Effect of condensed molasses solubles on intake, growth performance, digestibility and certain rumen parameters of sheep

by

Keara O'Reilly

Submitted in partial fulfilment of the requirements for the degree

MSc (Agric) Animal Science: Animal Nutrition

Department of Animal and Wildlife Sciences

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

October 2017

Table of Contents

Declaration

I Keara O'Reilly declare that the dissertation, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: ....

Date: ....

Acknowledgements

I would like to acknowledge the following people and thank them for their contribution to my studies and trial:

My supervisor, Prof W.A. van Niekerk, for providing me with the opportunity to do this study and for all his assistance. I would like to thank him for all the support and guidance he has given me during the course of both my undergraduate and post graduate career and for all his patience.

Voermol Feeds for their funding of this project and providing me with the opportunity to do this trial. Thank you to Mr U. Müller from Voermol Feeds for all your help during the trial and assisting with any questions I had.

Mr R.J. Coertze for his assistance with the statistical analysis of the data. All his time and patience is much appreciated.

To all the lecturers, staff and students at the University of Pretoria who assisted, supported and helped me complete this project, it is much appreciated.

The staff at the Hatfield experimental farm for the assistance when required and giving me the opportunity to use all the facilities I required.

My fiancé, Mr M. Nell, for all his support, help and for all the hours of assisting with the sampling, cleaning and looking after the animals.

My parents for giving me the opportunity to be in the position to complete this degree and for all their support.

Summary

Effect of condensed molasses solubles on intake, growth performance, digestibility and certain rumen parameters of sheep

By

Keara O'Reilly

Supervisor: Professor W.A. van Niekerk

Department: Animal and Wildlife Sciences

Faculty: Natural and Agricultural Sciences

                University of Pretoria

                Pretoria

Degree: MSc (Agric) Animal Science: Animal Nutrition

With a shortage of, and an increase in the price of conventional feedstuff for livestock, combined with the restrictions on the disposal of by-products from industrial plants, this has lead to by-product materials being commonly used as raw materials in ruminant diets. Condensed molasses solubles (CMS) is a by-product derived from the fermentation of molasses during the production of ethanol. In this trial sugarcane CMS was used to replace molasses in a high concentrate sheep ration. The experimental diets were based on an iso-nitrogenous and iso-energetic basis with only the inclusion levels of CMS differing between the diets. The treatments were 0% CMS (control), 4% CMS, 8% CMS and 12% CMS; thus the inclusion of CMS was 0%, 4%, 8% and 12% on an as is basis in the diets respectively. The control diet (0% CMS) contained no CMS but 8% molasses. Experiment 1, a 4 x 4 Latin Square design with four rumen cannulated Merino wethers was conducted to determine the effect of different inclusion levels of CMS on intake, apparent total tract digestibility, rumen fermentation, apparent nitrogen retention and microbial nitrogen production. Feed intake did not differ (P >0.05) between treatments. Organic matter digestibility was lower (P <0.05) in the 0% CMS and 4% CMS treatments compared to the 12% CMS treatment. Rumen fermentation, apparent nitrogen retention and microbial nitrogen production showed no differences (P >0.05) between treatments; however some experimental error may have influenced the microbial nitrogen production results. Experiment 2, a randomised complete block design with 200 South African Mutton Merino lambs (27.6 kg ± 4.8 kg) was conducted to access the effects of different inclusion levels of CMS on intake, growth performance and carcass characteristics of the lambs under practical feedlot conditions. The 0% CMS treatment had a lower (P <0.05) average daily gain and final live body weight compared to the 4% CMS treatment. The 0% CMS treatment had a higher (P <0.05) feed conversion ratio compared to the 4% CMS, 8% CMS and 12% CMS

treatments. Feed intake did not differ (P >0.05) between treatments. The 0% CMS treatment also had lower (P <0.05) carcass traits compared to the 4%, 8% and 12% CMS treatments. One of the concerns with using CMS was the high levels of sulphur. Liver samples were taken and analysed for copper to determine if the sulphur had reduced the absorption of the copper in the body. The copper concentration in the livers of 0% CMS treatment was lower (P <0.05) than the 8% CMS treatment. All the treatments had copper concentrations that fell within the range of normal liver copper values, thus assuming that sulphur did not have an adverse effect. The results suggest that condensed molasses solubles can be included up to 12% on an as is basis to replace molasses in a high concentrate diet without having an adverse effect on intake, growth performance, digestibility and certain rumen parameters of sheep. Further research needs to be conducted into including CMS at higher levels.

Abbreviations

ARC	Agricultural Research Council		m	meter
°C	degrees Celsius		mg	milligram
ADF	acid detergent fibre		MJ	Megajoule
ADG	average daily gain		mL	millilitre
ANOVA	analysis of variance		mmol	millimole
$BW^{0.75}$	metabolic body weight		N	nitrogen
CMS	condensed molasses solubles		NDF	neutral detergent fibre
CP	crude protein		NH_3-N	ammonia nitrogen
Cu	copper		nm	nanometer
DM	dry matter		NPN	non-protein nitrogen
DMD	dry matter digestibility		NRC	National Research Council
DMI	dry matter intake		OM	organic matter
DOMI	digestible organic matter intake		OMD	organic matter digestibility
EE	ether extract		OMI	organic matter intake
FCR	feed conversion ratio		PEM	polioencephalomalacia
FI	feed intake		ppm	parts per million
g	gram		RDP	rumen degradable protein
GE	gross energy		S	sulphur
H_2SO_4	sulphuric acid		SAS	statistical analysis system
H_3PO_4	phosphoric acid		SE	standard error
IVOMD	*in vitro* organic matter digestibility		TMR	total mixed ration
K	potassium		USA	United States of America
kg	kilogram		USDA	United States Department of Agriculture
L	litre		VFA(s)	volatile fatty acid(s)
ME	metabolisable energy			

List of tables

List of figures

Chapter 1

Introduction

With a considerable growth in the human population resulting in an increase in the demand for products of animal origin (Webb & Erasmus, 2013), as well as an increasing shortage of conventional feedstuff for livestock (Maneerat *et al.*, 2015) using by-products as an alternative to replace some of the conventional feedstuffs is becoming increasingly important. Feeding by-products to animals reduces the dependence of livestock on food that can be consumed by humans and utilises products that would have usually gone to waste (Bampidis & Robinson, 2006; López-Campos *et al.*, 2011). Conventional animal feeds are often expensive (Whitney *et al.*, 2014), thus the utilization of agro-industrial by-products in livestock feeds may be economically viable (Mirzaei-Aghsaghali & Maheri-Sis, 2008).

Ethanol production is continually on the rise (RFA, 2016). In 2015, the ethanol industry produced approximately 40 million metric tons of animal feed through the provision of by-products as an end product of certain industrial production processes (RFA, 2016). In the United States of America, the renewable fuel sector is one of the largest animal feed processing sectors (RFA, 2016). During ethanol production sugars are converted to ethanol and the remaining nutrients are concentrated in the by-product (NRC, 1981). The by-product will reflect the nutrient content of the original feed (NRC, 2007). The problem with using by-products in animal feed is its variable composition which needs to be accounted for. The by-products vary in nutrient composition as the producer does not have standards for the by-product which needs to be fulfilled, but is more interested in the end product from which the by-product originates.

Condensed molasses soluble (CMS) is a by-product derived from the fermentation of molasses during the production of ethanol (Karalazos & Swan, 1977). Condensed molasses solubles can be used as a raw material in ruminant feeds to replace molasses. Condensed molasses solubles have a very good mineral content, but adverse effects may be caused by high levels of sulphur and potassium (Stemme *et al.*, 2005). Feed intake may also be affected by feeding high levels of CMS (López-Campos *et al*, 2011). The main problems with the use of CMS in livestock feeds is the low dry matter content, high sulphur and variable composition (2017, U. Muller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380). With large quantities of CMS currently available and the ever growing ethanol industry which produces CMS as a by-product; as well as the environmental regulations regarding the dumping of this by-product (Potter *et al.*, 1985b), and with the increasing cost of molasses (Wagner *et al.*, 1983) investigation into using CMS in ruminant feed is important, so that this potentially useful livestock feed can be utilized instead of wasted. The use of this by-product in ruminant diets will also result in lower feed costs and producers will increase their profit, assuming that the efficiency of the animals does not decrease.

The aim of this study was to determine the effect of different inclusion levels of condensed molasses solubles when replacing molasses in a high concentrate diet on feed intake, growth performance, digestibility and certain rumen parameters of sheep.

Hypothesis

H_0: There is no optimum inclusion level of CMS in the diet (between 0% - 12% on an as is basis) that will increase average daily gain and decrease the feed conversion ratio.

H_1: There is an optimum inclusion level of CMS in the diet (between 0% - 12% on an as is basis) that will increase average daily gain and decrease the feed conversion ratio.

H_0: There is no optimum inclusion level of CMS in the diet (between 0% - 12% on an as is basis) that will effect dry matter intake.

H_1: There is an optimum inclusion level of CMS in the diet (between 0% - 12% on an as is basis) that will effect dry matter intake.

H_0: There is no optimum inclusion level of CMS in the diet (between 0% - 12% on an as is basis) that will increase apparent total tract digestibility, apparent nitrogen retention and microbial protein production.

H_1: There is an optimum inclusion level of CMS in the diet (between 0% - 12% on an as is basis) that will increase apparent total tract digestibility, apparent nitrogen retention and microbial protein production.

H_0: There is no optimum inclusion level of CMS in the diet (between 0% - 12% on as is basis) that will not have an effect on certain rumen parameters.

H_1: There is an optimum inclusion level of CMS in the diet (between 0% - 12% on an as is basis) that will not have an effect on certain rumen parameters.

Chapter 2

Literature Review

2.1 Overview of molasses

Molasses is a by-product of the sugar industry (Nguyen & Gheewala, 2008). The two types of molasses originating from sugar production are sugarcane molasses (originating from sugarcane) or sugar beet molasses (originating from sugar beet) (McDonald *et al.*, 2011). The nutrient composition will vary depending on the substrate of origin. Molasses is high in sugar, but low in fat and fibre and has a dry matter content of approximately 75% (NRC, 2007). Molasses has a high mineral content; especially potassium, sulphur and sodium (NRC, 2007).

Molasses is used to enhance the quality of pelleted feed (McDonald *et al.*, 2011) and reduces the dustiness of feeds with fine particles. Molasses increases the palatability of the diet due to the high levels of sugar. Molasses is commonly included in feedlot diets for these reasons. Molasses also acts as a carrier for substances such as urea, and is used as a binding agent in feed blocks (McDonald *et al.*, 2011).

Molasses serves as a cheap source of carbohydrates for ruminants (Karalazos & Swan, 1977). In tropical countries where sugarcane is produced in large quantities and the price of conventional grains is increasing, molasses can be used in ruminant diets (El Khidir & Thomsen, 1982). In tropical and sub-tropical countries, the grazing is usually of poor quality and molasses can be included in a supplement as a source of readily fermentable energy (McDonald *et al.*, 2011). When supplementing animals fed on poor quality roughages the presence of the water soluble carbohydrates in the molasses allows for a source of readily fermentable carbohydrates to be available to the rumen microorganisms. If there are adequate amounts of rumen degradable protein and readily fermentable carbohydrates, rumen microbial production can be optimised (Hall & Huntington, 2008). Molasses can also be used as an additive for silage. In treated silage, molasses reduces the pH and ammonia levels by increasing the lactic acid content (McDonald *et al.*, 2011).

When feeding molasses there may be some disadvantages, especially when including molasses at high levels in the diet. Polioencephalomalacia (PEM) has been commonly associated with molasses-urea feedlot diets (Mella *et al.*, 1976). Polioencephalomalacia is a nuerological disorder in ruminants that may be caused by thiamine deficiency, acute lead poisoning, water deprivation-sodium ion toxicosis and high sulphur intake (Gould, 1998). Feeding high levels of molasses to ruminants can result in PEM. Inclusion of high levels of molasses in a diet can also lead to diarrhoea due to the high levels of potassium (McDonald *et al.*, 2011).

2.2 Condensed molasses solubles

Condensed molasses solubles (CMS) is commonly referred to as vinasse in Europe (Weigand & Kirchgessner, 1980; Waliszewski *et al.*, 1997; Stemme *et al.*, 2005; Fernández, *et al.*, 2009; Yalcin *et al.*, 2010; López-Campos *et al.*, 2011). Condensed molasses solubles is a by-product derived from the fermentation of molasses during the production of ethanol (Karalazos & Swan, 1977). This product can be used as an excellent raw material in animal feeds, as it is a natural product from the sugar industry (Weldeman *et al.*, 1995) and is used primarily in ruminant feed. Condensed molasses solubles is a dark brown syrup. It is an ideal binding agent, reduces dustiness in feed, enhances pellet quality and improves palatability (Weldeman *et al.*, 1995). Spray dried CMS, also known as EC feed, is a spray dried form of CMS that is a fine, free flowing, light brown powder which is hygroscopic in nature (Weldeman *et al.*, 1995; Waliszewski *et al.,* 1997). Spray dried CMS has the same nutrient composition as CMS, with slightly higher calcium content due to the addition of hydrated lime. Figure 2.1 shows the basic process of the conversion of sugarcane into molasses and molasses into CMS.
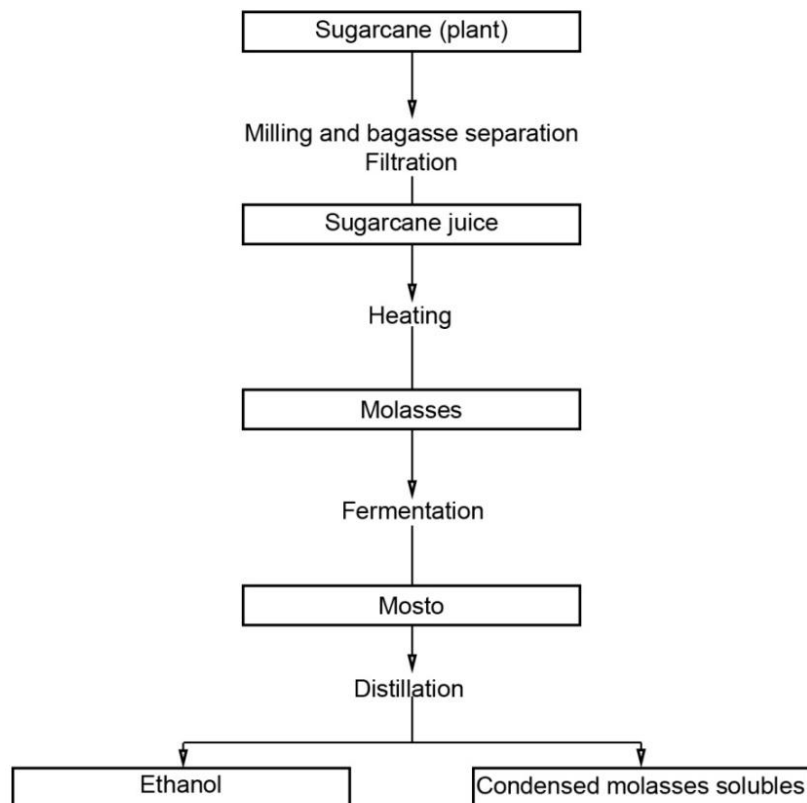
Figure 2.1 A flow diagram displaying the basic process of the production of condensed molasses solubles (Adapted from Christofoletti *et al.*, 2013)

Nutrient composition of condensed molasses solubles

There is little information known about the nutrient composition of CMS (Wagner *et al.*, 1983; Waliszewski *et al.*, 1997) and investigations into the nutritive value of this by-product are rare (Stemme *et al.*, 2005). Condensed molasses solubles have a variable nutrient composition which is dependent on the

substrate material used (sugarcane, sugar beet or citric origin), the production process, the auxiliary chemicals added during processing (nitric or sulphuric acid) and the removal of ingredients (depotassification) (Stemme *et al.*, 2005; López-Campos *et al.*, 2011). Due to the extensive withdrawal of sugar from molasses during the conversion of molasses to ethanol, the result is an increasing concentration of the other dry matter components in the remaining condensed residue (Weigand & Kirchgessner, 1980).

The dry matter (DM) of condensed molasses solubles is very low and CMS needs to be evaporated due to its high nutrient content (Waliszewski *et al.*, 1997). Varying ranges of dry matter values have been reported for CMS. Waliszewski *et al.* (1997) reported dry matter values of 520 – 670 g/kg of solids; while Potter *et al.* (1985a) reported values of 533 g/kg and 526 g/kg solids for two batches of sugarcane CMS that were included in a cattle ration. The high moisture content of CMS can be a problem as it may turn the feed mouldy. This was noted in a trial conducted by Pienaar (2016), where 2-3 weeks after delivery the feed turned mouldy due to the high moisture content of the CMS included in the feed.

Condensed molasses solubles are low in fermentable carbohydrates due to the extensive withdrawal of the sugar during the fermentation of molasses to ethanol (Karalazos & Swan, 1977; Weigand & Kirchgessner, 1980; Waliszewski *et al.*, 1997), with the sugar composing approximately 4% of the dry matter of CMS (Stemme *et al.*, 2005). Most of the fermentable sugars are utilized by the microorganisms during the production of ethanol (Potter *et al.*, 1985a). Condensed molasses solubles have a very low fibre and ether extract concentration and the contribution of fibre and ether extract by CMS to the diet is almost negligible (Weigand & Kirchgessner, 1980; Waliszewski *et al.*, 1997; Yalcin *et al.*, 2010; López-Campos *et al.*, 2011).

Condensed molasses solubles are characterised by a high crude protein content that mainly consists of non-protein nitrogen (NPN) (Weigand & Kirchgessner, 1980). The high NPN accounts for the low contribution of true protein (Weigand & Kirchgessner, 1980). The nitrogen (N) compounds are mostly non-essential amino acids, mainly glutamic and aspartic acid, as well as ammonia and betaine (Weigand & Kirchgessner, 1980; Waliszewski *et al.*, 1997; Iranmehr *et al.*, 2011; Zali *et al.*, 2017).

Condensed molasses solubles have a very good mineral content but adverse effects may be caused by high levels of sulphur (S) and potassium (K) (Stemme *et al.*, 2005). The main concern with the higher ash content of CMS is the high sulphur and potassium content; however, CMS also increases the content of minerals such as calcium, sodium, magnesium and iron (Waliszewski *et al.*, 1997). The high ash value can cause a problem as it results in the diminution of crude protein and energy, while increasing minerals such as potassium and sulphur in the diet (Weigand & Kirchgessner, 1980). Yalcin *et al.* (2010) stated that in Turkey and some other countries it was forbidden to use CMS in animal feeds due to the high ash content, however these days it is used by many countries around the world as a feed binder and dust reducer.

Molasses and condensed molasses solubles

Due to the extensive withdrawal of sugar from molasses during the conversion of molasses to ethanol, CMS contains a lower sugar content than molasses, but this also results in an increase in concentration of the other dry matter components (such as NPN and ash) when compared to molasses (Weigand &

Kirchgessner, 1980).Table 2.1 shows the composition of both sugarcane molasses and sugarcane CMS. This is a running average of results obtained from the proximate analysis of various batches of sugarcane molasses and sugarcane CMS over the past few years. This literature was provided by Voermol Feeds.

Table 2.1 A comparison of the composition of sugarcane molasses and sugarcane condensed molasses solubles (2017, U. Muller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380)

Chemical composition[1]	Molasses	CMS
Moisture (%)	22.00	50.00
Dry matter (%)	78.00	50.00
Protein (%)	5.68	4.00
TDN (%)	77.27	64.00
ME (MJ/kg)	11.02	9.20
Sugar (%)	64.77	60.00
Ash (%)	13.64	28.00
Calcium (%)	0.77	1.50
Phosphorus (%)	0.40	0.40
Potassium (%)	3.98	8.00
Sulphur (%)	0.61	2.42
Brix	84.00	52.00
PH	5.00	4.70

[1]Chemical composition on a dry matter basis

From Table 2.1 it can be seen that CMS has a tendency for a lower dry matter, sugar and energy content than molasses. The most noticeable difference is the increased ash content in CMS as opposed to molasses. Both sulphur and potassium are noticeably higher. Brix refers to the specific gravity of the molasses; the higher the brix, the higher the dry matter of the substance (2016, U. Muller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380). When formulating diets including CMS as a replacement for molasses, it is essential to account for the differences in the nutrient composition between the two raw ingredients (Stemme *et al.*, 2005). Karalazos & Swan (1977) reported a much higher rumen ammonia nitrogen concentration for sheep fed 20% beet CMS as opposed to 20% molasses (29.0 mg/100 mL as opposed to 11.6 mg/100 mL). The reason for this was that the higher nitrogen contribution in the diets from the CMS as opposed to the molasses was not accounted for and diets were not iso-nitrogenous. The crude protein concentrations of the diets were 21.5% and 13.6% for the beet CMS and molasses diets respectively.

Both CMS and molasses have the potential to reduce dust in the feed and increase the pellet quality of mixed feeds, as well as increase the palatability of the diet (Potter *et al.*, 1985a; Waliszewski *et al.*, 1997; Yalcin *et al.*, 2010). For ruminants CMS can be considered as a replacement for molasses, due to its molasses like properties (Waliszewski *et al.*, 1997), as long as the difference in contribution of the nutrients between CMS and molasses are accounted for (Stemme *et al.*, 2005). Condensed molasses solubles have been found to replace molasses without having an adverse effect. Potter *et al.* (1985a) found that when feeding steers finishing diets with 5% CMS, 5% molasses or a 5% CMS and molasses mixture there were no

significant differences in the average daily gains, feed intake, feed conversion ratios or dressing percentages of the animals.

Types of condensed molasses solubles

The three types of condensed molasses solubles are sugarcane CMS, sugar beet CMS (referred to as beet CMS) and citric CMS. Sugarcane CMS and beet CMS are the most common.

Table 2.2 Comparison between the nutrient composition of sugarcane, beet and citrus molasses (adapted from NRC, 2007)

Parameter	Sugarcane molasses	Sugar beet molasses	Citrus molasses
Dry Matter (%)	76.00	77.00	65.00
Organic Matter (%)	88.00	88.00	92.00
Crude protein (%)	6.00	9.00	10.00
Nuetral detergent fibre (%)	0.00	0.00	0.00
Acid detergent fibre (%)	0.00	0.00	0.00
Ether extracts (%)	0.80	0.20	0.30
Ash (%)	12.00	12.00	8.00
Calcium (%)	0.97	0.12	1.90
Phosphorus (%)	0.10	0.03	0.17
Potassium (%)	3.70	6.00	0.20
Sulphur (%)	0.53	0.60	0.23
Metabolisable energy (Mcal/kg)	2.70	2.70	2.80

*All values are according to the NRC (2007)

Table 2.2 is a comparison of the three different types of molasses (NRC, 2007); sugarcane, beet and citrus. The by-product of a process will reflect the nutrient content of the original feed (NRC, 2007). Thus, it can be assumed, for example if beet molasses has a higher potassium value than sugarcane molasses, then ultimately beet CMS will have a higher potassium value than sugarcane CMS. Sugarcane CMS has a lower protein content as opposed to beet CMS (Stemme *et al.*, 2005). Beet CMS contains large amounts of nitrogen as betaine (Stemme *et al.*, 2005; López-Campos *et al.*, 2011). Beet CMS also has a higher potassium content compared to sugarcane CMS (Weigand & Kirchgessner, 1980). Depotassification of beet CMS is essential, as excess potassium in the diet can disturb the mineral balance and inhibit the absorption of magnesium (Kania *et al.*, 1983). Sugarcane CMS, beet CMS and citric CMS have been used in trials and included at various levels fed to ruminants with varying results (Karalazos & Swan, 1977; Chen *et al.*, 1981; Potter *et al.*, 1985b; Fernández *et al.*, 2009).

2.3 Condensed molasses solubles and animal performance

Animal performance

Due to the large nutrient variation among CMS, it is important to know the nutrient composition of the CMS before including it in diets (Stemme *et al.*, 2005). Condensed molasses solubles should be analysed using the basic proximate analysis and the diet formulated according to the varying contribution of the nutrients in CMS.

The studies reviewed have shown varying results when including CMS in high concentrate diets fed to ruminants. A possible reason for the varying results in these reviewed studies may be that most of the diets were formulated on an iso-nitrogenous basis, but the authors did not stipulate anything about formulating for diets to be iso-energetic within each trial or accounting for the different contribution of nutrients that CMS provides. Condensed molasses solubles tend to be low in metabolisable energy (Weigand & Kirchgessner, 1980; Waliszewski *et al.*, 1997). Therefore, if the authors did not take this into account the results may not be as a result of the inclusion of CMS but rather of the varying nutrient composition between diets. In some studies feeds were not iso-energetic and/or iso-nitrogenous. The basal diets also differed between studies (Yeo *et al.*, 2004). Another factor was that in the studies CMS replaced different ingredients within the concentrate diets depending on the trial. For example, Potter *et al.* (1985a) replaced molasses with sugarcane CMS, but Potter *et al.* (1985b) replaced corn and/or soybean meal with sugarcane CMS. Different types of CMS were used between trials. Some trials used sugarcane CMS (Potter *et al.*, 1985a; Potter *et al.*, 1985b), some used beet CMS (Karalazos & Swan, 1977; López-Campos *et al.*, 2011) and one trial even used citrus CMS (Chen *et al.*, 1981). The different types of CMS have a varying nutrient composition (Stemme *et al.*, 2005; López-Campos *et al.*, 2011), and thus cannot be compared. More research needs to be conducted into including CMS in ruminant diets.

López-Campos *et al.* (2011) conducted a study with Merino lambs fed 0%, 10% or 20% beet CMS as a replacement for molasses and found a decrease in average daily gain as inclusion levels of CMS increased. The author hypothesized that the decrease in daily body weight gains could have been due to the reduction of energy in the diets containing CMS. The diets were not formulated to account for the different supply of nutrients in CMS, resulting in the energy, protein and mineral contents differing between diets (López-Campos *et al.,* 2011). Potter *et al.* (1985a) reported that when feeding steers finishing diets with 0% CMS or molasses (control), 5% sugarcane CMS, 5% sugarcane molasses or a 5% CMS-molasses mixture on a DM basis there were no significant differences in average daily gain. Average daily gains were 0.89 kg, 0.79 kg, 0.92 kg and 0.94 kg for the control, 5% molasses, 5% sugarcane CMS and 5% CMS-molasses mixture respectively. However, when steers were fed diets containing 0% or 10% sugarcane CMS on a DM basis, average daily gains decreased for steers fed the 10% CMS diets (Potter *et al.*, 1985a). The average daily gains were 0.97 kg and 1.14 kg for the 0% CMS and 10% CMS treatments respectively. Tillman & Kidwell (1951), Chen *et al.* (1981) and Yalcin *et al.* (2010) found no significant differences in average daily gains when including CMS in cattle diets.

