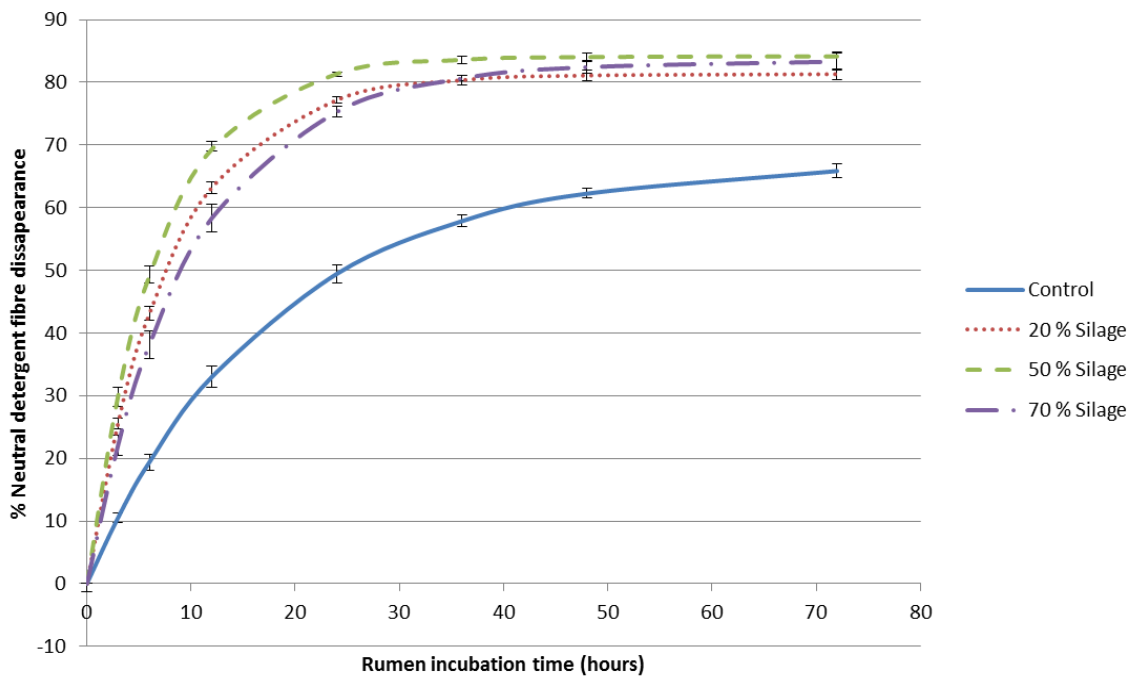


Figure 6.2 *In vitro* neutral detergent fibre disappearance of experimental diets containing 0, 20, 50 and 70% maize silage

Conclusion

Results obtained from this study showed that the 20% silage diet, which had the lowest ADF and NDF fraction, had the highest apparent DM and OM digestibility. The control diet with the highest ADF and NDF fraction, on the other hand, had the lowest DM and OM digestibility. Regarding the digestibility of DM and OM, it can therefore be concluded that generally an increase in the fraction of ADF and NDF will result in lower apparent digestibility. Crude protein digestibility was higher for the 20% silage and the control diet containing no silage. It was observed that the CP digestibility decreased as the silage inclusion level increased. The decreased CP digestibility was due to an increase of the NDIN fraction as the amount of silage in the diet increased. More CP was therefore unavailable for digestion due to the increased NDIN fractions in the higher silage based diets. Acid detergent and neutral detergent fibre digestibility was not negatively affected by silage inclusion level, however, and was higher than that of the control diet.

Lambs receiving the 20% silage diet recorded the highest daily DMI, which again confirms the palatability effect that the silage had at lower inclusion levels (Chapter 5). Methane production as a calculated value was therefore higher for the 20% silage diet than for the control diet. This may not be the case as individual raw materials were not taken into account and this could have had a substantial effect on the total methane production of the composite feed. It is known that methane production will decrease when silage inclusion level increases. Although peNDF was not determined for the silage in this study, it is well documented that including roughage and silage with a longer particle length will increase the peNDF content of a diet. The higher peNDF fraction of the 20% silage diet seemed to have had a positive effect on retention time and therefore on the digestibility of the diet.

The current study showed that maize silage inclusion of 20% will not only stimulate intake in lambs, but also improve the digestibility of nutrients, probably due to more appropriate retention time. Silage-based diets will therefore record less loss of energy via methane, urine and faeces production. Further research is needed, however, to measure the specific effect of silage inclusion on passage rate and other topical issues such as its contribution to methane production.

