THE EFFECT OF FINE PARTICLE REMOVAL FROM GROUND FORAGE SAMPLES ON *IN SACCO* DRY MATTER AND NEUTRAL DETERGENT FIBRE DISAPPEARANCE VALUES

by

Claudia Isabell Mack

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Animal Sciences)

at

Stellenbosch University

Department of Animal Sciences

Faculty: AgriSciences

Supervisor: Prof CW Cruywagen

Date: *March* 2011

Pectora roborant cultus recti

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: February 2011

Copyright © 2011 Stellenbosch University

All rights reserved

Abstract

Title: The effect of fine particle removal from ground forage samples on *in sacco* dry matter and neutral detergent fibre disappearance values

Name: Claudia Isabell Mack

Supervisor: Prof. C.W. Cruywagen

Institution: Department of Animal Sciences, Stellenbosch University

Degree: MScAgric

In vitro and *in situ* methods using the *in sacco* technique have a wide application in ruminant nutrition as they allow the degradability and quality of forages and ruminant diets to be determined quicker and at a lower cost than *in vivo* methods. These trials make use of artificial fibre bags, made of polyester (dacron) or nylon which are available in variable pore sizes. Results from such degradability trials are of great value to feed formulation programs such as AMTS.cattle and CPM Dairy and the more accurate the results are obtained from such trials the more accurate feed formulation models are enabling the ruminant nutritionist to formulate the best diet possible to reach the genetic potential of ruminants. The accepted method for *in sacco* trials (NRC, 2001) requires that the feed samples are ground through a 2 mm screen. This usually results in a variety of particle sizes, including a significant amount of extremely fine particles. Research has, however, shown that these fine particles can potentially be washed out of the dacron bags that are used in *in sacco* degradability trials. This would result in an over-estimation of the soluble and rapidly fermentable nutrient fractions. The objective of this study was to determine the effect of fine particle removal from ground forage samples on the chemical composition and *in vitro* dry matter (DM) and neutral detergent fibre (NDF) degradability of forages. Lucerne hay, oat hay and wheat straw samples were sourced from seven different locations in the Western Cape. Samples were milled through a 2 mm screen and then sieved through either 150 µm, 125 µm or 106 µm. All fractions were analysed for DM, crude protein (CP), NDF, fat and ash. Based on the NDF content of the original samples, four samples from each forage type were selected for *in vitro* trials to determine DM and NDF disappearance over time. Samples were incubated for 0, 6, 24 and 48 hours in an ANKOM Daisy II incubator. Significant variation occurred within forage types in terms of chemical composition. Fine particle

removal had no effect on the NDF content of lucerne hay and wheat straw, but sieved oat hay fractions had a higher NDF content than the un-sieved samples. The NDF content was on average 635.9 for the sieved OH fractions, whereas the NDF content of the un-sieved samples was 606.8. The CP content of sieved oat hay (61.4 on average) and wheat straw fractions (47.7 on average) were lower than the un-sieved fractions (65.7 for OH and 55.4 for WS), whereas for lucerne hay, sieving had no effect on CP content.

Dry matter and NDF disappearances were significantly higher for the un-sieved samples than for the sieved fractions for all three forage types at all incubation times, which indicates an over-estimation of the soluble and readily digestible forage fractions. Compared to sieved samples, DMD values at 0 hours (washing only) of the un-sieved samples were, on average, 13.8% higher for lucerne hay, 27.3% for oat hay and 44.7% for wheat straw. At 48 h, the over-estimation of lucerne DMD for the un-sieved samples was between 4.0% (compared to 106 μm sieve) and 7.3% (compared to 150 μm sieve). This over-estimation in the un-sieved samples was carried over to all four time points. No significant differences between the fractions (150, 125 and 106) were found within a forage type at all incubation times. The estimated degradation rates and the predicted digested proportions were also significantly higher for the un-sieved fractions compared to the sieved fractions. It was concluded that fine particle removal from forage samples would result in more accurate estimations of *in sacco* nutrient degradability.

Uittreksel

Titel: Die invloed van fyn materiaal verwydering uit gemaalde ruvoere op *in sacco* droë materiaal en neutraal-onoplosbaare vesel verdwyningswaardes

Naam: Claudia Isabell Mack

Studieleier: Prof. C.W. Cruywagen

Instansie: Departement Veekundige Wetenskappe, Universiteit Stellenbosch

Graad: MScAgric

In sacco in vitro- en *in situ*-metodes word dikwels toegepas in die studie van herkouervoeding aangesien hierdie metodes vinniger, meer effektief en meer ekonomies is as *in vivo*-metodes. Hierdie studies maak gebruik van kunsveselsakkies, gemaak van poliëster (dacron) of nylon wat beskikbaar is in verskeie poriegroottes. Resultate van sulke verteringsproewe is belangrik vir toepassing in voerformuleringsprogramme soos AMTS.cattle en CPM Dairy. Die resultate wat deur hierdie studies verkry word, is belangrik vir akkurate voerformulering deur formulerings-modelle en stel die herkouervoedingkundige in staat om die ideale voer te formuleer vir die manifestering van die dier se genetiese potensieaal. Die aanvaarde *in sacco*-metode (NRC, 2001) vereis dat voermonsters deur 'n 2 mm sif gemaal word wat 'n groot verskeidenheid partikelgroottes tot gevolg het met 'n beduidende hoeveelheid baie fyn materiaal. Navorsing het getoon dat hierdie baie fyn partikels uit die dacronsakkies gewas kan word tydens *in sacco* verteringstudies, met die gevolg dat die oplosbare en vinnig-verteerbare fraksie oorskat kan word. Die doel van die huidige studie was om die invloed van die verwydering van fyn partikels op die chemiese samestelling van ruvoermonsters te bepaal, asook die *in vitro* droë materiaal (DM) en neutraal-onoplosbaare vesel (NDF) verteerbaarheid daarvan. Monsters van lusernhooi, hawerhooi en koringstrooi, afkomstig van sewe verskillende lokaliteite in die Wes-Kaap, is deur 'n 2 mm sif gemaal en sub-monsters is deur 'n reeks siwwe met poriegroottes van 150 µm, 125 µm of 106 µm gesif. Al die fraksies is geanaliseer vir DM, ruproteïen (RP), NDF, vet en as. Vier monsters van elke voertipe is op grond van die NDF-inhoud geselekteer vir *in vitro*-studies om die DM- en NDF- verteerbaarheid oor tyd te bepaal. Monsters is vir 0, 6, 24 of 48 uur geïnkubeer. Die resultate het getoon dat daar betekenisvolle variasie in chemiese samestelling binne ruvoertipes voorgekom het. Die verwydering van die fyn partikels het geen invloed die

NDF-inhoud van lusernhooi en koringstrooi gehad nie. Wat hawerhooi betref, was die NDF-inhoud van die gesifte monsters egter betekenisvol hoër in vergelyking met die ongesifte monsters. Die NDF inhoud was gemiddeld 635.9 vir die gesifte monsters en vir die ongesifte monsters 606.8. Sifting het geen invloed op die RP-inhoud van lusernhooi gehad nie, maar vir hawerhooi (61.4 gemiddeld) en koringstrooi (47.7 gemiddeld) was die RP-inhoud van die gesifte monsters betekenisvol laer as dié van die ongesifte monsters (65.7 vir hawerhooi en 55.4 vir koringstrooi).

In vergelyking met die gesifte monsters, was die *in vitro* DM- en NDF-verteerbaarhede betekenisvol hoër vir die ongesifte monsters vir al drie ruvoertipes by alle inkubasietye. Hierdie resultate bevestig ‘n oorskatting van oplosbare en maklik verteerbare fraksies in gemaalde voermonsters. In vergelyking met die gesifte monsters, was die DMV-waardes van die ongesifte monsters by 0 ure (slegs gewas) gemiddeld 13.8% hoër vir lusernhooi, 27.3% vir hawerhooi en 44.7% vir koringstrooi. Na 48 h inkubasie was die oorskatting van lusern DMV vir die ongesifte monsters tussen 4.0% (vergeleke met die 106 µm sif) en 7.3% (vergeleke met 150 µm sif). Die oorskatting is oorgedra na al vier inkubasietye. Die resultate het geen noemenswaardige verskille tussen die fraksies (150 µm, 125 µm en 106 µm poriegroottes) van ‘n ruvoertipe by enige inkubasietyd aangedui nie. Die beraamde verteringstempo’s en verteerde fraksies was ook aansienlik hoër vir die ongesifte monsters in vergelyking met die gesifte monsters. Die gevolgtrekking is gemaak dat die verwydering van fyn partikels uit gemaalde ruvoermonsters die akkurate bepaling van *in sacco* verteerbaarheidswaardes verhoog.

Acknowledgements

I would like to thank the following people and organizations for their help and support. Without them this study would have been impossible and I would like to express my sincerest gratitude to each one of them:

- Professor C.W. Cruywagen, for his academic and moral support and his guidance throughout my postgraduate and undergraduate studies.
- The Hennie Steenberg Trust Fund for funding my studies.
- DAAD for providing me with a bursary throughout the years of my post-graduate studies.
- Ms. B. Ellis and the technical staff of the Department of Animal Sciences, Stellenbosch University, for their assistance during this study.
- Mr. I. Stevens and the technical staff of the Welgevallen Experimental Farm, Stellenbosch University, for their assistance during this study.
- The entire academic staff of Department of Animal Sciences, Stellenbosch University, for their assistance and guidance.
- Ms. A Botha for her guidance and support.
- My family for their support, guidance, encouragement and patience throughout my undergraduate and post-graduate studies. Without them this work would not have been possible.
- My friends for their help and moral support.

Table of Contents

Declaration ii
Abstract iii
Uittreksel v
Acknowledgements vii
Table of contents viii

Chapter 1: General introduction 1
1.1. References 4

Chapter 2: Literature review 6
2.1. Introduction 6
2.2. The Importance of forages in dairy cow nutrition 7
2.2.1 Defining forages 7
2.2.2 Nutritional composition and quality of forages 8
2.3. Rumen microbes 10
2.3.1 Rumen bacteria 12
2.3.2 Rumen protozoa 13
2.3.3 Rumen fungi 13
2.3.4 Improvement of ruminal cellulose digestion through manipulation of the microbes 14
2.4. Fibre 14
2.5. Neutral detergent fibre (NDF) 15
2.6. Physical effective fibre and particle size 18
2.7. *In sacco methods (both in vitro and in situ)* for determining ruminal DM and NDF digestion 19
2.8. Particle washout from dacron bags 21
2.9. Conclusion 23

2.10. References 25

Chapter 3: Chemical composition and sieve fractions of forages sourced from different locations in the Western Cape **31**

Abstract 31
3.1 Introduction 32
3.2. Materials and methods 33
3.2.1 Sample preparation 33
3.2.2 Chemical analysis of forages 33
3.3. Statistical analysis 35
3.4. Results and discussion 35
3.4.1 The chemical composition of forages ground through a 2 mm screen 35
3.4.2 The effect of different sieve sizes on particle distribution 37
3.4.3 The effect of sieving on NDF and CP content of forages 38
3.5. Conclusion 40
3.6. References 42

Chapter 4: The effect of fine particle removal on *in vitro* dry matter and NDF disappearance of forages **44**

Abstract 44
4.1 Introduction 45
4.2 Methods and materials 46
4.2.1 Sample preparation 46
4.2.2 Preparation of the *in vitro* medium and the reducing solution 47
4.2.3 Collection and preparation of rumen fluid 48
4.2.4 *In vitro* incubation of the forage samples 49
4.2.5 Zero hours 49

4.2.6 Analysis of the residues 50
4.2.7 Estimating the Dry Matter (DM) degradability 50
4.2.8 Estimating the neutral detergent fibre (NDF) degradability 51
4.2.9 Lignin 51
4.2.10 Rate calculator 51
4.2.11 Statistical analysis 52
4.3 Results and discussion 52
4.3.1 *In Vitro* Dry Matter Degradability 52
4.3.2 *In Vitro* NDF Degradability 56
4.3.3 Rate of NDF disappearance and proportion digested 60
4.4 Conclusion 62
4.5 References 64

Chapter 5: General conclusion 67
5.1 References **69**

CHAPTER 1

GENERAL INTRODUCTION

According to the Department of Economic and Social Affairs/ Population Division of the United Nations (2004), the world population is estimated to grow from 6.1 billion, which was established in the census in 2000, to 8.9 billion in the year 2050 (United Nations, 2004). The Food and Agricultural Organization of the United States estimated that in the year 2010 about 925 million people were undernourished. This is said to be an improvement to the number in the previous year. However, this is still a very high number (Food and Agricultural Organization of the United States, 2010).

Animal products contribute to one-sixth of the energy and one-third of the protein supplies required by humans. The animal protein and energy sources are made up of meat, milk and milk products. Animal products are also an excellent source of some vitamins, for example vitamin A, thiamin, niacin and riboflavin; and minerals such as calcium, iron and zinc. Wealth and religious views play a very big role in defining the protein intake of a human population (McDonald *et al.,* 2002). With this rapidly rising population growth there is a huge demand for an increase in animal protein production.

Chalupa *et al.* (1996), state that the modern consumer desires animal products that are low in fat and that contain more protein. Dairy cattle farmers are forced to increase the productivity of their cows and the quality of their animal products in order to remain compatible in the market. Running a profitable dairy farm is thus a balancing act between increasing the production and efficiency of the herd without jeopardizing animal health and causing damage to the environment.

According to Fernandez *et al.* (2004), when the genetic potential of dairy cattle for milk production increases, nutritional management becomes the essential factor in order to maximize the production and profit of the herd. They further state that increasing the energy intake of the dairy cows is a very important management strategy for attaining maximal profit. Strategies to

increase the energy intake of ruminants are processing the feeds by either grinding or pelleting them, as this increases the passage rate of the feeds ingested (Bourquin *et al.,* 1994).

Forage diets are not able to meet the energy demands of high producing dairy cows and it is essential to supplement those diets with energy and protein rich feeds (Holtshausen, 2004). As the concentrate level in a feed increases, the proliferation of fibrolytic bacteria is stunted and the rumen microbes shift to the degradation of the more readily digestible carbohydrates. Cell wall digestion may be therefore significantly decreased (Bourquin *et al.,* 1994). Low fibre diets cause a fall in the rumen pH, low acetate to propionate ratios and lowered milk fat concentrations. Feeds that are high in concentrates and low in fibre are also closely related with physiological disorders such as laminitis and acidosis (Santini *et al.,* 1983). The physically effective fibre (peNDF) is related to the physical properties (i.e. particle size, shape, moisture, etc.) of fibrous feedstuffs that promote the chewing activity of the ruminant animal and the formation of the rumen mat (Mertens, 1997). It is therefore essential that dairy cow rations contain a sufficient amount of physically effective fibre in order to promote chewing and saliva production. This will in turn prevent the pH from falling below 6 and keep the rumen healthy and functional (Plaizier, 2004). The latter further states that the NRC (2001) "recommend a minimum dietary NDF level of 25% DM, of which 75% must be from forage sources, in order to provide sufficient rumen buffering".

In order to improve animal productivity it is necessary to acquire a better understanding of the digestive function of ruminants (Baldwin & Allison, 1983). This and a thorough knowledge of the chemical composition and digestibility of raw materials is essential for the formulation of optimal and refined diets which may increase productivity and profitability of a dairy enterprise.