During most of the studies reviewed, feed intake was not affected when CMS was included at varying levels of the diet (Tillman & Kidwell, 1951; Chen *et al.*, 1981; Potter *et al.,* 1985a; Potter *et al.*, 1985b; Leontowicz *et al.*, 1994). There were reports of significantly higher feed to gain ratios in ruminants fed increasing levels of CMS in their diets (Potter *et al.*, 1985b; Leontowicz *et al.*, 1994; López-Campos *et al.*, 2011), this was hypothesized to be as a result of the lower average daily gains (López-Campos *et al.*, 2011). Chen *et al.* (1981), Potter *et al.* (1985a) and Yalcin *et al.* (2010) reported no significant differences in feed conversion ratio (feed to gain) when condensed molasses solubles were included in the diets.

Most of the carcass parameters and characteristics were not affected by the addition of CMS in the diets at varying inclusion levels (Chen *et al.*, 1981; Yalcin *et al.*, 2010; López-Campos *et al.,* 2011). Chen *et al.* (1981) reported that there was a tendency for a slightly higher dressing percentage in the animals fed diets with an inclusion of a 5% citrus CMS-molasses mixture compared to those fed diets with either 10% citrus CMS or 10% molasses included, but no reason was given for this.

Karalazos & Swan (1977) fed wethers a barley based concentrate diet containing either no molasses and beet CMS (control), 10% sugarcane molasses, 10% beet CMS, 20% sugarcane molasses or 20% beet CMS on a DM basis as a replacement for a proportion of the barley. The author found no differences in apparent dry matter digestibility, organic matter digestibility or nitrogen balance between treatments. Chen *et al.* (1981) also reported no differences in nitrogen retention when wethers were fed concentrate diets with citrus CMS included at levels up to 20% DM.

Karalazos & Swan (1977) reported no significant differences in the rumen pH or volatile fatty acid production when wethers were fed the control diet, 20% sugarcane molasses, or 20% beet CMS diet. The rumen ammonia values were much higher when wethers were fed the 20% beet CMS diet. Ammonia nitrogen values were 5.50, 11.6 and 29.0 mg/100mL for the control, 20% sugarcane molasses and 20% beet CMS diets respectively. The crude protein values of the diets were 12.6%, 13.6% and 21.5% for the control, 20% sugarcane molasses and 20% beet CMS diets respectively. The authors hypothesized that the difference in ammonia nitrogen was possibly due to the differences in nitrogen intake, as diets were not formulated on an iso-nitrogenous basis due to the high levels of nitrogen in the CMS (Karalazos & Swan, 1977).

Chen *et al.* (1981) reported no significant differences in the total volatile fatty acid production when feeding wethers a diet with 0%, 10% or 20% citrus CMS on a DM basis in place of corn and/or soybean meal. In agreement, Potter *et al.* (1985b) reported no differences in volatile fatty acid production when feeding steers a concentrate diet including 0%, 5%, 10% and 15% sugarcane CMS on a DM basis substituted for corn and/or soybean meal. However, with increasing levels of CMS, Chen *et al.* (1981) reported a decrease in the acetate to propionate ratio; while Potter *et al.* (1985b) reported an increase in the acetate to propionate ratio.

Sulphur in condensed molasses solubles

Sulphur is one of the main factors that limits the inclusion of ethanol by-products in high concentrate finishing feeds for ruminants (Drewnoski *et al.*, 2014). Due to the high levels of sulphur in CMS (Hannon & Trenkle, 1990; Stemme *et al.*, 2005) its usage is limited. Sulphuric acid is often added to reduce the pH during the fermentation of sugarcane molasses to ethanol (Barrera *et al.*, 2013). In studies conducted including CMS in ruminant diets results have shown that sulphur does not have an effect on animal performance (Hannon & Trenkle, 1990; Stemme *et al.*, 2005). However, Stemme *et al.* (2005) reported cases of watery faeces in pigs when inclusion levels of CMS in the diets were increased and the author attributed this to the laxative effect of the sulphur in the CMS.

When ruminants are exposed to higher than recommended sulphur levels in the diet, feed intake and growth is reduced. The first site to be affected by the high levels of sulphur is the rumen where sulphide is generated (Suttle, 2010). Sulphate is reduced to sulphide by sulphate producing bacteria in the rumen (Drewnoski *et al.*, 2014). Figure 2.2 shows the formation of sulphide in the rumen.
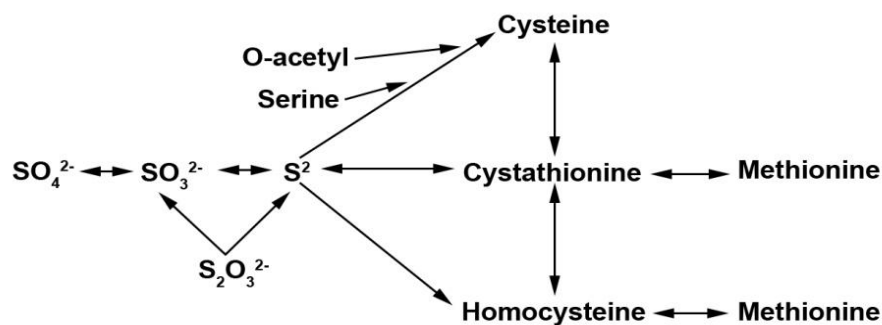
Figure 2.2 The formation of sulphide in the rumen (Kincaid, 1988)

Exposure to high levels of sulphur in the diet can induce copper deficiency in ruminants (Suttle, 2010). The reduction of sulphate and the degradation of sulphur amino acids by the rumen microorganisms in the rumen environment results in the production of sulphide (Spears, 2003). The reduction of sulphate to sulphide in the rumen is demonstrated in Figure 2.2. The sulphide binds to copper forming insoluble copper sulphide in the gut, reducing copper availability (Spears, 2003). When sulphur and molybdenum are present together in a diet, sulphur is reduced to sulphide and the sulphide and molybdenum bind forming thiomolybdate (Suttle, 2010). The thiomolybdate forms a complex with copper rendering the copper unavailable for absorption; this could result in copper deficiency (Suttle, 2010). There is then a decline in the copper concentration of the liver, as the liver is a storage organ for copper (Suttle, 2010).

Another problem that could arise due to high sulphur levels in the diet is polioencephalomalacia (Drewnoski *et al.*, 2014). In feedlot diets where the sulphur is already high, the addition of CMS will increase the risk of PEM. Sulphide production in the rumen by sulphide producing bacteria is a normal product of rumen microbial metabolism; however, when there are high levels of sulphur intake and excessive production and absorption of sulphide, the sulphide becomes a problem to ruminants (Gould, 1998). The

eructed sulphide gas is inhaled by the animal allowing it to enter the brain and cause necrosis (Drewnoski *et al.*, 2014). In a study, Gooneratne *et al.* (1989) induced PEM in lambs when including levels of 0.63% sulphur in their diet.

High levels of sulphur in the diet can also decrease feed intake and growth rate (Suttle, 2010). Zinn *et al.* (1997) reported a decrease in average daily gains, feed intake and feed efficiency when sulphur was increased from 0.20% to 0.25% on a DM basis in a finishing diet fed to heifers. Hannon & Trenkle (1990) reported that cattle on diets containing CMS at levels of 2.5% and 5.0% had a reduced feed intake. The author hypothesised that the decrease in intake was not due to decreased palatability of the feed as the reduction in intake only appeared after a few days. Thus, it was concluded that the decrease in feed intake may have been due to the high levels of sulphur in the diet due to the inclusion of CMS. No other studies reviewed, that involved the inclusion of CMS in high concentrate diets, reported similar results.

Nitrogen in condensed molasses solubles

Due to the high nitrogen (especially NPN) and mineral content of CMS, it has been suggested that CMS could be a possible non-protein nitrogen supplement for ruminants (Hannon & Trenkle, 1990). Other NPN sources added to ruminant diets to replace protein, most commonly urea, may be limiting in nutrients such as sulphur, phosphorus and potassium (Hannon & Trenkle, 1990). The role of protein supplementation, with a protein or non-protein nitrogen source, is to provide a supply of ammonia to the ruminant that is essential for the normal functioning of cellulolytic microbes in the rumen (Van Niekerk, 1975). Along with adequate fermentable energy, protein supplementation increases digestibility and feed intake of poor quality roughages.

Fernández *et al.* (2009) conducted a trial with sheep that were fed sugar beet pulp supplemented with 0%, 7% and 14% CMS. When offered a choice between the three CMS supplemented levels the sheep showed a preference for the 7% and 14% CMS inclusion. The author suggested that this may be due to the increasing rumen degradable protein (RDP) when the sheep consumed the diets containing CMS, as sugar beet pulp is very low in RDP. Therefore, the additional NPN in the CMS could have increased the digestibility of the straw and increased feed intake (López-Campos *et al.,* 2011).

Hannon & Trenkle (1990) conducted an *in vitro* and *in vivo* study using CMS to replace urea. Results from the *in vitro* study showed that CMS could potentially replace urea as a NPN source. In contradiction to that, in the *in vivo* growth and digestion study with cattle when CMS was added at 5%, results indicated that CMS was inferior to urea. The authors hypothesized that the decrease in feed intake and digestibility may have been due to the high levels of sulphur in the CMS and concluded that CMS could be a supplemental source of nitrogen for ruminants if the level of sulphur could be reduced (Hannon & Trenkle, 1990). Therefore, further research needs to be conducted into using CMS as a NPN supplement.

2.4 High concentrate diets and animal performance

Feed intake and digestibility

For efficient production high levels of voluntary intake are necessary, the more the animal eats the more it can produce (Forbes, 2000). The breaking down of feed particles is referred to as digestion (McDonald *et al.*, 2011). Factors affecting digestibility are ration composition, feed processing, animal factors and level of feeding (McDonald *et al.*, 2011). When feeding high concentrate diets the rumen pH is decreased and this can have a negative associative effect on the digestibility of fibre (McDonald *et al.*, 2011). When the rumen pH falls below 6, cellulolytic bacteria are inhibited, thus decreasing fibre digestibility (Owens & Goetsch, 1988). Grains are usually crushed, ground or pelleted to increase digestibility (McDonald *et al.*, 2011). As the level of feeding increases, the rumen retention time decreases, thus digestibility decreases (Galyean & Owens 1991). Galyean *et al.* (1979) fed steers a corn based diet at different levels of feed intake from maintenance level to twice x maintenance. As the dry matter increased the dry matter digestibility and organic matter digestibility decreased. When the dry matter intake was 2.558 kg/day dry matter digestibility was 85.7% and organic matter digestibility was 86.4%; when the dry matter intake was increased to 5.332 kg/day dry matter digestibility was 77.6% and organic matter digestibility was 78.2%.

Rumen fermentation

Rumen microorganisms ferment feed in the rumen and produce volatile fatty acids, ammonia nitrogen, methane and other by-products (Owens & Goetsch, 1988). During ruminal digestive processes protozoa and fungi are involved, however ruminal bacteria are responsible for most of the fermentation of feed in the rumen (Huntington, 1997). When feeding a concentrate as opposed to a forage based diet, there is an increase in the number of amylolytic bacteria and lactic acid producing bacteria and a decrease in the amount of fibrolytic bacteria in the rumen (Bevans *et al.*, 2005).

Volatile fatty acids are the main source of energy for ruminants (Fahey & Berger, 1988). As the proportion of concentrates (grain) in the diet increases, an increased amount of fermentable carbohydrates enters the rumen. The rapid fermentation of fermentable carbohydrates by the rumen microbes leads to volatile fatty acid accumulation in the rumen and a reduced rumen pH. Volatile fatty acid production in the rumen is higher with concentrate diets as opposed to forage based diets. When feeding concentrates, rumination times are reduced which decreases the amount of saliva produced and thus reduces the buffering action of saliva; forages also usually exert some buffering action which concentrates do not; (Owens & Goetsch, 1988), thus contributing to the decrease in rumen pH when concentrate diets are fed. Concentrate diets are more rapidly fermented than forage diets and the energy is more readily available (Owens & Goetsch, 1988).

The presence of increased fermentable carbohydrates in the rumen results in a shift from acetate (fibrololytic) to propionate (amylolytic) producing bacteria in the rumen (Thomas & Rook, 1981). As seen in Table 2.3, as the amount of concentrate in a diet increases, the amount of acetate produced in the rumen decreases and the amount of propionate increases (Fahey & Berger, 1988), resulting in decreased acetate to propionate ratio. When feeding high concentrate diets to productive animals, the shift from acetate to

propionate production in the rumen results in increased productivity. Propionate production is more efficient and provides more energy than acetate (Schelling, 1984). Propionate is also a glucogenic precursor, which is transported from the rumen to the liver to undergo gluconeogenesis where it is converted to glucose (McDonald *et al.*, 2011). Propionic acid yields 13.5 moles of ATP per mole of propionate, while acetic acid only yields 8 moles of ATP per mole of acetate, making propionate a better source of energy for the ruminant (McDonald *et al.*, 2011).

Table 2.3 The proportions of volatile fatty acid production with different forage to concentrate ratios (Fahey & Berger, 1988)

	Molar ratios		
Forage : Concentrate ratio	Acetate	Propionate	Butyrate
100 : 0	71.4	16.0	7.9
75 : 25	68.2	18.1	8.0
50 : 50	65.3	18.4	10.4
40 : 60	59.8	25.9	10.2
20 : 80	53.6	30.6	10.7

Rumen ammonia nitrogen is derived from the microbial degradation of dietary protein and non-protein nitrogen (Owens & Zinn, 1988). Rumen ammonia nitrogen and fermentable energy are utilised by rumen microbes for microbial protein synthesis. Microbial nitrogen production is lower in forage based diets as opposed to concentrate based diets (Ramos *et al.*, 2009; Ma *et al.*, 2014). The reason for this is possibly due to the increased amounts of fermentable carbohydrates available for microbial synthesis in concentrate based diets.

Nitrogen balance

The nitrogen balance or apparent nitrogen retention is the most common index to determine the protein status of a ruminant (Owens & Zinn, 1988). Nitrogen balance or apparent nitrogen retention is calculated as the difference between nitrogen intake and nitrogen output (Owens & Zinn, 1988; Morgan & Whitmore (unpublished), cited by McDonald *et al.,* 2002). Cole (1999) reported that as the crude protein concentration of the feed offered to lambs increased, the nitrogen intake, faecal nitrogen, urinary nitrogen and nitrogen retention increased. Nitrogen retention is an indicator of the protein status of the animal and the amount of protein available for growth (Tripathi *et al.*, 2007). Figure 2.3 depicts the fate of dietary crude protein and indicates the processes during which nitrogen losses can occur.
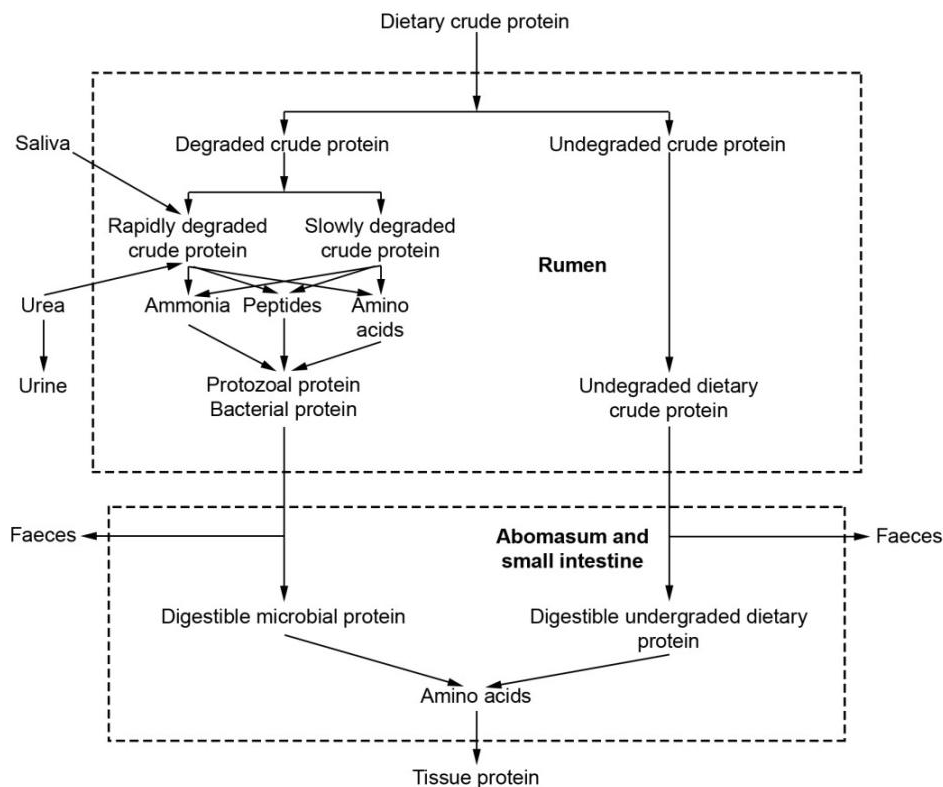
Figure 2.3 The fate of dietary crude protein in the ruminant animal (McDonald *et al.*, 2011)

2.5 Feedlot parameters

Performance parameters

To determine the performance of growing animals parameters such as weight gain, average daily gain, feed intake and feed conversion ratio are measured and recorded. Animals that grow quickly and can be marketed sooner are more profitable (Voermol, 2010). Average daily gain is calculated as the weight gained over the number of days. Factors that can affect average daily gain are diet, management, health and environmental factors. Fluharty & McClure (1997) reported that lambs fed a concentrate diet containing higher levels of protein had an increased average daily gain as opposed to lambs fed diets containing lower levels of protein

Feed intake is either measured as feed intake on an as is basis or feed intake on a dry matter basis. When feedlot producers want to know the actual amount of feed required per animal in order to do a cost analysis, feed intake on an as is basis is used (Fernández & Woodward, 1999; ARC, 2002; Davis, 2003). Dry matter intake indicates the amount of nutrients that the animals actually consume. The feed conversion ratio is calculated as the amount of feed required for 1 kg of gain (Beauchemin *et al.*, 1997; Hendriks *et al.*, 2013). The feed conversion ratio is one of the main factors determining profitability of a feedlot (Voermol, 2010).

Carcass parameters

South African consumers have a preference for lean and tender meat (Webb & O'Neill, 2008). Therefore producers aim to produce a younger carcass with a moderate amount of carcass fat at slaughter (Webb, 2015). Both the age and fat content of an animal influence the tenderness of the meat (Webb & O'Neill, 2008). In South Africa carcasses are classified according to the South African Red Meat Classification System (Webb, 2015). Table 2.4 shows how the carcasses are classified in South Africa.

Table 2.4 The South African Carcass Classification System for cattle and small stock (Webb, 2015)

South African Red Meat Classification System				
Age category	A = 0 permanent incisors	AB = 1-2 permanent incisors	B = 3-6 permanent incisors	C >6 permanent incisors
Rollermark	AAA	ABAB	BBB	CCC
Colour of rollermark	Purple	Green	Brown	Red
Carcass fat codes: 0 - no fat; 1 - very lean; 2 - lean 3 - medium, 4 - fat; 5 - slightly overfat; 6 – excessively over fat				
Conformation scores: 1 - very flat to 5 - very round				

The carcass classification groups carcasses in categories based on physical and compositional attributes (Webb, 2015), whereas with carcass grading carcasses are graded in order of merit. Carcasses are classified according to age, subcutaneous fat thickness and conformation (Webb, 2015). In South Africa age or maturity is determined by the dentition of the animal (Strydom, 2011). The fatness of a carcass is determined by the percentage of subcutaneous fat or the fat thickness (mm).

When carcasses are classified according to the South African Red Meat Classification System as seen in Table 2.4, South African consumers have a preference for an A2 to an A3 type of carcass (Webb, 2015). This means that carcasses from lambs with a lean to medium amount of fat and fewer than one permanent incisor are the most desirable by consumers and worth the most money (Brand *et al.*, 2017).

In a feedlot it is required that the lambs are slaughtered after a certain number of days on feed or at a targeted body weight (Sheridan *et al.*, 2003). In South Africa the feedlot industry tends to slaughter animals after a fixed amount of days on feed rather than a pre-determined body weight (Brand *et al.*, 2017). To achieve the required carcass classification, lambs need to be slaughtered at a certain body weight before they begin to deposit fat rather than muscle and become classed as over fat (Brand *et al.*, 2017). The carcass weight is directly correlated to the final live body weight of the animal (Cloete *et al.*, 2004). Once the lamb has been slaughtered the head, feet, wool, skin and the internal organs from the abdominal and thoracic cavity are removed, leaving the carcass of the lamb (Kirton *et al.*, 1995). Hot carcass weight is the weight of the carcass measured directly after slaughter and evisceration. The cold carcass weight is the weight recorded once the carcass has been chilled (Cloete *et al.*, 2004). The difference between the hot and cold carcass mass is the fluid and blood loss during chilling.

Dressing percentage is the proportion of final live body weight that is converted into carcass (Warmington & Kirton, 1990). Factors affecting the dressing percentage of lambs are live body weight, fat cover, gut fill, wool weight and breed (Kirton *et al.*, 1984). As the live body weight of a lamb increases so does the dressing percentage (Kirton *et al.*, 1984). Dressing percentage is influenced by the level of gut fill when the final live body weight is recorded (Sheridan *et al.*, 2003). An increase in gut fill when the final live body weight is recorded results in a decrease in dressing percentage. Kirton *et al.* (1995) also reported a lower dressing out percentage with heavier wool weights, as a lower proportion of the lambs live body weights were converted into carcass.

Chapter 3

Materials and Methods

3.1 Introduction

The trial consisted of two experiments; experiment one was a 4 x 4 Latin Square design, with four rumen cannulated Merino wethers, and experiment two was a feedlot trial (randomised complete block design), with 200 South African Mutton Merino lambs.

Both experiments took place at the small stock section on the Hatfield Experimental Farm of the University of Pretoria; South Street, Hatfield, Pretoria. The laboratory analyses were done at the Nutrilab of the Department of Animal and Wildlife Sciences at the University of Pretoria.

The experimental protocol and trial was approved by the Animal Ethics Committee of the University of Pretoria, ethics approval project number EC015-16. The trial was carried out following the approved research protocol.

3.2 Experimental diets

The trial consisted of four experimental diets differing in inclusion levels of sugarcane condensed molasses solubles. The control diet (0% CMS) contained 8% molasses but 0% CMS. Inclusion levels of CMS were 4% in treatment 1 (4% CMS), 8% in treatment 2 (8% CMS) and 12% in treatment 3 (12% CMS) on an as is basis, with no molasses included. Using these experimental diets, investigation into whether CMS could potentially replace molasses in high concentrate sheep diets and determining at what level the optimum inclusion level would be, took place.

The experimental diets were a total mixed ration (TMR), formulated as a feedlot lamb diet according to the National Research Council (NRC, 2007). Diets were formulated on an iso-energetic and iso-nitrogenous basis so that the differences in results were not due to differing metabolisable energy and crude protein contents of the experimental feed. The diets were formulated and ingredients were included in such a way that accounted for the variation in the contribution of nutrients between molasses and different inclusion levels of CMS. For example, the diets containing higher levels of CMS, such as treatment 3 (12% CMS), will require less minerals than the control (0% CMS) due to the higher ash content of CMS compared to molasses. Thus, the nutrient composition between diets was similar and the differences in results could be based on the differing inclusion levels of CMS. Table 3.1 shows the ingredient composition of the four experimental diets. All the diets contained Monensin-Na at 18ppm on an as is basis.

The TMR was in a pelleted form which prevented selective feeding and decreased the amount of feed that was wasted (Esplin *et al.*, 1957; Tag Eldin *et al.*, 2011). The pelleted feed also prevented dustiness and the CMS was able to enhance the manufacturing process of the pellets (López-Campos *et al.*, 2011). Pelleting the feed allowed more accurate measurements of feed intake, as the feed was easier to weigh back and was less likely to be lost to wind or birds as opposed to a mash diet.

Kalori 3000 (Reg. No. V2809, Act 36 1947), also known as EC feed, is a spray dried form of CMS that is a fine, free flowing, light brown powder which is hygroscopic in nature (Weldeman *et al.*, 1995; Waliszewski *et al.,* 1997). Kalori 3000 has the same nutrient composition as CMS, with slightly higher calcium content due to the addition of hydrated lime. Kalori 3000 has a very low moisture content of approximately 4%, thus overcoming the problem of feed turning mouldy when CMS is included at high levels in the feed due to the high moisture content of CMS (2016, U. Muller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380). Kalori 3000 was included at 6% (which is equivalent to 12% CMS on an as is basis) in treatment 3 (12% CMS) to investigate whether higher inclusions of CMS would affect results (2016, U. Muller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380). The spray dried form of CMS avoided the problem of high levels of CMS turning the feed mouldy, but still allowed investigation into the effects when including higher levels of CMS.

The four experimental diets were formulated, manufactured and supplied by Voermol feeds (Maidstone Village, Tongaat, 4380, KwaZulu-Natal, South Africa). Each experimental diet was formulated and manufactured as one batch. All the experimental feed was delivered at the same time and stored in a shed at the small stock section of the Hatfield Experimental Farm. Therefore, there were no differences within treatments between the batches. The same feed was used for both the 4 x 4 Latin Square design and the feedlot trial.

Table 3.1 Ingredient composition of the four experimental diets differing in inclusion levels of CMS

Ingredients (%)[2]	Treatment[1]			
	0% CMS	4% CMS	8% CMS	12% CMS
CMS[3]	0.00	4.00	8.00	0.00
Kalori 3000[4]	0.00	0.00	0.00	6.00
Molasses	8.00	0.00	0.00	0.00
Water	2.90	1.60	0.00	3.80
Maize meal	45.80	54.10	53.50	53.20
Bagasse	16.90	15.20	13.80	13.40
Brewers grain	15.00	15.00	15.00	15.00
Cotton seed oilcake meal	7.60	6.20	6.10	5.80
Urea	0.83	0.83	0.83	0.83
Ammonium sulphate	0.50	0.50	0.50	0.50
Salt	0.42	0.42	0.42	0.42
Minerals and additives	2.05	2.10	1.90	1.00

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3

[2]Ingredients (%) - Ingredient composition of the experimental diets on an as is basis in %

[3]Condensed molasses solubles - Included at different levels in the experimental diets on an as is basis to investigate if CMS can replace molasses and determine the optimum level

[4]Kalori 3000 - Spray dried form of CMS (V no. 2809), 6% Kalori 3000 is equivalent to 12% CMS on an as is basis

3.3 4 x 4 Latin Square design – Experiment 1

3.3.1 Experimental design and animals

Four rumen cannulated Merino wethers, approximately 36 months old with a mean body weight of 75.5 kg (±5.3 kg) were used in a 4 x 4 Latin Square design trial. The trial lasted 88 days consisting of 4 x 22 day experimental periods. Each experimental period was 22 days, of which the first 14 days was an adaptation period to the new experimental diet and the last eight days were for sample and data collection (Osuji *et al.*, 1993). The trial took place from the middle of June to the middle of September 2016. For the duration of the trial the average minimum temperature was approximately 2.5ºC and the average maximum was approximately 26.0ºC.