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Chapter 7

The effect of Maize silage-based diets on the chemical composition, physical characteristics and fatty acid composition of Merino lamb meat

Abstract

A study was conducted to determine whether silage-based diets could be used as a finishing feed for Merino lambs. Four diets were formulated on an iso-nitrogenous basis to contain 158 g/kg \pm 0.27 crude protein (CP) and a metabolisable energy content of 10.7 MJ/kg \pm 0.86 on a dry matter (DM) basis. The diets included a control diet with no silage and three treatment diets containing 20, 50 or 70% silage on an DM basis. The neutral detergent fibre (NDF) content of the respective diets was 421.7, 242, 316.7 and 341.8 g/kg. The lambs were fed *ad libitum* for 60 days before being slaughtered. The *M. longissimus dorsi* was removed from the left side of each carcass, after pH and temperature readings were taken at the abattoir, it was used to determine the proximate chemical composition, physical meat quality characteristics and fatty acid content. The experimental diets did not have an effect on the proximate chemical composition of the meat. Colour differences were found. However no clear pattern could be established. There were no differences in fatty acid composition. It can be concluded that maize silage can be included up to 70% in the finishing diets of Merino lambs with no adverse effect on the meat quality.

Key words: long-chain fatty acids; maize silage; Merino lambs

Introduction

Silage is widely used as a cost-effective source of roughage in dairy cattle nutrition in South Africa. It is not that commonly used in sheep production systems, however. European countries and Australia, on the other hand, make use of silage in their sheep enterprises to improve pasture utilisation, increase stocking rate, for use as a drought feed and also for the finishing of lambs in a feedlot (Marley *et al.*, 2007; Stanley, 2003). The same principles can, theoretically, be applied to South Africa, not only to optimise pasture utilisation but also to optimise sheep production to meet the ever-growing demand for animal protein. Sheep production systems in South Africa traditionally are extensive and sheep are more sensitive to silage quality than cattle, giving the impression that silage is not a practical or effective roughage source for sheep. However, increasing feed prices and practices like conservation farming, compel farmers to find ways to optimise their production systems. Using silage is proposed as one such option, but effects thereof on the meat quality first have to be determined. Factors affecting meat quality of ruminants can be divided into animal related factors and environmental factors. The animal factors are directly related to the animal and include the age, breed and sex of the animal. Environmental factors, on the other hand, include all the factors that are not animal-related, like diet, weather and processing of the meat (Priolo *et al.*, 2001). Of these, diet has the biggest effect, especially on tenderness and flavour, which, in turn, is due to the fat content of the meat (Wood *et al.*, 1999).

Fat and long-chain fatty acids affect the taste, nutritional value and storage stability of meat and therefore play an important role in the acceptability of meat by the consumer (Webb & O'Neill, 2008; Webb & Casey, 1995). Research has shown that acceptance by the consumer of ruminants raised on different production systems vary (Kerth *et al.*, 2007); the main contributing factor to this

is meat flavour resulting from differences in the fatty acid profile. Fatty acids of animal origin typically have more than 12 carbon atoms and are referred to as long-chain fatty acids. They can be classified according to their carbon bonds as saturated (SFA), monounsaturated (MUFA) or polyunsaturated fatty acids (PUFA) (Webb & O'Neill, 2008). Omega-3 (n-3) and Omega-6 (n-6) fatty acids are polyunsaturated fatty acids and are also regarded as dietary essential fatty acids. These fatty acids have to be included in the diet of monogastric animals (including humans) and do not play an important role in the immune response only, but also act as carriers for fat soluble vitamins (Hwang, 1990; Webb & O'Neill, 2008). The optimum ratio of PUFA: SFA for meat is no less than 0.4 and less than four for n-6/n-3. A feed that will change the concentration of the flavour precursors will have an effect on the flavour and therefore on the consumable product (Duckett & Kuber, 2001; Melton, 1990). Pasture-finished ruminants will have a higher concentration of n-3 PUFA and ruminants finished on concentrate diet will have a higher n-6 PUFA concentration (Enser *et al.*, 1998). The reason for this is that grass has a higher precursor fatty acid for α -linolenic acid (18:3), whereas concentrate diets have a higher precursor fatty acid for linoleic acid (18:2) (Marmer *et al.*, 1984). Finishing studies with beef cattle, such as the one by O'Sullivan *et al.* (2002), showed that maize silage resulted in a significant lower 18:3 content than grass silage and will therefore have an higher n-6/n-3 ratio. This will have an effect on consumer acceptability since meat with a higher n-3 content, and therefore lower n-6/n-3 content, is favoured by the consumer. Stanley (2003), however, stated that feeding high-silage diets will have no adverse effect on meat quality.