The digestibility of a feed gives a good indication of its energy content. The digestibility of a feed can be determined with *in vitro* methods in the laboratory. *In vitro* methods are much faster and cheaper than *in vivo* methods (Holecheck *et al.,* 1982; Kitessa *et al.,* 1999). Cruywagen *et al.* (2003) found that when grinding feed samples through a 1 or 2 mm screen, the samples often contain particles of such small size that they are able to pass through the pores of the dacron bags which in turn leads to an "overestimation of the soluble fraction".

The objective of this study was to test different mesh sizes in order to find an optimal size that will remove very fine particulate matter from feed samples. This mesh should at the same time

result in a uniform NDF and nutrient distribution in both the fraction that remains on top (the coarse fraction) and below the mesh (the fine fraction). The results of these studies could render useful information for the standardization of *in sacco* and *in vitro* procedures. Further, the effect of fine particle removal on the chemical composition of forages, their *in vitro* dry matter disappearance (DMD) and their neutral detergent fibre disappearance (NDFD) was to be studied.

1.1. References

Baldwin, R.L. & Allison, M.J., 1983. Rumen metabolism. J. Anim. Sci. 57: 461 - 477.

Bourqin, L.D., Titgemeyer, E.C., Van Milgen, J. & Fahey, Jr., G.C., 1994. Forage level and particle size effects on orchardgrass digestion by steers: II. Ruminal digestion kinetics of cell wall components. J. Anim. Sci. 72: 759 - 767.

Chalupa, W., Galligan, D. T. & Ferguson, J. D., 1996. Animal nutrition management in the 21 st century: dairy cattle. Anim. Feed Sci. Technol. 58: 1 - 18.

Cruywagen, C.W., Bunge, G.A. & Goosen, L., 2003. The effect of milling on physical material lost through Dacron bags of 53 micron pore size. J. Anim. Sci. 81 (suppl. 1)/ J. Dairy Sci. 86 (Suppl. 1):287.

Food and Agricultural Organization of the United States, 2010. Available at: http://www.fao.org/hunger/en/ (Accessed 29 September 2010).

Fernandez, I., Martin, C., Champion, M. & Michalet-Doreau, B., 2004. Effect of corn hybrid and chop length of whole-plant corn silage on digestion and intake by dairy cows. J. Dairy Sci. 87: 1298 - 1309.

Holecheck, J.L., Vavra, M. & Pieper, R.D., 1982. Methods for determining the nutritive quality of range ruminant diets: a Review. J. Anim. Sci. 54: 363 - 376.

Holtshausen, L., 2004. Effect of nonfiber carbohydrates on product yield and fiber digestion in fermentations with mixed ruminal microbes. PhD thesis, University of Florida, Gainesville, Florida, USA.

Kitessa, S., Flinn, P.C. & Irish, G.G., 1999. Comparison of methods used to predict the *in vivo* digestibility of feeds in ruminants. Aust. J. Agric. Res. 50: 825 - 841.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 2002. Evaluation of food: Digestibility. In: Animal Nutrition. (6th ed.). Pearson Education Ltd., Edinburgh Gate, Harlow, Essex, UK. pp. 483 - 487.

Mertens, D.R., 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80: 1463 - 1481.

National Research Council, 2001. Nutrient requirements of dairy cattle. (7thRev. ed.) National Academy. Press, Washington, D.C., USA.

Plaizier, J.C., 2004. Replacing chopped alfalfa hay with alfalfa silage in barley grain and alfalfa-based total mixed rations for lactating dairy cows. J. Dairy Sci. 87: 2495 - 2505.

Santini, F.J., Hardie, A.R. & Jorgensen, N.A., 1983. Proposed use of adjusted intake based on forage particle length for calculation of roughage indexes. J. Dairy Sci. 66: 811 - 820.

United Nations, 2004. World population to 2300. Department of Economic and Social Affairs/ Population Division. Copyright © United Nations 2004.
Available at:
http://www.un.org/esa/population/publications/longrange2/WorldPop2300final.pdf
(Accessed 29 September 2010).

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Ruminants have evolved anatomically to utilize fibrous feedstuffs in order to meet their nutritional needs. Their ability of obtaining energy from cellulose gives them a greater advantage than that of their non-ruminant counterparts (Van Soest, 1994). The digestive enzymes in the ruminant's compound digestive system alone are not able to digest the ß-linked polysaccharides like cellulose, which are abundant in fibrous feeds (McDonald *et al.,* 2002).

The consumption of forages involves complex interactions between the digestive tract of the ruminant animal, the microorganisms living in it symbiotically and the plants being consumed (Mertens & Ely, 1982). The reticulorumen houses a variety of bacteria, protozoa and fungi that are able to digest the cellulose contained in forages and other fibrous feeds which are consumed by the ruminant (McDonald *et al.,* 2002). The cellulose fermentation is an intricate process that includes attachment of the microbes to the substrate, hydrolysis and fermentation of the cellulose to volatile fatty acids, carbon dioxide and methane (Mouriño *et al.,* 2001). The rumen provides the perfect location for microbial fermentation. It remains at an almost constant temperature of 39 °C, it is buffered by bicarbonate-containing saliva and is a completely anaerobic environment allowing the microbial population to proliferate and carry out their function (Russel & Hespell, 1981). Upon washed out from the rumen, the ruminal bacteria constitute a source of microbial protein, which together with feed protein that has not been digested in the rumen, is used as a source of peptides and amino acids by the animal (Poos-Floyd *et al.,* 1985).

According to Allen (2000), the most important limitation to milk yield is the energy intake of high producing dairy cows. This in turn is dependent on the energy content of the feed and dry matter intake (Allen, 2000). However, forage diets are not able to meet the energy demands of high producing dairy cows and it is therefore necessary to supplement those diets with feeds that are rich in protein and energy (Holtshausen, 2004). High concentrate diets are often the

culprit for subclinical acidosis if not fed together with forages. Subclinical acidosis leads to lowered fibre digestion, unsatisfactory feed intake, low milk fat and health problems, for example laminitis (Maekawa *et al.,* 2002). It is therefore essential that these high energy diets contain enough physical effective fibre in order to promote rumination, saliva production and subsequent rumen buffering. The size of the particles, the neutral detergent fibre (NDF) content and the ratio of forage to concentrate, determines the physical effective fibre (peNDF) of a diet (Plaizier, 2004).

The main aim of every dairy cattle farmer should be to maximize cow performance and efficiency without putting the cows' health at risk by following good nutritional management. Good nutritional management is only possible with improving the understanding of the digestive function in ruminants (Baldwin & Allison, 1983). The *in vitro* and *in sacco* methods are commonly used to evaluate feedstuffs and to study the digestive functions of the rumen (Udén, 1992). However, these procedures often lead to contradicting results and therefore, it is essential to find a way to standardize *in sacco* procedures to minimize the overestimation of the soluble fraction and to improve their accuracy (Kitessa *et al,*. 1999).

2.2. The Importance of forages in dairy cow nutrition

2.2.1 Defining forages

Animal feeds are grouped into forages and concentrates. As described by Van Soest (1994), "concentrates are high-quality, low-fibre feeds such as cereals and milling by-products that contain a high concentration of digestible energy per unit weight and volume". Forages can be defined as the fraction of plants, other than grain, that are edible and suitable to provide nutrients to grazing animals or, that can be harvested for feeding (Forage & Grazing Terminology Committee, 1991). According to Van Soest, (1994), most forages fed to ruminants are angiosperms and they can be grouped into grasses (grass and grass-like plants), legumes (herbaceous legumes), forbs (non-legume broad leafed herbs) and browse (woody plants, shrubs and trees). Furthermore, forages are described by the percentage of cell walls they contain (Van Soest, 1994). The following table depicts different forage types.

Table 2.1 Feed types that fall within the definitions of forage (Wilkins, 2000).

Forage	Feed types
Herbage	Leaves, stems, roots of non-woody species, including sown and permanent grassland and crops that may be grazed or cut
Hay and silage	
Browse	Buds, leaves and twigs of woody species
Straw	

2.2.2 Nutritional composition and quality of forages

Forages vary widely in their nutritional value and therefore each forage type contributes differently to production of the animal (Forage & Grazing Terminology Committee, 1991). The following table depicts the metabolisable energy and crude protein contents of a few forages.

Table 2.2 Energy (MJ/kg DM) and protein (g/kg DM) content of different classes of forages (Wilkins, 2000).

Forage class	Metabolisable energy MJ kg^{-1}DM	Crude protein g kg^{-1}DM
Temperate grasses, hays and silages	7.0-13.0	60-250
Tropical grasses	5.0-11.0	20-200
Maize silage	10.0-12.0	60-120
Cereal straw	5.0-8.0	20-40
Root crops	11.0-14.0	40-130
Kale and rape	9.0-12.0	140-220

The quality of forages is an essential factor which will determine the productivity of the ruminant animal. One factor influencing the quality of forages is their fibre content. Fibre contributes to the bulky part of the forage which needs to be broken down in the rumen. The rumen microbes obtain their energy from fibre and therefore, it is essential for rumen functionality. The rumen microbes are not able to break down the parts of fibre which contain lignin. However, lignin is an essential component of forages as it plays a role in promoting rumination (Van Soest, 1994). Kalscheur *et al.,* (1997) state that if cows are fed diets that are low in forage and do not contain a buffer, a change in the rumen function can result. This altered rumen function results from a lowered rumen pH which causes lowered fibre digestibility. According to Beauchemin & Rode (1997), a minimum amount of forage fibre is necessary to avoid milk fat depression, to insure rumen health and longevity of the cows. These authors further state that if a dairy cow diet does not contain sufficient forage fibre, rumination and salivation is decreased, which in turn leads to reduced fibre digestion and lower ratios of acetate to propionate. Sub-acute and acute acidosis is common with low forage fibre diets.

An important measure for forage quality is rate of digestion. Forages that have a high rate of digestion also have a high rate of intake (Holecheck *et al.,* 1982). The less lignified forages are, the higher their NDF digestibility is (Oba & Allen, 1999). Forages that contain high fibre percentages are regarded as poor quality forages. Their fermentation rates are so low, that the rumen microbes' maintenance requirements are hardly met, which lowers the energy output of the animal itself (Van Soest, 1994). The voluntary feed intake of forages is regarded as a crucial factor when assessing the quality of forages (Blümmel & Becker, 1997).

Factors like age and maturity, soil quality and environment affect the fibre content of the forages and thus their nutritive quality. Young plants contain less structural carbohydrates like hemicellulose, cellulose and lignin compared to plants of higher maturity (McDonald *et al.,* 2002). The older a forage gets, the more lignified it becomes and the lower the leaf: stem ratio is. This in turn lowers the forages' nutritional value and the digestibility of the mature forages. There are however some exceptions to this rule. Maize and some other crops do not show a decline in their nutritive value as they mature. But this is related to the increase in starch content of maize, because the fibre degradability is lower in more mature maize plants. One can thus conclude that seed crops and cereals are at their ultimate maturity when harvested and straw, bran, husks and hulls are at their lowest quality at the stage where the reach maturity (Van Soest, 1994).

The environmental factors affecting forage quality range from temperature, light and water to soil fertility. Diseases and stresses like grazing can also affect the plant composition and thus forage quality. High temperature causes the forages to mature earlier which results in the accumulation of structural carbohydrates, especially lignin, which in turn lower their digestibility. When forages are exposed to increasing light their cell wall components decrease as more non-structural carbohydrates (for example sugars), amino acids and organic acids are formed. When water availability is limiting, the growth rate of the forages is reduced, which increases their digestibility. Cloudy weather accompanied by a lot of moisture causes the production of forages of low quality. These generalizations however can differ from forage to forage (Van Soest, 1994). The soil composition, i.e. its mineral composition greatly influences the composition and the yield of a plant or forage and its digestibility (Morrison, 1959).

2.3. Rumen microbes

Ruminant diets mainly consist of forages and roughages that have a high fibre contents. However, the ruminant's digestive enzymes are not able to digest the ß-linked polysaccharides, for examples cellulose, which is abundant in forages. The rumen hosts a vast number of anaerobic bacteria, protozoa and fungi that ferment the feed particles into volatile fatty acids and microbial protein (McDonald *et al.,* 2002). The variety of species in these microbial groups differ biochemically and morphologically and their substrate utilization spectra for carbohydrates overlap (Leedle *et al.,* 1982). The microbiota in the rumen forms the link between the ruminant and the diet it consumes (Weimer *et al.*, 1999). Allen & Mertens (1988) state that the digestion of fibre takes place in a "complex ecosystem that is influenced by dynamic interactions among the diet, microbial population and animal". A large part of the cell walls that are present in forage based diets that ruminants consume are made up of cellulose and therefore, cellulolytic microbes play an important role in ruminant nutrition. Research has shown that several rumen fungi and protozoa are able to digest cellulose. However, the ruminal cellulolytic bacteria are the most dominant microbes in the rumen that digest cellulose (Weimer, 1996). Each microbial species ferments different substrates and yields different types and quantities of fermentation products, which affect the composition of the milk that cows produce and the feed efficiency (Weimer *et al.,* 1999). The diet that the ruminant consumes affects the number of the ruminal microbes and the relative populations of the individual species. Concentrate feeds will therefore result in higher numbers of lactobacilli (McDonald *et al.*, 2002), and high fibre diets are associated with an increase in the population of the cellulolytic bacteria, which can be

associated with the increased cellulose concentration in the rumen. Nevertheless, each cow has its own unique composition of microbes in the rumen (Weimer *et al.,* 1999). According to Tajima *et al.* (2001), the state of health, use of antibiotics, geographic position and season also affect and change the microbial composition of the animal. Leedle *et al*. (1982) state that the feeding regime, frequencies of feeding and the levels of feed intake also determine which microbial species will be present in the rumen.

The rumen pH greatly affects the functionality of the ruminal microbes and in turn will affect fibre digestion (Weimer *et al.,* 1999). As quoted by Mouriño *et al.* (2001) a low pH decreases the extent of fibre digestion *in vitro*. Research has shown that the dominant cellulolytic bacteria do not proliferate when the pH falls below 6. This lowered cellulose digestion at low pH may be explained by the failure of the bacteria to adhere to the substrate and by inhibited cellulose hydrolysis (Mouriño *et al.,* 2001). Low rumen pH in the rumen results from the production of lactic acid that is the end product of starch and sugar digestion.

The microbes in the rumen closely interact with each other. It is distinguished between cooperative and competitive interaction. There are numerous types of cooperative interaction of which only a few will be mentioned for the purpose of this literature review. Certain combinations of rumen microbes may interact in such a way as to improve the degree of cell wall digestion. Some microbes hydrolyze complex carbohydrates but do not use the hydrolytes they generate. Other microbes may then utilize these nutrients for growth. This phenomenon is also known as cross-feeding (Baldwin & Allison, 1983). As quoted by Calitz (2009), *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* ferment hemicellulose to soluble sugars, which are then used as nutrients by *Butyrivibrio fibrisolvens.* Other microbial species may even remove substances that have an inhibiting effect on other microbes. Cooperative interactions are usually beneficial for both microbes involved. Competitive interaction on the other hand involves one microbe having an adverse effect on another microbe's functionality. *Streptococcus bovis* for example, produces excessive amounts of lactate which has an inhibiting effect on other microbial species in the rumen (Baldwin & Allison, 1983). Protozoa engulf a large part of the ruminal bacterial population which can change the patterns of fermentation in the rumen (Russel & Hespell, 1981).