In this experiment two factors other than treatment had an influence over the response; therefore treatments had to be blocked to control these two factors which were animal effect and experimental period effect (Kuehl, 2000). The experimental treatments were randomised in a 4 x 4 Latin Square design arrangement. Each experimental diet (treatment) appears once in each experimental period (row) and once for each animal (column).

The four treatments were:
1.   0% CMS (Control)
2.   4% CMS (Treatment 1)
3.   8% CMS (Treatment 2)
4.   12% CMS (Treatment 3)

Animals were allocated to their experimental diets according to a 4 x 4 Latin Square design layout as shown in Table 3.2.

Table 3.2 The 4 x 4 Latin Square design layout of the experimental diets allocated to the rumen cannulated wethers during the different experimental periods

Experimental period[2]	Animal[1]			
	1302	1314	3222	1309
1	A	B	C	D
2	C	D	A	B
3	B	C	D	A
4	D	A	B	C

[1]The numbers of the four rumen cannulated wethers used for the 4 x 4 Latin Square design

[2]Each experimental period consists of 22 days (14 days adaptation and 8 days collection)

A = 0% CMS (Control)

B = 4% CMS (Treatment 1)

C = 8% CMS (Treatment 2)

D = 12% CMS (Treatment3)

Before the trial began wethers were weighed using a Tal-Tec (model TT40) livestock scale and treated for internal parasites (Osuji *et al.*, 1993) using Gardal® 10% (MSD Animal Health, South Africa), which was diluted and administered according to the instructions on the bottle. Throughout the trial animals were monitored using the FAMACHA chart and treated accordingly (Bath *et al.*, 1996). All wethers were injected subcutaneously with a vitamin A, D and E complex before the trial began. Wethers hooves were trimmed and the wool around the rumen cannulae was sheared to prevent flies and decrease the chances of any infections occurring.

3.3.2 Adaptation period

Each adaptation period to the new experimental diet was 14 days (Osuji *et al.*, 1993; Olson *et al.*, 1999; Vyas *et al.*, 2014; Van Eeden, 2016). This was to ensure a consistent daily feed intake was achieved and that a stable microbial population was established in the rumen of the wethers on their new allocated experimental diet. From day 1 - 11 of each adaptation period sheep were housed in individual pens outside under shelter to ensure that they had access to sun and shade. The pens were 3.5 x 2 m with concrete floors, feed troughs and automatic water troughs. The pens were directly next to each other so that the wethers could still interact with each other socially. Wethers were fed twice daily at 08:00 and 16:00, the same schedule that was used during the sample collection periods. Every morning before the wethers were fed, the pens, feed troughs and water troughs were cleaned. Each wethers rumen cannula was cleaned and fly spray was applied and each sheep was checked over. If required maintenance tasks such as hoof trimming, dosing and shearing around the rumen cannulae were done at the beginning of the adaptation period of each experimental period. Sheep had *ad libitum* access to their assigned experimental diets and water throughout the trial. During the adaptation period wethers were given access to a salt lick *ad libitum*.

Before feeding on the morning of day 12, three days before collection and sampling began, each wether was weighed and placed in their respective metabolism crates and faecal bags were fitted (Dentinho *et al.*, 2014; Van Eeden, 2016). This was done to ensure that the sheep adapted to their metabolism crate and faecal bag before the sample collection period began. The metabolism crates were next to each other and wethers were able to see each other at all times, to ensure no unnecessary stress was induced, due to their social nature and flock instinct. Before feeding at 08:00 and 16:00 their feacal bags were emptied. Before the morning feed the orts were emptied, water troughs were cleaned and filled and the rumen cannulae were cleaned.

3.3.3 Sample collection period

On day 15 of each experimental period the sample collection period began. This consisted of eight days, in which data and samples of feed, orts, faeces, urine and rumen fluid were collected from all the wethers which were housed in their metabolism crates. There were four sample collection periods throughout the trial in which sampling and procedures remained consistent throughout.

Feed and ort collections
Wethers were fed their allocated experimental diets twice a day at 08:00 and 16:00, as these were their usual feeding times and any change in routine may have affected feed intake. The feed was weighed and

the quantity given to each sheep was recorded. The wethers had *ad libitum* access to feed.

Feed samples were taken daily for each experimental diet, throughout the experiment. These samples were pooled per treatment and placed in an airtight sealed plastic bag and stored at -20ºC in a freezer. By sampling feed every day any "between bag" differences for a given diet were accounted for. Thus, at the end of the experiment there was a representative composite sample of each treatment.

Every morning before feeding from day 16 to 21 the orts in the feed trough from the previous day were collected and weighed and the weight was recorded (Osuji *et al.*, 1993). The orts from the trough were mixed thoroughly and a 10% sample was collected and placed in an airtight sealed plastic bag and stored at -20ºC in a freezer. The remaining orts were discarded. Care was taken to ensure that none of the orts were left in the trough as this would have affected the digestibility calculations. This same procedure was repeated for each animal each day during each sample collection period. The orts samples were composited for each animal (treatment) per sample collection period.

Faecal collections
Every morning from day 17 to 22 after the orts were collected and before feeding, the faeces from the faecal bag was collected and weighed out and the weight was recorded (Osuji *et al.*, 1993; Olson *et al.*, 1999). The faeces from the bag was mixed thoroughly and a 10% sample was collected and placed in an airtight sealed plastic bag and stored at -20ºC in a freezer (Osuji *et al.*, 1993). The remaining faecal matter was discarded. Care was taken to ensure that none of the faeces were lost during collection and the bags were completely emptied and sealed after the collection as this would have affected the digestibility results. The same procedure was repeated every afternoon before the wethers were fed their afternoon feed. This same procedure was repeated for each animal each day during each sample collection period. The faecal samples were composited for each animal (treatment) per sampling period. The length of the faecal collection period was sufficient enough to account for the within day and daily variation in faecal excretion (Galyean *et al.*, 1986).

Urine collection
As described by Chen & Gomes (1995), total urine collection was done daily (every 24 hours) during the sample collection periods by attaching a metal urine pan to the metabolism crate. The urine from the urine pan drained into and collected in a container which contained 100 mL of 10% sulphuric acid (H_2SO_4). This was to ensure that the pH of the urine was less than 3, to preserve the urine and prevent ammonia nitrogen loss (Chen *et al.*, 1992; Osuji *et al.*, 1993) by bacterial destruction of purine derivitives (Chen & Gomes, 1995). The container was covered with a layer of cheese cloth to ensure that the urine was not contaminated by any solids such as wool or faeces. The urine in the container was collected every morning and the pH was measured with a Crison pH meter (which was calibrated each time before collections using buffer solutions of pH 4, 7 and 9). Additional H_2SO_4 was added, when required, to obtain a pH of 3 or less. The urine was then measured and the total amount was recorded. A 50 mL sub-sample was taken for nitrogen analysis, placed in a container and stored at -20ºC in a freezer for further analysis.

As described by Osuji *et al.* (1993) the remaining urine was then diluted to a volume of 4000 mL using water. This was mixed thoroughly to ensure that the urine and water were homogenous throughout the mixture. The urine was diluted to prevent precipitation of uric acid (Chen *et al.*, 1992; Osuji *et al.*, 1993). A 50 mL sub-sample of the diluted urine was then taken for analysis of purine derivatives, placed in a container and stored at -20ºC in a freezer for further analysis.

The remaining urine was then discarded and a 100 mL of 10% H_2SO_4 was added to the clean container and placed back under the metabolism crate to collect the urine for the next 24 hours. The same procedure was repeated for each animal each day during each sample collection period. The respective urine samples taken each day were composited for each animal (treatment) per sample collection period.

Rumen fluid collection

Rumen fluid was collected to analyse the effect of different inclusion levels of CMS in the diet on rumen fermentation. This was done by measuring the rumen pH and analysing the volatile fatty acid (VFA) and ammonia nitrogen (NH_3-N) production in the rumen. Rumen fluid was collected twice a day for four days, from day two to five of each sample collection period from each animal, according to the collection times in Table 3.3. The rumen fluid was collected at different times, to ensure that there was a representative pooled sample over a 24 hour period to account for the diurnal variation (Mentz *et al.*, 2015).

Table 3.3 Rumen fluid collection days and times during each sample collection period from all wethers

Sampling period	Animal	Day of sampling period	Time of rumen fluid collection[1]	
			Morning	Afternoon
1, 2, 3, and 4	1302, 1314, 3222, 1309	2	06:00	18:00
1, 2, 3, and 4	1302, 1314, 3222, 1309	3	03:00	15:00
1, 2, 3, and 4	1302, 1314, 3222, 1309	4	00:00	12:00
1, 2, 3, and 4	1302, 1314, 3222, 1309	5	09:00	21:00

[1] Rumen fluid was collected at different times to account for diurnal variation

During each collection time rumen fluid was collected through the rumen cannula from various regions of the rumen (cranial, caudal, dorsal and ventral regions) to ensure that a representative sample of the entire rumen was obtained (Vyas *et al.*, 2014; Mynhardt *et al.*, 2016). The rumen fluid was squeezed through four layers of cheese cloth to ensure that only the rumen fluid and no solid rumen content was collected. All of the solid rumen content was placed back into the rumen of the animal to ensure the rumen fluid collections had no effect on the digestibility measurements. The rumen cannula of the wether was cleaned and any rumen fluid spillage was cleaned up immediately after collections to ensure rumen fluid did not contaminate the urine by dripping into the urine pan.

Directly after the rumen fluid was collected the pH was measured using a Crison pH meter (which was calibrated each time before collections using buffer solutions of pH 4, 7 and 9) and the results recorded. The

rumen fluid samples were then preserved for VFA and NH_3-N analysis. For VFA determination, samples were preserved with 4 mL of 25% phosphoric acid (H_3PO_4) solution per 20 mL of rumen fluid as described by Webb (1994). For NH_3-N determination, samples were preserved using 5 mL of 50% H_2SO_4 solution per 30 mL of rumen fluid as described by Broderick & Kang (1980). These samples were then stored at -20ºC in a freezer for further analysis. This same procedure was repeated for each animal at each collection time during the sample collection period. The respective rumen fluid samples taken at each collection time were composited for each animal (treatment) per sample collection period.

Water intake

Every morning after feeding the remaining water was measured and the volume recorded. The water trough was then cleaned and filled with a measured amount of water which was recorded. Every afternoon before feeding a known volume of water was added to each water trough to ensure that wethers had *ad libitum* access to water. This volume was also recorded. Thus, the total water intake per wether could be calculated.

At the end of the sampling period wethers were taken out the metabolism crates and weighed and placed into their individual pens outside. They were then assigned to their new experimental diets according to Table 3.2 and the adaptation phase was then repeated.

3.3.4 Laboratory analysis

Feed, orts and faeces

The composited feed samples, one per experimental diet, and composited orts samples that were pooled for each animal (treatment) per sample collection period, were each mixed thoroughly and representative sub-sample of each sample was taken using the manual cone and quartering method (Faithfull, 2002). The sub-samples were milled using a Retsch ultra-centrifugal mill (model SM100) fitted with a 1mm screen. The milled feed and orts samples were then placed in containers for proximate analysis.

The composited faecal samples that were pooled for each animal (treatment) per sample collection period were thawed. A 2 g sub-sample of each faecal sample was weighed out into a tinfoil container in duplicate and the initial dry matter of the faeces was determined by placing the 2 g sub-samples in an oven at 105ºC for 24 hours according to the AOAC (2000) procedure 934.01. The remaining amount of each faecal sample was dried in an oven at 55ºC for 48 hours (Mahgoub *et al.*, 2000). Once dried at 55ºC, the faecal samples were then milled using the Retsch ultra-centrifugal mill (model SM100) fitted with a 1mm screen and the milled samples were placed in containers for proximate analysis.

The milled feed, orts and faecal samples were then analysed for dry matter and ash. In the case of the faeces the second dry matter was determined, as the initial dry matter had already been determined. The dry matter was determined by placing 2 g of the milled sample in duplicate in an oven at 105ºC for 24 hours following the AOAC (2000) procedure 934.01. The ash concentration of the samples was determined by placing the dried samples from the dry matter analysis (in the case of the faecal samples, the second dry matter analysis) in a muffle furnace for two hours at 250ºC and a further four hours at 600ºC, following the

AOAC (2000) procedure 942.05. The ash content was used to determine organic matter (OM) of the samples using the equation OM (%) = 100 - %Ash.

Feed, faecal and ort samples were analysed for crude protein (CP) by weighing out 0.2 g of each milled sample in duplicate and following the Dumas combustion method, using the Leco-TruMac Nitrogen determinator apparatus, according to the AOAC (2000) procedure 968.06. Crude protein was calculated as N x 6.25% (McDonald *et al.*, 2011).

The milled feed samples were analysed for acid detergent fibre (ADF), neutral detergent fibre (NDF) and ether extract (EE). The feed samples were analysed for ADF and NDF according to the method described by Goering & Van Soest (1970). The samples were analysed for ether extract according to the AOAC (2000) procedure 920.39.

Feed samples were analysed for calcium, phosphorus, sulphur and potassium. Before any specific mineral analysis began samples underwent mineral digestion using nitric and perchloric acid according to the AOAC (2000) procedure 935.13. The feed samples were analysed for calcium according to the method described by Giron (1973) and phosphorus according to the AOAC (2000) procedure 965.17. Sulphur and potassium was measured with a spectrometer (model Agilent 725, 700 series) at wavelengths of 182 nm and 766.5 nm for sulphur and potassium respectively, according to the Agricultural Research Council (ARC, unpublished).

In vitro organic matter digestibility (IVOMD) of each of the feed samples was determined as described by Tilley & Terry (1963) modified by Engels & Van der Merwe (1967). The rumen fluid used for the *in vitro* analysis was obtained from two rumen cannulated wethers kept at the University of Pretoria Hatfield experimental farm. The artificial saliva and urea solutions were prepared at the Nutrilab of the University of Pretoria. The *in vitro* incubation was done using the IncoShake apparatus which maintained a constant temperature of 38 - 39ºC throughout incubation. Bomb calorimetery was used to determine gross energy (GE) of the feed samples using a water bomb calorimeter (Cal2K). The metabolisable energy (ME) of the feed was calculated using the equation ME (MJ/kg DM) = GE x (0.82xIVOMD) (Robinson *et al.*, 2004).

Urine

The composited urine samples that were preserved for nitrogen analysis were thawed at ambient temperature. Once thawed, 0.2 g was weighed out in duplicate for each sample and analysed for nitrogen according to Dumas combustion method, using the Leco-TruMac Nitrogen determinator, according to AOAC (2000) procedure 968.06.

The composited urine samples preserved for determination of microbial protein production analysis were thawed at ambient temperature. Samples were analysed for purine derivatives; allantoin, uric acid, xanthine and hypoxanthine (Tamminga & Chen, 2000). Samples were analysed using high performance liquid chromatography (2016, Z. Apostolides, Pers. Comm., Department of Biochemistry, University of Pretoria, Private bag X20, Hatfield, 0028). Total purine derivative excretion (mmol/day) was calculated and

used for the estimation of microbial nitrogen production (Chen & Gomes, 1995). Microbial nitrogen production was then calculated following the calculations described by Chen *et al.* (1992) and Chen & Gomes (1995).

Rumen fluid

The composited rumen fluid samples from each animal (treatment) per sample collection period that were preserved for NH_3-N analysis, as well as the samples preserved for VFA analysis were thawed overnight at 4ºC in a fridge. The samples for NH_3-N analysis were analysed based on the phenol-hypochlorite reaction using the spectrophotometer (Specord 200) at 600 nm to read the tubes, following the procedure of Broderick & Kang (1980). The samples for VFA analysis were analysed based on the gas chromatography method using the gas chromatograph (model Shimadzu GC 2010_Tracera) following the procedure of Webb (1994).

3.3.5 Calculations and parameters measured

Using the data collected during the sample collection period and the laboratory results, the following calculations were made and parameters derived for each animal given their allocated treatment for each sample collection period, thus four results per treatment for each parameter were obtained.

Feed intake

Using the data collected for feed intake and the dry matter and organic matter laboratory results of the feed and orts, the following feed intake parameters were derived:

- Dry matter intake/day
- Organic matter intake/day
- Digestible organic matter intake/day
- Feed intake (FI) per kg metabolic body weight ($BW^{0.75}$) was calculated as (Mahgoub *et al.*, 2000):

$$FI \ (g/kg \ BW^{0.75}) = FI \ (g) \ / \ ((wethers \ weight \ (kg))^{0.75})$$

The weight of each wether was recorded before going into the metabolism crate and when being removed from the crate at each experimental period. The wethers' weight for that experimental period was then calculated by taking the average of those two weights.

Digestibility

Data and laboratory results from the feed, orts and faecal samples were used to determine apparent total tract dry matter and organic matter digestibility. The following general equations were used to determine the digestibility.

The general equation to determine the digestibility coefficient (McDonald *et al.*, 2011):

$$\text{Digestibility coefficient} = \frac{\text{nutrient consumed} - \text{nutrient in faeces}}{\text{nutrient consumed}}$$

For example (Osuji *et al.*, 1993):

$$\text{Apparent dry matter digestibility coeffecient} = \frac{(DM \ feed \ (g) - DM \ orts \ (g)) - DM \ faeces \ (g)}{DM \ feed \ (g) - DM \ orts \ (g)}$$

Digestibility coefficients for other nutrients were calculated by multiplying the dry matter values of feed, orts and faeces by the percentage of nutrients in the feed, orts and faeces on a DM basis respectively, and using the same equation as above (Osuji *et al.*, 1993).

Nitrogen Balance

The data recorded and nitrogen analysis from the feed, orts, faeces and urine was used to determine N intake, N excretion (N in faeces and N in urine) and apparent N retention, thus determining the apparent N balance by following the below calculations adapted from Morgan and Whitmore (unpublished), as cited by McDonald *et al.* (2002).

Daily N intake:

Daily N intake (g/day) = (feed offered (g DM/day) x N in feed (g/kg DM)) – (orts remaining (g DM/day) x N in orts (g/kg DM))

Daily N output:

Daily faecal N (g/day) = faeces collected (g DM/day) x N in faeces (g/kg DM)

Daily urinary N (g/day) = urine collected (g/day) x N in urine (g/kg)

Daily N output (g/day) = daily faecal N (g/day) + daily urinary N (g/day)

Daily N balance:

Apparent N retention (g/day) = daily N intake (g/day) – daily N output (g/day)

Rumen fermentation

From the rumen fluid collections and analysis the following parameters for rumen fermentation were derived:

- Diurnal and average rumen pH
- Average rumen ammonia nitrogen production
- Rumen volatile fatty acid production:
    Average volatile fatty acid production
    Percentage of acetate, propionate, butyrate, iso-butyrate and valerate
    Acetate : Propionate ratio

Microbial nitrogen production

The parameters measured were total purine derivatives (mmol/day) and microbial nitrogen (g N/day). Purine derivitives were used to calculate daily microbial nitrogen supply according to Chen & Gomes (1995). Daily microbial nitrogen production was calculated using the model $y = 0.84x + (0.150 \, BW^{0.75} \, e^{-0.25x})$; where y represented the purine derivatives in the urine and x represented total microbial protein (Chen & Gomes, 1995).

3.3.6 Statistical analysis

The linear statistical model for the 4 x 4 Latin Square design used in this experiment is:

$y_{ij} = \mu + p_i + \gamma_j + T_k + e_{ij}$

where: i, j, k = 1, 2, 3, 4   (Kuehl, 2000)

Where:

y_{ij} = the observation on the experimental unit in the ith row and the jth column of the design

μ = the mean

p_j = the experimental period effect (row effect)

γ_j = the animal effect (column effect)

T_k = the effect of the kth treatment (treatment 1 to 4)

e_{ij} = the random, independent experimental errors with mean 0 and variance σ^2

Data was analysed statistically as a 4 x 4 Latin Square design using the GLM model (SAS, 2017). For repeated period measurements the repeated measures analysis of variance (ANOVA) with the GLM model was used (SAS, 2017). Least square means and standard errors (SE) were calculated. Significance between means was declared at $P < 0.05$ using the Fischers test (Samuels, 1989).

The assumption is that there is no interaction between the treatments and the rows and columns (Kuehl, 2000).

3.4 Feedlot trial – Experiment 2

3.4.1 Animals and processing

In the feedlot trial 210 Mutton Merino lambs (wethers and ewes) with an average mean body weight of 27.6 kg (±4.8 kg) were purchased from a breeder and transported to the University of Pretoria Hatfield experimental farm. Upon arrival the lambs had *ad libitum* access to milled lucerne and *Eragrostis curvula* hay as well as water. Lambs were allowed to rest in a large pen over night.

The next day, following procedures used at commercial feedlots, the lambs were processed (2015, A. Le Riche, Pers. Comm., Cavalier, Private bag X680, Cullinan, 1000). Each individual lamb was assigned and tagged with a unique three digit ear tag. Each lamb was weighed twice using a Tal-Tec (model TT40) livestock scale and the average weight of each individual lamb was then recorded and used for blocking the animals when assigning them to treatments, as described in the experimental design (Section 3.4.2). Lambs were treated for internal parasites with Gardal® 10% (MSD Animal Health, South Africa), which was diluted and administered according to the instructions on the bottle. Lambs were vaccinated against clostridial and pasteurella infections using Multivax-P Plus (MSD Animal Health, South Africa). Each lamb received 2 mL of the vaccine through a subcutaneous injection. The animals did not receive hormonal growth implants. The tails of the lambs were docked to prevent blowfly strikes and reduce urine and faecal soiling. French *et al.* (1994) conducted a controlled field study with over 3000 lambs on seven farms that showed that lambs with

docked tails had consistently lower blowfly strikes than lambs with undocked tails and performance was not different between lambs with docked or undocked tails.

The day after processing lambs were assigned into blocks, pens and treatments as described in the experimental design below. Lambs were tagged with a second coloured ear tag corresponding to their treatment. The colours of the ear tags were red for the control, yellow for treatment one, green for treatment two and blue for treatment three. The lambs were then placed in their assigned pens and began with adaptation to the new high concentrate diet. Of the 210 lambs, ten were outliers and were pulled from the experiment during blocking, thus there were 200 lambs for the trial.

The feedlot trial period was 54 days. The entire trial took place over 72 days; three days for arrival and processing, 14 days for adaptation, 54 days on feed and one day to slaughter the lambs.

3.4.2 Experimental design

The experimental design was a randomised complete block design which was used to control and reduce the experimental error (Kuehl, 2000). Using the individual body weights recorded during processing, the 200 lambs were divided into five homogenous blocks according to weight and gender. Each block, which consisted of 40 lambs, was then divided into four pens of 10 lambs each. Each pen consisted of seven wethers and three ewes. Pens within a block had similar weights (±0.18 kg). Each pen within a block was randomly assigned to one of the four treatments, so that each treatment occurred once within each block. This was done by assigning a different permutation randomly to each block (Kuehl, 2000). This resulted in a complete randomised block design with a total of 20 pens and four treatments, five pens (replications) per treatment. Five replications per treatment were included to make provision for variation in feed intake, feed efficiency and growth performance of animals within a treatment, thus increasing the accuracy of the experiment. The pens were treated as the experimental units (Kuehl, 2000).

Treatments were:
1. 0% CMS (Control)
2. 4% CMS (Treatment 1)
3. 8% CMS (Treatment 2)
4. 12% CMS (Treatment 3)

Figure 3.1 shows the random assignment of treatments to the pens within a block. The average body weight (kg) of each pen which was used to block lambs into homogenous blocks according to weight is displayed in Figure 3.1.

Figure 3.1 The randomised assignment of the treatments in the randomised complete block design

3.4.3 Housing

After the lambs were processed and allocated to their treatment and pens, they were placed in their pens (10 animals per pen). Pens were approximately 13 x 4.5 m in size. There were automatic watering systems and the feed troughs were 4.5 m in length. This allowed 45 cm feeding space per lamb, which was adequate feeding space for each lamb, as seen in Figure 3.2. There was shelter over the feed bunk to prevent feed from getting wet in the event of rain. The shelter also extended into the pen to allow space for lambs to go for shade or protection in the event of adverse weather conditions. Figure 3.3 shows the pens that were used during the trial.

The trial was held from the beginning of July to the middle of September 2016. For the duration of the trial the average minimum temperature was 2.7ºC and the average maximum was 27.3ºC. Since the trial was run from winter and into spring the weather conditions varied from cold to hot with wind, rain and sun.

Figure 3.2 Photo of feedlot lambs eating their pelleted feed from the feed troughs
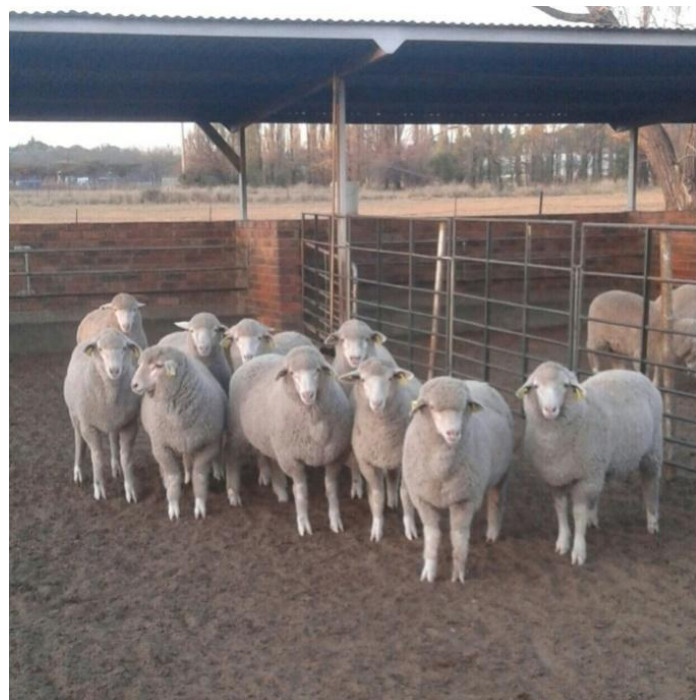
Figure 3.3 Photo of the pens used during the trial

3.4.4 Adaptation/transition period

The lambs were transitioned onto their high concentrate experimental diet over a period of 14 days. They were offered *ad libitum* access to milled *Eragrostis curvula* hay during the first 10 of the 14 days and the TMR feedlot ration was increased by 100 g/day/lamb (1 kg/pen), until a feeding level of approximately 4 - 4.5% of body weight was reached (Voermol, 2010). The adaptation period was required to prevent the

occurrence of metabolic disorders while the ruminal microorganisms were adapting to the increased amount of readily fermentable carbohydrates present in high concentrate diets (Brown *et al.*, 2006). When changing from forage based to a concentrate based diet, there is a shift from fibrolytic to amylolytic bacteria in the rumen, increased VFA production and a drop in ruminal pH, which makes animals susceptible to sub-acute or clinical acidosis if there is an abrupt change in diet without an adaptation period (Bevans *et al.*, 2005). The 14 days adaptation allowed a smooth transition for all lambs.