The aim of this study was therefore to determine whether maize silage-based diets will have a significant effect on proximate chemical composition, physical meat quality characteristics and fatty acid composition of Merino lambs.

Materials and methods

Animals and sampling

Forty Merino lambs were sourced from Ouplaas in Bredasdorp in the Southern Cape of South Africa and transported to Welgevallen experimental farm, Stellenbosch, South Africa where the study was conducted. Animals were fed one of four dietary treatments containing 0 (control), 20, 50 or 70% silage in an effort to determine the effect of silage on the meat characteristics of such lambs. For the complete experimental design and analytical methodologies, please see Chapter 3. Table 3.1 containing the experimental diets, is repeated in this chapter for the sake of convenience as Table 7.1.

Table 7.1 Physical and chemical composition of four diets fed to the Merino lambs

DM Basis, g/kg	Maize silage inclusion level			
Physical composition, g/kg	Control	20%	50%	70%
Maize silage (pH 3.62)	0	200	500	700
Yellow Maize	545	513	303	144
Lucerne meal	150	0	0	0
Cottonseed oil cake	60	100	103	104
Molasses (meal)	88	50	40	0
Salt	5	5	5	5
Sodium bicarbonate	20	20	20	20
Ammonium chloride	5	5	5	5
Limestone	18	18	18	18
MCP	3	3	3	3
Urea	5	5	2	0
min/vit	1	1	1	1
Oat hay	100	80	0	0
Total, g/kg	1000	1000	1000	1000
Chemical composition, DM				
Dry matter g/kg	909.2	778.8	357.5	281.8
Organic matter g/kg	900.1	938.6	937.5	933.8
Ether extract g/kg	19.2	32.8	31.2	36.8
Crude Protein g/kg	165.2	159.0	154.5	153.3
Acid detergent fibre g/kg	204.1	121.6	184.6	204.1
Neutral detergent fibre g/kg	421.7	242.0	316.7	341.8
ME (MJ/kg)	10.3	10.6	10.3	10.0

Processing of lambs

After having been fed the respective treatment diets for 60 days, the lambs were slaughtered on the same day at Roelcor Abattoir, Malmesbury, South Africa. The animals' final live weights were determined before transportation. They were penned on arrival and fasted for 16 h, with *ad libitum* access to fresh water. Lambs were electrically stunned for four seconds at 200 volts before being suspended and exsanguinated to bleed. Muscle pH (pH₄₅) and temperature (temp₄₅) readings were taken 45 minutes *post mortem* between the 1st and 6th lumbar vertebrae of the right *Longissimus dorsi* (LD) muscle. Carcasses were suspended in a cooler at 2°C for 48h. A second pH (pH₄₈) and

temperature (temp₄₈) reading, adjacent to the 1st, was taken after 48 hours *post mortem*. After this, the left LD was removed from the carcass for later meat quality analysis.

Physical analysis

The physical analysis was done after the left LD had been removed from the carcass and transported to the laboratory. Analysis done on the LD included drip loss, cooking loss, meat colour, Warner-Bratzler shear force (WBSF) and measuring of back-fat thickness.

Drip loss was determined by cutting the LD into 1.5 cm steaks, transversely to the grain, in one fluid motion. The steaks were weighed and suspended in an inflated plastic bag, caution was exercised to ensure that the meat would not come into contact with the bag. The bags with the steaks inside were suspended in a fridge at $\pm 4^{\circ}\text{C}$ for 24h. The steaks were removed from the bags, after the storage period, and gently blotted dry with a paper towel and weighed back again.

For determining the cooking loss, 1.5 cm steaks were cut again, perpendicular to the longitudinal axis of the muscle, in one fluid motion. The steaks were weighed and placed in marked, thin-walled, plastic bags and submerged in a water bath at 80°C for 60 minutes. Water that accumulated in the bag was removed after cooking and the steaks were stored in a fridge at 4°C until equilibrium had been reached. Steaks were then removed from the plastic bags, blotted dry with a paper towel and weighed.

Warner-Bratzler shear force measurements were taken on the steaks used for the determination of cooking loss. The samples were cut perpendicular to the longitudinal axis of the muscle using a Warner-Bratzler Shear attachment, with a circular cross section of 1.27 cm \varnothing blade. This blade is fitted to an electrical scale which is programmed to measure the maximum force (measured in kilograms) taken to cut through the meat. Maximum shear force values to shear a cylindrical core (1.27 cm \varnothing) of cooked meat (at a crosshead speed of 3.33 mm.s⁻¹) were recorded for each sample and a mean was calculated for each individual animal (Honikel, 1998).