2.3.1 Rumen bacteria

Every mL of the ruminal contents contains 10^9-10^{10} bacteria. The diet of the ruminant animal affects the bacterial species and the total number of bacteria present in the rumen (McDonald *et al.*, 2002). The following table depicts a summary of the major culturable bacterial species in the rumen and their substrates.

Table 2.3 The major ruminal bacteria grouped according to their functionality and their substrates (Adapted from Baldwin & Allison, 1983 and McDonald *et al.,* 2002).

Bacterial species	Substrates
(Holo) Cellulolytic	
Fibrobacter succinogenes	Cellulose, glucose
Bacteriodes succinogenes	Starch, pectin
Ruminococcus albus	Cellobiose, xylan
Ruminococcus flavefaciens	Cellulose, xylan
Amylo- and Dextrinolytic	
Bacteroides amylophilus	Pectin
Streptococcus bovis	Starch, soluble sugars, protein
Succinimonas amylolytica	
Succinivibrio dextrinosolvens	Pectin
Saccharolytic	
Bacteroides ruminicola	Starch, pectin, protein
Butyrivibrio fibrisolvens	Cellulose, starch, protein
Megasphaera elsdenii	Lactate, protein, glucose
Selemonas ruminantium	Starch, lactate
Prevotella ruminicola	Glucose, xylan, starch

The most dominant culturable cellulolytic bacteria found in the rumen are *Fibrobacter succinigenes, Ruminococus flavefaciens,* and *Ruminococcus albus.* These bacteria are nutritionally specialized to only use cellulose and the products that result from its hydrolysis as substrates. The substrate cell wall structure and the rumen environment, which constantly flows and houses a large micro flora, set limits to the digestive capabilities of the cellulolytic bacteria.

The microbes that are found attached to the fibre particles and which are not very mobile have thus evolved to digest cellulose very fast (Weimer, 1996).

2.3.2 Rumen protozoa

Protozoa are less abundant in the rumen than bacteria (Baldwin & Allison, 1983). These ciliates amount to only 10^6 cells per ml rumen contents (McDonald *et al.,* 2002). But due to their larger size in comparison to bacteria, they make up about half of the total microbial mass in the rumen (Baldwin & Allison, 1983). According to Van Soest (1994), protozoa engulf bacteria and any particle that matches the size of a bacterium. Examples of these are starch, chloroplasts, proteins and plastic particles. This predation on ruminal bacteria is believed to lower the efficiency of the rumen. Some protozoan species can utilize cellulose; however, this plays a minor role in the rumen metabolism. Protozoa are classified as fermentative anaerobes which release "acetate, butyrate, lactate, carbon dioxide, and hydrogen" when fermenting their substrates. An important function of protozoa is that they prevent a rapid decline in rumen pH due to lactate production from rapid starch degradation, by engulfing starch and by grazing on starch-digesting bacteria (Russell & Hespell, 1981). As quoted by Calitz (2009) they also remove lignin from carbohydrates, which makes the carbohydrates more available for hydrolysis by microbial enzymes, thus improving cellulose digestion. However, the turnover of protozoa is very slow and their outputs are relatively small in comparison to rumen bacteria output (Van Soest, 1994).

2.3.3 Rumen fungi

Rumen fungi have only been discovered in the 1970s and further research is needed (Van Soest, 1994). So far 12 species of fungi have been cultured, most of which belong to the genus *Neocallimastix* (McDonald *et al.,* 2002). These strict anaerobes play an important role in digestion of feeds in the rumen. Van Soest (1994) states that the rumen fungi release a "more soluble cellulose complex than" the one released by the bacteria in the rumen. Their rate of digestion was found to be slower than that of cellulose fermenting bacteria; nevertheless, the extent of digestion is not altered. Fungi are also able to attach to coarser cellulose particles and are able to digest them faster than rumen bacteria do (Van Soest, 1994). Fungi do not digest lignin. However, their rhizoids and sporangia filaments enter fibrous structures and make parts of lignin more soluble by releasing enzymes, which give rumen bacteria access to the cellulose

and hemicellulose. The nutrients which are released are subsequently carried to the sporangium (Van Soest, 1994).

2.3.4 Improvement of ruminal cellulose digestion through manipulation of the microbes

The feeding practices of today often render new challenges to the rumen microbes. Thus it is of great interest to the animal nutritionist to manipulate the microflora of the rumen in order to improve the productivity of the ruminant animal. The structural carbohydrates of plants, for example cellulose, are the feedstuffs that greatly contribute to meeting the energy requirements of the ruminant animal. Only a few ruminal bacteria have evolved to digest cellulose, however, as stated by Weimer (1998), the number of those bacteria is sufficient for the fibre digestion, but rather the accessibility of fibre is limiting. A way of improving cellulose digestion in the rumen would be to make the fibre more accessible to the cellulolytic bacteria, rather than producing a new “hypercellulolytic” strain (Weimer, 1998). Researchers are busy with engineering ruminal bacteria which are tolerant to lower pH into bacteria that are able to digest cellulose as it seems to be a fruitless endeavor to improve the functionality of existing cellulolytic bacteria (Weimer, 1996). In their paper, Weimer *et al.,* (1999) state that it is not an easy undertaking to introduce genetically engineered cellulolytic bacteria into the rumen as the bacteria that are native in the rumen often inhibit the engineered strains (Weimer, 1998). Weimer (1996) reported that providing proper feeding management strategies, that ensure a healthy rumen environment for the rumen microbes, may greatly improve the performance of the animal.

2.4. Fibre

According to Mertens (1997), fibre can be defined nutritionally “as the slowly digestible or indigestible fraction of feeds that occupies space in the gastrointestinal tract of the animal”. Jung (1997) states that fibre is the term used to describe the plant cell wall of forages fed to livestock. Plant cell walls are complex structures made up of lignin, hemicelluloses, cellulose, pectin, protein, lignified nitrogenous substances, waxes, cutin and mineral components. The insoluble parts of the cell walls are constituted by lignin, cellulose and hemicelluloses which are covalently cross-linked (Van Soest, 1994). Mammals do not produce the enzymes to hydrolyze the *ß*1-4 linked polysaccharides that are abundant in cell walls. Therefore, they rely on microorganisms that reside in the gastrointestinal tract that are able to digest these

polysaccharides into nutrients that can be absorbed by the animal (Jung, 1997). Fibre stimulates sufficient rumination and saliva production and rumen buffering (Sudweeks *et al.,* 1981). It further helps to form the ruminal mat that acts as a filter which prevents feed particles to pass out of the rumen too quickly and thus the subsequent nutrient loss. The fibre requirement of ruminants is affected by the size of the rumen, level of intake and production and particle size (Van Soest *et al.,* 1991).

2.5. Neutral detergent fibre (NDF)

In ruminant nutrition, neutral detergent fibre (NDF) is regarded as "the best single chemical predictor" for voluntary feed intake (Allen, 1996). Dairy cow diets need to contain enough NDF in order to keep the rumen healthy and to ensure high milk production. It is common practice to formulate dairy diets to a "specific forage NDF content" as it is known that NDF affects rumination and the subsequent rumen pH. Next to the NDF contents of a forage, its NDF digestibility is as important as it directly influences the performance of the animal and it goes hand in hand with determining the quality of the forage (Oba & Allen, 1999). NDF is considered as the part of forages or diets that causes rumen fill (Dado & Allen, 1995).

Several different methods for forage fibre analysis are available, but in ruminant nutrition the neutral detergent fibre method (NDF) developed by Van Soest is now widely used (Jung, 1997). The term neutral detergent fibre describes the residue that remains after extracting a forage sample with a boiling neutral solution of sodium lauryl sulphate and ethylenediaminetetraacetic acid (EDTA). This residue is made up of mainly lignin, cellulose and hemicelluloses (McDonald *et al.,* 2002). The extraction with neutral detergent quantifies the percentage of cell wall and non-cell wall components in forages, where the cell wall components are slowly digestible by rumen microbes and the non-structural ones are easily digestible by rumen microbes. Pectin is a cell wall component; it is, however, extracted during the NDF procedure (Chalupa *et al.,* 1996). The solubility in detergent and the nutritional availability of pectin result from its lack of covalent bonding with the lignified matrix, as pectin is located in the middle lamella in the cell wall (Van Soest, 1994). The following table depicts the plant components that are soluble and insoluble in neutral detergent solution.

Table 2.4 Forage fraction classification using the method of Van Soest (Van Soest & Wine, 1967).

Fraction	Components
Cell contents (soluble in ND-solution)	Lipids
	Sugar, organic acids
	Water-soluble matter
	Pectin, starch
	Non-protein nitrogen
	Soluble protein
Cell wall contents (insoluble in ND-solution)	
1. Soluble in AD-solution	Fibre-bound protein
	Hemicellulose
2. ADF	Cellulose
	Lignin
	Lignified nitrogen
	Silica

ND-solution = neutral detergent solution; AD-solution = Acid detergent solution

Due to the fact that most of the pectin contained in the forages is solubilized, NDF underestimates the actual cell wall concentration in certain forages (Jung, 1997). However, this and other short comings this method may have, is not regarded as problematic when "fibre is defined as the incompletely digestible fraction of feeds" (Jung, 1997). In addition for analyzing forages for fibre, the neutral detergent method can also be used for feeds that contain starch. In this case amylase needs to be added to the neutral solution (McDonald *et al.,* 2002). For the optimal removal of starch, it is essential to add 2-ethoxyethanol to the neutral solution. Due to certain health risks 2-ethoxyethanol has now been replaced by triethylene glycol (Van Soest *et al.,* 1991). The table below depicts NDF and non fibre carbohydrate (NFC) percentages of different forage and concentrate types.

Table 2.5 Feed carbohydrate fractions (Sniffen, as cited by Chalupa *et al.,* 1996).

Feedstuff	NDF	NFC
Forages		
Alfalfa hay, early vegetative	35.8	25.5
Alfalfa hay, late vegetative	40.0	24.7
Alfalfa hay, early bloom	43.7	24.1
Alfalfa hay, mid bloom	46.9	23.8
Grass hay, late vegetative	57.0	10.3
Grass hay, pre bloom	62.2	8.4
Grass hay, early bloom	65.4	8.2
Grass hay, mid bloom	67.2	8.7
Corn silage, well eared	45.0	39.7
Corn silage, few ears	55.0	29.0
Concentrates		
Barley	28.3	55.4
Beet pulp	54.0	33.3
Brewers grains	46.0	13.5
Citrus pulp	21.1	60.7
Corn and cob meal	26.0	59.4
Corn distillers grains	42.5	12.3
Corn gluten feed	41.3	27.1
Corn gluten meal	14.0	14.3
Corn grain	9.0	74.3
Corn hominy	27.4	55.9
Cottonseed meal	34.0	13.3
Linseed meal	25.0	28.7
Corn grain	32.2	47.1
Peanut meal	14.0	26.3
Rapeseed meal	0	50.1
Sorghum grain	8.7	73.8
Soybeans	0	32.9
Soybean meal (44% CP)	14.0	27.3
Soybean meal (48% CP)	10.0	27.4
Soy bean hulls	69.9	14.3
Sunflower meal	40.0	26.6
Wheat grain	14.0	71.0
Wheat bran	51.0	20.6
Wheat middlings	37.2	35.6

NDF = neutral detergent fibre; NFC = Non-fibre carbohydrate.

From the table above one can see that grasses have a higher NDF content than legumes. Concentrates are in general higher in NFC's and contain lower NDF percentages.

2.6. Physical effective fibre and particle size

Research has shown that the physical characteristics of fibre greatly affect the rumen environment and subsequent animal health, fermentation and absorption of nutrients and milk fat production (Mertens, 1997). The physical effectiveness of fibre (peNDF) affects chewing and salivation (Maekawa *et al.,* 2002), mainly due to differences in particle length (Allen, 1997).

As previously discussed, ruminant diets are, in general, formulated on the basis of neutral detergent fibre (NDF). The NDF however, is only a measure of the "chemical characteristics" of forage and does not account for the physical properties such as particle size and particle density. This may become a problem when formulating diets, when the forages are milled very finely. Other factors affecting the physical characteristics of forages and feeds for dairy cattle are the ratio of forage to concentrate, the type of forage and concentrate used in the formulation, and the type of feed processing.

The peNDF is thus linked to the physical properties, like for example particle size, shape, moisture, fragility, the relationship of eating time to rumination and preservation of fibrous feedstuffs that promote chewing activity and formation of the rumen mat. Moreover, the peNDF of forage is determined by its physical effective factor (pef) and NDF content. The pef is derived from the effect of a given forage on chewing activity of a ruminant. A laboratory method has been developed to determine peNDF, where the proportion of particles that remain on top of a 1.18 mm sieve in a vertical shaker is multiplied by the NDF content (Mertens, 1997).

When too little effective fibre is included in dairy cow rations, mild and borderline acidosis often result. This is due to changed fermentation which results from reduced rumination, which again leads to reduced salivation. Therefore, not enough buffering saliva is released into the rumen which leads to a fall in the ruminal pH (Yang & Beauchemin, 2007). Lowered rumen pH leads to a cascade of events like low milk fat percentages due to low ratios of acetate to propionate in the rumen (Mertens, 1997), lowered appetite, reduced rumen motility, stunted microbial yield and lowered fibre digestion. In acute cases low rumen pH can even lead to liver abscesses, liver ulcers, and laminitis; and in some cases even death of the animal (Allen, 1997). Therefore dairy

cow rations should always be balanced for peNDF in order to promote chewing activity as this aids in fine tuning the ration. Further, when formulating diets, the peNDF can be utilized to set the lower limit of the fibre to carbohydrate ratio (Mertens, 1997).

Particle size plays a significant role in dry matter intake. When distension in the rumen is the limiting factor for feed intake, decreased particle size by chopping the forages more finely can increase the dry matter intake (Allen, 2000). However, voluntary dry matter intake is also affected by the particle fragility and the digestibility of the NDF of the forages as this determines the filling effect of forages. Milling and pelleting decreases the particle size and lowers the volume of the feedstuffs and therefore decreases retention time. This in turn increases the feed intake of the ruminant (Allen, 1996). This higher feed intake and reduced retention time in the rumen could potentially reduce the time for microbial fibre and organic matter digestion (Firkins *et al.,* 1986). Rumination is also decreased with decreasing particle size which can lead to a decreased rumen pH as discussed above.

As quoted by Yang & Beauchemin (2007) long forage particles play a crucial role in in promoting the rumen walls to contract and subsequently mix the rumen contents. It has been reported that when feeding long forage particles, a larger proportion of the starch gets digested in the large intestine rather than in the rumen, which lowers the risk of rumen acidosis. Research has shown that increased particle size of the forages also increase the forage digestibility and the production of microbial protein (Yang *et al.,* 2002). Thus it is beneficial to increase the percentage of forage and the milling size to reduce the acidosis risk in high producing dairy cows (Yang & Beauchemin, 2007). Care needs to be taken to not feed feeds where the particle size is too long, as this promotes sorting of the ration by the animal. A “Penn State Particle Separator” can assist in measuring the particle size of feedstuffs (Heinrichs & Kononoff, 2002).