3.4.5 Feedlot phase

Feed was weighed into buckets on a daily basis and the weight was recorded. Lambs were fed their allocated experimental diet throughout the day to ensure that there was always *ad libitum* fresh feed available to maximise feed intake. The amount of feed offered per pen was recorded. Feed bunk management practices were kept as close as possible to commercial feedlots to reduce variability in feed intake. By decreasing variability in feed intake the risk of decreased performance and metabolic disorders such as acidosis occurring was reduced (Schwartzkopf-Genswein *et al.*, 2003).

Once every seven days orts were weighed back and sampled (Price *et al.*, 2009; Pienaar *et al.*, 2012; Brand *et al.*, 2013; Van de Vyver *et al.*, 2013). Each pen's orts were weighed back and the amount remaining was recorded. The weekly feed intake for each pen was determined by subtracting the amount of feed offered to that pen for the week from the amount of orts remaining in the feed trough at the end of the week, on weigh back day. Orts were sampled from the feed trough by collecting sub-samples from different areas within the feed trough to get a representative sample of the orts. These samples were then placed in an airtight sealed plastic bag, labelled with the pen number, dated and stored at -20ºC in a freezer for further dry matter analysis to determine dry matter intake (DMI).

Once every seven days lambs were weighed using the Tal-Tec (model TT40) livestock scale and each individual lambs' weekly weight was recorded (Price *et al.*, 2009; Pienaar *et al.*, 2012; Brand *et al.*, 2013; Van de Vyver *et al.*, 2013; Whitney *et al.*, 2014). Each pen (consisting of 10 lambs) was individually taken to the crush each week at the same time of day, weighed and returned to their pen directly afterwards so that they still had the remainder of the day to eat, ensuring that this activity did not interfere with their feeding routine and feed intake. The weights of the individual animals within a pen were averaged to get a weekly pen weight and average daily gain (ADG). Using the weekly feed intakes and ADG the weekly feed conversion ratio (FCR) of each pen was calculated.

Management of the animals, feeding and daily activities were kept as close as possible to simulate normal standard feedlot conditions. It was ensured that animals had *ad libitum* access to feed and fresh water. Water troughs were cleaned as required. Each morning and afternoon the pens were walked and animals were observed for discomfort or signs of presenting acidosis, bloat or any other disturbance. Any sick or injured animal was recorded and treated. There was no morbidity or mortality. Faeces were observed for any diarrhoea, digestive disturbances or the presence of internal parasites.

3.4.6 Slaughtering and carcass data collection

After 54 days on feed, lambs were each weighed twice the day before slaughter and the average of the two weights for each lamb was recorded as the final live body weight. Lambs were not fasted or sheared before weighing. The lambs were transported to Cavalier Abattoir (Pty) Ltd; Section 83, Farm Tweefontein, Boekenhoutskloof, Cullinan. All the lambs from the trial were transported to the abattoir and slaughtered together on the same day. Therefore, there were no differences between days on feed or slaughtering processes between blocks, treatments or pens. Lambs were slaughtered following the standard procedures used in South African abattoirs (2016, A. Snyman, Pers. Comm., Cavalier, Private Bag X680, Cullinan, 1000).

Carcasses were tagged with the animals' unique three digit ear tag number, as shown in Figure 3.4, so that the data obtained from the carcass could be traced back to the animal, to ensure that the treatment and pen the animal came from, could be identified. During the slaughtering process the hot carcass weight and carcass classification of each lamb was recorded. Carcass classification was done according to the South African Red Meat Classification System (Webb, 2015), following the Agricultural Product Standards Act (Act 119 of 1990) (National Department of Agriculture, 1990). Livers from selected animals were collected, tagged with the animals' unique three digit ear tag number and frozen at -20ºC in a freezer for further dry matter and copper analysis in the laboratory (Van Ryssen, 2000).

The rumens and remaining livers were also tagged with the animals' unique three digit ear tag number so that they could be observed. Rumens were cut open, the rumen content was emptied and rumens were washed (Rezac *et al.*, 2014a). The rumens were then observed for any damage to the rumen wall or any lesions as this would indicate if incidents of ruminal acidosis had occurred and assist in assessing the nutritional health of the animals (Rezac *et al.*, 2014b). Livers were also observed for any abscesses or abnormalities. No significant observations were made and no problems were encountered, therefore the data from this was not recorded or used. Rezac *et al.* (2014a) reported a significant decrease in performance and an increase in liver abscesses in feedlot cattle with severe rumen lesions. For this reason it was important to include this in the slaughtering process even if the information was not used.

The carcasses were chilled in a fridge at the abattoir. After 24 hours the carcasses were weighed again and cold carcass weight was recorded (Cloete *et al.*, 2004). Using the hot carcass weight and cold carcass weight, chilling loss could be calculated.

Figure 3.4 Photo of the carcasses tagged with the animals unique ear tag number and slaughter information

3.4.7 Laboratory analysis

The dry matter content of the feed and orts was determined following the AOAC (2000) 934.01 procedure. These values were used to calculate dry matter feed intake.

The 40 livers (10 livers per treatment) collected during slaughtering were thawed overnight at 4ºC in a fridge. Samples were taken from pre-determined areas (right and caudate lobe). A 2 g sub-sample was taken from the sample and weighed out into a tinfoil container in duplicate. The initial dry matters of the livers were determined by placing the 2 g sub-samples in an oven at a 105ºC for 24 hours according to the AOAC (2000) procedure 934.01. The remaining amount of each sample was dried in an oven at 55ºC for 48 hours. After 24 hours the livers were rotated so that they dried completely through. Once dried, the samples were then milled using a blender and placed in a container for further analysis. Milled liver samples underwent acid mineral digestion according to the AOAC (2000) procedure 935.13. The copper content of the livers was determined with the Varian SpectrAA atomic absorption spectrometer, using the certified beef liver standard supplied by the China National Centre for Iron and Steel, code number NCS ZC71001 as the control. The second dry matter of the milled liver samples was determined following the AOAC (2000) 934.01 procedure.

3.4.8 Calculations and measured parameters

Using the data collected during the feedlot phase and slaughtering process and the laboratory results, the following calculations were made and parameters derived.

Performance data and feed intake

- Weekly weights were determined by weighing lambs once every seven days. The weight gained for the 54 days on feed was determined by subtracting the final live body weight (before being transported to the abattoir) from the starting live body weight. The weights of the lambs within a pen were averaged to determine the pen weight, as the pens were the experimental units.

Using the data collected and DM results from the laboratory the feed intake and dry matter intake was determined by subtracting the orts from the feed offered.

- Dry matter intake per kilogram metabolic body weight was calculated as demonstrated by Mahgoub *et al.* (2000):

  DMI (g/kgW$^{0.75}$) = DMI (g) / ((lambs weight (kg))$^{0.75}$)

- The average daily gain was calculated weekly, as well as an average for the overall trial using the calculation:

  ADG (kg/lamb/day) = (end weight - initial weight) / number of days

- The feed conversion ratio was calculated weekly as well as an average for the overall trial using the calculation (Beauchemin *et al.*, 1997; Hendriks *et al.*, 2013):

  FCR = (FI or DMI (kg)) / (Weight gain (kg))

Carcass parameters

- Hot carcass weight was determined by weighing the carcasses directly after slaughter and evisceration.

- Cold carcass weight was determined by weighing the carcasses after they had been chilled at -4ºC in a fridge for 24 hours after slaughter (Cloete *et al.*, 2004).

- Dressing percentage was calculated using the following formula:

  Dressing percentage (%) = (hot carcass weight (kg) / final live body weight (kg)) *100
  (Notter *et al.*, 1991; Beauchemin *et al.*, 1997; Avendaño-Reyes *et al.*, 2006; Ekiz *et al.*, 2012)

- Chilling loss percentage was calculated using the following formula:

  Chilling loss (%) = ((hot carcass weight (kg) - cold carcass weight (kg))/ hot carcass weight (kg)) *100 (Rodríguez *et al.*, 2008; López-Campos *et al.*, 2011)

- Carcass classification was done according to the South African Red Meat Classification System which accounts for age, subcutaneous fat thickness and conformation score (Webb, 2015).

Liver data

- Liver DM (%) was determined in the laboratory.
- The liver copper (mg Cu/kg DM) was calculated.

3.4.9 Statistical analysis

The linear model for a randomised complete block design is in this experiment is:

$y_{ij} = μ + T_i + p_j + e_{ij}$

where: i = 1, 2, 3, 4   j = 1, 2, 3, 4, 5   (Kuehl, 2000)

Where:

y_{ij} = the response of the experimental unit with the ith treatment in the jth block

$μ$ = the general mean

T_i = the treatment effect

p_j = the block effect (represents the average deviation of the units in block j from the general mean)

e_{ij} = the experimental error, assumed to be independent with mean 0 and common variance $σ^2$

Data was analysed statistically with the GLM model (SAS, 2017) as a randomised complete block design for the average effects over time. For repeated period measures the repeated measures analysis of variance with the GLM model was used (SAS, 2017). Least square means and standard error were calculated. Significance between means was declared at P <0.05 using the Fischers test (Samuels, 1989). The starting live body weight was included as a covariate against average daily gain and final live body weight.

The pens were treated as the experimental units. There were no interactions between treatments and blocks as the treatment and block effects were assumed to be additive (Kuehl, 2000). The random allocation of treatments to experimental units justifies the assumption of independent experimental error (Kuehl, 2000).

Chapter 4

Results and Discussion
4 x 4 Latin Square design

4.1 Introduction

To access the effect of different inclusion levels of CMS in a high concentrate diet on intake, digestibility, rumen fermentation and microbial nitrogen production, a 4 x 4 Latin Square design trial was conducted. The results of the 88 day 4 x 4 Latin Square design trail will be given. Parameters such as feed intake, apparent total tract digestibility, apparent nitrogen retention, rumen fermentation and microbial nitrogen production will be discussed. There were very few metabolism and rumen fermentation studies that could be found with sheep consuming a high concentrate diet to compare values of this trial with, as most the metabolism and rumen fermentation studies involving sheep were done with sheep receiving poor quality roughages and given supplements to improve the utilization of poor quality roughages. Thus, values would differ with these two types of diets (high forage versus high concentrate) (Galyean & Owens, 1991). Results from trials using various types of CMS at different inclusion levels of concentrate diets fed to ruminants were also compared. What is important to note is that most of the trials reviewed involving CMS were based on diets that were not iso-nitrogenous and/or iso-energetic. In the majority of the reviewed trials CMS is also not replacing molasses, but rather a portion of the concentrate. The type of CMS (sugarcane, beet or citrus) included in the diets also differed between the trials reviewed.

This chapter will begin with the results and discussion of the proximate analysis conducted on the four experimental diets. This analysis of the experimental diet will apply to Chapter 5 as well, as the same experimental diets from the same batch of feed were used to complete both experiments within the trial.

4.2 Experimental diets

The ingredients used in the experimental diets in this trial are representative of ingredients commonly used in the formulation of sheep feedlot diets in South Africa (Smith, 2008; Brand *et al.*, 2013; Van de Vyver *et al.*, 2013; Brand *et al.*, 2017). The main difference was the roughage used in this trial. In common South African sheep feedlot diets the roughage used is lucerne, however in this trial sugarcane bagasse was used, as seen in Table 3.1. Bagasse is the fibrous residue remaining after the sugarcane stalk has been crushed and the juice extracted (Pandey *et al.*, 2000). According to the NRC (2007) sugarcane bagasse contains 86% NDF and 59% ADF, whereas lucerne (mature Alfalfa hay) contains 55% NDF and 45% ADF. The higher NDF and ADF values of the sugarcane bagasse could be the reason for the slightly higher NDF and ADF concentration of the experimental diets in this trial as opposed to the NDF and ADF concentrations commonly formulated for in feedlot diets (2017, U. Muller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380). Crude protein concentrations were within the ranges of common South African feedlot diets (Smith, 2008; Van de Vyver *et al.*, 2013; Brand *et al.*, 2017).

A proximate analysis was conducted on the four experimental diets as described in Section 3.3.4.

Table 4.1 shows the chemical composition of the four experimental diets determined by the proximate analysis.

Table 4.1 Chemical composition of experimental diets differing in inclusion levels of CMS

Nutrients[2]	Treatment[1]			
	0% CMS	4% CMS	8% CMS	12% CMS
Dry matter (g/kg)	900.10	902.10	897.60	894.50
Organic matter (g/kg DM)	927.58	934.13	927.94	911.40
Crude protein (g/kg DM)	157.03	160.92	161.64	163.09
Neutral detergent fibre (g/kg DM)	357.20	353.20	338.60	329.60
Acid detergent fibre (g/kg DM)	197.00	211.00	175.00	173.20
Ether extract (g/kg DM)	43.18	46.46	45.60	44.37
Ash (g/kg DM)	72.42	65.87	72.07	88.60
Calcium (g/kg DM)	12.20	11.22	12.09	10.39
Phosphorus (g/kg DM)	3.88	3.83	3.87	3.46
Sulphur (g/kg DM)	2.44	2.36	2.48	3.29
Potassium (g/kg DM)	6.90	5.74	7.42	9.07
[3]Gross energy (MJ/kg DM)	18.01	18.30	18.32	17.86
[4]Metabolisable energy (MJ/kg DM)	11.70	12.03	12.18	11.98
IVOMD (%DM)	79.20	80.17	81.07	81.84

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3

[2] Nutrients in experimental diet determined by proximate analysis

[3]Gross energy (MJ/kg) - Determined using bomb calorimetry

[4]Metabolisable energy (MJ/kg) = 0.82 x (GExIVOMD) (Robinson *et al.*, 2004)
IVOMD - *in vitro* organic matter digestibility

As shown in Table 4.1, diets were formulated on an iso-nitrogenous and iso-energy basis, so that the effects of different inclusion levels of CMS in the diets could be investigated. The dry matter content of the diets also remained consistent for all experimental diets. The variation in the contribution of nutrients from CMS as opposed to molasses was also taken into account and diets were formulated accordingly, so that all diets had a similar nutrient composition (2017, U. Muller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380). One of the main concerns of replacing molasses with CMS was the high levels of sulphur in CMS (Hannon & Trenkle, 1990; Stemme *et al.*, 2005). This was accounted for in the formulation of the diets; however the 12% CMS diet had a slightly higher sulphur value than the other treatments. The sulphur content of the 12% CMS diet was 3.29 g/kg or 0.329% of the feeds dry matter. This value was slightly higher than the maximum recommended inclusion level of sulphur which is 0.3% of the dry matter content of the feed when a concentrate diet is fed (NRC, 2005). Weiss (2012) stated that dairy cattle may be able to handle diets with up to 0.5% sulphur when fed moderate starch diets. Sheep are usually known to tolerate greater amounts of minerals (López-Campos *et al.*, 2011) and usually have a higher requirement for sulphur than cattle (Suttle, 2010). Therefore, it was assumed that the slightly higher sulphur value in the 12% CMS treatment would not cause a problem. The effect of the high sulphur value was evaluated and will be discussed in Chapter 5.

4.3 The effect of different inclusion levels of condensed molasses solubles on feed intake and apparent total tract digestibility

Table 4.2 shows the feed intake and feed intake per kilogram metabolic body weight calculated on a dry matter basis. Feed intake is a function of metabolic requirements and has been related to metabolic body weight (Van Soest, 1994). Therefore, feed intake per kilogram metabolic body weight was also calculated to account for the differences in size and weight between the four wethers. The water intake of the wethers is also shown.

Table 4.2 The effect of different inclusion levels of CMS on mean (±SE) feed intake, feed intake per kilogram metabolic body weight and water intake in sheep

Parameters	Treatment[1]				$\pm SE$[2]
	0% CMS	4% CMS	8% CMS	12% CMS	
Feed intake DM (g/day)					
DMI	1 050	1 262	1 247	1 187	92.8
OMI	976	1 182	1 158	1 080	85.5
DOMI	713	863	859	831	68.2
Feed intake DM per day (g/kgBW$^{0.75}$)					
DMI	41	47	47	45	3.6
OMI	38	44	43	41	3.3
DOMI	28	32	32	32	2.6
Water intake (l/day)					
Water intake	2.907[b]	3.454[a]	3.363[ab]	3.044[ab]	0.1571

[a,b,c] Means within a row with different superscript differ significantly (P <0.05)

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3

[2]±SE - Standard error

DMI - dry matter intake, OMI - organic matter intake, DOMI - digestible organic matter intake

BW$^{0.75}$ - metabolic body weight

The dry matter intake (DMI), organic matter intake (OMI) and digestible organic matter intake (DOMI), calculated as total intake per day (g/day), did not differ (P >0.05) between treatments. These results were in agreement with Karalazos & Swan (1977) and Chen *et al.* (1981) who also reported no significant differences in dry matter intake or organic matter intake when including either beet or citrus CMS at levels of up to 20% on a DM basis in concentrate diets fed to sheep. Potter *et al.* (1985b) also found no significant differences in dry matter intake when including sugarcane CMS up to levels of 15% on a DM basis to replace corn and/or soybean in a diet fed to cattle. The DMI, OMI and DOMI calculated per kilogram metabolic body weight, also did not differ (P >0.05) between treatments. When digestible organic matter intake was calculated per kilogram metabolic body weight the 4% CMS, 8% CMS and 12 % CMS treatments all had the same intake which was 32 g/kg BW$^{0.75}$, while the 0% CMS treatment tended to have the lowest intake. In this trial it was noted that with the 0% CMS treatment the pellets would break up and crumble, turning into a powdery mash with few pellets remaining intact. This could have been the reason for the tendency of a slightly lower intake in the 0% CMS treatment. In support of this, Casey & Webb (1995) found lower feed intakes in wethers when fed mash as opposed to pelleted diets.

As mentioned, CMS has a high mineral concentration (Leontowicz *et al.*, 1994; Stemme *et al.*, 2005; Fernández *et al.*, 2009). Due to the high mineral content of CMS (especially sulphur and potassium) it could be expected that animals may experience diarrhoea when fed increasing levels of CMS (Leontowicz *et al.*, 1994; Stemme *et al.*, 2005). It is important to note that no wethers experienced any diarrhoea or wet faeces throughout this trial. This was in agreement with Chen *et al.* (1981), Stemme *et al.* (2005) and Yalcin *et al.* (2010) who reported no changes in the faeces of ruminant animals when fed diets containing CMS. Chen *et al.* (1981) reported no laxative effect when citrus CMS was included up to 20% on a DM basis in diets fed to sheep. One of the main homeostatic mechanisms animals use to adapt to varying levels of mineral intake is through urinary excretion of minerals (especially sodium and potassium) (Miller, 1975). In a study, Fisher *et al.* (1994) included potassium in a dairy cow TMR at a low, medium or high level. The TMR contained 1.6%, 3.1% and 4.6% potassium for the low, medium and high levels respectively. Urine output and water intake of the dairy cows consuming the TMR that contained medium and high levels of potassium was significantly higher compared to dairy cows that consumed the TMR with the lower inclusion level of potassium. Due to the high level of minerals in CMS, which could lead to diarrhoea or increased urination, both leading to an increase in water requirements, it was decided that water intake would be an important parameter to evaluate in this trial.

Water intake differed (P <0.05) between the 0% CMS treatment and the 4% CMS treatment. The 0% CMS treatment had a lower (P <0.05) water intake than the 4% CMS treatment. The water intake of the 0% CMS treatment did not differ (P >0.05) from the 8% CMS and 12% CMS treatment. The water intake of the 4% CMS treatment also did not differ (P >0.05) from the 8% CMS and 12% CMS treatment. The only difference (P <0.05) in water intake was between the 0% CMS and 4% CMS treatment and there was no tendency for water intake to increase in the diets containing higher levels of CMS. Thus, the difference in water intake could have been be attributed to the tendency of the 0% CMS treatment to have a lower feed intake than the 4% CMS treatment, rather than due to the differing inclusion levels of CMS. Utley *et al.* (1970) reported that when water intake was restricted there was a decrease in feed intake. Chen *et al.* (1981) reported no significant differences in water intake when diets containing 0%, 10% and 20% citrus CMS on a DM basis were fed to sheep.

Table 4.3 shows the apparent total tract dry matter and organic matter digestibility.

Table 4.3 The effect of different inclusion levels of CMS on mean (±SE) apparent total tract digestibility in sheep

Parameters	Treatment[1]				±SE[2]
	0% CMS	4% CMS	8% CMS	12% CMS	
Dry matter digestibility (%)	70.52[ab]	69.83[b]	72.33[ab]	73.54[a]	1.061
Organic matter digestibility (%)	73.56[b]	73.17[b]	74.98[ab]	77.05[a]	1.000

[a,b,c] Means within a row with different superscript differ significantly (P <0.05)

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3
[2]±SE - Standard error

There were no differences (P >0.05) between the 0% CMS, 8% CMS and 12% CMS treatments, as well as the 0% CMS, 4% CMS and 8 CMS% treatments for apparent total tract dry matter digestibility (DMD). The DMD of the 4% CMS treatment differed (P <0.05) from the 12% CMS treatment. The 12% CMS treatment had a higher (P <0.05) DMD than the 4% CMS treatment. The apparent total tract organic matter digestibility (OMD) of the 0% CMS and 4% CMS treatments differed (P <0.05) from the 12% CMS treatment, with the 12% CMS treatment having a higher (P <0.05) OMD than the 0% CMS and 4% CMS treatments. However, there were no differences (P >0.05) between the 0% CMS, 4% CMS and 8% CMS diets, as well as the 8% and 12% CMS diets for OMD.

Galyean & Owens (1991) stated that as the level of intake increases, digestibility tends to decrease. It was not expected that there would be differences (P <0.05) in digestibility between the treatments in this trial, as the intake between treatments did not differ (P >0.05). A possible reason for the significant differences in digestibility between the treatments could be due to the differing NDF and ADF concentrations in the diets. An increase in the fibre content of the diet leads to a decrease in the digestibility (McDonald *et al.*, 2011). As seen in Table 3.1 the inclusion of bagasse was decreased slightly as the levels of CMS increased. Given that bagasse has a very high NDF and ADF content (NRC, 2007), as discussed earlier in this chapter, the NDF and ADF content of the diets increased with the slightly higher inclusion levels of bagasse. In the 4% CMS treatment the inclusion level of bagasse was 15.20% on an as is basis and the ADF concentration was 211.00 g/kg DM, while in the 12% CMS treatment the inclusion of bagasse was 13.40% on an as is basis and the ADF concentration was 173.20 g/kg DM. The DMD was 69.83% and 73.54% and the OMD was 73.17% and 77.05% for the 4% CMS and 12% CMS treatments respectively. The 0% CMS and 4% CMS diets have a higher NDF and ADF content. This could be a possible reason for the lower (P <0.05) DMD for the 4% CMS and the lower (P <0.05) OMD for the 0% CMS and 4% CMS treatments.

Yang *et al.* (2001) reported that when increasing the amount of forage in the total mixed ration for dairy cows, and thus increasing the concentration of ADF in the diet from 17.4% to 23.9% and NDF from 31.7% to 37.8%, the apparent total tract dry matter digestibility and organic matter digestibility decreased significantly. Apparent total tract dry matter digestibility decreased from 63.2% to 59.1% and organic matter digestibility decreased from 64.7% to 60.7%. Thus, we can assume that in this study the possibility of the lower DMD and OMD was due to the increasing NDF and ADF concentration of the diet.

In contrast to this trial, Karalazos & Swan (1977) reported no significant differences in digestibility when beet CMS was included up to 20% on a DM basis in the diets of sheep. The values ranged from 73.0% - 73.6% for DMD and 75.7 – 76.6% for OMD. In this trial values ranged from 69.83 – 73.54% for DMD and 73.17 – 77.05% for OMD. Therefore, even though there were differences (P <0.05) in this current trial, the digestibility values still fell within range of that reported by Karalazos & Swan (1977).

4.4 The effect of different inclusion levels of condensed molasses solubles on rumen fermentation

Table 4.4 shows the results obtained during the rumen fermentation study. Results from the rumen pH, volatile fatty acid production and rumen ammonia nitrogen are shown.

Table 4.4 The effect of different inclusion levels of CMS on mean (±SE) rumen fermentation parameters in sheep

Parameters	Treatment[1]				±SE[2]
	0% CMS	4% CMS	8% CMS	12% CMS	
Average rumen pH	5.92	5.75	5.77	5.76	0.086
Average NH$_3$-N (mg NH$_3$-N/100ml)	19.74	20.01	16.74	15.19	1.410
VFA production					
Total VFA (mmol/l)	81.32[ab]	89.29[ab]	79.96[b]	96.39[a]	4.594
Acetate (%)	58.52	53.99	54.46	53.15	2.123
Propionate (%)	22.20	25.09	24.84	25.34	3.127
Butyrate (%)	15.04	16.15	15.67	17.17	1.738
Isobutyrate (%)	1.23	1.13	1.32	1.23	0.068
Valerate (%)	1.91	2.80	2.68	2.39	0.406
Acetate:Propionate ratio	2.70	2.24	2.32	2.40	0.288

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

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3

[2]±SE - Standard error

VFA - volatile fatty acids, NH$_3$N - ammonia nitrogen

Rumen pH

There were no differences ($P > 0.05$) in the average rumen pH between any of the treatments. These results were in agreement with Karalazos & Swan (1977) that also reported no significant differences in the rumen pH when a concentrate diet was fed to wethers containing either no molasses and beet CMS (control), 20% molasses or 20% beet CMS on a DM basis. In that trial the pH values reported were 5.42, 5.49 and 5.60 for the control, 20% molasses and 20% beet CMS diets respectively. These were slightly lower than the average rumen pH values in this trial which were 5.92, 5.75, 5.77 and 5.76 for the 0% CMS, 4% CMS, 8% CMS and 12% CMS treatments respectively; however the sheep in that trial where fed a barley based diet where in this trial a maize based diet was fed (Table 3.1). Barley is highly fermentable in the rumen, whereas maize has a slower rate of fermentation in the rumen (Ørskov, 1986; Nikkhah, 2012), therefore the inclusion of barley in a diet usually results in a lower rumen pH as opposed maize.

The 4% CMS, 8% CMS and 12% CMS treatments had a similar average rumen pH (5.75-5.77), with the 0% CMS treatment tending to have a slightly higher average rumen pH. This could be due to the animals in the 0% CMS treatment tending to display a lower feed intake. In agreement with this, Robinson *et al.* (1986) conducted a study on Fresian cows and found that a decrease in feed intake resulted in a direct increase in rumen pH. Rumen pH values rose from 5.99 to 6.27 when cows consumed 24.0 kg/day and 19.0 kg/day respectively. An increase in feed intake of a high concentrate diet leads to an increase in the amount of fermentable carbohydrates in the rumen and a decrease in pH (Owens & Goetsch, 1988).

There is a considerable fluctuation in the rumen pH over a 24 hour period which is influenced by the intake of fermentable carbohydrates (Zebeli *et al.*, 2010). Figure 4.1 shows the fluctuation in rumen pH of wethers over a 24 hour period. There was a similar pattern between all the treatments with the rumen pH dropping after feeding, as the VFA's accumulate, and reaching a peak at around midnight. Feeding took place at 08:00 and 16:00 and the drop in rumen pH was noted at the 09:00 and 18:00 collections respectively.