Freshly cut steaks were allowed to bloom on a bench for 60 minutes at 4°C before colour measurements were taken. Measurements were taken with a calibrated Colour-guide 45^o/0^o colorimeter (BYK-Gardner GmbH, Gerestried, Germany) which measured the surface colour according to the CIE – Lightness (CIE L*), green-red value (CIE a*) and blue-yellow value (CIE b*) described by Honikel (1998). Values could only be taken in duplicate due to the size of the steaks. The CIE a* and CIE b* values were used to calculate the Chroma value (saturation/colour intensity) and the hue-angle (colour definition) according to the following formulas:

$$\text{Hue-angle } (^{\circ}) = \tan^{-1} (b^*/a^*)$$

$$\text{Chroma } (C^*) = (a^{*2} + b^{*2})^{-0.5}$$

Proximate chemical analysis

The left LD muscle was used in both proximate chemical analysis and physical analysis. All visible fat was removed before proximate chemical analysis. Each LD muscle was individually homogenised and the mincer cleaned with hot soapy water after every sample. The proximate analysis done included the determination of moisture, ash, protein and fat content according to the AOAC methods (AOAC, 2002). The moisture content was determined by weighing a sample into a clean, dry porcelain crucible and drying it in an oven for 24 hours at 100°C before weighing it

(Method 934.01, AOAC, 2002). Ash determination was done on an already moist free sample as described by AOAC Method 942.05. The samples were burned to ash in an oven at 500°C for 6 hours, left to cool down in the oven for 2 hours. The samples were then transferred to a desiccator for 30 minutes before it was weighed again. Nitrogen analysis was performed according to the combustion method (Method 992.15) with a Leco FP-528 N and protein Analyser (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI, USA), calibrated with EDTA. Fat content of the sample was determined by ether extraction, chloroform: methanol (2:1), according to Lee *et al.* (1996).

Protein, energy and fat content is expressed on an As Is basis rather than an DM basis due to the fact that meat consumed is not expressed by the amount of dry tissue eaten but rather per serving expressed on an As Is basis (Rousseau, 2006).

Fatty acid composition

Fatty acid analysis was done on the left LD muscle according to the modified method of Folch *et al.* (1957). The method was adapted as follows. A meat sample was thawed, homogenised and extracted with a chloroform:methanol (2:1 v/v) solution to break the bonds between the lipids and the other compounds. The chloroform:methanol (CM 2:1) solution contained 0.01% Butylated hydroxytoluene (BHT) that acted as an antioxidant. A Polytron mixer was used to mix the sample with the CM 2:1 extraction solvent for 40 seconds; care was taken not to overheat the sample. The acid used for the internal standard to quantify the individual acids was heptadecanoic acid (C17:0). A transmethylating agent (methanol:sulphuric acid; 19:1; v/v) was added to a sub-sample of extracted lipids after which it was left to cool down. The resulting fatty acid methyl esters (FAMES) were then extracted with water and hexane. The top hexane phase was transferred to a Kimax tube (125 x 16 mm) with a screw cap and dried under nitrogen in a 45°C water bath. The FAMES were purified and analysed by gas chromatography. The apparatus used was a Thermo Finnigan Focus GC (Thermo Electron S.p.A., Strada Rivoltana, 20090 Rodana, Milan, Italy). GC column conditions were as follows:

- Column: BPX70, 60 m x 0.25 mmID, 0.25 µm (SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134, Australia)
- Initial temperature: 60°C, 5 min
- Rate 1: 7°C/min
- Final temperature: 160°C
- Detector: 260°C
- Injector: 220°C
- Split flow: 20:120
- Carrier: Hydrogen, 20 ml/min
- Injector volume: 1 µL
- Run time: 45 min

Statistical analysis

The effect of silage-based diets on proximate chemical composition, physical meat quality characteristics and fatty acid composition of Merino lambs was analysed by using PROC GLM and PROC ANOVA of Statistica (Data analysis software system, Statsoft Inc. 2011, version 10.0). The Least Square Means were calculated for all effects tested and significance declared at $P \leq 0.05$.

Results and discussion

The effect that the four experimental diets had on the proximal chemical composition of the LD is expressed in Table 7.2. No significant differences induced by the experimental diets were recorded between moisture, ash, protein, fat and energy content of the different treatment groups ($P > 0.05$). Cloete *et al.* (2012) found similar values for moisture, protein and ash content in a study done on LD of Merinos kept on pasture (73.3%, 22.9%, and 1.07% respectively). The fat percentage reported by these authors, however, was lower (2.19%) than found in this study, which resulted in a lower calculated energy value. One would expect the fat content to be higher, due to the fact that the Merinos, in the study by Cloete *et al.* (2012), were slaughtered at 20 months of age whereas the lambs in the current study were slaughtered at approximately five months. This, however, was not the case and can be explained by the fact that the Merinos were raised on pasture alone and did not receive any supplementary feeding. The Merino lambs in the current study received a more balanced concentrate diet (Table 7.1). Although the protein and energy content could not be optimised due to the restriction caused by the high silage (70%) diets, raw materials such as cottonseed oil cake and yellow maize were still included. This, together with the necessary minerals, vitamins and optimum housing conditions, resulted in better growth and therefore higher live weights and fat content at a younger age.