2.7. *In sacco* methods (both *in vitro* and *in situ)* for determining ruminal DM and NDF digestion

The ruminal degradability of ruminant feeds can be used to derive energy contents. A feed’s degradability can be determined *in vivo* by making use of live animals or *in vitro*, which is done in a laboratory. The preferred method to measure the digestibility (ruminal or total tract) of

feedstuffs is the *in vivo* method (Kitessa *et al.,* 1999). However, *in vivo* methods are very work and time consuming (McDonald *et al.,* 2002). Laboratory methods are less expensive and less time consuming (Kitessa *et al.,* 1999). Throughout the last century many *in vitro* and *in situ/ in sacco* methods have been developed in order to estimate the ruminal in *vivo* fermentation process (Pienaar *et al.,* 1989). The *in vitro* methods, which are also known as micro methods (Holecheck *et al.,* 1982), mimic digestion in the rumen (Ørskov *et al.,* 1980) and include the *in vitro* gas production procedure (which measures fermentation) and the traditional two stage *in vitro* fermentation in rumen liquor which has been described by Tilley and Terry (1963).

In the *in vitro* gas production system, the amount of gas produced by fermentation is recorded either manually of automatically. The amount of gas released is in proportion to the amount of feedstuff that is being fermented. This method can be applied to measure the extent of fermentation and the rate thereof (Bunge, 2006). As quoted by Malan (2009), the gas production system also describes how the rumen microbes react to the substrate they are incubated with and it also can be used as a measure of the rate of volatile fatty acid and microbial protein production. A great advantage of this method is that a large number of samples can be incubated at the same time (McDonald *et al.,* 2002).

Several *in vitro* procedures exist, however the two-stage technique developed by Tilley and Terry (1963) is regarded as the standard procedure. In this method the feed samples are incubated with rumen liquor for a period of 48 hours and which is then followed by the addition of an acid-pepsin solution and subsequent incubation for another 48 hours (Holecheck *et al.,* 1982; Kitessa *et al.,* 1999). The function of the acid is to kill off the rumen bacteria and the pepsin digests the undigested protein remaining in the residue (McDonald *et al.,* 2002). The advantage of the *in vitro* two-stage procedure over the nylon bag *in situ* method is that more samples can be analyzed at the same time and the former method is more accurate in predicting the digestibility of a feedstuff. Further, research has shown that rumen liquid from any species can be utilized, provided they receive a diet which is similar to the diet that is being analyzed (Holecheck *et al.,* 1982). The two-stage procedure however is not very suitable for analyzing straws and feeds of lower qualities. Goering and Van Soest (1970) modified the two-stage method of Tilley and Terry (1963) to determine the dry matter digestibility. The feed samples are incubated with rumen liquor and a medium that contains a macro- and mineral solution and a buffer solution. Resazurin is added to the medium as an indicator and a reducing solution is added to the medium to remove the oxygen. The residue is then extracted with a

neutral detergent solution. The results obtained with the method modified by Goering and Van Soest (1970) estimate the true dry matter digestibility, where the method proposed by Tilley and Terry (1963) measures the apparent dry matter digestibility of feedstuffs (Ammar *et al.,* 2004). ANKOM technology Corp., Macedon, NY developed the DAISY II incubator. In this incubator several different feeds can be incubated at the same time in order to analyze *in vitro* dry matter digestibility. This technology renders more accurate results and is less labour intensive than the old *in vitro* methods. Feed samples that need to be analyzed are weighed into filter bags, of which a maximum number of 25 are incubated in a digestion vessel containing rumen fluid and a buffered medium. The DAISY II incubator rotates the digestion vessels at a constant temperature of 39°C (Holden, 1999).

In the *in sacco* method, also called *in situ* method, feed samples are weighed into filter bags which are then inserted into the rumen via a ruminal cannula. These porous bags are made of either polyester (such as dacron) or nylon. After the incubation period the bags are removed from the rumen and fermentation is stopped by killing the rumen bacteria by either placing the bags into a 75% ethanol solution or by placing the sample bags into ice. Subsequently the samples are washed with water, then dried at 65°C (Holecheck *et al.,* 1982) and weighed in order to determine the dry matter loss (Kitessa *et al.,* 1999). This method, however, has a few shortcomings, being that the feed samples are not subjected to particle breakdown by rumination and the samples are not able to leave the rumen once they are small enough (Dewhurst *et al.,* 1995). Furthermore, the *in sacco* method often leads to an overestimation of the fermentable fraction of the samples due to fine particle washout out of the bags (Ørskov *et al.,* 1980; Dewhurst *et al.,* 1995). Finally, the *in sacco* method requires surgically modified animals which results in high costs and has risen potential animal welfare problems (Kitessa *et al.,* 1999).

2.8. Particle washout from dacron bags

As reported by Ørskof *et al.* (1980), one of the disadvantages of the nylon bag technique used to evaluate feedstuffs is that not necessarily complete degradation into simpler compounds is measured with this technique, but rather the breakdown of particles until they are small enough to pass through the dacron bag pores. Thus when grinding feed samples through a 1 or 2 mm screen, the samples often contain particles of such small size that they are able to pass through the pores of the dacron bags which in turn leads to an overestimation of the soluble fraction and

the underestimation of the non-fermentable fraction (Dewhurst *et al.,*1995; Cruywagen *et al.,* 2003).

Research has shown that with increasing incubation time of samples, the fineness of the particles has a smaller effect on the dry matter disappearance. This can be ascribed to an increase in surface area with a decrease in particle size, and therefore increasing the sites of attachment for rumen microbes which will lead to faster degradation of the smaller particles. This increased surface area seems to only affect the initial rate of digestion. Other researchers, however, did not find any differences in dry matter disappearance when grinding forage samples through screens of different sizes. Nevertheless, it was found that there was a great amount of particle washout through the pores of the dacron bags. This can be corrected for the washout of particles by preparing a second set of the samples and then soaking and washing them in water, after which they are dried (Ørskov *et al.,* 1980). Kitessa *et al.* (1999) state that some researchers, when studying the effect of particle size on dry matter disappearance, obtained results that contradict each other. Other researchers incubated samples of different forage types *in situ* that were milled through the same mesh size and they also obtained results that differed greatly from each other. This can be ascribed to different particle size distributions of different forage types after grinding them through the same screen size. When incubating these samples, weight losses from the dacron bags can be attributed to smaller particles being washed out through the pores of the bags, as already discussed above, which leads to these contradicting results.

It is essential to find a way to standardize *in sacco* procedures to minimize the overestimation of the soluble fraction. Nocek (1998), quoted by Kitessa *et al.* (1999), proposed that protein supplements should be ground through a 2 mm sieve and a 5 mm sieve should be used for feeds that are high in fibre in order to gain uniformity so that different feed types can be compared to each other. Cruywagen *et al.* (2003) ran two trials where they studied the effect of milling on particle loss through dacron bag pores. In the first trial, they milled Lucerne hay and wheat straw through a mesh of 1 mm. Subsequently they sieved the samples through a mesh with a pore size of 60 µm. NDF analysis was done on the fraction of the samples that remained on the top of the sieve, on the material that fell through the sieve, and as well on the un-sieved samples. All samples were also washed with water in a washing machine to quantify the dry matter losses through washout. In the second trial the authors used a sieve of 250 µm instead of the 60 µm sieve and analyzed an additional forage type, namely *Eragrostis curvula*. The

samples were analyzed in the same way as the samples in the first trial. Both trials rendered results that proved that the sieve size affects the distribution of NDF in the fractions that arise (i.e. the coarse material remaining on the top and the fine material collecting at the bottom of the sieve). With the 60 µm sieve the fine forage fraction that collected below the sieve contained a higher amount of NDF than the coarse material that remained on top of the sieve and the un-sieved samples. In contrast to the results obtained with the 60 µm, when using the 250 µm sieve, the coarse material that remained on the top of the sieve contained the higher NDF content among the three different treatments. The authors concluded from their studies that sieve sizes ranging in between 60 µm and 250 µm could possibly render a uniform distribution of the nutrients in the fine and coarse material which can then be regarded as representative of the original sample. However, more research is required to shed light on this issue.

The nature of the dacron bags, (i.e. the materials used), the weaving structure, the shape and the size of the pores can also have an effect on the extent of particle washout from the pores and which will render contradicting *in vitro* and *in situ* results. It is recommended to use bags that have a uniform pore size and/or that will not deform and change the pore size during incubation. Reusing bags is also not advised. The right pore size needs to be found that will allow enough rumen microbes to enter the bags upon incubation, but will prevent excessive washout of indigestible material through the pores. The pore size that is recommended ranges between 35 and 50 µm (Kitessa *et al.,* 1999). In their study, Cruywagen *et al.* (2003) used dacron bags with a pore size of 53 µm.

2.9. Conclusion

Ruminants have evolved to have a strong symbiotic relationship with the rumen microbes that live in their reticulorumen. This microbiota allows ruminant animals to utilize fibrous feedstuffs as their source of nutrients. Still, forages alone do not suffice in meeting the energy requirements of high producing dairy cows. Therefore dairy cattle are fed energy rich concentrate feeds.

These concentrate feeds are usually low in fibre which greatly reduces rumination and saliva production which can lead to health problems, such as subclinical acidosis and laminitis. The low pH which results from the decreased production of buffering saliva leads to decreased milk production, lowered milk fat percentage, decreased fibre digestion and lowered intake.

Nonetheless, it should be of great interest to the dairy farmer to maximize the energy intake of his cows in order for them to be able to reach their genetic potential.

Particle size and the physical effectiveness of fibre play a critical role in dry matter intake, fermentation, “ensuring optimal feed utilization” and rumen health. It is, however, a balancing act to formulate diets with the optimal particle size and particle distribution that meet all the requirements of a high producing dairy cow.

In vitro and *in sacco* methods are research tools that are widely used to evaluate feedstuffs, and to study the digestive functions of the rumen. Contradicting results have been obtained in the past and soluble fractions have often been overestimated. It is therefore essential to find a way to standardize *in sacco* procedures to minimize the overestimation of the soluble fraction, to improve their accuracy and to find a suitable sieve aperture for sample preparation, which will ensure the even NDF distribution in the feed or forage sample.

2.10. References

Allen, M.S. & Mertens, D.R., 1988. Evaluating constraints on fiber digestion by rumen microbes. J. Nutr. 118: 261 - 270.

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74: 3063 - 3075.

Allen, M.S., 1997. Relationship between fermentation acid production in the rumen and the requirement for physical effective fiber. J. Dairy. Sci. 8: 1447 - 1462.

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83: 1598 - 1624.

Ammar, H., Ranilla, M.J. & López, S., 2004. Seasonal variations in the chemical composition and in vitro digestibility of some Spanish leguminous shrub species. Anim. Feed Sci. Technol. 115 (3): 327 - 340.

Baldwin, R.L. & Allison, M.J., 1983. Rumen metabolism. J. Anim. Sci. 57: 461 - 477.

Beauchemin, K.A. & Rode, L. M., 1997. Minimum versus optimum concentrations of fiber in dairy cow diets based in barley silage and concentrates of barley or corn. J. Dairy Sci. 80: 1629 - 1639.

Blümmel, M. & Becker, K., 1997. The degradability characteristics of fifty-four roughages and the roughage neutral detergent fibres as described by *in vitro* gas production and their relationship to voluntary feed intake. Brit. J. Nutr. 77: 757 - 768.

Bunge, G.A., 2006. The effect of supplemental biotin in dairy cow diets on forage fermentation characteristics. MSc (Agric) thesis, Stellenbosch University, South Africa.

Calitz, T., 2009. The effect of acid buf and combinations of acid buf and sodium bicarbonate in dairy cow diets on production response and rumen parameters. MSc (Agric) thesis, Stellenbosch University, South Africa.

Chalupa, W., Galligan, D. T. & Ferguson, J. D., 1996. Animal nutrition management in the 21 st century: dairy cattle. Anim. Feed Sci. Technol. 58: 1 - 18.

Cruywagen, C.W., Bunge, G.A. & Goosen, L., 2003. The effect of milling on physical material lost through Dacron bags of 53 micron pore size. J. Anim. Sci. 81 (suppl. 1)/ J. Dairy Sci. 86 (Suppl. 1):287.

Dado, R.G. & Allen, M.S., 1995. Intake limitations, Feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. J. Dairy Sci. 78: 118 - 133.

Dewhurst, R.J., Hepper, D. & Webster, A.J.F., 1995. Comparison of in sacco and in vitro techniques for estimating the rate and extent of rumen fermentation of a range of dietary ingredients. Anim. Feed Sci. Tech. 51: 211 - 229.

Firkins, J.L., Berger, L.L., Merchen, N.R. & Fahey, G.C.,Jr, 1986. Effects of forage particle size, level of feed intake and supplemental protein degradability on microbial protein synthesis and site of nutrient digestion in steers. J. Anim. Sci. 62: 1081 - 1094.

Forage & Grazing Terminology Committee, 1991. Vegetation terms. In: Terminology for grazing lands and grazing animals. Pocahontas Press Inc., Blacksburg, Virginia, USA.

Goering, H.K. & Van Soest, P.J., 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications). Agricultural Handbook Number 379. ARS-USDA, Washington, D.C., USA.

Heinrichs, J.A. & Kononoff, P.J., 2002. Evaluating particles size of forages and TMRs using the new Penn State Forage Particle Size Separator. The Pennsylvania State University, University Park, PA.

Holden, L.A., 1999. Comparison of methods of *in vitro* dry matter digestibility for ten feeds. J. Dairy Sci. 82: 1791 - 1794.

Holecheck, J.L., Vavra, M. & Pieper, R.D., 1982. Methods for determining the nutritive quality of range ruminant diets: a Review. J. Anim. Sci. 54: 363 - 376.

Holtshausen, L., 2004. Effect of nonfibre carbohydrates on product yield and fibre digestion in fermentations with mixed ruminal microbes. PhD thesis, University of Florida, Gainesville, Florida, USA.

Jung, H.J.G., 1997. Analysis of fiber and cell walls in ruminant nutrition. J. Nutr. 127: 810S - 813S.

Kalscheur, K.F., Teter, B.B., Piperova, L.S. & Erdman, R.A., 1997. Effect of dietary forage concentration and buffer addition on duodenal flow of trans-$C_{18:1}$ fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80: 2104 - 2114.

Kitessa, S., Flinn, P.C. & Irish, G.G., 1999. Comparison of methods used to predict the *in vivo* digestibility of feeds in ruminants. Aust. J. Agric. Res. 50: 825 - 841.

Leedle, J.A.Z., Bryant, M.P. & Hespell, R.B., 1982. Diurnal variations in bacterial number and fluid parameters in ruminal contents of animals fed low- or high-forage diets. Appl. Environ. Microbiol. 44: 402 - 412.

Maekawa, M., Beauchemin, K.A. & Christensen, D.A., 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J. Dairy Sci. 85: 1165 - 1175.

Malan, M., 2009. The effect of sugar, starch and pectin as microbial energy sources on *in vitro* forage fermentation kinetics. MSc (Agric) thesis, Stellenbosch University, Stellenbosch, South Africa. Pp.11 - 12.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 2002. Evaluation of food: Digestibility. In: Animal Nutrition. (6th ed.). Pearson Education Ltd., Edinburgh Gate, Harlow, Essex, UK. pp. 246 - 263.

Mertens, D.R. & Ely, L.O., 1982. Relationship of rate of digestion to forage utilization– a dynamic model evaluation. J. Anim. Sci. 54, 4: 895 - 905.

Mertens, D.R., 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80: 1463 - 1481.

Mouriño, F., Akkarawongsa, R. & Weimer, P.J., 2001. Initial pH as a determinant of cellulose digestion rate by mixed ruminal microorganisms in vitro. J. Dairy Sci. 84: 848 - 859.