The main symptom of sub-acute acidosis is variable and reduced feed intake (Nocek, 1997; Owens *et al.*, 1998; Cooper *et al.*, 1999). There is no agreement on the definition or exact pH values defining sub-acute ruminal acidosis (Plaizier *et al.*, 2008). A rumen pH of 5.6 and 5.2 are usually the values considered for sub-acute acidosis and clinical acidosis respectively (Owens *et al.*, 1998). Gozho *et al.* (2005) defined sub-acute ruminal acidosis as a set time that the rumen pH remains between 5.2 – 5.6. Enemark (2009) used a threshold pH of 5.5 as an indicator for sub-acute ruminal acidosis. In this current trial the rumen pH was only taken at set times of three hour intervals during rumen fluid collections and was not recorded at minute intervals using a submersible pH meter and data logger. Thus, the time that the rumen pH was between a pH of 5.2 and 5.6 could not be accurately predicted. Therefore, a value of pH 5.5 or less was used to indicate sub-acute acidosis. As seen in Figure 4.1, the only treatment that reached a pH of 5.5 or below was the 4% CMS treatment. The 4% CMS treatment only dropped to a pH level of 5.5 twice, at 09:00 and 18:00, which was probably due to those times being the collections after feeding at 08:00 and 16:00, but the pH did not remain below 5.5. It is assumed that sub-acute acidosis did not present a problem in any of the treatments after analysing these results of the rumen pH and feed intake.
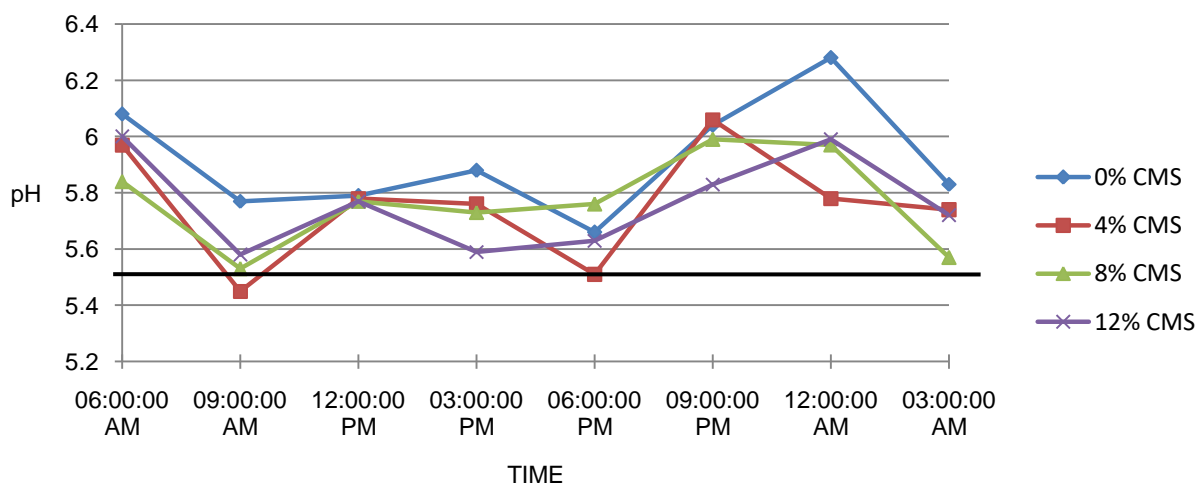
Figure 4.1 The rumen pH of sheep over a 24 hour period fed different inclusion levels of CMS

Volatile fatty acids
The total volatile fatty acid production differed (P <0.05) between the 8% CMS treatment and the 12% CMS treatment. There were however no differences (P >0.05) between the 0% CMS, 4% CMS and 8% CMS treatments, as well as no differences (P >0.05) between the 0% CMS, 4% CMS and 12% CMS treatments. The difference (P <0.05) between the 8% CMS and 12% CMS treatments was not expected as the dry matter intake (in Table 4.2) and rumen pH (in Table 4.4) showed no differences (P >0.05) between these two

treatments. It could be suggested that the lower VFA production in the 8% CMS could have been due to an experimental period effect. Thus, due to one experimental period having significantly different values the average mean value for that treatment became significantly different. It was noted that during the fourth experimental period the wether on the 8% CMS treatment had a lower intake and slightly higher rumen pH value when compared to the other wethers and the values obtained in the previous experimental period from that wether. When the data was statistically analysed and it was noted that 8% CMS treatment had a lower (P <0.05) total VFA production, the raw data from the trial and laboratory analysis was reviewed. When reviewing the data, total volatile fatty acid production for the 8% CMS treatment over the four experimental periods was 92.45 mmol/L, 86.41 mmol/L, 82.30 mmol/L and 58.70 mmol/L for experimental period 1, 2, 3 and 4 respectively. It can be assumed that the lower total VFA production may have been due to the wether in round 4 (who specifically in that round had a lower intake) rather than a treatment or diet effect. It was expected that the 0% CMS treatment would have had a slightly lower VFA production as there was less substrate present in the rumen for fermentation due to a slightly lower feed intake, this was not the case.

Karalazos & Swan (1977) reported no significant differences in total VFA production between sheep fed diets with no molasses and beet CMS (control), 20% molasses or 20% beet CMS on a DM basis. In agreement with Karalazos & Swan (1977), Chen *et al.* (1981) found no significant differences when sheep where fed diets containing 0%, 10% or 20% citrus CMS on a DM basis. Karalazos & Swan (1977) reported VFA values that ranged from 75.1 – 86.9 mmol/L and Chen *et al.* (1981) reported values from 78.3 – 85.0 mmol/L; while in this trial values ranged from 79.96 – 96.39 mmol/L. Even though the values reported in this trial had a wider range, the values were still in agreement with Karalazos & Swan (1977) and Chen *et al.* (1981). Potter *et al.* (1985b) also reported no significant differences in the total volatile fatty acid production when cattle were fed a finishing diet containing either 0%, 5%, 10% or 15% sugarcane CMS.

There were no differences (P >0.05) between any of the treatments for the proportions of acetate, propionate, butyrate, isobutyrate, valerate or isovalerate. This is in agreement with Karalazos & Swan (1977) who also reported no significant differences in the proportions of acetic, propionic and butyric acid when beet CMS was included in the diets up to 20% on a DM basis. In contrast, Chen *et al.* (1981) reported a significant decrease in the proportion of acetic acid and an increase in the proportion of propionic acid and valeric acid with no differences reported in butyric acid when citrus CMS was included in the diet at 0%, 10% and 20% on a DM basis. Potter *et al.* (1985b) reported an increase in acetic and a decrease in propionic acid when feeding cattle diets containing up to 15% sugarcane CMS. The reasons for the differences between the trials were not accounted for.

The acetate to propionate ratio also did not differ (P >0.05) between treatments. The acetate to propionate ratio was in accordance with other results from sheep fed high concentrate diets (Merchen & Berger, 1985). The 0% CMS treatment tended to have the highest acetate to propionate ratio. This was expected as the 0% CMS treatment had a tendency for a slightly higher pH. A lower pH leads to a shift from acetate to propionate producing bacteria in the rumen (Thomas & Rook, 1981). Thus, there is an increase in propionate production at the expense of acetate production (Schelling, 1984). Russell, (1998) reported that

when the rumen pH decreased from 6.5 – 5.3, the acetate to propionate ratio decreased from 1.2 – 0.6, when cracked maize was incubated *in vitro.*

Rumen ammonia nitrogen

The rumen ammonia nitrogen values did not differ (P >0.05) between treatments. There was a large range between average rumen ammonia nitrogen concentrations (15.19 - 20.01 mg NH_3N/100ml). The rumen ammonia nitrogen values were 19.74, 20.01, 16.74 and 15.19 mg NH_3N/100 mL for the 0% CMS, 4% CMS, 8% CMS and 12% CMS treatments respectively. Ammonia released from non-protein nitrogen or the degradation of feed protein in the rumen cannot be converted to microbial protein unless a source of fermentable energy is available (Bartley & Deyoe, 1981). An increase in ammonia concentration in the rumen is expected if a soluble protein or NPN source is consumed and there is not enough fermentable energy for microbial protein production (Bartley & Deyoe, 1981; Detmann *et al.,* 2014). It is possible that the slightly higher NDF and ADF value (thus lower fermentable carbohydrates) in the 0% and 4% CMS treatment (due to the higher bagasse and/or slightly lower maize meal as shown in Table 3.1) could be the reason for the tendency of the 0% CMS and 4% CMS treatment to have a slightly higher ammonia nitrogen value, as the amount of degradable protein may have exceeded the amount of available fermentable carbohydrates, and the excess degradable protein was converted into ammonia nitrogen (McDonald *et al.,* 2011). In the case of the 8% and 12% CMS treatments it could be possible that there was an increase in the amount of fermentable carbohydrates, so more ammonia nitrogen was converted into microbial protein, thus the ammonia concentration still increased due to microbial activity, but not as much as the 0% and 4% CMS treatments (Karalazos & Swan, 1977; Bartley & Deyoe, 1981).

Karalazos & Swan (1977) reported a significant increase in rumen ammonia nitrogen for diets containing 20% beet CMS on a DM basis. Ammonia nitrogen values were 5.50, 11.6 and 29.0 mg/100ml for sheep fed concentrate diets with inclusions of no molasses and beet CMS (control), 20% molasses or 20% beet CMS respectively. The author hypothesized that the differences in the rumen ammonia nitrogen was due to the difference in the crude protein content of the diet containing beet CMS due to the high N levels in CMS, as diets were not formulated on an iso-nitrogenous basis. The crude protein levels in the diets were 12.6, 13.6 and 21.5% of the diets DM for the 0% beet CMS and molasses, 20% molasses and 20% beet CMS diets respectively.

4.5 The effect of different inclusion levels of condensed molasses solubles on apparent nitrogen balance

Table 4.5 shows the apparent nitrogen retention of wethers which was calculated as the difference between nitrogen intake and nitrogen output (McDonald *et al.,* 2002). Total nitrogen excretion, as well as faecal and urinary nitrogen excretion was calculated.

Table 4.5 The effect of different inclusion levels of CMS on mean (±SE) apparent nitrogen balance in sheep

Parameters	Treatment[1]				±SE[2]
	0% CMS	4% CMS	8% CMS	12% CMS	
N intake (g N/day)	26.40	32.71	32.40	31.26	2.424
N excretion (g N/day)	23.43	24.95	28.07	25.45	1.420
N faeces (g N/day)	6.05	7.28	7.22	6.91	0.495
N urine (g N/day)	17.38[b]	17.67[ab]	20.85[a]	18.54[ab]	1.000
Apparent N retention (g N/day)	2.97	7.77	4.33	5.81	1.719

[a,b,c] Means within a row with different superscript differ significantly (P <0.05)
[1]Treatment - Percentage of CMS on an as is basis included in experimental diets
0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3
[2]±SE - Standard error
N – nitrogen

There were no differences (P >0.05) observed between treatments for nitrogen intake. This was to be expected as the experimental diets were formulated on an iso-nitrogenous basis and feed intake did not differ (P >0.05) between treatments.

Faecal nitrogen excretion did not differ (P >0.05) between treatments. For urinary nitrogen excretion there were no differences (P >0.05) between the 0% CMS, 4% CMS and 12% CMS treatments, as well as no differences (P >0.05) between the 4% CMS, 8% CMS and 12% CMS treatments. The urinary nitrogen excretion differed (P <0.05) between the 0% CMS treatment and the 8% CMS treatment. The 0% CMS treatment had a lower (P <0.05) urinary nitrogen than the 8% CMS treatment. The reason for a higher urinary nitrogen excretion is usually as a result of a higher nitrogen/crude protein intake (Merchen, 1988). Devant *et al.* (2000) compared high and low protein concentration cattle rations and reported that the high protein treatments had a significantly higher urinary nitrogen excretion as opposed to the low protein treatments, with urinary nitrogen excretion being 40.6 and 21.7 g N/day respectively. This was probably not the case in this trial as diets were iso-nitrogenous and there were no differences (P >0.05) in nitrogen intake between treatments. A possibility for the higher urinary nitrogen excretion in this trial was probably due to the increased quantity of urine produced by the 8% CMS treatment. As seen in appendix A, the 0% CMS treatment had a lower (P <0.05) urinary output than the 8% CMS treatment. Therefore, the higher urinary nitrogen excretion in the 8% CMS treatment was possibly due to the increased amount of urine produced, as urinary nitrogen excretion is calculated as daily urinary N (g/day) = urine collected (g/day) x N in urine (g/kg) (from Morgan & Whitmore (unpublished), as cited by McDonald *et al.*, 2002). In appendix A it can also be noted that the 4% CMS treatment had a lower (P <0.05) urinary excretion than the 8% CMS treatment as well, but the urinary N excretion in the 4% treatment does not differ (P >0.05) from the 8% CMS treatment. The reason for this was probably due to the tendency of a slightly higher N intake of the 4% CMS compared to the 0% CMS treatment. In Table 4.5 it is noted that the 0% CMS treatment had a nitrogen intake of 26.40 g/day, whereas the 4% CMS treatment had a nitrogen intake of 32.71 g/day, therefore it is expected that there will be a slightly higher urinary N excretion from the 4% CMS treatment even if the quantity of urine was slightly lower. As mentioned earlier, in most circumstances as the amount of nitrogen intake increases, the amount of nitrogen excreted in the urine increases (Merchen, 1988). The total amount of nitrogen excreted did not differ (P >0.05) between treatments.

The apparent nitrogen retention did not differ (P >0.05) between treatments. This was in agreement with the study of Karalazos & Swan (1977) who also found no significant differences in the nitrogen balance when sheep were fed a high concentrate diet containing either no molasses and beet CMS (control), 10% molasses, 20% molasses, 10% beet CMS or 20% beet CMS on a DM basis. The author reported values of 5.11, 3.98, 5.48, 5.51 and 4.62 g N/day for the control diet, 10% molasses, 20% molasses, 10% beet CMS and 20% beet CMS diets respectively. These are within range of the values from this trial which were 2.97, 7.77, 4.33 and 5.81 for the 0% CMS, 4% CMS, 8% CMS and 12% CMS diets respectively. Cole (1999) reported that an increase in nitrogen intake resulted in a direct increase in nitrogen retention when feeding lambs concentrate diets with differing levels of protein. That trend was seen in this trial.

Chen *et al.* (1981) also reported no significant differences for the nitrogen intake, faecal nitrogen or nitrogen balance of lambs fed diets containing 0%, 10% or 20% citrus CMS on a DM basis. The author also reported differences in urinary nitrogen; however in contradiction to this study they reported significantly lower urinary nitrogen excretion with increasing levels of CMS.

4.6 The effect of different inclusion levels of condensed molasses solubles on microbial nitrogen production in the rumen

Table 4.6 The effect of different inclusion levels of CMS on mean (±SE) purine derivative production and rumen microbial nitrogen production in sheep

Parameters	Treatment[1]				±SE[2]
	0% CMS	4% CMS	8% CMS	12% CMS	
Total purine derivatives (mmol/day)	18.08	51.64	11.17	20.51	11.416
Microbial N (g N/day)	15.86	42.59	10.25	13.48	10.368

[a,b,c] Means within a row with different superscript differ significantly (P <0.05)

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3

[2]±SE - Standard error

N - nitrogen

There were no differences (P >0.05) in total purine derivatives or microbial nitrogen production between the treatments. The high standard error, lack of significant differences and the value of the 4% CMS treatment (42.59 g/day), which is much higher than the rest of the treatments may have been due to experimental error. To analyse the purine derivatives and determine the microbial nitrogen in the rumen a total urine collection was done according to Chen & Gomes (1995). The rumen fluid collections took place during the same period that the urine was being collected. When opening and closing the rumen cannulae for collections or when the sheep lay down the rumen cannulae may have leaked, the rumen fluid would have fallen in the total urine collection and increased the amount of microbes in the urine. Thus, when analysing the urine for purine derivatives and calculating microbial nitrogen production, the microbes may have not only been from the urine but also originated from the rumen fluid which leaked into the urine pan where the urine was being collected. This could be a potential reason for the higher purine derivative and microbial nitrogen values obtained in the 4% CMS treatment.

The purine derivative and microbial nitrogen values of the 0% CMS, 8% CMS and 12% CMS were within range of values reported for sheep fed TMR diets. Ramos *et al.* (2009) reported microbial nitrogen values of 14.4 g N/day and 16.9 g N/day when Merino sheep were fed a high concentrate diet containing either grass hay or alfalfa hay as the roughage. Ma *et al.* (2014) fed four sheep either a 40:60 or 60:40 concentrate:forage diet with either a high or low level of undegradable dietary protein and reported microbial nitrogen values of 11.75 - 17.02 g N/day.

No studies conducted with ruminants fed high concentrate diets with inclusions of CMS and the effect on purine derivative excretion or microbial nitrogen production were found. Fernández *et al.* (2009) reported microbial nitrogen values of 7.7 g N/day and 6.3 g N/day when sheep were fed sugar beet pulp containing 0% beet CMS or 13% beet CMS on a DM basis respectively. It was suggested that those results were not compared to this study as sugar beet pulp is a roughage and the animals in this trial were fed a concentrate based diet. Studies have shown that when the concentrate:roughage ratio of a diet increases, so does the microbial nitrogen (Ramos *et al.*, 2009; Ma *et al.*, 2014). This is possibly due to the decreased amount of fermentable carbohydrates present in roughage as opposed to a high concentrate diet (Ma *et al.*, 2014).

Chapter 5

Results and Discussion
Feedlot Trial

5.1 Introduction

In order to access the effects of different inclusion levels of condensed molasses solubles in a high concentrate diet in a practical application, a feedlot trial was conducted. This chapter will cover the results obtained during the 54 day feedlot trial, slaughtering process and liver analysis. Parameters such as growth performance, feed intake, feed conversion ratio, carcass characteristics and liver analysis will be discussed. A copper analysis was conducted on the livers of the lambs to assess if there were any effects due to the high levels of sulphur in the CMS. The results obtained from this trial were compared to other South African lamb feedlot trials with animals fed feedlot diets of a similar nutrient composition. Results from trials using various types of CMS at different inclusion levels of concentrate diets fed to sheep or cattle were also compared. What is important to note is that most of the trials reviewed involving CMS were based on diets that were iso-nitrogenous but not iso-energetic. Therefore, the results reported tend to be more a result of the differing energy contents between the diets. In the majority of the reviewed trials CMS is also not replacing molasses but rather a portion of the concentrate. The types of CMS included in the diets also differed between the trials reviewed. The chemical analysis of the feed used in the trial was discussed in Chapter 4, so it will not be discussed again here as the same batch of experimental feed was utililised.

5.2 The effect of different inclusion levels of condensed molasses solubles on growth performance, feed intake and feed efficiency

Table 5.1 The effect of different inclusion levels of CMS on mean (±SE) growth performance, feed intake and feed conversion ratio of feedlot lambs

Parameters	Treatment[1]				±SE[2]
	0% CMS	4% CMS	8% CMS	12% CMS	
Growth performance					
Starting live body weight (kg)	31.13[ab]	30.89[ab]	31.39[b]	30.53[a]	0.213
Final live body weight (kg)	45.74[b]	47.26[a]	47.12[ab]	46.34[ab]	0.452
Weight gained (kg)	14.61[b]	16.37[a]	15.73[ab]	15.81[ab]	0.392
ADG	0.271[b]	0.303[a]	0.291[ab]	0.293[ab]	0.0073
Feed intake/day/lamb					
Feed intake, as is basis (kg)	1.664	1.720	1.689	1.677	0.0250
Dry matter intake (kg)	1.498	1.552	1.516	1.501	0.0224
Dry matter intake (g/kg $BW^{0.75}$)	96.33	98.23	96.00	96.65	0.969
FCR					
FCR (as is basis)	6.16[b]	5.68[a]	5.81[a]	5.73[a]	0.112
FCR (dry matter basis)	5.54[b]	5.12[a]	5.21[a]	5.13[a]	0.101

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

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3

[2]±SE - Standard error

ADG - average daily gain (kg/lamb/day), $BW^{0.75}$ - metabolic body weight, FCR – feed conversion ratio

Table 5.1 summarises the growth performance, feed intake and feed conversion ratio of the feedlot lambs recorded during the 54 day feeding period.

Starting live body weight

The starting live body weights of lambs at the commencement of the 54 day feeding trial were very similar (31.0 kg ± 0.45 kg). There were no differences (P >0.05) in the starting live weight between the 0% CMS, 4% CMS and 8% CMS treatments, as well as no differences (P >0.05) between the 0% CMS, 4% CMS and 12% CMS treatments. The weights of the lambs in the 8% CMS treatment differed (P <0.05) from the weight of the lambs in the 12% CMS treatment, with the 8% CMS treatment having a higher starting live body weight. This had no effect on any of the results, as the starting live body weight was included as a covariate against average daily gain and final live body weight. The dry matter intake per kilogram metabolic body weight was also calculated to account for this significant difference in the starting live body weight.

Average daily gains and body weight gained

The mean body weight gained per lamb in this trial was 15.88 kg which is in agreement with a study by Sheridan *et al.* (2003) who reported a body weight gain of 15.7 kg over a 56 day feeding period when South African Mutton Merinos were fed a high energy diet (12.7 MJ/kg). The mean average daily gain for this trial was 0.290 kg/lamb/day which is in agreement with the findings of Sheridan *et al.* (2003) that reported an ADG of 0.281 kg/lamb/day. The results for the average daily gains in this trial are comparable to the results of other South African feedlot studies. Gouws *et al.* (2016) reported a maximum ADG of 0.302 kg/lamb/day and a minimum ADG of 0.289 kg/lamb/day when lambs were fed a feedlot diet, while in this trial the maximum ADG was 0.303 kg/lamb/day and the minimum ADG was 0.271 kg/lamb/day. Price *et al.* (2009) reported average daily gains that ranged from 0.298 – 0.340 kg/lamb/day when South African Mutton Merino lambs were fed a feedlot finisher diet. The higher average daily gains reported by Price *et al.* (2009) could be due to the lambs in that study receiving hormonal growth implants (Zeraplix) at the start of the study, as opposed to the lambs in this study who did not receive hormonal growth implants. In a study by Allen (2015), feedlot lambs that received Zeranol implants tended to have higher average daily gains than those that did not receive implants.

There were differences (P <0.05) in body weight gained and average daily gains between the 0% CMS treatment and the 4% CMS treatment, with the 0% CMS treatment having a lower (P <0.05) body weight gain and average daily gain compared to the 4% CMS treatment. Body weight gained and average daily gains of the 0% CMS treatment did not differ (P >0.05) from the 8% CMS and 12% CMS treatments. The body weight gained and average daily gains of the 4% CMS treatment also did not differ (P >0.05) from the 8% CMS and 12% CMS treatments. Figure 5.1 shows the average cumulative body weight gain over the 54 day feeding period. From day 21 the 0% CMS treatment has a tendency for the lowest average gain and the 4% CMS treatment for the highest average gain, with the trend remaining consistent throughout the remainder of the 54 days.
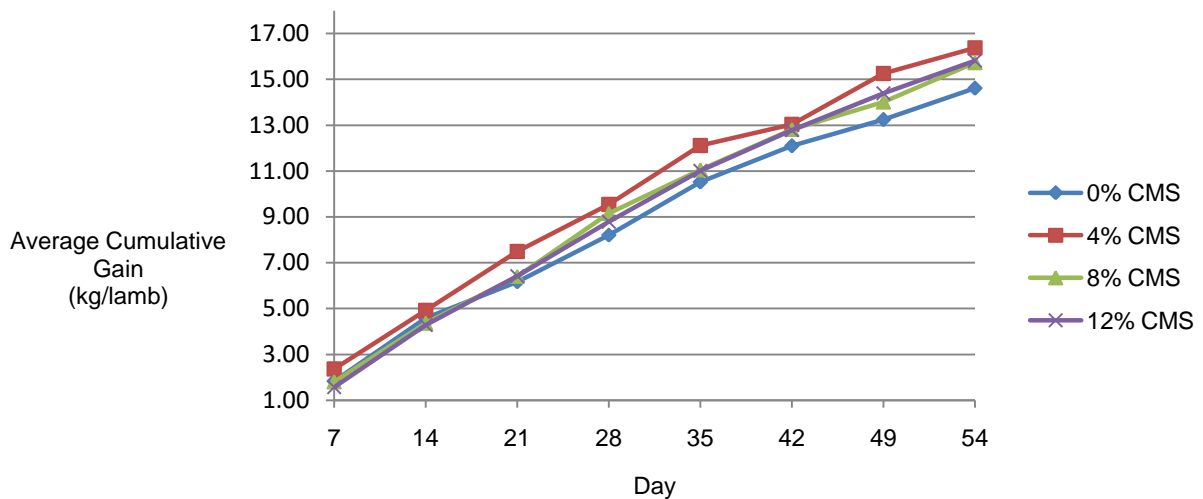
Figure 5.1 The average cumulative body weight gain of feedlot lambs fed different inclusion levels of CMS over the 54 day feeding period

The results from this trial contradict other trails where various types of condensed molasses solubles were included at different levels in the concentrate diets fed to ruminants. López-Campos *et al.* (2011) did a study with Merino lambs fed 0%, 10% or 20% beet CMS on an as is basis as a replacement for molasses and found a decrease in ADG as inclusion levels of CMS increased. The author hypothesized that the decrease in daily body weight gains could have been due to the reduction in energy in the diets containing CMS. The diets were not formulated to account for the different supply of nutrients in CMS, resulting in the energy, protein and mineral contents differing between diets (López-Campos *et al.,* 2011). Potter *et al.* (1985b) replaced maize and/or soybean meal with 0%, 5%, 10% and 15% sugarcane CMS on a DM basis in diets fed to steers and reported a tendency for decreasing average daily gains when CMS was included. Potter *et al.* (1985a) found that when feeding steers finishing diets with 5% sugarcane CMS, 5% sugarcane molasses or a 5% sugarcane CMS and sugarcane-molasses mixture on a DM basis there were no significant differences in average daily gain. However, when steers were fed diets containing 0% or 10% sugarcane CMS on a DM basis, average daily gains decreased for steers fed the 10% CMS diets (Potter *et al.*, 1985a). Tillman & Kidwell (1951), Chen *et al.* (1981), Leontowicz *et al.* (1994) and Yalcin *et al.* (2010) found no significant differences in average daily gains of cattle fed diets containing CMS. The reason for the differing results in these reviewed studies may be that most of the diets were formulated on an iso-nitrogenous basis, but it was not stated if diets were formulated on an iso-energetic basis or accounted for the different contribution of nutrients that CMS provides. Condensed molasses soluble tends to be low in metabolisable energy (Weigand & Kirchgessner, 1980; Waliszewski *et al.*, 1997). Therefore, if authors did not take this into account the results may not be as a result of the inclusion of CMS but rather of varying nutrient composition between diets. Leontowicz *et al.* (1994) stated that in fattening bulls replacing 40% of the concentrate with beet CMS did not significantly affect the body weight gains; however, the slightly lower body weight gains and significantly lower dressing percentages and fat deposition in the bulls fed beet CMS was due to the deficient energy in the diets containing CMS. In this current trial diets were based on an iso-nitrogenous and iso-energetic basis with only the inclusion levels of CMS differing, as opposed to the above reviewed studies

which could explain the differences in the average daily gains and body weight gains obtained. Another reason for the contradiction in results from this trial could be that in the reviewed trials CMS was not always replacing one ingredient consistently throughout all the studies. For example, Potter *et al.* (1985a) replaces molasses with sugarcane CMS, but Potter *et al.* (1985b) replaced corn and/or soybean meal with sugarcane CMS. The type of CMS used in the reviewed trials was also of different origins (sugarcane, beet or citrus), which means that they had different compositions and this could have contributed to the contradiction of results between trials.