Table 7.2 Proximate chemical composition (on an As Is basis) of *M longissimus dorsi* of the Merino lambs fed four different diets (mean \pm SD)

	Maize silage inclusion level				P-value
	Control	20%	50%	70%	
Moisture (g/kg)	728.9 \pm 0.41	727.7 \pm 0.23	727.8 \pm 0.23	729.6 \pm 0.30	0.958
Ash (g/kg)	14.4 \pm 0.18	16.3 \pm 0.36	18.0 \pm 0.29	20.4 \pm 0.24	0.599
Protein (g/kg)	248.8 \pm 0.30	248.3 \pm 0.31	251.7 \pm 0.35	249.0 \pm 0.33	0.863
Fat (g/kg)	38.7 \pm 0.28	43.2 \pm 0.24	37.8 \pm 0.28	36.8 \pm 0.23	0.335
Energy (kJ) ¹	444.6	446.3	449.2	443.8	0.923

¹Total energy (kJ) = (g protein in 100g sample x 17) + (g fat in 100g sample x 37) (As gazetted: SA Act No 54 of 1972)

The effects that the different experimental diets had on the physical characteristics of the LD of the Merino lambs are summarised in Table 7.3. The pH of meat plays an important role in meat quality, especially the ultimate pH (pH₄₈), which will have an influence on meat colour, flavour and

tenderness (Jensen *et al.*, 2004). There were no significant differences in pH₄₅ or pH₄₈ of the four groups. In the current study, pH₄₈ ranged between 5.57 and 5.61, which, according to Jensen *et al.* (2004), falls in the optimum range of 5.5-5.6. A low ultimate pH not only prevents microbial growth, but the lactic acid/lactate also has a positive effect on the meat flavour. The pH₄₈ value recorded by Cloete *et al.* (2012) for sheep kept on pasture, however, is higher (pH 5.83) than the expected value. The higher ultimate pH value could be due to sheep kept on the pasture not being accustomed to human contact. This would result in higher stress levels during handling, transportation and slaughter than in sheep raised in individual pens and having daily human contact. It has also been shown that animals raised in the pasture have higher ultimate pH than concentrate-finished animals, due to the lower energy content of their diet (Priolo *et al.*, 2001)

Table 7.3 Physical characteristics of the *M. longissimus dorsi* for the Merino lambs fed a control diet or one of three silage based diets containing 20, 50 or 70% silage (mean \pm SD)

	Maize silage inclusion level				P-value
	Control	20%	50%	70%	
pH ₄₅	6.5 \pm 0.10	6.7 \pm 0.05	6.7 \pm 0.08	6.7 \pm 0.05	0.25
Temperature ₄₅ (°C)	27.2 \pm 0.90	28.7 \pm 0.81	28.2 \pm 0.91	27.6 \pm 0.57	0.59
pH ₄₈	5.6 \pm 0.02	5.6 \pm 0.01	5.6 \pm 0.01	5.6 \pm 0.01	0.19
Temperature ₄₈ (°C)	5.4 \pm 0.09	5.5 \pm 0.11	5.3 \pm 0.07	5.2 \pm 0.09	0.26
Drip loss (%)	1.2 \pm 0.06	1.0 \pm 0.05	1.2 \pm 0.10	1.1 \pm 0.05	0.18
Cooking loss (%)	23.3 ^a \pm 0.86	19.8 ^{bc} \pm 0.57	21.0 ^{bc} \pm 0.65	21.5 ^{ac} \pm 0.67	0.009
Shear force (kg/1.27 cm diameter)	2.8 \pm 0.20	2.6 \pm 0.20	2.6 \pm 0.26	2.8 \pm 0.19	0.81
L* value	40.4 \pm 0.59	38.6 \pm 0.58	38.6 \pm 0.60	39.6 \pm 0.89	0.17
a* value	12.3 ^b \pm 0.40	13.6 ^a \pm 0.18	13.1 ^{ab} \pm 0.31	12.3 ^b \pm 0.20	0.006
b* value	11.9 ^a \pm 0.22	11.3 ^{ab} \pm 0.24	11.9 ^a \pm 0.34	10.8 ^b \pm 0.34	0.026
Hue angle (°)	44.0 ^a \pm 1.04	39.6 ^b \pm 0.61	42.2 ^{ab} \pm 0.95	41.2 ^b \pm 1.06	0.015
Chroma	17.2 ^{ab} \pm 0.33	17.7 ^a \pm 0.24	17.7 ^a \pm 0.36	16.4 ^b \pm 0.25	0.012
Fat thickness (2nd -3rd last thoracic vertebrae)	0.7 \pm 0.12	1.0 \pm 0.08	0.8 \pm 0.08	0.8 \pm 0.08	0.248
Fat thickness (4th -5th lumbar vertebrae)	0.3 \pm 0.06	0.4 \pm 0.08	0.5 \pm 0.09	0.4 \pm 0.07	0.329