Morrison, F.B., 1959. Feeds and feeding: A handbook for the student and stockman. (22nd ed.). Eds. The Morrison Publishing Company. Clinton, Iowa, USA. 1165 pp.

National Research Council, 1989. Nutrient requirements of dairy cattle. (6th Rev. ed.). National Academy Press, Washington, D.C., USA.

Oba, M. & Allen, M.S., 1999. Evaluation of the Importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82: 589 - 596.

Ørskov, E.R., Hovell, F.D.DeB. & Mould, F., 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5:3: 195 - 231.

Pienaar, J.P., Roux, C.Z. & Cronjé, P.B., 1989. Comparison of *in vivo* and *in sacco* methods to estimate the mean retention time of fermentable organic matter in the rumen. S. Afr. J. Anim. Sci. 19(2); 71 - 75.

Plaizier, J.C., 2004. Replacing chopped alfalfa hay with alfalfa silage in barley grain and alfalfa-based total mixed rations for lactating dairy cows. J. Dairy Sci. 87: 2495 - 2505.

Poos-Floyd, M., Klopfenstein, T. & Britton, R.A., 1985. Evaluation of laboratory techniques for predicting ruminal protein degradation. J. Dairy. Sci. 6: 829 - 839.

Russel, J.B. & Hespell, R.B., 1981. Microbial rumen fermentation. J. Dairy Sci. 64: 1153 - 1169.

Sniffen, C.J., 1988. Proc. Application of nutrition in dairy practice. American Cyanamid CO., Wayne, NJ.

Sudweeks, E.M., Ely, L.O., Mertens, D.R. & Sisk, L.R., 1981. Assessing minimum amounts and form of roughages in ruminant diets: Roughage value index system. J. Anim. Sci. 53: 1406 - 1411.

Tajima, K., Aminov, R.I., Nagamine, T., Matsui, H., Nakamura, M. & Benno, Y., 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl. Environ. Microbiol. 67: 2766 - 2774.

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

Udén, P., 1992. The influence of leaf and stem particle size *in vitro* and of sample size in sacco on neutral detergent fibre fermentation kinetics. Anim. Feed Sci. Technol. 37: 85 - 97.

Van Soest, P.J. & Wine, R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Contamination of Plant Cell-wall Constituents. J. Assoc. Off. Anal. Chem. 50: 50 - 55.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583 - 3597.

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

Weimer, P.J., 1996. Why don't ruminal bacteria digest cellulose faster? J. Dairy Sci. 79: 1496 - 1502.

Weimer, P.J., 1998. Manipulating ruminal fermentation: a microbial ecological perspective. J. Anim. Sci. 76: 3114 - 3122.

Weimer, P.J., Waghorn, G.C., Odt, C.L. & Mertens, D.R., 1999. Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. J. Dairy Sci. 82: 122 - 134.

Wilkins, R.J., 2000. Forages and their role in animal systems. In: Forage evaluation in ruminant nutrition. Eds. Givens, D.I., Owen, E., Axford, R.F.E., Omed, H.M. CAB International, Wallingford, UK. pp. 114.

Yang, W. Z., Beauchemin K. A. & Rode L. M., 2002. Effects of particle size of alfalfa-based dairy cow diets on site and extent of digestion. J. Dairy Sci. 85(8), 1958 - 1968.

Yang, W.Z. & Beauchemin, K.A., 2007. Altering physical effective fiber intake through forage proportion and particle length: chewing and ruminal pH[1]. J. Dairy Sci. 90: 2826 - 2838.

CHAPTER 3

Chemical composition and sieve fractions of forages sourced from different locations in the Western Cape

Abstract

The aim of the study was firstly to determine the variation in chemical composition that occurs within forage types sourced from various locations in the Western Cape, South Africa. A second objective was to find a sieve with a mesh size that would remove very fine particles from ground (2 mm) forage samples to be used in in sacco trials, but that would still render a nutrient distribution of the sieved sample that would be comparable to that of the original ground sample. The first study comprised of the proximate analysis (moisture, ash, fat, crude protein and NDF) of three forage types, namely lucerne hay, oat hay and wheat straw. Based on the NDF results, samples from five localities of each of the three forage types were then chosen and sieved through sieves with mesh sizes of 106 µm, 125 µm or 150 µm. Lucerne hay and oat hay showed significant variation in terms of NDF and crude protein content, whereas the variation between wheat straw samples for these parameters was much smaller. The variation amongst lucerne and oat hay samples can be explained by factors such as harvesting stage, soil types, rainfall, and other environmental and management factors. Wheat straw, on the other hand, being a crop residue, is probably not so much affected by environmental factors as lucerne and oat hay. For lucerne hay, the NDF and CP contents of the sieved fractions did not differ from the original sample. For oat hay, the NDF content of the sieved samples was higher than that of the original samples, whereas the CP content of the sieved samples was lower than that of the original samples. The NDF content of wheat straw samples did not differ between the sieved and original samples, whereas the CP content of the sieved samples were lower than that of the original samples. It was concluded that variation in chemical composition occurs between forage samples sourced from different locations. Regarding fine particle removal, it was concluded that mesh sizes between 106 µm and 150 µm can be used to remove fine particles before in sacco trials, but that sieving may change the NDF content of oat hay and the CP content of both oat hay and wheat straw. Sieving did not affect the NDF or CP content of lucerne hay.

3.1. Introduction

It is essential to have a good knowledge of the nutritive value of the feedstuffs used in animal production in order for the livestock to be able to reach their genetic potential. Chemical analysis has been used since the early nineteenth century to determine the nutritional value and the quality of feedstuffs (Ørskov *et al.,* 1980). The Weende system of proximate analysis is one of the oldest systems used to determine the feed value of raw materials and it has wide applications in human and animal nutrition.

Today, *in vitro* and *in sacco* digestibility procedures are very popular and are reliable tools for testing feeds for quality and nutritional composition (Udén, 1992) as the rate and extent of fermentation of organic matter are imperative determinants of both factors (Dewhurst *et al.,* 1995). Furthermore, these methods aid in getting a better knowledge of the digestive processes and rumen functionality of livestock. Raw materials can be evaluated on a large scale at a lower cost in comparison to *in vivo* digestibility studies (Udén, 1992). Results from such digestibility trials are of great value to feed formulation programs such as CPM Dairy and AMTS.cattle. However, research has shown that feeds used for *in vitro* studies, which are ground through 1 or 2 mm screens, may contain particles that are small enough to leave the pores of the dacron bags which could lead to an overestimation of the soluble fraction of the feedstuff being analysed. These small particles could, however, be removed from the sample by sieving it through a sieve with a suitable mesh size. There is thus a need to standardize *in vitro* procedures (Dewhurst *et al.,* 1995; Cruywagen *et al.,* 2003).

The objectives of this study were (i) to determine the variation in chemical composition that exists within forage types sourced from various locations, and (ii) to determine the effect of fine particle removal on the consistency of chemical composition of the retained material, especially in terms of crude protein (CP) and NDF contents. The hypothesis was that fine particle removal would not affect the CP and NDF composition of forages. This study was executed in the Department of Animal Sciences of the Stellenbosch University and the forages used in this study were chosen with further *in vitro* trials in mind.

3.2. Materials and methods

3.2.1 Sample preparation

Three forage types, namely lucerne hay *(Medicago sativa)*, oat hay *(Avena sativa)* and wheat straw *(Triticum aestivum)* were chosen for this research project. Lucerne hay represents a high quality forage, oat hay a medium quality and wheat straw a low quality forage. For each of these forage types, a sample was obtained from seven different locations in the Western Cape.

Whole bales of each forage sample were milled through a farm hammer mill set at a chop length of 5 cm. Representative samples of each forage were then taken and milled through a 2 mm screen with a laboratory hammer mill (Scientific RSA, supplied by Centrotec, Cape Town) Samples (20 g at a time) of each forage were taken for sieving through screens of different mesh sizes to remove fine particles. Mesh sizes were 150 µm, 125 µm and 106 µm. For each forage, the different sieves were stacked in a horizontal shaker (Retsch AS 200, supplied by Wirsam Scientific, Cape Town) and a collection pan was placed beneath each sieve to collect the fine particles from the respective sieves. Samples were sieved for ten minutes, with the amplitude setting at 80. For each sample, the amount that remained on the top of the sieve and the amount that collected in the pan were weighed to calculate the proportion of fine particles that was removed by each sieve. The course material remaining on the top of the sieves were retained for all the trials in this study. The original forage samples and their fractions were place into sealable plastic containers and stored in a cool room at approximately 4° C until used.

3.2.2 Chemical analysis of forages

Representative samples of all the original un-sieved samples (three forages, seven locations) were analysed for dry matter (DM) crude protein (CP) neutral detergent fibre (NDF), crude fat (as ether extract, (EE)) and ash to determine the level of variation that occurs between samples from different locations. Furthermore, the three sieved fractions of five of the seven locations of each of the three forages, thus 45 samples more, were also selected for NDF and CP analyses. The reason for selecting only five locations was because *in vitro* trials were later done on all the fractions of these samples (i.e. 60 samples, also including the un-sieved samples) and the facilities that were available for *in vitro* trials limited the number of samples that could be selected. Selection was based on the outcome of the first NDF analyses and samples were

chosen to cover the range of variation that was observed in terms of NDF content. For NDF analysis, two methods (described later) were used. The Fibertech apparatus allows for only six samples to be analysed at a time. This method was only used to determine the NDF content of the original un-sieved samples for the purpose of comparing samples from different locations. The ANKOM NDF method allows for 24 samples to be analysed simultaneously and since 930 samples had to be analysed for NDF after the *in vitro* trials, the ANKOM method was the one of choice. Both methods were used on all the original un-sieved forage samples, which allowed for a comparison between the methods.

Moisture was determined by weighing ca. 2 g of the sample into porcelain crucibles and then placing them in a force draught oven set at 100° C for 24 hours (AOAC, 2002; Official Method 934.041). Dry matter values were calculated and tabulated as g/kg.

The ash content was determined by ashing 2 g of the samples in a muffled furnace for 6 h at a temperature of 500°C (AOAC, 2002; Official Method 942.05). After 6 h, the furnace was turned off and allowed to cool down over-night before removing the crucibles and placing them in a desiccator until weighed. Ash content was used to calculate organic matter (OM) by subtracting ash from DM. The OM values were tabulated as g/kg DM.

The total nitrogen content was determined with the aid of a LECO FP-528, Protein/ Nitrogen Determinator (Leco Corporation, St. Joseph, USA). Approximately 0.15 g per sample was accurately weighed into the appropriate tin foil cups. The foil cups were then carefully closed and the ends twisted together. The samples were combusted in the Leco furnace and the nitrogen content (%N) was recorded. Crude protein content was calculated by multiplying the nitrogen content with 6.25 (AOAC, 2002; Method 990.03). All values were tabulated as g/kg DM.

Total fat content was determined as ether extract (AOAC, 2002, Method 920.39) with a Tecator Soxtec System HT 1043 Extraction Unit. Approximately 2 g of the samples were weighed into aluminium thimbles and the fat in the samples was extracted with diethyl ether ($C_4H_{10}O$). Values were tabulated as g/kg DM.

Neutral detergent fibre (NDF) was determined in two ways, as already mentioned. In the first method (further referred to as NDF1), the Fibertech System M 1020 Hot Extractor (SMM Instruments Pty. Ltd., Cape Town, South Africa) was used. Approximately 1 g of each forage

sample was accurately weighed into glass crucibles and placed into the Fibertech apparatus where the samples were analysed with NDS solution according to the method of Robertson & Van Soest (1981). In the second method (further referred to as NDF2), an ANKOM Fiber analyzer (Ankom® Technology Corp., Macedon, NY, USA) was used. This procedure requires the use of the Akom®$^{200/220}$ Fiber Analyzer, Ankom Technology- F57 filter bags, neutral detergent solution, sodium sulfite (Na_2SO_3) and heat heat-stable alpha-amylase which was added during rinsing. This procedure is also based on the method of Van Soest *et al.* (1991).

3.3. Statistical analysis

Regarding the first objective of the trial, the purpose was not to compare the chemical composition of the different forages with each other, but only to determine the variation within forage types sourced from various locations. Only the mean values (± SE) and the coefficients of variation pertaining to the seven localities (DM, OM, EE, CP and NDF 1) and 5 localities (for NDF 2) of the three forage types are thus reported in the Tables 3.1 and 3.2. Regarding the second objective, the effect of the sieving treatments within forages were analysed by means of a main effects ANOVA, with the aid of Statistica 9 (2010). Main effects were sieve size and locality. A Bonferroni post-hoc test was done to separate treatment means that differed. Significance was declared at $P \leq 0.05$.

3.4. Results and discussion

3.4.1 The chemical composition of forages ground through a 2 mm screen

The mean values of the chemical composition of the un-sieved samples (± SE and the coefficients of variation) are given in Table 3.1. For lucerne hay, the biggest variation appeared to occur for EE, CP and NDF 1 and 2. The coefficient of variation (CV) for the NDF content of lucerne hay for both methods (Fibertech and Ankom) was quite large. A high variation for in the NDF content of lucerne hay sourced from different locations can be expected due to factors such as harvesting at different growth stages, i.e. age and maturity (McDonald *et al.,* 2002), and due to different soil types and environmental factors, (for example temperature, rain fall, irrigation, light and soil fertility). Older plants have a higher fibre content which is also more lignified than in younger plants. High temperatures increase the accumulation of structural

carbohydrates, thus increasing the NDF content of forages (Van Soest, 1994). Water also drives NDF content, according to Van Soest (1994). The high variation which appeared to occur for CP can also be explained by environmental factors that could have differed between the localities and harvesting at different growth stages. The high CV observed in terms of EE may also be ascribed to differences in environmental and management factors, but a high CV for EE was also observed for the other forages. Therefore, variation as a result of experimental error in EE analysis cannot be ruled out. Regarding DM and OM, lucerne hay showed a very low variation within the seven localities.

Table 3.1 The mean chemical composition (g/kg DM ± SE) of the un-sieved forage samples sourced from seven localities which were used in the trial. All values (except DM) are on a DM basis.

Feedstuff		DM	OM	EE	CP	NDF 1	NDF 2
n=7							
LUC	*Mean± SE*	887.8±3.2	808.6±6.5	14.8±0.8	160.4±7.1	529.7±29.4	469.8±27.7
	CV	0.97	2.13	14.59	11.72	14.68	13.20
OH	*Mean± SE*	899.2±2.9	846.3±8.4	24.3±5.0	60.2±4.8	664.2±22.7	606.8±9.5
	CV	0.86	2.63	54.36	20.98	9.04	3.52
WS	*Mean± SE*	903.4±3.2	865.0±4.0	14.8±2.9	55.3±1.7	827.6±13.5	807.8±14.2
	CV	0.95	1.24	51.02	8.34	4.32	3.93

DM = dry matter; OM = organic matter; EE = ether extract; CP = crude protein; NDF = neutral detergent fibre; LUC = lucerne hay; NDF 1= Fibertech method; NDF 2= ANKOM method; SE = standard error; CV = coefficient of variation.