For efficient production high levels of voluntary intake are necessary, as generally the more the animal eats the more it can produce (Forbes, 2000). Therefore, we can expect animals with a higher growth rate to consume more feed. Since there were no differences (P >0.05) in feed intake between the treatments and the dry matter intakes were very similar (1.525 kg ± 0.027 kg) as shown in Table 5.1, it was expected that there would be no differences in ADG and body weight gained between treatments, since the treatments were formulated on an iso-nitrogenous and iso-energetic basis. As seen above, after reviewing studies of cattle and sheep fed concentrate diets with varying inclusion levels of CMS, it was not expected that an increase would be seen in ADG and body weight gains of lambs fed treatments containing CMS as opposed to molasses. However, as shown in Table 5.1 the 0% CMS treatment had a lower (P <0.05) body weight gain and average daily gain than the 4% CMS treatment. In this trial it was noted that with the control diet (0% CMS) the pellets would break up and crumble and not keep its structure, turning into a powdery mash feed with few pellets remaining intact. This allowed the lambs fed the 0% CMS treatment to select feed as it was often crumbles of feed. Thus, instead of eating the entire pellet they could select certain parts of the feed (NRC, 2007). The pellet quality of the other treatments (4% CMS, 8% CMS and 12% CMS) was superior and pellets did not crumble or deteriorate. In studies comparing mash and pelleted diets; increased growth rates, increased feed intake and improved feed efficiencies have been noted when pelleted diets were fed to feedlot lambs as opposed to ground/mash diets (Esplin *et al.*, 1957; Fontenot & Hopkins, 1965; Casey & Webb, 1995). It was hypothesized that this was due to lambs being unable to select specific feed components within the diet (Casey & Webb, 1995). In a study using high and low energy diets in a pelleted and mash form, Casey & Webb (1995) reported that there were significant differences in the average daily gains when wethers were fed pelleted as opposed to mash diets. The mean ADG of the wethers fed the pelleted diets was 0.283 kg/wether/day and the mean ADG of the wethers fed the mash diets was 0.204 kg/wether/day. Therefore, a reason for the lower (P <0.05) average daily gain and body weight gained in the 0% CMS treatment compared to the 4% treatment could be due to the poor pellet quality and lambs selecting certain feed components within the experimental diet.

Final live body weight
The mean final live body weight of the lambs at the end of this 54 day trial before they were slaughtered was 46.62 kg and ranged between 45.74 − 47.26 kg as seen in Table 5.1. These results are in agreement with common South African values for final live body weights of South African Mutton Merino lambs before slaughter (Gouws *et al.*, 2016; 2017, A. Le Riche, Pers. Comm., Cavalier, Private bag X680, Cullinan, 1000). The final live body weight of the lambs in this trial were slightly lower than the findings of Price *et al.* (2009) that reported a mean value of 48.7 kg when South African Mutton Merino lambs were fed

a feedlot finisher diet; and they were slightly higher than the findings of Pienaar *et al.* (2012) which reported a mean value of 44.5 kg when South African Mutton Merino lambs were fed a standard feedlot diet. The reason for the differences in final live body weight could be due to the different lengths of time that the lambs were on feed, the sex of the lambs in the different trials or the season during which the trial was conducted. In this trial the lambs were on feed for a period of 54 days, whereas in the other two trials lambs were on feed for 60 days (Price *et al.*, 2009) and 47 days (Pienaar *et al.*, 2012) respectively. As days spent in the feedlot increases, the final live body weight increases (Brand *et al.*, 2017).

The final live body weights of the 0% CMS treatment differed (*P* <0.05) from the 4% CMS treatment. The lambs fed the 0% CMS treatment had a lower (*P* <0.05) final live body weight than the lambs fed the 4% CMS treatment. This was due to a lower (*P* <0.05) body weight gain and ADG throughout the trial in the lambs fed the 0% CMS treatment compared to the 4% CMS treatment, as seen in Table 5.1. The trend of lambs that have the lower average daily gain resulting in the lower final live body weight and the higher average daily gains resulting in the higher final live body weight was noted in studies by Fluharty & McClure (1997), Price *et al.* (2009), Pienaar *et al.* (2012) and Gouws *et al.* (2016). The final body weight of the 0% CMS treatment did not differ (*P* >0.05) from the 8% CMS and 12% CMS treatments. The final live body weight of the 4% CMS treatment also did not differ (*P* >0.05) from the 8% CMS and 12% CMS treatments. The reason for these two statistical results could possibly be due to the findings that there were no differences (*P* >0.05) in the body weight gained and ADG throughout the trial between the 0% CMS, 8% CMS and 12% CMS treatments and between the 4% CMS, 8% CMS and 12% CMS treatments respectively, as shown in Table 5.1. Figure 5.2 shows that from approximately 21 days on feed the 0% CMS treatment tended to have a lower body weight and this trend continued throughout the remainder of the 54 day period.
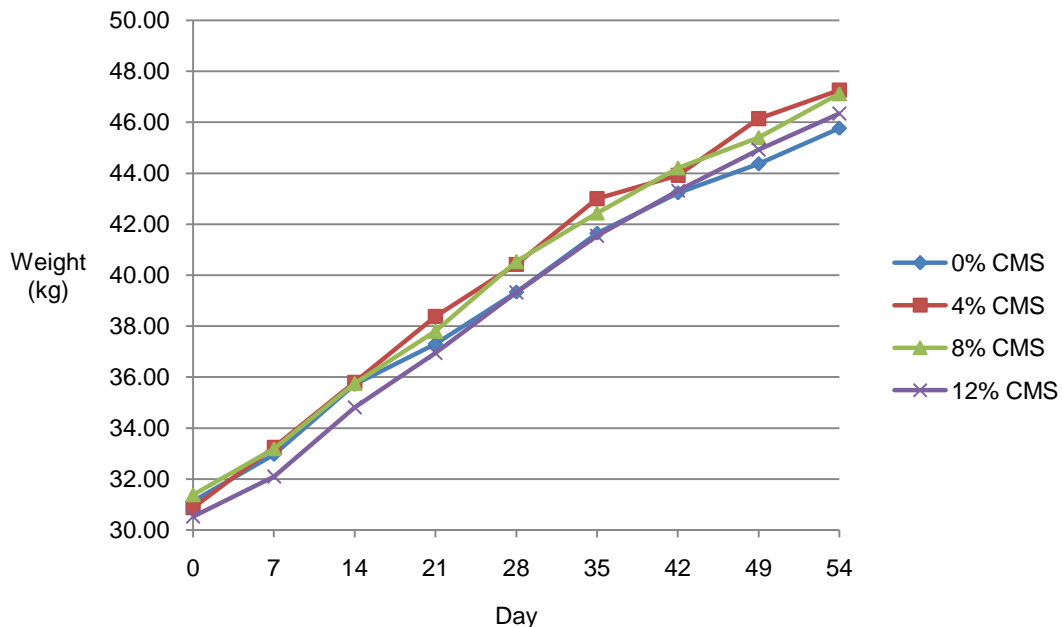

Figure 5.2 The weekly body weights of feedlot lambs fed different inclusion levels of CMS over the 54 day feeding period
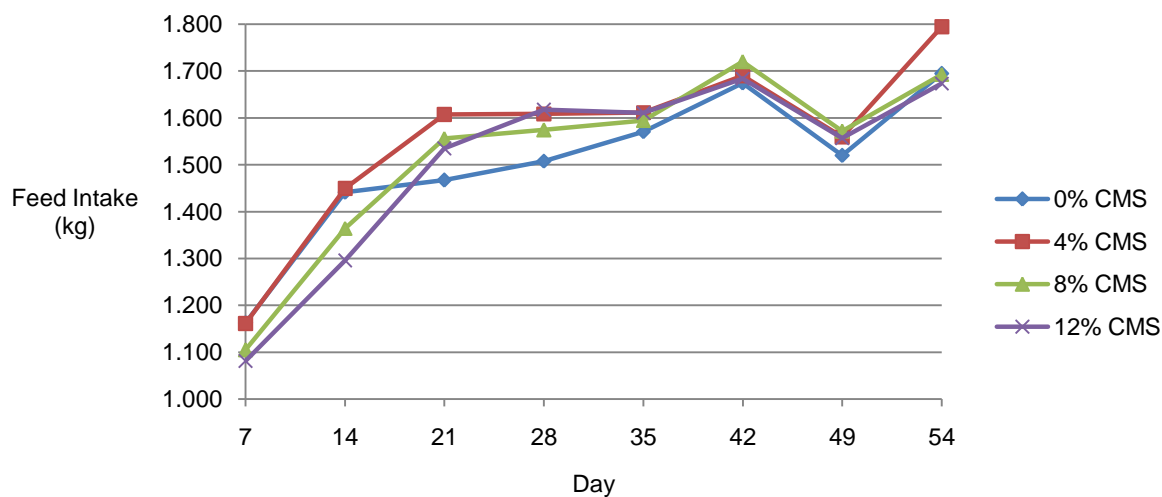
Feed intake

Feedlot producers want to know the actual amount of feed required per animal in order to do a cost analysis, therefore feed intake on an as is basis was calculated (Fernández & Woodward, 1999; ARC, 2002; Davis, 2003). The dry matter intake indicates the amount of nutrients that the animals actually consume. The mean feed intake on an as is basis for the trial was 1.688 kg/lamb/day, ranging from 1.664 – 1.720 kg/lamb/day. Representative with this trial, Sheridan *et al.* (2003) reported a cumulative feed intake of 99.84 kg over a 56 day feeding period when South African Mutton Merinos were fed a high energy diet (12.7 MJ/kg), which is a mean feed intake of 1.783 kg/lamb/day. The mean dry matter intake in this trial was 1.517 kg/lamb/day. This value is representative of the dry matter intake values obtained from other South African lamb feedlot studies. Mean dry matter intake values of 1.477 kg/lamb/day was reported by Price *et al.* (2009), 1.622 kg/lamb/day by Pienaar *et al.* (2012) and 1.592 kg/lamb/day by Gouws *et al.* (2016).

In agreement with the results obtained in the 4 x 4 Latin Square design (Chapter 4), there were no differences (P >0.05) between treatments for feed intake on an as is basis or feed intake on a dry matter basis. These results suggest that the CMS did not have an effect on the palatability of the diets, due to the lower dry matter and sugar content in CMS as opposed to molasses (Karalazos & Swan, 1977; Stemme *et al.*, 2005). It also suggests that the high ash content in the feed (López-Campos *et al.*, 2011), had no adverse effects on feed intake as may have been expected (2016, U. Muller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380). There were also no differences (P >0.05) between treatments when dry matter intake was calculated per kg metabolic body weight. Therefore, the difference (P <0.05) in starting live body weight had no effect on feed intake. Figure 5.3 shows the cumulative dry matter intake (kg/lamb) over the 54 day period. There are no evident differences at any point for any of the treatments, therefore it can be assumed that the lambs adapted to all treatments with no problems or metabolic disturbances. Variable feed intake could have indicated sub-acute acidosis in the animals, as variation in intake between days or depression of feed intake is a symptom of sub-acute acidosis (Nocek, 1997; Owens *et al.*, 1998). This did not seem to be the case in this trial.
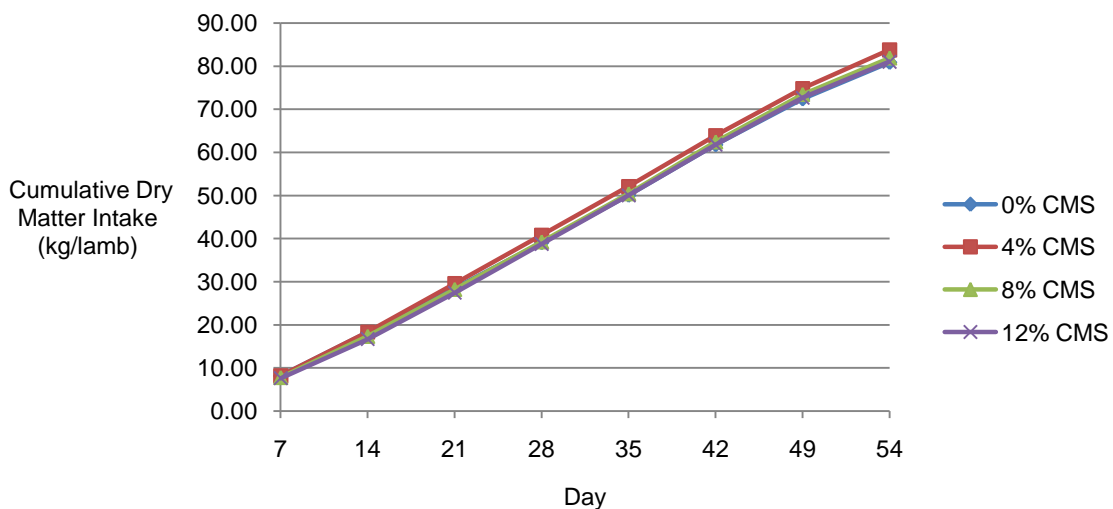
Figure 5.3 The cumulative dry matter intakes of feedlot lambs fed different inclusion levels of CMS over the 54 day feeding period

In Figure 5.4 it was noted that the weekly feed intakes of all the treatments follow a similar trend over the 54 day period. Therefore, if there were changes in the feed intake between the weeks it can be assumed that it was an environmental effect rather than a treatment effect due to the inclusion of CMS. For example, the reason for the decrease in feed intake in all the treatments at 49 days was probably due to a sudden increase in the daily temperatures. That week fell within the first week of September, and the average maximum temperature during the day was approximately 29.0ºC, whereas in the previous week the average maximum temperature during the day was approximately 23.7ºC. When there is sudden high temperature or a heat wave; or animals experience heat stress, a coping mechanism is to lower feed intake, thus decreasing metabolic heat production (Mitlöhner *et al.*, 2001). Figure 5.4 also shows the increasing feed intake with increasing days on feed. Brand *et al.* (2017) reported an increase in feed intake with increasing age of the lambs.

Figure 5.4 The weekly feed intakes of feedlot lambs fed different inclusion levels of CMS over the 54 day feeding period

The results from this trial are in agreement with Tillman & Kidwell (1951), Chen *et al.* (1981), Potter *et al.* (1985a), Potter *et al.* (1985b) and Leontowicz *et al.* (1994) that all reported no significant differences in dry matter intake when various types of CMS was included at different levels in ruminant diets. López-Campos *et al.* (2011) reported a decrease in the amount of concentrates consumed when beet CMS was included at 20% as opposed to 0% and 10% in the diet on an as is basis. The author hypothesized that this could be due to the high levels of potassium or minerals in the diet.

Feed efficiency

In South Africa the measure of feed efficiency is commonly calculated as the feed conversion ratio. The feed conversion ratio is calculated the amount of feed (kg) required for an animal to gain a kilogram of body weight (Beauchemin *et al.*, 1997; Hendriks *et al.*, 2013). The mean feed conversion ratio in this trial was 5.85 and 5.25 on an as is basis and dry matter basis respectively. These values are within normal range for South African Mutton Merino lambs (A. Le Riche, Pers. Comm., Cavalier, Private bag X680, Cullinan, 1000). Sheridan *et al.* (2003) reported a feed conversion ratio of 6.47 on an as is basis when South African Mutton Merinos were fed a high energy diet (12.7 MJ/kg) over a 56 day feeding period. This is slightly higher

than the value from this trial. The reason for this could be that Sheridan *et al.* (2003) reported a slightly higher feed intake than this trial with a similar body weight gain, thus a slightly higher FCR can be expected. The feed conversion ratio on a dry matter basis fell within the range of other South African lamb feedlot trials; Price *et al.* (2009) with a mean FCR of 4.62 and Gouws *et al.* (2016) with a mean FCR of 5.52. The probable reason for the lower FCR reported by Price *et al.* (2009) when South African Mutton Merino lambs were fed a feedlot finisher diet, could again be due to the lambs in that study receiving hormonal growth implants (Zeraplix) at the start of the study, as opposed to the lambs in this study who did not receive hormonal growth implants. In a study by Hutcheson *et al.* (1992) feedlot lambs that received Zeranol implants had an improved feed efficiency of 13% compared with lambs that did not receive implants.

The 0% CMS treatment differed (P <0.05) from the 4% CMS, 8% CMS and 12% CMS treatments for FCR on both an as is and dry matter basis. The 0% CMS had a higher (P <0.05) FCR than the 4% CMS, 8% CMS and 12% CMS treatments. There were no differences (P >0.05) in FCR between the 4% CMS, 8% CMS and 12% CMS treatments on an as is or dry matter basis. Feed conversion ratio is a measure of efficiency, and efficiency is highly associated with growth rate (Hendriks *et al.*, 2013). Therefore, it is expected that the animals with a higher average daily gain, but consuming the same/similar amounts of feed will have an improved FCR. This could have been the situation in this study leading to differences between the feed conversion ratios. There were no differences (P >0.05) in the feed intake; however average daily gains did differ, thus leading to differences in FCR. There were no differences (P >0.05) in dry matter feed intake between the 0% CMS and 4% CMS treatments, but the 0% CMS treatment had a lower (P <0.05) ADG than the 4% CMS treatment. Thus, the 0% CMS resulted in an expected higher (P <0.05) FCR than the 4% CMS treatment (5.45 as opposed to 5.12). What is interesting to note is that the 8% CMS and 12% CMS treatments did not differ (P >0.05) for feed intake or average daily gain from the 0% CMS treatment; however, the FCR of the 8% CMS and 12% CMS is lower (P <0.05) than the 0% CMS. The reason for this could be that the dry matter intakes for 0% CMS, 8% CMS and 12% CMS treatments, were 1.498, 1.501 and 1.516 kg/lamb/day, which was a mean dry matter intake of 1.507 ± 0.009 kg/lamb/day, which were very similar. The average daily gains for the 0% CMS, 8% CMS and 12% CMS treatments were 0.271, 0.291 and 0.293 kg/lamb/day which showed that 0% CMS treatment had a tendency to have a lower ADG. Therefore, when combined, the similar feed intake and higher average gains in the 8% and 12% CMS treatments, probably resulted in the lower (P <0.05) feed conversion ratios for the 8% CMS and 12% CMS treatments when compared to the 0% CMS treatment.

Chen *et al.* (1981), Potter *et al.* (1985a) and Yalcin *et al.* (2010) reported no differences in feed conversion ratio (feed to gain) when condensed molasses solubles were included at different levels in the diet fed to steers. Potter *et al.* (1985b) and Leontowicz *et al.* (1994) reported significantly higher feed to gain ratios in cattle fed increasing levels of sugarcane and beet CMS respectively, substituted for concentrates. López-Campos *et al.* (2011) reported that for lambs fed diets with beet CMS included at 0%, 10% and 20% on an as is basis, their feed to gain ratio increased as the levels of CMS increased. This was as a result of the lower average daily gains (López-Campos *et al.*, 2011). The probable reason for the differing outcomes

of the feed conversion ratios between the reviewed studies and this current trial were due to the differences in average daily gains between the reviewed studies and this current trial.

5.3 The effect of different inclusion levels of condensed molasses solubles on carcass parameters

Table 5.2 shows the results of the carcass parameters recorded and calculated after the lambs were slaughtered.

Table 5.2 The effect of different inclusion levels of CMS on mean (±SE) carcass parameters of feedlot lambs

Parameters	Treatment[1]				±SE[2]
	0% CMS	4% CMS	8% CMS	12% CMS	
Hot carcass weight (kg)	20.99[b]	22.52[a]	21.98[a]	21.95[a]	0.241
Cold carcass weight (kg)	20.64[b]	22.18[a]	21.63[a]	21.58[a]	0.242
Dressing percentage (%)	45.87[c]	47.52[b]	46.75[a]	47.41[ab]	0.238
Chilling loss (%)	1.68	1.50	1.59	1.70	0.066
[3]Carcass classification	A2.40[b]	A2.71[a]	A2.77[a]	A2.67[a]	0.065

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

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3

[2]±SE - Standard error

[3]Carcass classification done according to the South African Red Meat Classification System (Webb, 2015)

Carcass weights

In this trial the mean hot carcass weight was 21.86 kg and the mean cold carcass weight was 21.51 kg. Values ranged from 20.99 – 22.52 kg and 20.64 – 22.18 kg for hot and cold carcass weights respectively. These carcass weights were in agreement with Pienaar *et al.* (2012) who reported a mean cold carcass weight of 22.5 kg for South African Mutton Merino lambs fed a standard feedlot diet. Gouws *et al.* (2016) reported a mean carcass weight of 23.05 kg for feedlot lambs; however, it was not stated if this was hot or cold carcass weight. Sheridan *et al.* (2003) reported a slightly higher mean cold carcass weight of 24.58 kg when South African Mutton Merinos were fed a high energy diet (12.7 MJ/kg) for 56 days, which could be due to the higher final live body weight recorded in that trial. Sheridan *et al.* (2003) reported a mean final live body weight of 49.05 kg, whereas in this trial the mean final live body weight was 46.62 kg. Unfortunately Price *et al.* (2009) did not report carcass weights so a comparison could not be made.

For both hot and cold carcass weights the 0% CMS treatment was lower ($P < 0.05$) than 4% CMS, 8% CMS and 12% CMS treatments. The reason for this could possibly be due to the differences ($P < 0.05$) in the final live body weights. Cloete *et al.* (2004) reported that a higher final live body weight will directly result in a higher carcass weight. This is shown in Figure 5.5 where the relationship between final live body weight, hot carcass weight and cold carcass weight can be seen. The 4% CMS treatment had a tendency to have the highest final live body weight and also showed a tendency to have the highest hot and cold carcass weight; whereas the 0% CMS treatment had a tendency to have the lowest weights. Webb & Casey (1995) found that lambs with a final body weight of 37.0 kg and 43.0 kg had carcass weights of 17.38 ± 1.31 kg and 20.53

± 1.04 kg. Therefore, since the lambs in the 0% CMS had a lower (*P* <0.05) live body weight, it could be a direct positive correlation to them having a lower (*P* <0.05) carcass weight compared to the 4% CMS treatment. There were no differences (*P* >0.05) in carcass weights between the 4% CMS, 8% CMS and 12% CMS treatments.
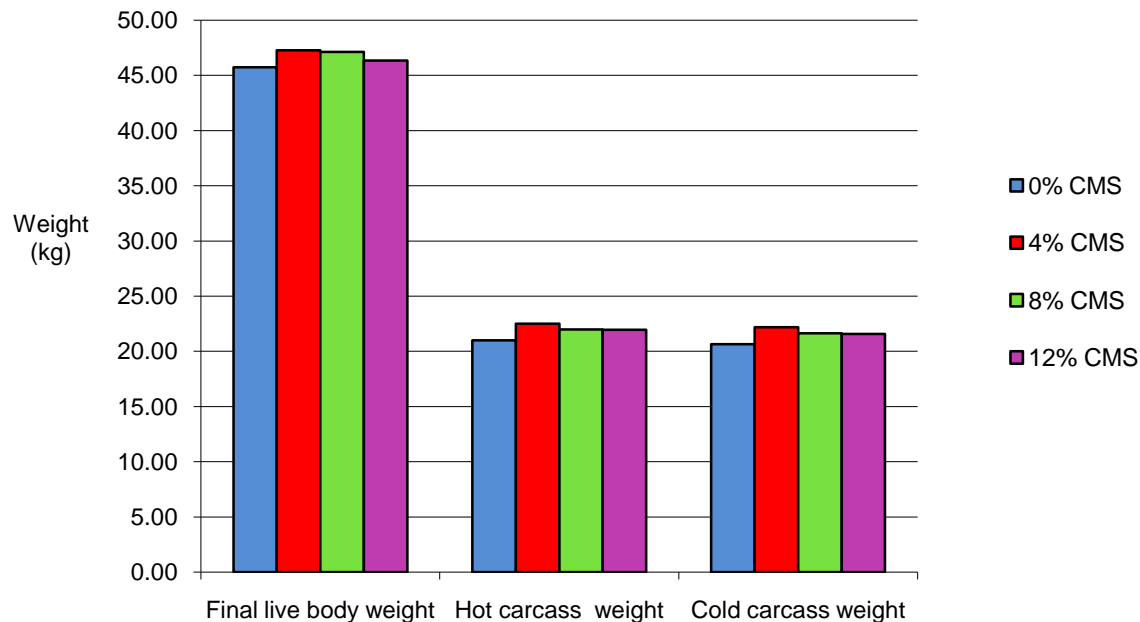
Figure 5.5 A comparison of the final live body weight, hot carcass weight and cold carcass weight of feedlot lambs fed different inclusion levels of CMS over the 54 day feeding period

López-Campos *et al.* (2011) conducted a study with Merino lambs fed 0%, 10% or 20% beet CMS as a replacement for molasses on an as is basis. The animals were grown until a fixed live body weight (approximately 25 kg) before they were slaughtered. Cold carcass weights were 11.7 kg, 11.7 kg and 11.6 kg for the 0%, 10% and 20% beet CMS fed lambs respectively, showing no differences between treatments (López-Campos *et al.*, 2011). It can be assumed that due to lambs being slaughtered at a set live weight, their carcass weights did not differ. Therefore, the difference in carcass weights of this current study, as seen in Table 5.2, were probably not due to the differing inclusion levels of CMS but rather higher final live body weights as discussed.