^{a,b,c} Means in the same row with different superscripts differ significantly ($P \leq 0.05$).

One of the main consumer preferences relating to the acceptability of cooked meat is the juiciness thereof. This, in turn, is directly related to the amount of water retained after the meat is cooked (Forrest, 1975). In this study, significant differences were found between the groups, with the 20%

silage diet recording the lowest ($19.78\% \pm 0.566$) and the control diet the highest ($23.31\% \pm 0.860$) percentage cooking loss ($P = 0.009$). Cooking loss in this study was lower than in the study done by Cloete *et al.* (2012), mainly due to the age difference previously mentioned. Schönfeldt *et al.* (1993), in a separate study on sheep and goats, found that meat of younger animals is juicier than that of older animals. Differences in cooking loss in the current study, although significant, do not present a clear pattern and is most likely do to the variation between individual animals.

According to Lawrie and Ledward (2006), the physical appearance of meat is the first factor determining consumer satisfaction. After this, juiciness, as described earlier, is a major contributing factor along with the tenderness of the meat. Meat tenderness can be described by the initial ease of penetration of the meat by the teeth during chewing, the ease with which meat breaks into fragments and the amount of residue that remains after chewing (Weir, 1960). Sensory panels are generally used to determine tenderness and, due to difficulties related to objective and chemical methods, physical methods like Warner-Bratzler shear force (WBSF) are also used (Lawrie & Ledward, 2006; Safari *et al.*, 2001). A WBSF value, according to Safari *et al.* (2001) as cited in Shorthose *et al.* (1986), of more than 5 kg will be regarded as tough by consumers. The WBSF values in this study were all below 5 kg and the experimental diets had no effect on the toughness of the meat ($P = 0.812$).

Colour of fresh-chilled or frozen meat plays an important role when the consumer decides which meat to buy. Meat with a bright red colour will be favoured over dark red meat (Jensen *et al.*, 2004). An L^* value greater than 34, according to Velasco *et al.* (2004) as cited in Hopkins (1996), will be acceptable to the consumer. No significant differences ($P = 0.175$) were found in this study between different groups and the L^* values were higher than 34, thus meeting consumer demands. The control and 70% diet, however, differed in green-red value (a^*) from the 20% diet ($P \leq 0.05$). There also were differences between the b^* value, Hue angle and Chroma value. It is common knowledge that different diets will have an effect on meat colour, especially the lightness thereof. Animals finished on pasture will have darker meat than the same animals finished on concentrate feeds (Díaz *et al.*, 2002; Priolo *et al.*, 2001). One would therefore expect that the meat would become darker as the inclusion of concentrate feeds is decreased. The results in the current study presented no exact pattern, however, and differences between the values were inconsistent.

Fat thickness in the current study was not affected by the different diets; this most likely was due to the diets having been formulated on an iso-nitrogenous basis and the animals being of the same breed, weight and age at slaughter (Table 7.1).

The effect that the experimental diets had on the fatty acid content of meat from the different groups is summarised in Table 7.4 and is expressed as g/100g of identified fatty acids. No significant differences were found between the SFA, MUFA or PUFA contents of the different groups ($P = 0.087$; $P = 0.107$; $P = 0.229$) and this correlates with the results obtained by Rousseau (2006) for the sheep receiving the control diet. The PUFA:SFA ratio is also in line with results obtained by Rousseau (2006), and no significant differences were found between groups ($P = 0.314$). Enser *et al.* (1998) stated that the n-6/ n-3 of pasture-fed ruminants is usually less than two and those fed concentrates can range from six to ten. In this study using silage-based diets, the observed ratio was in the order of seven and this corresponds well with literature on animals fed concentrate-based diets. It should be kept in mind that the treatment diets in the study did contain varying proportions of concentrates, as reported in Chapter 3. The current study found no differences between the groups, not even between the control diet and the high silage diet (70%).

This can be explained by the study done by O'Sullivan *et al.* (2002), which found that beef cattle receiving maize silage diets had a significantly lower n-3 content than cattle finished on grass silage.