For oat hay, the biggest variation within the seven localities appeared to occur for EE, CP and NDF 1, whereas the variation for DM and OM was very low. For CP, the high variation observed can be ascribed to environmental factors that may differ in between the seven localities and different harvesting methods (i.e. harvesting at different growth stages). It is quite well known that time of harvesting oats for hay production in the Western Cape is extremely variable, therefore variation in terms of NDF and CP content could generally be expected. The extremely high CV in EE content, however, probably arises from analytical errors. Wheat straw showed the biggest variation for EE. As mentioned for the other forages, this was probably due to errors

in chemical analysis. Ether extract values for all the forages were low and because EE was not important in the current study, the variation in EE results are of no real concern. Variation in terms of the other nutrients was quite low, which could have been expected because wheat straw is the crop residue remaining after harvesting the ripe cereal grain.

It appears that the values for NDF 2 (Ankom filter bag method) are lower than those for NDF 1 (Fibertech method) for all three forage types. This may be explained by the fact that sodium sulphite was used in the Ankom method. As reported by Ferreira and Mertens (2007), sodium sulphite can solubilize lignin. Therefore, the addition of sodium sulphite in the Ankom method could have under-estimated the NDF content. In the current study, both α-amylase and sodium sulphite were used in the bag method (NDF 2). In the study of Ferreira and Mertens (2007), higher NDF values were also reported for the crucible NDF method when sodium sulphite was omitted, compared to the filter bag method where both α-amylase and sodium sulphite were used. Ferreira and Mertens (2007) used maize silage as substrate, therefore the use of α-amylase was required.

3.4.2 The effect of different sieve sizes on particle distribution

The amount (percentage) of material that remained on top of the sieves after sieving through 106 µm, 125 µm and 150 µm are depicted in Table 3.2.

Table 3.2 The percentage material from three forage types which remained on top of the screen when sieved into three fractions.

	106 µm	**125 µm**	**150 µm**
Sample	**CM, %**	**CM, %**	**CM, %**
Lucerne (Mean ± SE)	86.1 ± 0.9	82.9 ± 1.0	79.5 ± 1.0
Oat hay (Mean ± SE)	86.4 ± 0.7	83.1 ± 0.9	80.5 ± 1.1
Wheat straw (Mean ± SE)	88.4 ± 0.7	85.3 ± 0.9	82.5 ± 0.9

CM= Coarse material remaining on top of screen; SE= standard error.

It can be seen from Table 3.2 that between 11 and 20% material losses occurred when ground samples were sieved to remove fine particles. The material, or at least a part of it, that passed through the sieves could potentially be washed out of dacron bags during *in sacco* trials and could thus be responsible for over-estimating the soluble fraction. The purpose of measuring the amount of material that remained on the top screen was merely to get an idea of material losses that occur with fine particle removal. Such information could help researchers to ensure that they sieve sufficient material to be used in trials or analyses. No statistical analyses were therefore done to compare sieve sizes in terms of material loss. However, one would expect that the finer the mesh, the less material would be lost.

Due to the high number of samples that were to be analysed for NDF in the *in vitro* trial (discussed in Chapter 4), it was decided to select five samples from the seven locations for each forage for the NDF2 analysis. For the *in vitro* trials discussed in Chapter 4 the number was later further reduced to four locations. The selection was based on NDF content and for each forage type the aim was to include four samples that covered the NDF spectrum that was observed in the seven original samples.

3.4.3 The effect of sieving on NDF and CP contents of forages

Table 3.3 shows the mean NDF and CP contents of the original (un-sieved) and sieved samples of lucerne, oat hay and wheat straw from five localities which were chosen according to their NDF contents as determined by the Fibertech method.

From Table 3.3 it can be seen that for lucerne hay, the NDF and CP contents in all the sieved fractions (150 µm, 125 µm and 106 µm) did not differ from the original un-sieved sample. This result was expected, namely a sample where only the finest particles, which may leave the Dacron bags during *in sacco* trials through particle wash-out, are removed. But such a sieved sample would still render a nutrient distribution that would be comparable to the original sample. As the chemical composition of all the fractions of lucerne hay did not differ from each other, any of the sieve sizes could be used.

Table 3.3 The mean NDF and CP content of the un-sieved and sieved samples (150 μm, 125 μm, 106 μm).

	Treatment					
n = 5	**2mm**	**150**	**125**	**106**	**SEm**	**P**
Luc						
NDF 2	469.8	471.0	472.2	471.9	6.56	0.994
CP	168.6	163.8	168.1	169.7	3.22	0.596
OH						
NDF 2	606.8 ^{a}	635.0 ^{b}	638.9 ^{b}	633.7 ^{b}	2.18	< 0.001
CP	65.7 ^{a}	60.2 ^{b}	62.1 ^{b}	61.8 ^{b}	0.72	0.001
WS						
NDF 2	807.8	806.6	814.5	810.1	0.51	0.704
CP	55.4 ^{a}	46.8 ^{b}	48.1 ^{b}	48.2 ^{b}	0.84	< 0.001

^{a,b} For each forage, values with similar superscripts within rows did not differ

CP = crude protein; NDF 2 = neutral detergent fibre as determined with the ANKOM method.

For oat hay, the results for all three fractions for NDF and CP differed significantly from the original un-sieved sample. The three fractions of oat hay nonetheless did not differ from each other. Although the difference in NDF content between the sieved samples and the un-sieved one was significant, the difference was less than 5%. Further research could be done to determine a sieve mesh size which would render a fraction that would be comparable to the un-sieved sample in terms of their nutrient composition.

Wheat straw showed no difference between the three factions from the original un-sieved sample for NDF, but conversely for CP the fractions differed significantly from the original samples. There was no difference, however, between sieve sizes. These results for crude protein for oat hay and wheat straw suggest that similar studies with protein sources could be of value for standardizing *in vitro* digestibility procedures.

In two trials run by Cruywagen *et al.* (2003), sieves with mesh sizes of 60 μm and 250 μm, respectively, were used to sieve forage samples. Subsequently, NDF was determined on all

fractions. Both trials rendered results indicating that sieve size affects the distribution of NDF in the different fractions (i.e. the coarse material remaining on the top and the fine material collecting at the bottom of the sieve). The authors concluded from their studies that sieve sizes ranging in between 60 μm and 250 μm could possibly render a uniform distribution of the nutrients in the fine and coarse material which can then be regarded as representative of the original sample. Together with the results obtained in the current study, it is evident that more research will, however, have to be done on this topic. Similar tests should be run by making use of screens with pore sizes ranging between 60 μm and 106 μm and between 150 μm and 250 μm in order to be able to find an optimal sieve mesh size that would remove very fine particles but still result in a sample with a nutrient distribution which is comparable to the un-sieved sample.

Throughout the research for this project no additional results pertaining to this topic could be found in the literature.

3.5. Conclusion

From our first study it was concluded that there is great variation between samples obtained from different localities. The cause for these differences can be due to harvesting at different growth stages, different soil qualities and environmental factors across localities.

In the second study, it was shown that between 11 and 20% of the sample can be classified as 'fine matter' which is able to wash out of Dacron bags, and therefore can lead to an overestimation of the soluble fraction.

The last study showed that for lucerne hay, these small particles could be removed from the sample by sieving it through a sieve with either a pore size of 106 μm, 125 μm, or 150 μm. The fractions that resulted did not differ from the un-sieved original sample. For oat hay, the results for all three fractions for NDF and CP differed significantly from the original un-sieved sample, it can therefore be concluded that more research would be needed in order to find a suitable mesh size for oat hay in order to be able to standardize *in vitro* procedures. For wheat straw it appeared that the NDF study rendered similar results to the study for lucerne hay. However, when testing the samples for CP, significant differences from the un-sieved sample compared to the three fractions were observed. It can therefore be concluded that more research will be

necessary in order to be able to find a sieve with a suitable pore size to be able to standardize *in vitro* procedures.

3.6. References

AOAC International, 2002. Official methods of analysis (17th ed). Association of Official Analytical Chemists, Arlington, Virginia, USA.

Cruywagen, C.W., Bunge, G.A. & Goosen, L., 2003. The effect of milling on physical material lost through Dacron bags of 53 micron pore size. J. Anim. Sci. 81 (suppl. 1)/ J. Dairy Sci. 86 (Suppl. 1):287.

Dewhurst, R.J., Hepper, D. & Webster, A.J.F., 1995. Comparison of in sacco and in vitro techniques for estimating the rate and extent of rumen fermentation of a range of dietary ingredients. Anim. Feed Sci. Technol. 51: 211 - 229.

Ferreira, G. and Mertens, D.R., 2007. Measuring detergent fibre and insoluble protein in corn silage using crucibles or filter bags. Anim. Feed Sci. Technol. 133, 335 - 340.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 2002. Evaluation of food: Digestibility. In: Animal Nutrition. (6th ed.). Pearson Education Ltd., Edinburgh Gate, Harlow, Essex, UK. pp.

Ørskov, E.R., Hovell, F.D.DeB. & Mould, F., 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5(3) 195 - 231.

Robertson, J.B. & van Soest, P.J., 1981. The detergent system of analysis and its application to human foods. In: Basic and clinical nutrition vol 3. The analysis of dietary fibre in food. Eds. James. W.P.T. and Theander, O. Dekker, NY. Pp. 158 - 276.

Statistica 9, 2010. StatSoft Inc., USA.

Udén, P., 1992. The influence of leaf and stem particle size *in vitro* and of sample size *in sacco* on neutral detergent fibre fermentation kinetics. Anim. Feed Sci. Tech. 37: 85 - 97.

Van Soest, P.J., 1967. Development of a comprehensive system of feed analyses and its application to forages. J. Anim. Sci. 26: 119 - 128.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583 - 3597.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant. (2nd ed.) Cornell University Press, Ithaca, New York, USA. 11 pp.

CHAPTER 4

The effect of fine particle removal on *in vitro* dry matter and NDF disappearance of forages

Abstract

The aim of this study was to determine the effect of fine particle removal on dry matter (DM) and neutral detergent fibre (NDF) degradability in forages. For this study, lucerne hay (LUC), oat hay (OH) and wheat straw (WS) samples and their fractions after sieving through 150, 125 or 106 µm sieves, were chosen from four localities, based on the NDF content of the un-sieved samples. For the in vitro degradability trial 0.5 g of each was weighed into small dacron bags and heat sealed. The forage samples were then incubated in an ANKOM Daisy II incubator for 6, 24 or 48 hours to determine the DM and NDF degradability. The samples were incubated in flasks containing 1076 ml buffered medium, 54 ml reducing solution and 270 ml rumen fluid. Rumen fluid was collected early in the morning from two lactating Holstein cows who received a commercial semi-complete lactating cow feed. The zero hour values were determined by washing in water. Following incubation, residues were analyzed for NDF using the ANKOM method and the NDF degradability was calculated. Dry matter and NDF disappearances were significantly higher for the un-sieved samples than for the sieved fractions for all three forage types at all incubation times, which indicates an over-estimation of the soluble and readily digestible forage fractions. Compared to sieved samples, DMD values at 0 hours (washing only) of the un-sieved samples were, on average, 13.8% higher for lucerne hay, 27.3% for oat hay and 44.7% for wheat straw. At 48 h, the over-estimation of lucerne DMD for the un-sieved samples was between 4.0% (compared to 106 µm sieve) and 7.3% (compared to 150 µm sieve). This over-estimation in the un-sieved samples was carried over to all four time points. No significant differences between the fractions (150, 125 and 106) were found within a forage type at all incubation times. The estimated digestion rates and the predicted digested proportions were also significantly higher for the un-sieved fractions compared to the sieved fractions. For 6 and 48 hours of incubation of lucerne hay, differences between the NDFD of samples sieved through 150 and 106 µ were recorded. This would suggest that fine particles that could potentially be washed out from dacron bags were still retained in the 106 µ sieved sample. It can thus be recommended to sieve samples through a 150 µm sieve which would remove most small particles of the sample, without altering the chemical composition of the sample.

4.1. Introduction

The digestibility of a ruminant feed gives a good indication of how much energy the feed contains. The preferred measures to determine the digestibility of feeds are *in vivo* methods which make use of live animals. However, *in vivo* methods are very time consuming and expensive. Today *in vitro* digestibility methods have a wide application in animal nutrition as the trials that make use of the methods are quick to run at and are less costly. Also a great number of samples can be run at the same time (Kitessa *et al.,* 1999). These micro methods (Holecheck *et al.,* 1982), mimic digestion in the rumen (Ørskov *et al.,* 1980) in a laboratory setup and include the *in vitro* gas production method and the traditional two stage *in vitro* fermentation in rumen liquor which has been developed by Tilley and Terry (1963). Not only the chemical composition of forages, but also the quality thereof and kinetic parameters can be determined by estimating the rate of *in vitro* digestibility (Holecheck *et al.,* 1982).

In sacco trials make use of artificial fibre bags, made of polyester (dacron) or nylon. These bags are helpful tools in determining feed quality and aid in improving the understanding of the processes that take place in the rumen (Ørskov *et al.,* 1980). Artificial fibre bags are also used in *in vitro* digestibility trials in the laboratory utilizing an ANKOM DAISY II incubator (Ankom Technology Corp., Macedon, NY). Results from such digestibility trials are of great value to feed formulation programs such as AMTS.cattle and CPM Dairy.

The accepted method for *in sacco* trials (NRC, 2001) requires that the feed samples are ground through a 2 mm screen. This usually results in a variety of particle sizes, including a significant amount of extremely fine particles. These fine particles can potentially be washed out of the dacron bags that are used in *in sacco* digestibility trials. This would result in an over-estimation of the soluble and rapidly fermentable nutrient fractions. A study was designed to test the hypothesis that the removal of particles <150 μm would lower the estimated DMD and NDF disappearance values obtained from *in sacco* trials. This study was executed in the Department of Animal Sciences of the Stellenbosch University.

4.2. Materials and methods

4.2.1 Sample preparation

Three forage types, namely lucerne hay *(Medicago sativa)*, oat hay *(Avena sativa)* and wheat straw *(Triticum aestivum)* were chosen for this trial. Lucerne hay represented a high quality forage, oat hay a medium quality and wheat straw a low quality forage. For each of these forage types a sample was obtained from seven different locations within the Western Cape.

Samples of all three forage types were milled and sieved as explained in Chapter 3. Coarse material remaining on top of the respective screens was used in this trial. Due to the high number of samples that were to be analysed for NDF in this trial, it was decided to select samples from four out of the seven locations for each forage for the *in vitro* trial. The selection was based on NDF content of each forage type and the aim was to include four samples that covered the NDF spectrum that was observed in the seven original samples.

Dacron bags (50 x 100 mm) with a nominal mean pore size of 53 micron, supplied by Bar Diamond (Parma, ID, USA), were used and the top half of the bags were cut off to reduce the size to 50 x 50 mm. The bags were dried at 100°C for twenty-four hours and then transferred to a desiccator to cool down for thirty minutes. Bags were then accurately weighed and the moisture free weight recorded. Directly after weighing, bags were labelled clearly with an acetone resistant permanent marker. Subsequently, approximately 0.5 grams of each forage sample were weighed into the dacron bags before they were heat sealed with an impulse bag sealer (Ankom® 1915/1920 Heat Sealer; Ankom® Technology Corp., Macedon, NY, USA). Blank bags that contained no substrates were also prepared to correct for microbial or other particulate residues from the rumen fluid.

The three different forages, namely lucerne hay, oat hay and wheat straw, were incubated separately. The *in vitro* runs for each forage type were repeated five times. For each incubation run, three bags were prepared for each forage sample (the un-sieved samples and the three fractions thereof) to be analysed, so that each sample could be incubated for each of the three time periods, namely 6, 24 and 48 hours. Separate incubation jars were used for each incubation time and a total of sixteen bags were evenly distributed into each jar, where each jar represented a time period as discussed above.