Dressing percentage
Dressing percentage is the proportion of final live body weight that is converted into carcass (Warmington & Kirton, 1990). In this study dressing percentage was calculated using hot carcass weight as a proportion of the final live body weight (Notter *et al.*, 1991; Beauchemin *et al.*, 1997; Avendaño-Reyes *et al.*, 2006; Ekiz *et al.*, 2012). The mean dressing percentage for this trial was 46.88%. This result is comparable with dressing percentages of South African Mutton Merino lambs fed standard feedlot diets in South African feedlots (2017, A. Le Riche, Pers. Comm., Cavalier, Private bag X680, Cullinan, 1000). In other South African feedlot trials Sheridan *et al.* (2003) reported a dressing percentage of 50.1% for South African Mutton Merinos fed for 56 days. Pienaar *et al.* (2012) reported a mean dressing percentage of 49.8%, which ranged from 48.4 – 50.7%, for South African Mutton Merino lambs fed a standard feedlot diet for 47 days. Both

studies reported slightly higher dressing percentages than this study. In neither of the studies the authors stated whether the final live weight was taken as a fasting weight and if lambs were sheared before final live weights were recorded. Dressing percentage is influenced by the level of gut fill when the final live body weight is recorded (Sheridan *et al.*, 2003). Kirton *et al.* (1995) conducted a study comparing the body and carcass composition of 7885 sheep. The sheep that were fasted overnight lost on average 2.3 – 2.9 kg of live weight (mainly gut fill) and this resulted in an average dressing percentage increase of 3.6 – 4.2%. Kirton *et al.* (1995) also reported a lower dressing percentage with heavier wool weights, as a lower proportion of their live body weights were converted into carcass. Therefore, if the animals final live body weight was recorded after being fasted or sheared in the studies by Sheridan *et al.* (2003) and Pienaar *et al.* (2012), it would be expected that they would have a higher dressing percentage than in this study, as the lambs in this study were not fasted or sheared before their final live body weight was recorded. Unfortunately Price *et al.* (2009) and Gouws *et al.* (2016) did not report dressing percentages in their studies, so no comparisons could be made.

The dressing percentage of the 0% CMS treatment differed (*P* <0.05) from the 4% CMS, 8% CMS and 12% CMS treatments, with 0% CMS displaying the lowest dressing percentage. It was also noted that the dressing percentage of the 4% CMS treatment was higher (*P* <0.05) than the 8% CMS treatment. However, there is no difference (*P* >0.05) between the 4% CMS and 12% CMS treatments and between the 8% CMS and 12% CMS treatments. These differences in the dressing percentage could have been as a result of the differences in final live body weights of the lambs. For example, the 0% CMS treatment had a tendency for the lowest final live body weight and had the lowest (*P* <0.05) dressing percentage. An increase in slaughter weight is usually as a result of an increased fat percentage (Webb & Casey, 1995). As the level of fat increases, so does dressing percentage (Warmington & Kirton, 1990; Webb 2015; Brand *et al.*, 2017). Studies by Solomon *et al.* (1980) investigated the effects of slaughter weight and breed on carcass properties of 33 lambs and reported that as the slaughter weight increased, there was an increase in the dressing percentage. Kemp *et al.* (1976) and Solomon *et al.* (1980) reported that as the carcass weights of lambs increased so did their dressing percentages, indicating that the heavier carcasses were fatter than lighter carcasses.

In studies by Chen *et al.* (1981), Yalcin *et al.* (2010) and López-Campos *et al.* (2011) no significant differences in the dressing percentage was found when CMS was included in the diets of sheep and cattle. Chen *et al.* (1981) reported a tendency for a slightly higher dressing percentage in cattle consuming diets containing a 5% citrus CMS and 5% molasses mixture on a DM basis as opposed to a 10% citrus CMS or 10% molasses mixture. Those results were in agreement with Potter *et al.* (1985b) who also reported a tendency of a slightly higher dressing percentage in steers fed diets with sugarcane CMS included at 5% of their diet on a DM basis.

Carcass classification

Carcass classification groups carcasses in categories based on physical and compositional attributes (Webb, 2015). According to Webb (2015, E.C. Webb, Pers. Comm., Department of Animal and Wildlife Sciences, University of Pretoria, Private bag X20, Hatfield, 0028) South African consumers have a

preference for an A2 to an A3 carcass when carcasses are classified according to the South Red Meat Classification System (Webb, 2015). An A2 carcass is a carcass that came from an animal with no permanent incisors which has a lean carcass and an A3 carcass is a carcass that comes from an animal with no permanent incisors which has a medium fat carcass (Webb, 2015). This is in agreement with Brand *et al.* (2017) who stated that the highest economic value comes from carcasses of lambs with a lean to medium amount of fat and less than one permanent incisor. In this trial, when the carcasses were classified using the South African Red Meat Classification system (Webb, 2015) according to Act 119 of 1990 (National Department of Agriculture, 1990) there was a mean carcass classification of A2.64. The carcass classification for each treatment, as shown in Table 5.2, was A2.40 for 0% CMS, A2.71 for 4% CMS, A2.77 for 8% CMS and A2.67 for 12% CMS treatments. Therefore, the carcasses from all the treatments fell into the category of an A2 to A3 carcass, which is the most desirable by South African consumers.

The carcass classification of the 0% CMS treatment differed (P <0.05) from the 4% CMS, 8% CMS and 12% CMS treatments. The 0% CMS treatment had a lower (P <0.05) carcass classification fat code than the 4% CMS, 8% CMS and 12% CMS treatments. This could be as a result of the dressing percentage of the 0% CMS treatment being significantly lower than the other treatments. In the past producers would produce a fatter carcass to increase their dressing percentage (Webb, 2015). Brand *et al.* (2017) reported that lambs with fatter carcasses have higher dressing percentages. Therefore a carcass containing less fat will result in a lower dressing percentage. Since the 0% CMS treatment had a lower (P <0.05) dressing percentage than the 4% CMS, 8% CMS and 12% CMS treatments, it is suggested that those lambs would also have a lower (P <0.05) fat code in the carcass classification. There were no differences (P >0.05) in carcass classification between the 4% CMS, 8% CMS and 12% CMS treatments.

The studies by Chen *et al.* (1981) and Potter *et al.* (1985b) were conducted in the United States of America (USA). In the USA marbling is an important quality parameter of beef carcasses and a carcass with a higher fat content is given the best grades according to the United States Department of Agriculture (USDA) quality carcass grading, as opposed to South Africa where consumers have a preference for a leaner carcass (Webb & O'Neill, 2008). In general, carcass quality traits such as marbling and the USDA quality grade were not significantly different between steers fed CMS at various inclusion levels (Chen *et al.*, 1981; Potter *et al.*, 1985b). However, in a study by Potter *et al.* (1985a) when sugarcane CMS was fed to steers at 10% of their diet, marbling and the carcass quality grade decreased. This was attributed to the steers having a lower dressing percentage and a leaner carcass at slaughter (Potter *et al.*, 1985b).

Chilling loss
Chilling loss was calculated as the difference between hot carcass weight and cold carcass weight as a proportion of the hot carcass weight (Rodríguez *et al.*, 2008; López-Campos *et al.*, 2011). There were no differences (P >0.05) in the chilling loss between treatments. An increase in carcass fat will result in increased insulation of the carcass, thus the carcass could retain more fluids resulting in a decreased chilling loss (Attah *et al.*, 2004). This was not the case in this trial because all the carcass classifications were between a 2 and a 3 fat code and a difference in chilling loss would only be expected if there was a larger difference between the fat content; for example a fat code of 1 and 5. Thus, even though carcass

classification differed (P <0.05), there were no differences (P >0.05) in chilling loss. This was in agreement with the findings of studies by Yalcin *et al.* (2010) and López-Campos *et al.* (2011) that found no differences in the chilling loss of the carcass between animals fed diets containing no CMS or CMS. In a study by López-Campos *et al.* (2011) no significant differences were noted between the lambs that were fed either 0%, 10% or 20% beet CMS on an as is basis and carcasses had chilling losses that ranged between 2.50 – 2.68%.

5.4 The effect of different inclusion levels of condensed molasses solubles on liver copper concentration

One of the main concerns with using condensed molasses solubles is the high levels of sulphur in CMS (Stemme *et al.*, 2005). Exposure to high levels of sulphur in the diet can induce copper deficiency in ruminants (Suttle, 2010). The high levels of sulphur reduce the absorption of copper in the animal and can result in a decrease in the copper concentration in the liver. The liver is a storage organ for copper (Suttle, 2010). Therefore, liver analysis can assist to determine the copper status of an animal (Van Ryssen, 2000) and ultimately the influence of the dietary sulphur.

Table 5.3 The effect of different inclusion levels of CMS on mean (±SE) dry matter and copper concentration in the livers of feedlot lambs

Parameters	Treatment[1]				±SE[2]
	0% CMS	4% CMS	8% CMS	12% CMS	
Dry matter (%)	30.16	30.12	29.73	29.67	0.379
Copper (mg Cu/kg DM)	141.96[a]	182.82[ab]	272.57[b]	172.69[ab]	35.154

[a,b,c] Means within a row with different superscript differ significantly (P <0.05)

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3

[2]±SE - Standard error

Cu - copper, DM - dry matter

Table 5.3 shows the effects of different inclusion levels of CMS on the dry matter content and copper (Cu) concentration in the livers of the feedlot lambs.

The concentration of copper in the livers ranged between 141.96 and 272.57 g Cu/kg dry matter of liver. Animals with liver copper concentrations between 75 – 300 mg Cu/kg DM are within the acceptable range (adapted from Puls, 1994 cited by Van Ryssen (unpublished)). Therefore, all the livers in this trial fell within the acceptable range for liver copper concentrations. The wide range of values (141.96 - 272.57 mg Cu/kg DM) and high standard error between treatments was probably due to sampling problems. There were fewer liver samples collected, sampled and analysed from each treatment than intended. This was due to logistical difficulties during the slaughtering process at the abattoir.

The copper concentration in the livers of the 0% CMS treatment differed (P <0.05) from the 8% CMS treatment. There were no differences (P >0.05) between the 0% CMS, 4% CMS and 12% CMS treatments, as well as no differences (P >0.05) between the 4% CMS, 8% CMS and 12% CMS treatments when analysing the copper concentrations in the livers of feedlot lambs. In this trial it was expected that there

would be a decrease in the copper concentration in the livers of the lambs fed higher levels of CMS (such as the 8% CMS and 12% CMS treatments) due to the increased sulphur concentrations in the condensed molasses solubles (Stemme *et al.*, 2005), however this was not seen. Therefore, it can be assumed that the differences noted in the liver copper concentration were due to sampling error and not treatment effect, as the 8% CMS treatment had a higher (P <0.05) liver copper concentration than the 0% CMS treatment.

Diets were formulated and ingredients added in such a way, that ensured the diets including CMS at higher levels did not have a significant increase in the concentration of sulphur. As shown in Table 4.1, the sulphur concentration of the experimental diets were 2.44, 2.36, 2.48 and 3.29 g/kg DM feed for the 0% CMS, 4% CMS, 8% CMS and 12% CMS treatments respectively. Therefore, the total sulphur concentration in the diet was probably too small to have an effect on the copper concentration in the liver. It can therefore be suggested that the higher levels of sulphur in CMS will not cause a problem or problems related to copper deficiencies when CMS is included in diets up to levels of 12% on an as is basis, as long as the increased levels of sulphur are accounted for during the formulation of feed.

Pienaar (2016) found no differences in the copper concentration in the livers of feedlot cattle fed diets containing 0%, 5%, 10% and 15% sugarcane CMS on an as is basis. Pienaar (2016) also reported that the copper concentration in the livers were above the marginal ranges where a copper deficiency may have occurred. Potter *et al.* (1985b) also found no significant differences in the liver copper concentration of steers when sugarcane CMS was included at 0%, 5%, 10% and 15% of their diet on a DM basis, with liver copper values of 207, 238, 293 and 245 ppm on a DM basis respectively.

The dry matter content of the livers in this trial averaged 29.92% ± 0.25%. According to Van Ryssen (2016) in a range of studies done, the mean liver dry matter of healthy sheep was 28.9% ± 1.7% (n = 69). This is in agreement with the results from this trial. There were no differences (P >0.05) between treatments for the dry matter content of the livers. There were no studies or literature found concerning the effect of CMS on liver dry matter content, therefore no comparison could be made.

Chapter 6

Conclusion

In the 4 x 4 Latin Square design there were no differences (P >0.05) between treatments for dry matter intake, organic matter intake or digestible organic matter intake, when calculated as g/day or g/kgBW$^{0.75}$. The 0% CMS treatment had a lower (P <0.05) water intake than the 4% CMS treatment. The 0% CMS and 4% CMS treatments had a lower (P <0.05) apparent total tract organic matter digestibility compared to the 12% CMS treatment. A reason for this could not be concluded, but it was suggested to be as a result of the slightly higher NDF and ADF concentrations in those diets. The only difference between the treatments for the rumen parameters measured was that the total volatile fatty acid production was lower (P <0.05) for the 8% CMS treatment compared to the 12% CMS treatment. It was concluded that this may have been due to experimental error. There were no differences (P >0.05) in rumen pH, proportions of volatile fatty acids, acetate:propionate ratio or rumen ammonia nitrogen between treatments. There were no differences (P >0.05) between treatments for apparent nitrogen retention, nitrogen intake, faecal nitrogen and total nitrogen excretion. However, urinary nitrogen was lower (P <0.05) in the 0% CMS treatment compared to the 8% CMS treatment. There were no differences (P >0.05) between treatments for microbial nitrogen production, however experimental error may have influenced the microbial nitrogen results.

In the feedlot trial the 0% CMS treatment had a lower (P <0.05) average daily gain, body weight gain and final live body weight compared to the 4% CMS treatment. It can be assumed that the lower ADG in the 0% CMS treatment may be attributed to the poor pellet quality of the 0% CMS diet and the lambs selecting feed. In agreement with the 4 x 4 Latin Square design there were no differences (P >0.05) in feed intake between the treatments. The feed conversion ratio was higher (P <0.05) in the 0% CMS treatment compared to the 4% CMS, 8% CMS and 12% CMS treatments. The 0% CMS treatment had a lower (P <0.05) carcass weight, dressing percentage and carcass classification compared to the 4% CMS, 8% CMS and 12% CMS treatments. The carcass traits seemed to be related to the final live body weight of the lambs. The dressing percentage of the 4% CMS treatment was higher (P <0.05) than the 8% CMS treatment. There were no differences (P >0.05) in chilling loss between the treatments. After analysing the results obtained in the 4 x 4 Latin Square design it can be assumed that the results obtained in the feedlot trial were not due to the differences in digestibility of the feeds, but rather the acetate to propionate ratio in the rumen and the apparent nitrogen retention by the animal. The 0% CMS treatment had a lower (P <0.05) body weight gain than the 4% CMS treatment and also tended to have a higher acetate to propionate ratio and lower nitrogen retention than the 4% CMS treatment. This is expected as propionate production is more efficient and provides more energy than acetate (Schelling, 1984) and nitrogen retention is an indicator of the protein status of the animal and the amount of protein available for growth (Tripathi *et al.*, 2007).

The copper concentration in the livers of 0% CMS treatment was lower (P <0.05) than the 8% CMS treatment. All the treatments had liver copper concentrations that fell within the normal acceptable range. Thus, it was assumed that the high sulphur concentration in the CMS should not have an effect on the animals.

When formulating diets including CMS, it is essential that the variations in the contribution of the nutrients from the CMS are accounted for and that the diets are then formulated accordingly. Although differences between the treatments for certain measured parameters were noted in the 4 x 4 Latin Square design and feedlot trial, there was not enough evidence to conclude that the differences were attributed solely to the treatments but other external factors may have influenced the results.

In conclusion, condensed molasses solubles can be included up to levels of 12% on an as is basis to replace molasses in a high concentrate diet without having an adverse effect on intake, growth performance, digestibility and certain rumen parameters in sheep.

Chapter 7

Critical Evaluation

Future research

As there were no adverse affects in this trial when sugarcane CMS was included up to levels of 12% on an as is basis in a high concentrate diet to replace molasses; it is recommended that investigation into increasing the inclusion levels of CMS above 12% on an as is basis in a similar trial is conducted.

Some of the literature reviewed included CMS at higher levels than 12%, but gave no reliable indication of the effects when including CMS at higher levels in ruminant diets, as the diets were not always formulated to account for the differences in the nutrient contribution from CMS. Therefore, the diets reviewed were often not based on an iso-nitrogenous and iso-energetic basis and the results were not necessarily due to the high CMS levels but rather the differing nutrient composition of the diets. This was even stated by the authors in some of the studies (Karalazos & Swan, 1977; Leontowicz *et al.*, 1994).

Investigation into using CMS in a supplement given to ruminants fed a medium or poor quality roughage is required. The high levels of NPN, sulphur and other minerals could make CMS an adequate source of NPN for diets that are low in rumen degradable protein (Leontowicz *et al.*, 1994; Stemme *et al.*, 2005; Fernández *et al.*, 2009).

4 x 4 Latin Square design

It is recommended that the digestibility trial and rumen fermentation evaluation is done in two separate parts during each sample collection period. For example, it would be better to collect the feed, orts, faecal and urine samples that are required for digestibility, apparent nitrogen retention and microbial nitrogen production determination over the first six days of the sample collection period; and then the rumen collections to analyse rumen pH, VFA production and NH_3-N production is done for the remaining two days. This will ensure that the rumen collections do not interfere with the digestibility and rumen microbial nitrogen production results. During the rumen fluid collections some of the solid rumen contents may have been lost, which could have resulted in a higher digestibility coefficient than expected. The other problem was that doing the rumen collections while doing total urine collection for rumen microbial nitrogen analysis, some of the rumen fluid may have fallen into the urine pan and ultimately contaminated the urine. Therefore, when analysing for purine derivatives in the urine to determine microbial nitrogen production, if any rumen fluid fell in it increased the results of the microbial protein production and this lead to inaccurate calculations of the microbial nitrogen production.

The collection of the urine for the purine derivative analysis to determine microbial nitrogen production in this trial was conducted following the total urine collection method as mentioned by Chen & Gomes (1995), as this is the preferred method for purine derivative analysis (Mentz *et al.*, 2015). In future, it may be better to conduct spot urine collection as described by Chen *et al.* (1995). Chen *et al.* (1995) conducted a study with sheep to evaluate the use of the ratio of the purine derivatives to creatinine in spot urine samples as an indicator of microbial protein supply. The authors concluded that the purine derivative:creatinine ratio (corrected for metabolic body weight) in spot urine could be used to evaluate microbial protein supply in

sheep fed *ad libitum* (Chen *et al.*, 1995). Mentz *et al.* (2015) found no differences in purine derivatives or microbial nitrogen when using either the total purine derivative or spot urine method when supplementing sheep fed poor quality roughage. The use of spot urine collection will prevent the contamination of urine by rumen fluid. This contamination distorts the amount of microbial nitrogen calculated from the purine derivatives analysed in the urine. This would be another way to overcome the rumen fluid contaminating the urine.

Feedlot trial

During the slaughtering process the number of livers collected was lower than intended due to logistical problems during the slaughtering. This led to a slightly wider range of copper values between treatments which could not be attributed to the treatments as there were not enough samples to confirm this. Therefore, more intense planning needs to be done in future to try and obtain and tag all the livers from all the lambs instead of set animals and more care needs to be taken when sampling the livers.

Diet

The control diet (0% CMS) was of a poor pellet quality. No differences (P >0.05) in intake were noted, but the 0% CMS treatment tended to have a slightly lower feed intake in both trials and a lower (P <0.05) ADG and body weight gained. This could have been due to the poor pellet quality as no other results or observations indicated that it was due to the inclusion of molasses as a replacement for CMS in the control diets, and the diets were formulated to be iso-nitrogenous and iso-energetic. Casey & Webb (1995) reported that the average daily gains and feed intake were lower when wethers were fed a mash as opposed to a pelleted diet. Therefore, pellet quality may have had an influence on the trial.

Using bagasse as an ingredient, which is high in NDF and ADF (NRC, 2007), resulted in a difference in NDF and ADF concentration between the experimental diets. Even when including a slightly higher amount of bagasse such as 1.8%, resulted in a 38.0 g/kg and 23.6 g/kg increase in the ADF and NDF content of the diet on a DM basis respectively. This may have influenced the apparent total tract digestibility values in the trial. Therefore, a better quality roughage, such as lucerne with a lower NDF and ADF content (NRC, 2007), or a mixture of lucerne and bagasse could have been used in the experimental diets.

Comparison of results to other trials using CMS

When comparing this trial to other trials that included CMS in total mixed rations and concentrate diets, it was problematic. There are only a few studies that were conducted using CMS with ruminants fed concentrate diets and not many recent publications. Most of the studies that were conducted used diets that were not formulated on an iso-energetic and/or iso-nitrogenous basis, thus it was not clear if the results in the reviewed studies were due to the CMS or differing concentrations of nutrients in the diet. In some cases CMS was used to replace various components of the diet. For example, CMS was used to replace parts of the concentrates (maize or soybean meal) in a diet fed in a study conducted by Potter *et al.* (1985b), whereas in other diets it was used to replace molasses (Karalazos & Swan, 1977; Potter *et al.* 1985a). The different studies used different types of CMS; some trials used sugarcane CMS (Potter *et al.* 1985a; Potter *et al.* 1985b), a few used beet CMS (Karalazos & Swan, 1977; Leontowicz *et al.*, 1994) and one trial used citrus CMS (Chen *et al.*, 1981). Since the different types of CMS (sugarcane, beet and citrus) have different

nutrient compositions, they cannot be compared. More research on using condensed molasses solubles as an ingredient in animal feed needs to be conducted.

References

Allen, J., 2015. Effects of dietary zilpaterol hydrochloride and a non-steroidal growth implant on growth and carcass characteristics of feedlot lambs. MSc (Agric) Animal Science: Production Physiology, dissertation, Dept. of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa.

AOAC, 2000. Official methods of analysis (17th ed.) Volume 1. Association of Official Analytical Chemists, Inc., Maryland, USA.

ARC, 2002. Feedlot Management: South African feedlot industry and economics of beef production. Available at: ARC: Animal Nutrition and Products Institute. Private bag X2, Irene, 0062, South Africa.

Attah, S., Okubanjo, A.O., Omojola, A.B. & Adesehinwa, A.O.K., 2004. Body and carcass linear measurements of goats slaughtered at different weights. Livest. Res. Rural Dev. 16, 1-6.

Avendaño-Reyes, L., Torres-Rodríguez, V., Meraz-Murillo, F.J., Pérez-Linares, C., Figueroa-Saavedra, F. & Robinson, P.H., 2006. Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 84, 3259-3265.

Bampidis, V.A. & Robinson, P.H., 2006. Citrus by-products as ruminant feeds: A review. Anim. Feed Sci. Technol. 128, 175-217.

Barrera, E.L., Spanjers, H., Dewulf, J., Romero, O. & Rosa, E., 2013. The sulfur chain in biogas production from sulfate-rich liquid substrates: a review on dynamic modeling with vinasse as model substrate. J. Chem. Technol. Biot. 88, 1405-1420.

Bartley, E.E. & Deyoe, C.W., 1981. Reducing the rate of ammonia release by the use of alternative non-protein nitrogen sources. In: Ruminant Nutrition. Eds: Haresign, W. & Cole, D.J.A., Butterworths, London, England.

Bath, G.F., Malan, F.S. & Van Wyk, J.A., 1996. The FAMACHA[©] ovine anemia guide to assist with the control of haemonchosis. Proceedings of the 7th Annual Congress of the Livestock Health and Production Group of the South African Veterinary Association, 5-7 June, Port Elizabeth, South Africa.

Beauchemin, K.A., Jones, S.D.M., Rode, L.M. & Sewalt, V.J.H., 1997. Effects of fibrolytic enzymes in corn or barley diets on performance and carcass characteristics of feedlot cattle. Can. J. Anim. Sci. 77, 645-653.

Bevans, D.W., Beauchemin, K.A., Schwartzkopf-Genswein, K.S., McKinnon, J.J. & McAllister, T.A., 2005. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. J. Anim. Sci. 83, 1116-1132.

Brand, T.S., Genis, M.P., Hoffman, L.C., Van de Vyver, W.F.J., Van der Walt, C. & Jordaan, G.F., 2013. Effects of dietary energy content and provision of β-adrenergic agonist on the production of feedlot lambs. S. Afr. J. Anim. Sci. 43, S135-S139.

Brand, T.S., Van der Westhuizen, E.J., Van der Merwe, D.A. & Hoffman, L.C., 2017. Effects of days in feedlot on growth performance and carcass characteristics of Merino, South African Mutton Merino and Dorper lambs. S. Afr. J. Anim. Sci. 47, 26-33.

Broderick, G.A. & Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy Sci. 63, 64-75.

Brown, M.S., Ponce, C.H. & Pulikanti, R., 2006. Adaptation of beef cattle to high-concentrate diets: Performance and ruminal metabolism. J. Anim. Sci. 84, E25-E33.

Casey, N.H. & Webb, E.C., 1995. Influence of dietary energy levels and form of diet on composition of fatty acids in subcutaneous adipose tissue of wethers. Small Rumin. Res. 18, 125-132.

Chen, M.C., Ammerman, C.B., Henry, P.R., Palmer, A.Z. & Long, S.K., 1981. Citrus condensed molasses solubles as an energy source for ruminants. J. Anim. Sci. 53, 253-259.

Chen, X.B., Chen, Y.K., Franklin, M.F., Ørskov, E.R. & Shand, W.J., 1992. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. J. Anim. Sci. 70, 1534-1542.

Chen, X.B. & Gomes, M.J., 1995. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivitives – An overview of the technical details. International feed resource unit. Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, England, occasional publications.

Chen, X.B., Mejia, A.T., Kyle, D.J. & Ørskov, E.R., 1995. Evaluation of the use of the purine derivative: creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: studies in sheep. J. Agric. Sci. 125, 137-143.

Christofoletti, C.A., Escher, J.P., Correia, J.E., Marinho, J.F.U. & Fontanetti, C.S., 2013. Sugarcane vinasse: Environmental implications of its use. Waste Manag. 33, 2752-2761.

Cloete, J.J.E., Hoffman, L.C., Cloete, S.W.P. & Fourie, J.E., 2004. A comparison between the body composition, carcass characteristics and retail cuts of South African Mutton Merino and Dormer sheep. S. Afr. J. Anim. Sci. 34, 44-51.

Cole, N.A., 1999. Nitrogen retention by lambs fed oscillating dietary protein concentrations. J. Anim. Sci. 77, 215-222.

Cooper, R.J., Klopfenstein, T.J., Stock, R.A., Milton, C.T., Herold, D.W. & Parrott, J.C., 1999. Effects of imposed feed intake variation on acidosis and performance of finishing steers. J. Anim. Sci. 77, 1093-1099.

Davis, J. (Ed)., 2003. Feedlotting Lambs: A producers guide. Landlinks Press, Collingwood, Australia.

Dentinho, M.T.P., Belo, A.T. & Bessa, R.J.B., 2014. Digestion, ruminal fermentation and microbial nitrogen supply in sheep fed soybean meal treated with *Cistus ladanifer* L. tannins. Small Rumin. Res. 119, 57-64.

Detmann, E., Valente, É.E., Batista, E.D. & Huhtanen, P., 2014. An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. Livest. Sci. 162, 141-153.

Devant, M., Ferret, A., Gasa, J., Calsamiglia, S. & Casals, R., 2000. Effects of protein concentration and degradability on performance, ruminal fermentation, and nitrogen metabolism in rapidly growing heifers fed high-concentrate diets from 100 to 230 kg body weight. J. Anim. Sci. 78, 1667-1676.