Table 7.4 Fatty acid composition (g/100 g of identified fatty acids) of the *M. longissimus dorsi* of the Merino lambs fed a control diet or one of three silage based diets containing 20, 50 or 70% silage (mean \pm SD)

	Maize silage inclusion level				P-value
	Control	20%	50%	70%	
SFA ¹	50.3 \pm 0.72	46.4 \pm 0.70	46.3 \pm 2.70	50.3 \pm 0.58	0.086
MUFA ²	35.7 \pm 0.94	36.7 \pm 0.97	42.1 \pm 3.71	36.3 \pm 0.51	0.107
PUFA ³	11.1 \pm 0.90	10.9 \pm 0.57	9.1 \pm 0.92	11.0 \pm 0.69	0.230
PUFA:SFA ⁴	0.2 \pm 0.02	0.2 \pm 0.01	0.2 \pm 0.02	0.2 \pm 0.02	0.314
Σ n-6 ⁵	9.5 \pm 0.78	9.5 \pm 0.51	7.8 \pm 0.79	9.2 \pm 0.65	0.277
Σ n-3 ⁶	1.3 \pm 0.11	1.2 \pm 0.09	1.1 \pm 0.13	1.5 \pm 0.09	0.128
(n-6)/(n-3) ⁷	7.1 \pm 0.30	7.8 \pm 0.45	7.3 \pm 0.43	6.4 \pm 0.48	0.129

¹Saturated fatty acids; ²Mono-unsaturated fatty acids; ³Polyunsaturated fatty acids; ⁴Ratio of polyunsaturated to saturated fatty acids; ⁵Ratio of omega-6 to omega-3 fatty acids.

Conclusion

The aim of this study was to determine the effect of silage-based diets on the chemical composition, meat quality characteristics and fatty acid composition of the meat of Merino lambs. The chemical composition of the *M. longissimus dorsi* of Merino lambs was not affected by inclusion level of maize silage. Physical meat quality characteristics, i.e. pH, meat temperature, drip loss and colour were also not affected by different inclusion levels of maize silage. There were significant differences in cooking loss between the control diet and the 20 and 50% silage diets, but not the 70% diet. On the other hand, the cooking loss from the 70% diet did not differ from the 20 and 50% silage diet. The difference found has therefore no clear pattern and is most likely due to individual variation between animals rather than the effect of the experimental diets. As the animals were slaughtered at the same age and weight and had no significant difference in fat content, the shear force did not differ between treatments ($P = 0.812$). Colour measurements were inconclusive, with no differences in L^* values, but differences between a^* , b^* , Hue angle and Chroma value were recorded. It was expected that the meat would become darker as the inclusion of concentrate feeds decreased, but there was no clear pattern between the differences and the L^* values were greater than 34 for all groups. It can therefore be concluded that high silage diets, unlike pasture, will not significantly affect the colour of meat and, therefore, the acceptability thereof for the consumer. The diets also had no effect on the fatty acid content of the meat and it was interesting to find that the silage-based diets had the same effect on n-6/ n-3 content as the control diet, unlike in pasture fed animals. Differences in fatty acid content may be found in older sheep with a higher fat content. Care should be taken, however, since the total fat content of the animal will also have an effect on the fatty acid content.

It was concluded, based on the results obtained in this study, that maize silage can be included to up to 70% in the finishing diets of Merino lambs without adversely affecting the proximate chemical composition, physical meat quality characteristics and fatty acid composition of the meat.

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Chapter 8

General conclusion

Silage was prepared from irrigated maize produced in the Western Cape (Faircape, Durbanville, South Africa). The maize was ensiled without the use of inoculants since lactic acid bacteria (LAB) are naturally present in the crop. The chopped maize was compacted into 220 L plastic drums and left to ferment for 60 days. The resultant silage of this study had a higher than normal CP value (111.2 g/kg DM) when compared to typical silage. The DM (270 g/kg) content was lower than the ideal and more in the range of high moisture maize silage. One of the main concerns regarding the low DM was the effect that it would have on the DMI of the lambs. A pH of 3.62 indicated that optimum amounts of lactic acid were produced, confirming that LAB was naturally present in the maize crop. The maize silage also contained normal levels of starch, propionic acid, butyric acid and low but normal levels of WSC. Levels of acetic acid, however, were higher than expected. This resulted in DM losses during fermentation, but the advantage thereof was improved aerobic stability of the silage during feed out. Ammonia nitrogen levels were at the upper limit but still within the normal range of what was expected. No excessive protein breakdown had therefore occurred. The silage produced during this study was of a good overall quality and suitable for use in ruminant nutrition.