4.2.2 Preparation of the *in vitro* medium and the reducing solution

The incubation medium, also known as buffer solution, was prepared as described by Van Soest & Robertson (1985). The solution was made up of distilled water, micro minerals, macro minerals, a buffer solution, tryptose and rezasurin. A reducing solution was also prepared to ensure that the medium was reduced when adding the rumen liquid inoculum. The rezasurin in the medium acted as an indicator to show when the incubation medium was reduced. The composition of the different solutions is indicated in Table 4.1.

Table 4.1 Composition of the different media used in the *in vitro* digestibility trial.

Reagent	Quantity
1 Litre Buffer solution:	
Distilled water	1000 mL
Ammonium bicarbonate (NH_4HCO_3)	4 g
Sodium bicarbonate ($NaHCO_3$)	35 g
1 Litre Macro-mineral solution:	
Distilled water	1000 mL
Di-sodium hydrogen orthophosphate anhydrous (Na_2HPO_4)	5.7 g
Potassium dihydrogen orthophosphate (KH_2PO_4) (anhydrous)	6.2 g
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.59 g
Sodium chloride (NaCl)	2.22 g
100 mL Micromineral solution:	
Calcium chloride dihydrate ($CaCl_2.2H_2O$)	13.2 g
Manganese chloride tetrahydrate ($MnCl_2.4H_2O$)	10 g
Cobalt (II) chloride hexahydrate ($CoCl_26H_2O$)	1 g
Ferric chloride hexahydrate ($FeCl_3.6H_2O$)	8 g

1 Litre Incubation medium:

Distilled water	500 mL
Tryptose	2.5 g
Micromineral	0.125 mL
Macromineral	250 mL
Buffer	250 mL
Rezasurin	1.25 mL

100 mL Reducing solution:

Beaker A:

Distilled water	50 mL
Cysteine hydrochloride ($C_3H_7NO_2$ HCl)	0.625 g
Potassium hydroxide (KOH) pellets	10

Beaker B:

Distilled water	50 mL
Sodium sulphide nonahydrate	0.625 g

After preparation, the medium was placed into a water bath with a temperature of 39°C until inoculating with rumen fluid. Keeping the medium at a temperature of 39°C also reduced the temperature shock to the rumen organisms when the rumen fluid was added to the *in vitro* medium. The pH of the medium was on average 7.3.

4.2.3 Collection and preparation of rumen fluid

The rumen liquor was collected at 7:00 on the morning of each incubation. Two ruminally cannulated Holstein cows were used as donors. The cows were housed at the Welgevallen Experimental Farm of the Stellenbosch University. Cows were kept confined and each received 25 kg/day of a commercial semi-complete lactating cow feed, supplied by Afgri Animal Feeds (Klipheuwel, South Africa), plus oat hay *add libitum.* The rumen fluid was collected and directly

squeezed through two layers of cheese cloth into a thermos flask which had been pre-rinsed with hot water in order to limit temperature loss. The thermos flask was filled to the brim and a handful of solid feed material from the rumen was pushed into the flask after which it was immediately closed. Back in the laboratory, the rumen fluid was emptied into a pre-warmed industrial blender and purged with carbon dioxide (CO_2). The blender was then sealed and the rumen liquid was blended at a low speed for 10 seconds after which it was filtered through four layers of cheese cloth into a pre-warmed thermos flask while purging with carbon dioxide (CO_2) to maintain anaerobic conditions. The thermos flask was then closed right away. The temperature of the rumen fluid was kept at 39°C and the pH averaged 6.4.

4.2.4 *In vitro* incubation of the forage samples

The ANKOM® Daisy^{II} incubator was used for the *in vitro* trials. Per incubation, three glass jars with a capacity of two litres each were used. Each jar represented one time interval, namely 6, 24 or 48 hours. As previously discussed, the three different forages, namely lucerne hay, oat hay and wheat straw, were incubated separately and the runs for each forage type were repeated five times. Once the sample bags were distributed into the glass vessels, 1076 ml of incubation medium and 54 ml of reducing solution were added to each vessel whilst purging with CO_2. The digestion vessels were then closed tightly and placed into the Daisy^{II} incubator with the temperature set at 39.5°C until the medium was completely reduced and clear. The incubation vessels were then taken from the incubator, opened and purged with CO_2. Whilst purging, 270 ml of rumen fluid was added to each vessel. They were then closed tightly and returned to the incubator for the pre-determined incubation times. After each time interval, one jar was removed and the bags were washed with water until the rinse water ran clear. The bags were then air dried overnight before transferring them to a force draught oven set at 60°C for three days. After the required time, the bags were placed in a desiccator for 30 minutes and then weighed accurately. Thereafter, the bags were further processed to determine the NDF content of the residues in order to calculate the NDF disappearance.

4.2.5 Zero hours

To determine the soluble and rapidly fermentable fractions, samples of all the different forage fractions were weighed into similar dacron bags and washed in water. The bags were then placed into a basin where they were soaked in water and washed gently by hand for five

minutes. Bags were then air dried and subsequently placed into a force draught oven set at 60°C for three days. After drying, the bags were placed into a desiccator to cool down for 30 minutes after which they were weighed accurately. Thereafter, the bags were further processed to determine the NDF content.

4.2.6 Analysis of the residues

The NDF content of all the residues from the *in vitro* incubations, as well as the residues from the samples that were washed (zero hours) were analysed by using the Akom®$^{200/220}$ Fiber Analyzer and the method was based on the method of Van Soest *et al.* (1991), as discussed in detail in Chapter 3. After the NDF procedure the bags were air dried and then placed into an oven set at 100°C for 24 hours. Thereafter, the bags were placed into a desiccator for 30 minutes and then weighed accurately.

4.2.7 Estimating the Dry Matter (DM) degradability

The dry matter (DM) degradability of the forages was calculated using the following equation:

$$DMD = 100 - \frac{[W_3 - (W_1 \times C_1)]}{W_2} \times 100 \qquad \text{Equation 1}$$

Where:

Where:	DMD	=	Apparent DM degradability (%)
	W_1	=	Bag weight (mg)
	W_2	=	Sample weight (mg DM)
	W_3	=	Weight of dried bag and residue after incubation (mg DM)
	C_1	=	Blank bag correction factor

4.2.8 Estimating the neutral detergent fibre (NDF) degradability

The NDF degradability of the forages was calculated as follows:

$$NDF = 100 - \frac{[W_3 - (W_1 \times C_1)]}{W_2} \times 100 \qquad \text{Equation 2}$$

Where: NDFD = Apparent NDF degradability (%)

W_1 = Bag weight (mg)

W_2 = Sample weight (mg NDF; DM)

W_3 = Dried weight of bag with residue after NDF treatment (mg NDF)

C_1 = Blank bag correction factor

4.2.9 Lignin

Acid detergent lignin (ADL) was determined by using the Akom®$^{200/220}$ Fibre Analyzer. An amount of 0.5 g of each sample was weighed into separate F57 ANKOM fibre analysis bags. Subsequently the filter bags were heat sealed and the acid detergent fibre (ADF) was determined by using the method of Goering and Van Soest (1970). Thereafter, the ADF residue was soaked in 72% sulphuric acid for three hours in order to dissolve the cellulose. ADL was determined according to the ANKOM method (ANKOM, 08/05).

4.2.10 Rate calculator

The NDF digestion rates were estimated with the aid of the Beta version 1b (2006) rate calculator, obtained from Dr M. Van Amburgh (personal communication). The NDF and lignin values of the different forage substrates, as well as the NDF disappearance values at 6 h and 24 h were used in the rate calculator.

4.2.11Statistical analysis

The DM and NDF disappearance data obtained at the different incubation times were subjected to a main effects ANOVA with the aid of Statistica 9 (2010). Main effects were treatment, locality and repetition. The means were separated with a Bonferoni post-hoc test and significance was declared at $P \leq 0.5$.

4.3. Results and discussion

4.3.1 *In Vitro* Dry Matter Degradability

Results on the effect of fine particle removal from forage substrates on *in vitro* DM disappearance values are indicated in Table 4.2. Dry forage samples are typically ground through a 1 or 2 mm screen in preparation of chemical analyses and *in sacco* degradability trials. After grinding, these samples are not homogenous in particle size, and they contain particles that vary in length, from longer than 2 mm to extremely fine and dusty particles that are only a few microns in diameter. Fine particles that are smaller than the pore diameter of dacron bags used in *in sacco* trials may result in washout losses and an over-estimation of nutrient losses due to microbial digestion. Small particles of DM that are lost through washout are no longer available to microbial fermentation. This general problem of over-estimation of the soluble fraction in *in situ* and *in vitro* nylon bag techniques is consistent with findings of Ørskov *et al.* (1980), Kitessa *et al.* (1999), Kamalak *et al.* (2005) and Karabulut *et al.* (2006).

From Table 4.2 it is evident that fine particle removal from forage substrates had a significant effect on *in vitro* DM disappearance of forages. In all the forages used, lower DM disappearance values were obtained where fine particles had been removed before *in vitro* incubation. This was observed for all the incubation times. There was no difference in DM disappearance between the three sieve sizes for any of the forages at any of the incubation times.

Compared to sieved samples, DMD values at 0 hours (washing only) of the un-sieved samples were, on average, 13.8% higher for lucerne hay, 27.3% for oat hay and 44.7% for wheat straw. This would indicate the magnitude by which the “soluble DM fraction” may be over-estimated in forages due to the washout effect of fine particles.

It can further be noted from Table 4.2 that the DMD for the different sieve fractions (150 µm, 125 µm and 106 µm) for all three forage types at all incubation times did not differ significantly from each other. Research has shown that grinding of forages increases the *in vitro* digestibility. This increased *in vitro* digestibility results from the decreased particle size due to grinding which causes an increase in the surface area of the forage to which the microbes can adhere (Mertens & Ely, 1982; Weakly *et al.,* 1983). Thus, in the current study one could have expected differences in the DMD between the different fractions as the 106 µm sieve would retain a lager variety of small particles than the 125 µm sieve. The 150 µm sieve would remove more small particles than the other two sieves. Thus, the 106 µm fraction may have been expected to have the highest DMD as the 106 µm fraction contains more small materials. Weakly *et al.* (1983), on the other hand, suggested that particles that are smaller than 0.6 mm would be more prone to clumping together, thus lowering the DMD. They further state, that "differences in particle hardness and wetability" could affect the DMD of samples.

The effects of fine particle removal on DM disappearance at various incubation times are also shown in Figure 4.1. As can be seen, the initial effect of DMD over-estimation at 0 h was carried over to the subsequent incubation times for all the forages. At 48 h, the over-estimation of lucerne DMD for the un-sieved samples was between 4.0% (compared to 106 µm sieve) and 7.3% (compared to 150 µm sieve). For oat hay, the over-estimation was between 4.6% (150 µm sieve) and 5.2% (125 µm sieve) and for wheat straw it was between 6.2% (106 µm sieve) and 9.2% (125 µm sieve). It can further be seen in Figure 4.1 that for oat hay and wheat straw, the over-estimation of DM disappearance appears to be larger from 0 to 6 hours of incubation. Over-estimation at short incubation times was also noted by Willman *et al.* (1996).

Table 4.2 The effect of fine particle removal by sieves with different mesh sizes (150 µm, 125 µm, 106 µm) on *in vitro* DM degradability of forages.

Time	Lucerne						Oat Hay						Wheat Straw					
	2mm	150	125	106	SEm	*P*	2mm	150	125	106	SEm	*P*	2mm	150	125	106	SEm	*P*
0 h	31.9^{a}	27.7^{b}	28.3^{b}	28.2^{b}	0.442	<0.001	39.7^{a}	29.0^{b}	28.8^{b}	28.7^{b}	1.795	0.004	15.1^{a}	10.2^{b}	10.7^{b}	10.4^{b}	1.040	0.025
6 h	44.5^{a}	42.2^{b}	42.4^{b}	41.9^{b}	0.692	0.045	37.1^{a}	32.1^{b}	32.1^{b}	31.9^{b}	0.208	<0.001	16.2^{a}	11.1^{b}	10.8^{b}	11.4^{b}	0.321	<0.001
24 h	66.9^{a}	63.1^{b}	64.1^{b}	64.2^{b}	0.361	<0.001	60.2^{a}	$55.5^{\mathbf{b}}$	55.9^{b}	55.8^{b}	0.382	<0.001	38.9^{a}	34.3^{b}	34.1^{b}	34.7^{b}	0.381	<0.001
48 h	68.3^{a}	63.6^{b}	65.0^{b}	65.6^{b}	0.720	<0.001	70.2^{a}	67.1^{b}	66.7^{b}	66.9^{b}	0.367	<0.001	54.9^{a}	50.9^{b}	50.3^{b}	51.7^{b}	0.285	<0.001

SEm = Standard error of the mean; DM = dry matter

$^{a, b, c}$Means in the same row with different superscripts, differed significantly

.

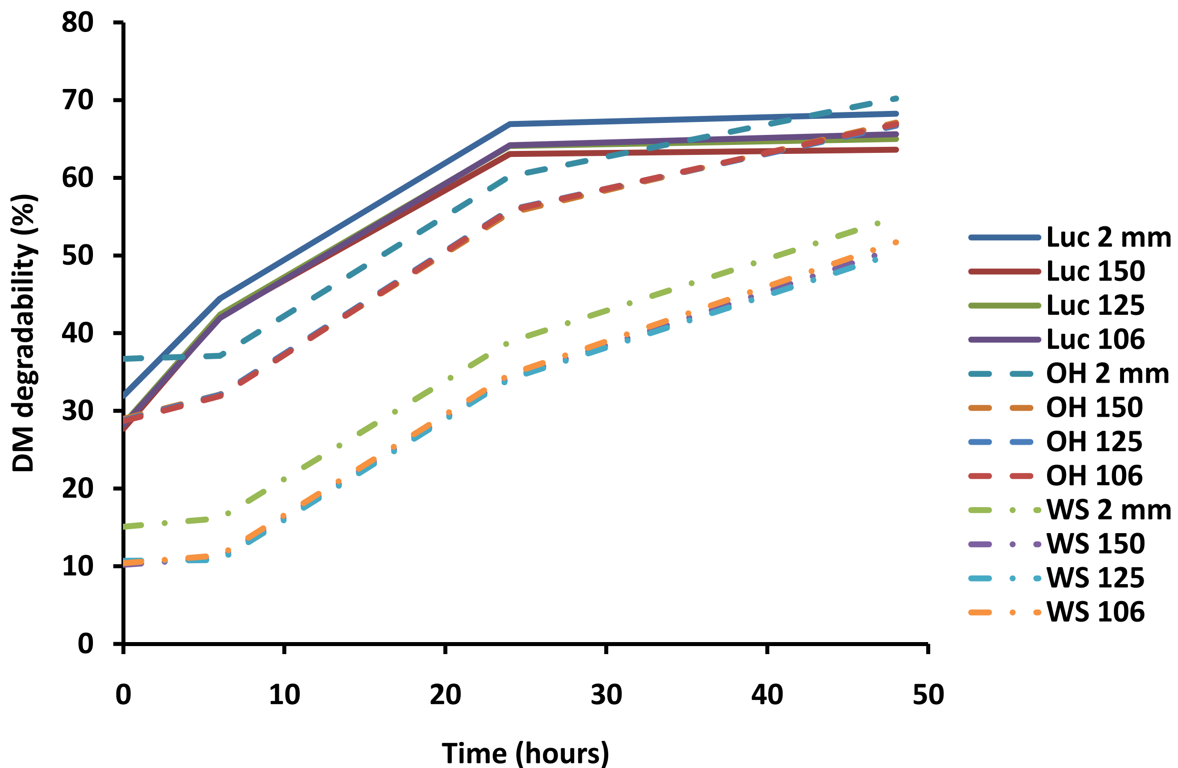

Figure 4.1 The effect of fine particle removal from forage samples on *in vitro* dry matter (DM) degradability.