Drewnoski, M.E., Pogge, D.J. & Hansen, S.L., 2014. High-sulfur in beef cattle diets: A review. J. Anim. Sci. 92, 3763-3780.

Ekiz, B., Yilmaz, A., Ozcan, M. & Kocak, O., 2012. Effect of production system on carcass measurements and meat quality of Kivircik lambs. Meat Sci. 90, 465-471.

El Khidir, O.A. & Thomsen, K.V., 1982. The effect of high levels of molasses in combinations with hay on digestibility of organic matter, microbial protein synthesis and volatile fatty acid production *in vitro*. Anim. Feed Sci. Technol. 7, 277-286.

Enemark, J.M.D., 2009. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. Vet. J. 176, 32-43.

Engels, E.A.N. & Van der Merwe, F.J., 1967. Application of an *in vitro* technique to South African forages with special reference to the effect to certain factors on the results. S. Afr. J. Agric. Sci. 10, 983-995.

Esplin, A.L., Garrigus, U.S., Hatfield, E.E. & Forbes, R.M., 1957. Some effects of pelleting a ground mixed ration on feed utilization by fattening lambs. J. Anim. Sci. 16, 863-871.

Fahey, G.C. & Berger, L.L., 1988. Carbohydrate nutrition of ruminants. In: The Ruminant Animal: Digestive Physiology and Nutrition. Ed: Church, D.C., Prentice Hall, New Jersey, USA. pp. 269-297.

Faithfull, N.T., 2002. Methods of agricultural chemical analysis: A practical handbook. CABI Publishing, Wallingford, England.

Fernández, M.I. & Woodward, B.W., 1999. Comparison of conventional and organic beef production systems I. feedlot performance and production costs. Livest. Prod. Sci. 61, 213-233.

Fernández, B., Bodas, R., López-Campos, Ó., Andrés, S., Mantecón, A.R. & Giráldez, F.J., 2009. Vinasse added to dried sugar beet pulp: Preference rate, voluntary intake and digestive utilisation in sheep. J. Anim. Sci. 87, 2055-2063.

Fisher, L.J., Dinn, N., Tait, R.M. & Shelford, J.A., 1994. Effect of level of dietary potassium on the absorption and excretion of calcium and magnesium by lactating cows. Can. J. Anim. Sci. 74, 503-509.

Fluharty, F.L. & McClure, K.E., 1997. Effects of dietary energy intake and protein concentration on performance and visceral organ mass in lambs. J. Anim. Sci. 75, 604-610.

Fontenot, J.P. & Hopkins, H.A., 1965. Effect of physical form of different parts of lamb fattening rations on feedlot performance and digestibility. J. Anim. Sci. 24, 62-68.

Forbes, J.M., 2000. Physiological and metabolic aspects of feed intake control. In: Farm Animal Metabolism and Nutrition. Ed: D'Mello, J.P.F., CABI Publishing, Wallingford, England. pp. 319-334.

French, N.P., Wall, R. & Morgan, K.L., 1994. Lamb tail docking: a controlled field study of the effects of tail amputation on health and productivity. Vet. Rec. 134, 463- 467.

Galyean, M.L., Wagner, D.G. & Owens, F.N., 1979. Level of feed intake and site and extent of digestion of high concentrate diets by steers. J. Anim. Sci. 49, 199-203.

Galyean, M.L., Krysl, L.J. & Estell, R.E., 1986. Marker-based approaches for estimation of fecal output and digestibility in ruminants. In: Feed Intake of Beef Cattle: Symposium. Ed: Owens, F.N., Oklahoma State Univ., Stillwater, MP. 121, 96-113.

Galyean, M.L. & Owens, F.N., 1991. Effects of diet composition and level of feed intake on site and extent of digestion in ruminants. In: Physiological Aspects of Digestion and Metabolism in Ruminants (1st ed.). Eds: Tsuda, T., Sasaki Y. & Kawashima, R., Academic Press, San Diego, USA.

Giron, H.C., 1973. Atomic Absorption Newsletter 12, 28. Perkin Elmer Atomic Spectrophotometer.

Goering, H.K. & Van Soest, P.J., 1970. Forage Fiber Analysis (Apparatus, reagents, procedures and some applications). Agriculture Handbook No. 379. ARS-USDA. U.S. Dept. Of. Agric. Washington, DC, USA.

Gooneratne, S.R., Olkowski, A.A. & Christensen, D.A., 1989. Sulfur-induced polioencephalomalacia in sheep: some biochemical changes. Can. J. Vet Res. 53, 462-467.

Gould, D.H., 1998. Polioencephalomalacia. J. Anim. Sci. 76, 309–314.

Gouws, R.F., Hagg, F.M., Van der Veen, R.H., Erasmus, L.J. & Holm, D.E., 2016. The potential of Calcified marine algae and/or Capsicum as natural alternatives to Monensin in lamb feedlot diets. ADSA, Joint annual meeting, 19-23 July, Salt Lake City, Utah, USA.

Gozho, G.N., Plaizier, J.C., Krause, D.O., Kennedy, A.D. & Wittenberg, K.M., 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J. Dairy Sci. 88, 1399-1403.

Hall, M.B. & Huntington, G.B., 2008. Nutrient synchrony: Sound in theory, elusive in practice. J. Anim. Sci. 86, E287-E292.

Hannon, K. & Trenkle, A., 1990. Evaluation of condensed molasses fermentation solubles as a nonprotein nitrogen source for ruminants. J. Anim. Sci. 68, 2634-2641.

Hendriks, J., Scholtz, M.M. & Neser, F.W.C., 2013. Possible reasons for differences in residual feed intake: An overview. S. Afr. J. Anim. Sci. 43, S103-S106.

Huntington, G.B., 1997. Starch utilization by ruminants: from basics to the bunk. J. Anim. Sci. 75, 852-867.

Hutcheson, J.P., Greene, L.W., Carstens, G.E & Byers, F.M., 1992. Effects of zeranol and two dietary levels of calcium and phosphorus on performance, carcass and bone characteristics, and calcium status in growing lambs. J. Anim. Sci. 70, 1346-1351.

Iranmehr, M., Khadem, A.A., Rezaeian, M., Afzalzadeh, A. & Pourabedin, M., 2011. Nutritional value of vinasse as ruminant feed. Krmiva. 53, 3-8.

Kania, B.F., Kulasek, G., Leontowicz, H., Krezmiński, R., Leontowicz, M. & Sobczak, E., 1983. Balance study on sheep fed with beet molasses solubles. S. Afr. J. Anim. Sci. 13, 21-22.

Karalazos, A. & Swan, H., 1977. The nutritional value for sheep of molasses and condensed molasses solubles. Anim. Feed Sci. Technol. 2, 143-152.

Kemp, J.D., Johnson, A.E., Stewart, D.F., Ely, D.G. & Fox, J.D., 1976. Effects of dietary protein, slaughter weight and sex on carcass composition, organoleptic properties and cooking losses of lambs. J. Anim. Sci. 42, 575-583.

Kincaid, R., 1988. Macro elements for ruminants. In: The Ruminant Animal: Digestive physiology and nutrition. Ed: Church, D.C., Prentice Hall, New Jersey, USA.

Kirton, A.H., Carter, A.H., Clarke, J.N. & Duganzich, D.M., 1984. Dressing percentages of lambs. Proceedings of the New Zealand Society of Animal Production. 44, 231-233.

Kirton, A.H., Carter, A.H., Clarke, J.N., Sinclair, D.P., Mercer, G.J.K. & Duganzich, D.M., 1995. A comparison between 15 ram breeds for export lamb production. 1. Liveweights, body components, carcass measurements and composition. New Zeal. J. Agr. Res. 38, 347-360.

Kuehl, R.O., 2000. Design of experiments: Statistical principles of research design and analysis (2nd ed.). Duxbury Thomson learning, Pacific Grove, California, USA.

Leontowicz, H., Krzemiński, R., Leontowicz, M., Kulasek, G., Tropilo, J. & Sobczak, E., 1994. Condensed beet molasses solubles for fattening bulls. J. Anim. Feed Sci. 3, 23-31.

López-Campos, Ó., Bodas, R., Prieto, N., Frutos, P., Andrés, S & Giráldez, F.J., 2011. Vinasse added to the concentrate for fattening lambs: Intake, animal performance, and carcass and meat characteristics. J. Anim. Sci. 89, 1153-1162.

Ma, T., Deng, K.D., Tu, Y., Jiang, C.G., Zhang, N.F., Li, Y.L., Si, B.W., Lou, C. & Diao, Q.Y., 2014. Effect of dietary concentrate:forage ratios and undegraded dietary protein on nitrogen balance and urinary excretion of purine derivatives in Dorper x Thin-tailed Han crossbred lambs. Asian Australas. J. Anim. Sci. 27, 161-168.

Mahgoub, O., Lu, C.D. & Early, R.J., 2000. Effects of dietary energy density of feed intake, body weight gain and carcass chemical composition of Omani growing lambs. Small Rumin. Res. 37, 35 - 42.

Maneerat, W., Prasanpanich, S., Tumwasorn, S., Laudadio, V. & Tufarelli, V., 2015. Evaluating agro-industrial by-products as dietary roughage source on growth performance of fattening steers. Saudi. J. Biol. Sci. 22, 580-584.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 2002. Animal Nutrition (6th ed.). Pearson Education Limited, Harlow, England.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A. & Wilkinson, R.G., 2011. Animal Nutrition (7th ed.). Pearson Education Limited, Harlow, England.

Mella, C.M., Perez-Oliva, O. & Loew, F.M., 1976. Induction of bovine polioencephalomalacia with a feeding system based on molasses and urea. Can. J. Comp. Med. 40, 104-110.

Mentz, A.M., Van Niekerk, W.A., Hassen, A., Coertze, R.J. & Gemeda, B.S., 2015. Effect of diets differing in rumen soluble nitrogen on utilization of poor-quality roughage by sheep. S. Afr. J. Anim. Sci. 45, 528-537.

Merchen, N.R. & Berger, L.L., 1985. Effect of salinomycin level on nutrient digestibility and ruminal characteristics of sheep and feedlot performance of cattle. J. Anim. Sci. 60, 1338-1346.

Merchen, N.R. 1988. Digestion, absorption and excretion in ruminants. In: The Ruminant Animal: Digestive Physiology and Nutrition. Ed: Church, D.C., Prentice Hall, New Jersey, USA. pp. 172-201.

Miller, W.J., 1975. New concepts and developments in metabolism and homeostasis of inorganic elements in dairy cattle: A Review. J. Dairy Sci. 58, 1549-1560.

Mirzaei-Aghsaghali, A. & Maheri-Sis, N., 2008. Nutritive value of some agro-industrial by-products for ruminants – A review. World J. Zool. 3, 40-46.

Mitlöhner, F.M., Morrow, J.L., Dailey, J.W., Wilson, S.C., Galyean, M.L., Miller, M.F. & McGlone, J.J., 2001. Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle. J. Anim. Sci. 79, 2327-2325.

Mynhardt, H., Van Niekerk, W.A., Erasmus, L.J., Hassen, A. & Coertze, R.J., 2016. Substitution of rumen degradable nitrogen with urea in sheep fed low quality *Eragrostis curvula* hay. Sci. Agric. 73, 498-504.

National Department of Agriculture, 1990. Agricultural Product Standards Act, 1990 Regulations regarding the classification and marketing of meat. Act No. 119 of 1990.

Nguyen, T.L.T. & Gheewala, S.H., 2008. Life cycle assessment of fuel ethanol from cane molasses in Thailand. Int. J. Life Cycle Assess. 13, 301-311.

Nikkhah, A., 2012. Barley grain for ruminants: A global treasure or tragedy. J. Anim. Sci. Biotechnol. 3. Available at: http://www.jasbsci.com/content/3/1/22.

Nocek, J.E., 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80, 1005–1028.

Notter, D.R., Kelly, R.F. & McClaugherty, F.S., 1991. Effects of ewe breed and management system on efficiency of lamb production: II. Lamb growth, survival and carcass characteristics. J. Anim. Sci. 69, 22-33.

NRC, 1981. Feeding value of ethanol production by-products. National Academies Press, Washington D.C., USA.

NRC, 2005. Mineral Tolerance of Animals (2nd ed.). National Academies Press, Washington D.C., USA.

NRC, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids. National Academies Press, Washington D.C., USA.

Olson, K.C., Cochran, R.C., Jones, T.J., Vanzant, E.S., Titgemeyer, E.C. & Johnson, D.E., 1999. Effects of ruminal administration of supplemental degradable intake protein and starch on utilization of low-quality warm-season grass hay by beef steers. J. Anim. Sci. 77, 1016-1025.

Ørskov, E.R., 1986. Starch digestion and utilization in ruminants. J. Anim. Sci. 63, 1624-1633.

Osuji, P.O., Nsahlai, I.V. & Khalili, H., 1993. Feed Evaluation ILCA Manual. International Livestock Centre for Africa. Addis Ababa, Ethiopia: Chapter 1 and 4.

Owens, F.N. & Goetsch, A.L., 1988. Ruminal fermentation. In: The Ruminant Animal: Digestive Physiology and Nutrition. Church, D.C., Prentice Hall, New Jersey, USA. pp. 145-171.

Owens, F.N. & Zinn, R., 1988. Protein metabolism of ruminant animals. In: The Ruminant Animal: Digestive Physiology and Nutrition. Church, D.C., Prentice Hall, New Jersey, USA. pp. 227-249.

Owens, F.N., Secrist, D.S., Hill, W.J. & Gill, D.R., 1998. Acidosis in cattle: a review. J. Anim. Sci. 76, 275-286.

Pandey, A., Soccol, C.R., Nigam, P. & Soccol, V.T., 2000. Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. Bioresour. Technol. 74, 69-80.

Pienaar, G.H., Einkamerer, O.B., Van der Merwe, H.J., Hugo, A., Scholtz, G.D.J. & Fair, M.D., 2012. The effects of an active live yeast product on the growth performance of finishing lambs. S. Afr. J. Anim. Sci. 42, 464-468.

Pienaar, G.A., 2016. The potential of condensed molasses soluble (CMS) to replace molasses in feedlot diets. MSc (Agric) Animal Science: Nutrition Science, dissertation, Dept. of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa.

Plaizier, J.C., Krause, D.O., Gozho, G.N. & McBride, B.W., 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J. 176, 21-31.

Potter, S.G., Ammerman, C.B., Henry, P.R., Becker, H.N. & Palmer, A.Z., 1985a. Effect of sugarcane condensed molasses solubles, sugarcane molasses and monensin on performance and volatile fatty acid production in finishing steers. Anim. Feed Sci. Technol. 12, 275-283.

Potter, S.G., Moya, A., Henry, P.R., Palmer, A.Z., Becker, H.N. & Ammerman, C.B., 1985b. Sugarcane condensed molasses solubles as a feed ingredient for finishing cattle. J. Anim. Sci. 60, 839-846.

Price, M.M., Einkamerer, O.B., De Witt, F.H., Greyling, J.P.C. & Fair, M.D., 2009. The effect of dietary ionophores on feedlot performance of lambs. S. Afr. J. Anim. Sci. 39, 141-144.

Puls, R., 1994. Mineral Levels in Animal Health. Diagnostic Data (2nd ed.). Sherpa International, Clearbrook, Canada.

Ramos, S., Tejido, M.L., Martínez, M.E., Ranilla, M.J. & Carro, M.D., 2009. Microbial protein synthesis, ruminal digestion, microbial populations, and nitrogen balance in sheep fed diets varying in forage-to-concentrate ratio and type of forage. J. Anim. Sci. 87, 2924–2934.

Rezac, D.J., Thomson, D.U., Bartle, S.J., Osterstock, J.B., Prouty, F.L. & Reinhardt, C.D., 2014a. Prevalence, severity, and relationships of lung lesions, liver abnormalities, and rumen health scores measured at slaughter in beef cattle. J. Anim. Sci. 92, 2595-2602.

Rezac, D.J., Thomson, D.U., Siemans, M.G., Prouty, F.L., Reinhardt, C.D. & Bartle, S.J., 2014b. A survey of gross pathologic conditions in cull cows at slaughter in the Great Lakes region of the United States. J. Dairy Sci. 97, 4227-4235.

RFA, 2016. Ethanol industry outlook. Available: https://www.ethanolrfa.org/wp-content/uploads/2016/02/Ethanol-Industry-Outlook-2016.pdf. (accessed 5 September 2017).

Robinson, P.H., Tamminga, S. & Van Vuuren, A.M., 1986. Influence of declining level of feed intake and varying propotion of starch in the concentrate on rumen fermentation in dairy cows. Livest. Prod. Sci. 15, 173-189.

Robinson, P.H., Givens, D.I. & Getachew, G., 2004. Evaluation of NRC, UC Davis and ADAS approaches to estimate the metabolizable energy values of feeds at maintenance energy intake from equations utilizing chemical assays and in vitro determinations. Anim. Feed Sci. Technol. 114, 75-90.

Rodríguez, A.B., Landa, R., Bodas, R., Prieto, N., Mantecón, A.R. & Giráldez, F.J., 2008. Carcass and meat quality of Assaf milk fed lambs: Effect of rearing system and sex. Meat Sci. 80, 225-230.

Russell, J.B., 1998. The importance of pH in the regulation of ruminal acetate to propionate ratio and methane production in vitro. J. Dairy Sci. 81, 3222-3230.

Samuels, M.L., 1989. Statistics for the life sciences. Collier MacMillan Publishers, London, England.

SAS, 2017. Statistical Analysis Systems user's guide (version 9.3). SAS Institute Inc., Cary, North Carolina, USA.

Schelling, G.T., 1984. Monensin mode of action in the rumen. J. Anim. Sci. 58, 1518-1527.

Schwartzkopf-Genswein, K.S., Beauchemin, K.A., Gibb, D.J., Crews, D.H., Hickman, D.D., Streeter, M. & McAllister, T.A., 2003. Effect of bunk management on feeding behavior, ruminal acidosis and performance of feedlot cattle: A review. J. Anim. Sci. 81, E149-E158.

Sheridan, R., Ferreira, A.V. & Hoffman, L.C., 2003. Production efficiency of South African Mutton Merino lambs and Boer goat kids receiving either a low or a high energy feedlot diet. Small Rumin. Res. 50, 75-82.

Smith, P.S., 2008. Dietary fibre requirements of feedlot lambs. MSc (Agric) Animal Science: Nutrition Science, dissertation, Dept. of Animal, Wildlife and Grassland Sciences, University of the Free State, Bloemfontein, South Africa.

Solomon, M.B., Kemp. J.D., Moody, W.G., Ely, D.G. & Fox, J.D., 1980. Effect of breed and slaughter weight on physical, chemical and organoleptic properties of lamb carcasses. J. Anim. Sci. 51, 1102-1107.

Spears, J.W., 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133, 1506S-1509S.

Stemme, K., Gerdes, B., Harms, A. & Kamphues, J., 2005. Beet-vinasse (condensed molasses solubles) as an ingredient in diets for cattle and pigs – nutritive value and limitations. J. Anim. Physiol. Anim. Nutr. 89, 179-183.

Strydom, P.E., 2011. Quality related principles of the South African beef classification system in relation to grading and classification systems of the world. S. Afr. J. Anim. Sci. 41, 177-193.

Suttle, N.F., 2010. Mineral Nutrition of Livestock (4th ed.). CABI Publishing, Wallingford, England.

Tag Eldin, H.A., Babiker, I.A. & El Khidir, O.A., 2011. Feedlot performance of Sudanese desert lambs fed with mash or pelleted diets of varying energy levels. J. Saudi Society. Agric. Sci. 10, 77-79.

Tamminga, S. & Chen, X.B., 2000. Animal-based techniques for the estimation of protein value of forages. In: Forage Evaluation in Ruminant Nutrition. Ed: Givens, D.I., Owen, E., Axford, R.F.E. & Omed, H.M., CABI Publishing, Wallingford, England. pp. 215-232.

Thomas, P.C. & Rook, J.A.F., 1981. Manipulation of rumen ferementation. In: Recent Developments in Ruminant Nutrition. Ed: Haresign, W. & Cole, D.J.A., Butterworths, London, England. pp. 157-183.

Tilley, J.M.A. & Terry, R.A., 1963. A two stage technique for the *in vitro* digestion of forage crops. J. Brit. Grassld. Soc. 18, 104-111.

Tillman, A.D. & Kidwell, J.F., 1951. The value of ammoniated condensed distillers molasses solubles as a feed for beef cattle. J. Anim. Sci. 10, 934-938.

Tripathi, M.K., Chaturvedi, O.H., Karim, S.A., Singh, V.K. & Sisodiya, S.L., 2007. Effect of different levels of concentrate allowances on rumen fluid pH, nutrient digestion, nitrogen retention and growth performance of weaner lambs. Small Rumin. Res. 72, 178-186.

Utley, P.R., Bradley, N.W. & Boling, J.A., 1970. Effect of restricted water intake on feed intake, nutrient digestibility and nitrogen metabolism in steers. J. Anim. Sci. 31, 130-135.

Van de Vyver, W.F.J., Beukes, J.A. & Meeske, R., 2013. Maize silage as a finisher for Merino lambs. S. Afr. J. Anim. Sci. 43, S111-S115.

Van Eeden, M., 2016. Effects of intermittent feed supplementation on certain rumen parameters and intake of sheep fed a medium quality roughage. MSc (Agric) Animal Science: Nutrition Science, dissertation, Dept. of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa.

Van Niekerk, B.D.H., 1975. Supplementation of grazing cattle. Proceedings: Seminar on potential to increase beef production in tropical America, Cali, Columbia, 83-94.

Van Ryssen, J.B.J., 2000. The multifactorial nature of trace nutrient nutrition and the supplementation of trace elements to livestock in South Africa. 38th Congress of the South African Society for Animal Science, Alpine Heath, KwaZulu-Natal, South Africa.

Van Ryssen, J.B.J., 2016. Bodily fluid tissue analyses to access the mineral nutritional status of livestock: Basic principles and considerations. RUVASA Congress, 10-12 March, Protea Ranch Hotel, Polokwane, South Africa, 80-88.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant (2nd ed.). Cornell University Press, Ithaca, New York.

Voermol, 2010. Market grain through lambs. Available: http://www.voermol.co.za/en/news/technical/market-grain-through-lambs/default.aspx. (accessed 20 May 2016).

Vyas, D., Uwizeye, A., Mohammed, R., Yang, W.Z., Walker, N.D. & Beauchemin, K.A., 2014. The effects of active dried and killed dried yeast on subacute ruminal acidosis, ruminal fermentation, and nutrient digestibility in beef heifers. J. Anim. Sci. 92, 724–732.

Wagner, J.J., Lusby, K.S. & Horn, G.W., 1983. Condensed molasses solubles, corn steep liquor and fermented ammoniated condensed whey as protein sources for beef cattle grazing dormant native range. J. Anim. Sci. 57, 542-552.

Waliszewski, K.N., Romero, A. & Pardio, V.T., 1997. Use of cane condensed molasses solubles in feeding broilers. Anim. Feed Sci. Technol. 67, 253-258.

Warmington, B.G. & Kirton, A.H., 1990. Genetic and non-genetic influences on growth and carcass traits of goats. Small Rumin. Res. 3, 147-165.

Webb, E.C., 1994. Synthesis of long chain fatty acids in ruminants and their effects on meat quality. PhD thesis (Animal Science), University of Pretoria, Pretoria, South Africa. (with modifications).

Webb, E.C. & Casey, N.H., 1995. Genetic differences in fatty acid composition of subcutaneous adipose tissue in Dorper and SA Mutton Merino wethers at different live weights. Small Rumin. Res. 18, 81-88.

Webb, E.C. & O'Neill, H.A., 2008. The animal fat paradox and meat quality. Meat Sci. 80, 28-36.

Webb, E.C. & Erasmus, L.J., 2013. The effect of production system and management practices on the quality of meat products from ruminant livestock. S. Afr. J. Anim. Sci. 43, 413-423.

Webb, E.C., 2015. Description of carcass classification goals and the current situation in South Africa. S. Afr. J. Anim. Sci. 45, 229-233.

Weigand, E. & Kirchgessner, M., 1980. Protein and energy value of vinasse for pigs. Anim. Feed Sci. Technol. 5, 221-231.

Weiss, W.P., 2012. Real world recommendations for vitamins and minerals. Penn State Dairy Cattle Nutrition Workshop, 12-14 November, Grantville, Pennsylvania, USA.

Weldeman, W., Smith, A. & Du Toit, S.W., 1995. CMS Report. Available at: WDS Bemarking BK. P.O. Box 7336, Stellenbosch, 7559.

Whitney, T.R., Lupton, C.J., Muir, J.P., Adams, R.P. & Stewart, W.C., 2014. Effects of using ground redberry juniper and dried distillers grains with solubles in lamb feedlot diets: Growth, blood serum, fecal, and wool. J. Anim. Sci. 92, 1119-1132.

Yalcin, S., Eltan, Ö., Karsli, M.A. & Yalcin, S., 2010. The nutritive value of modified dried vinasse (ProMass) and its effects on growth performance, carcass characteristics and some blood biochemical parameters in steers. Revue. Méd. Vét. 161, 245-252.

Yeo, J.M., Jeong, S.G., Kim, H.S., Ahn, B.S., Kim, C.H. & Shin, H.T., 2004. The effects of condensed molasses solubles (CMS)/molasses mixtures on ruminal microbial protein synthesis. J. Anim. Sci. Technol. 46, 61-68.

Yang, W.Z., Beauchemin, K.A. & Rode, L.M., 2001. Effects of grain processing, forage to concentrate ratio, and forage particle size on rumen pH and digestion by dairy cows. J. Dairy Sci. 84, 2203-2216.

Zali, A., Eftekhari, M., Fatehi, F. & Ganjkhanlou, M., 2017. Effect of vinasse (condensed molasses solubles) on perormance and meat chemical composition of Holstein male calves. Ital. J. Anim. Sci. 16, 515-520.

Zebeli, Q., Mansmann, D., Steingass, H. & Ametaj, B.N., 2010. Balancing diets for physically effective fibre and ruminally degradable starch: A key to lower the risk of sub-acute rumen acidosis and improve productivity of dairy cattle. Livest. Sci. 127, 1-10.

Zinn, R.A., Alvarez, E., Mendez, M., Montaño, M., Ramirez, E. & Shen, Y., 1997. Influence of dietary sulphur level on growth performance and digestive function in feedlot cattle. J. Anim. Sci. 75, 1723-1728.

Appendix A

Table A1 The effects of different inclusion levels of CMS on mean (±SE) urine output in cannulated wethers

Parameters	Treatment[1]				±SE[2]
	0% CMS	4% CMS	8% CMS	12% CMS	
Urine output (g/day)	721.71[b]	690.23[b]	977.996[a]	885.68[ab]	65.410

[a,b,c] Means within a row with different superscript differ significantly (P <0.05)

[1]Treatment - Percentage of CMS on an as is basis included in experimental diets

0% CMS = Control; 4% CMS = Treatment 1; 8% CMS = Treatment 2; 12% CMS = Treatment 3

[2]±SE - Standard error