The production study showed that DMI of Merino lambs were negatively affected at 50 and 70% inclusion levels of maize silage. The lambs receiving the control and 20% silage diet recorded the highest cumulative feed intakes. The FCR, however, was higher for the control and 20% silage diet. It must be stated again that one of the aims was to determine the effect of silage inclusion levels on intake. Diets were therefore formulated on an iso-nutrient basis, except for fibre (NDF). The poor FCR was related to the low quality of the control feed and the concentrate in the 20% silage diet rather than the silage itself. The low quality of the control feed was confirmed by the low *in vitro* digestibility thereof. Due to the limited numbers of cannulated animals available, the effect of the 0, 50 and 100% maize silage on rumen pH can only be taken as an indication of what can be expected. It was interesting to observe that the 100% silage diet, even though it had the lowest pH, had the best rumen pH profile. This was ascribed to the higher rumination and salivation effect, resulting in more bicarbonate-containing saliva reaching the rumen to buffer the drop in pH. It is therefore of importance for future research to focus on the effect that silage-based diets have on rumen pH. If possible, automated pH loggers should also be used, so that the rumen would not be exposed to oxygen every hour, thereby sustaining the anaerobic conditions to obtain more accurate results.

The *in vivo* digestibility study showed that the DM and OM digestibility declined as the fibre fraction increased with increased levels of silage in the diet. It was also evident that CP digestibility decreased as the silage inclusion level increased. This was a result of the increase of NDIN in the higher silage diets. The lower DM and OM digestibility as well as the increase of NDIN fraction in diets containing more than 50% silage diet makes it inefficient for the production of mutton. The 20% silage diet recorded the highest energy retention, due mainly to a significantly higher DM intake and a total excretion that did not differ from the other silage-based diets. One of the concerns during the calculation of energy retention was the calculation of energy lost as methane. It was assumed that eight percent of the gross energy had been lost in the form of methane. This, however, is not completely true since different feedstuffs produce different amounts of methane. It would be interesting to determine the contribution of silage to greenhouse gas production,

especially when one considers the whole process; from ensiling to rumen fermentation. In this current study, the DMI of the 20% silage diet was higher than that of the control diet, therefore the gross energy intake and calculated methane production were also higher. It would therefore be important for future research to determine the effect of silage-based diets on methane production, since it may lower the total amount of methane produced by ruminants in a feedlot. Another factor to take into account for future research is the effect of silage inclusion level on peNDF content and the effect thereof on the retention time and subsequent digestibility of nutrients. Given a diet with a relatively low peNDF level, the literature indicates that the retention time of nutrients will increase as peNDF is increased. The peNDF in the current study was not taken into account but, based on the digestibility results, it was concluded that the 20% treatment had a positive effect on the retention time and therefore on digestibility. A too high indigestible fibre content, on the other hand, will increase retention to such an extent that animal production is impaired. Results obtained from the *in vitro* digestibility study regarding the digestibility of DM and NDF correlated with those found in the *in vivo* study and therefore supports the notion that low silage inclusion levels will not have a negative effect on DM or NDF digestibility. It was evident from the digestibility study that the 20% silage diet not only stimulated DMI but also improved the digestibility of nutrients.

Literature was cited that stated that silage-based diets will not have an effect on meat quality. It is generally accepted, however, that meat from ruminants finished in a feedlot with high concentrates will differ from those finished on pasture. This made it necessary to study the effect of silage on meat quality since silage is more of a roughage-based diet than a concentrate one. No differences between the experimental diets were found for the most important meat attributes. The diets, again, were of low nutritive value due to the restriction brought about by the 70% silage diet. Lambs had the same growth rate and were slaughtered at the same age and fat content. The conclusion of the meat quality study was that silage inclusion of up to 70% will not have a negative effect on meat quality. This, however, is not completely true. If the 70% silage diet were to be compared with a commercial diet with high nutrient specifications and high concentrate inclusion levels, results would be different.

The conclusion of the current study is that the best inclusion level of silage in a diet for the finishing of Merino lambs that stimulates intake is 20%, on a DM basis. The 20% inclusion level will stimulate DMI and have no negative effect on meat quality and may even improve the retention time and, therefore, digestibility. The 50% silage diet on the other hand had a better FCR even though it had a lower DMI than the 20% diet. Optimum inclusion of silage for the finishing of Merino lambs is therefore between 20 and 50%. The next step in investigating the suitability of maize silage in sheep nutrition will be to optimise nutrients for high production based on the 20 and 50% silage based diets and to compare it with a commercial feedlot diet. Factors to be taken into account in the next study should be the effect of silage-based diets on methane emission and also on the effect thereof on retention time.