Poppi *et al.* (2000) stated that the "the most important factor influencing the production response of an animal is the total quantity of nutrients absorbed". Therefore, the feed intake and the digestibility thereof are considered as the major variables that are used in any system used for feed evaluation. Per definition, the 'potential digestibility' of a feed or forage is the fraction that disappears after an extended period of incubation (*in sacco* or *in vitro*). Conversely, the fraction of a feed or forage that is unavailable to the rumen microorganisms is regarded as the 'indigestible fraction'.

When fine particles are not removed before *in sacco* trials, rumen degradability values can be significantly overestimated due to particle washout. Modern dynamic feed formulation models, for example AMTS.cattle and CPM Dairy, rely on accurate degradation rates (kd) because digestibility is a function of kd/(kd+kp), where kd equals the rate of degradation (%/h) and kp equals the rate of passage from the rumen. It is therefore essential to prevent over-estimations

of the digestible fractions during *in vitro* and *in sacco* trials. This could be accomplished by removing fine particles from the samples by sieving through a sieve with a suitable mesh size.

Although the purpose of this study was not to compare the different forages it would appear from Table 4.2 and Figure 4.1 that the three forage types differed from each other regarding degradability. At 0 hours the particle washout for both lucerne hay and oat hay appeared to be much higher than for wheat straw. It appeared that lucerne generally had the highest DMD, followed by oat hay and wheat straw. The apparent differences can be ascribed to differences in chemical composition of the three forages. Wheat straw had the highest NDF content (Chapter 3, Table 3.1) which is less fermentable by the microorganisms in the rumen fluid, and also the lowest crude protein content, whereas lucerne hay had the lowest NDF and highest crude protein contents. The NDF content of oat hay was intermediate between lucerne hay and wheat straw, which was reflected in its intermediate degradability. However, these differences may also be explained due to difference in fragility among forages and differences among nutrient distributions in the fractions. Karabulut *et al.* (2006) reported similar findings for both lucerne hay and wheat straw. It appeared (Figure 4.1) that the DMD of lucerne hay started to decrease faster from 24 hours than that of the other forages. A possible explanation could be that lucerne hay has a larger dry matter fraction that is soluble and rapidly fermentable than oat hay and wheat straw. However, once this rapidly soluble fraction has disappeared, the degradability of lucerne hay would level out (as can be seen from Figure 4.1) with an increase in the lignified fractions which comprises the indigestible part of lucerne hay. According to Dehority & Johnson (1961) the lignin percentage in a plant increases as the plant reaches maturity. Subsequently, the digestible cellulose fraction would decrease.

4.3.2 *In Vitro* NDF Degradability

Results on the effect of fine particle removal from forage substrates on *in vitro* NDF disappearance are shown in Table 4.3. It is apparent that fine particle removal from forage substrates had a significant effect on NDF disappearance. For all three forages used in this study, lower NDF disappearance values were noted where the fine particles were removed by sieving prior to *in vitro* incubation. The NDFD values differed significantly from the original samples at all incubation times for all three forages. Compared to the average values of the sieved samples, NDFD values at 0 hours (washing only) of the un-sieved samples were 142% higher for lucerne hay, 79% higher for oat hay and 173% higher for wheat straw. This is an

indication of the magnitude by which the 0 hours NDF disappearance may be over-estimated in forages due to the washout effect of fine particles. Theoretically, 0 hours NDFD should be close to zero. It can also be noted that in the case of lucerne hay, differences between sieve size treatments were observed at 6 and 48 hours of incubation. Lower NDFD values were observed for the 150 µm sieve than for the 106 µm sieve. This would suggest that more fine particles were still retained in the 106 µm sieved sample. Nevertheless, for lucerne hay the 150 µm fraction did not differ from the 125 µm and the 125 µm fraction in turn did not differ from the 106 µm fraction for both 6 and 48 hours of incubation. This is in accordance with research findings of Mertens & Ely (1982), who state that a decreased particle size due to grinding causes increases the *in vitro* digestibility of forages due to an increase in surface area for microbial attachment. Conversely, no significant differences in NDFD between the different fractions were noted in the results presented in Table 4.3 for oat hay and wheat straw at all incubations times. This could be explained by different particle size distributions within the samples and possibly also due to different brittleness of the different forage types.

The effects of fine particle removal on NDFD disappearance at various incubation times are also shown in Figure 4.2.

As can be seen in Figure 4.2, the initial effect of NDF over-estimation at 0 h was carried over to the subsequent incubation times for all the forages. At 48 h, the over-estimation of lucerne NDFD for the un-sieved samples was between 12.3% (compared to 106 µm sieve) and 22.3% (compared to 150 µm sieve). For oat hay the over-estimation was between 4.6% (150 µm sieve) and 5.9% (106 µm sieve) and for wheat straw it was between 4.6% (106 µm sieve) and 6.7% (125 µm sieve). It can further be noted from Figure 4.2 that at 0 hours, the NDF disappearance values ranged between 0 and 11% for all three forages and their fractions, whereas the DM disappearance values depicted in Figure 4.1 ranged between 10 and 40%. The DM fraction, of course, includes a variety of soluble and rapidly fermentable nutrients. An interesting observation is that, from 24 hours of incubation onwards, the NDFD of lucerne hay starts to plateau, a phenomenon that was also observed in DMD (Figure 4.1). An explanation could be that, from the two digestion pools (fraction of degradable NDF and the undegradable fraction or potentially digestible and indigestible fractions (Smith *et al.,* 1970)), lucerne hay has a large fast fermentable pool, where the readily fermentable DM digests relatively quickly. Once all the readily digestible particles have disappeared, rumen microbes start to digest the less digestible carbohydrates from the slow degradable NDF pool. As soon as the indigestible fraction is

reached, DMD is extremely low (Van Soest *et al.*, 2005). Lignin makes up most of the indigestible component of the indigestible fraction, thus explaining the DMD and NDFD curves to flatten off in lucerne hay. On the contrary, the NDF disappearance of oat hay and wheat straw continued to increase at 48 hours, with wheat straw appearing to increase almost linearly. This might be due to NDF that was enclosed in structural components such as lignin, which had been made available to digestion after an increased time of incubation. Lignin is covalently bound to cellulose and hemicellulose, and these linkages are the ones retarding degradability by ruminal microbes. As can be seen from Figure 4.2, the NDFD of the sieved fractions for each forage sample were very close together, thus further underlining the results demonstrated in Table 4.3., where it was noted that the sieved fractions for each forage type did not differ from each other throughout all incubations times. It is thus evident, that when fine particles are not removed from forage samples prior to *in sacco* trials, neutral detergent degradability values can be significantly overestimated.

Table 4.3 The effect of fine particle removal by sieves with different mesh sizes (150 μm, 125 μm, 106 μm) on *in vitro* NDF degradability (NDFD) of forages.

	Lucerne						Oat Hay						Wheat Straw					
Time	**2mm**	**150**	**125**	**106**	**SEm**	***P***	**2mm**	**150**	**125**	**106**	**SEm**	***P***	**2mm**	**150**	**125**	**106**	**SEm**	***P***
0 h	10.8^{a}	4.0^{b}	4.7^{b}	4.7^{b}	0.986	0.003	8.1^{a}	4.8^{b}	4.8^{b}	4.0^{b}	0.610	0.005	8.2^{a}	2.5^{b}	3.6^{b}	2.9^{b}	1.153	0.022
6 h	19.6^{a}	11.6^{b}	13.9^{bc}	14.9^{c}	0.769	<0.001	12.7^{a}	8.0^{b}	8.3^{b}	7.6^{b}	0.333	<0.001	13.8^{a}	8.9^{b}	9.1^{b}	9.4^{b}	0.532	<0.001
24 h	46.0^{a}	38.2^{b}	40.0^{b}	40.6^{b}	0.657	<0.001	44.8^{a}	40.5^{b}	41.0^{b}	40.5^{b}	0.534	<0.001	37.4^{a}	33.5^{b}	33.8^{b}	34.2^{b}	0.484	<0.001
48 h	49.4^{a}	40.4^{b}	42.2^{bc}	44.0^{c}	1.095	<0.001	59.1^{a}	56.5^{b}	56.3^{b}	55.8^{b}	0.501	<0.001	60.9^{a}	57.2^{b}	57.1^{b}	58.2^{b}	0.313	<0.001

SEm = Standard error of the mean; NDF = neutral detergent fibre

$^{a, b, c}$Means in the same row with different superscripts, differed significantly.

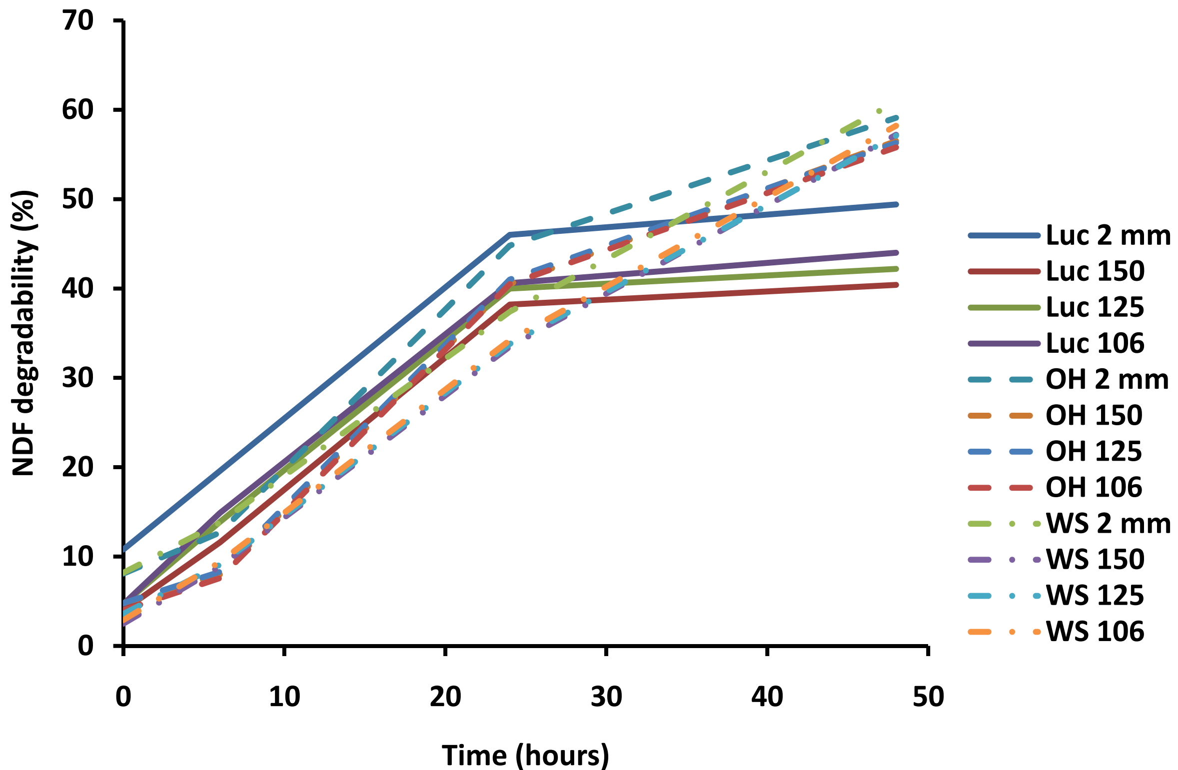

Figure 4.2 The effect of fine particle removal on *in vitro* neutral detergent fibre (NDF) degradability of forages.

4.3.3 Rate of NDF disappearance and proportion digested

The NDF digestibility values, as well as the NDF and lignin contents of the different forages and fractions, were used in the Van Amburgh Rate Calculator to estimate NDF digestion rates and proportion of NDF eventually digested. The results are presented in Table 4.4.

Rates of digestion are essential parameters in determining the quality of forage NDF and are not highly correlated with lignin contents, especially after 48 hours of incubation (Van Soest *et al.,* 2005). Applied to i*n vivo* conditions, the rate and extent of degradation in the rumen of the animal are important determinants of the nutrients that are absorbed into the bloodstream of the ruminant (Kamalak *et al.,* 2005). It is not clear yet whether the rates of digestion of feeds are affected by the chemical composition of feeds or whether the methods available are not able to detect "the characteristics that control digestion rate". Nevertheless, it can be assumed that the

morphological tissue types in plants, animal and microbial factors are capable of changing digestion rates. The potential extent of digestion is closely related to the "chemical composition, plant morphology and crystallinity of the plant", where lignin puts the upper limit to the potential extent of digestion (Mertens & Ely, 1982). Vanzant *et al.* (1998) state that the latest predictive models used in ruminant nutrition, rely on accurate "quantitative information regarding rates and extents of ruminal degradation". The authors' further report that *in situ* methods used in order to obtain these rates, vary greatly within and between laboratories, leading to large variation between results. Standardization of *in vitro* and *in situ* procedures is thus regarded as essential.

Table 4.4 The effect of fine particle removal in ground forages on estimated rates of NDF disappearance and predicted digested proportions, using the Van Amburgh rate calculator.

Item	Sieve size				SEM	*P*
	2 mm	150 µm	125 µm	106 µm		
Lucerne						
Rate (%/h)	8.93[a]	5.85[b]	6.43[b]	6.58[b]	0.294	<0.001
Proportion digested (%)	63.3[a]	53.0[b]	54.9[b]	55.1[b]	1.382	0.002
Oat hay						
Rate (%/h)	3.80[a]	3.38[b]	3.48[b]	3.45[b]	0.087	0.032
Proportion digested (%)	43.0[a]	40.1[b]	40.8[b]	40.7[b]	0.531	0.019
Wheat straw						
Rate (%/h)	3.75[a]	3.30[b]	3.35[b]	3.33[b]	0.083	0.012
Proportion digested (%)	42.2[a]	39.7[b]	39.6[b]	40.2[b]	0.685	0.082

From Table 4.4 it can be seen that fine particle removal had a significant effect on the estimated rate of NDF digestion (k_d) and the proportion digested in all three forages. For all three forage types significantly lower digestion rates were estimated when fine particles had been removed prior to *in vitro* incubation. Compared to the average value of the sieved samples, NDF digestion rates of the un-sieved samples were over-estimated by 42.6% for lucerne, 8.1% for oat hay and 7.2% for wheat straw. Regarding the proportion NDF digested, the over-estimations of the un-sieved samples were 16.6, 4.8 and 4.5% for lucerne hay, oat hay and wheat straw, respectively. The over-estimations were especially apparent for lucerne hay. Although the effect of fine particle removal on estimated rates of NDF digestion and proportion of NDF digested was also significant in oat hay and wheat straw, the magnitude of the over-estimations was much smaller in these forages than in the case of lucerne hay. A possible explanation might be

that, despite lucerne hay's higher lignin content, the composition of lucerne hay NDF is such that it is more readily digestible than that of oat hay and wheat straw.

Processing of forages by grinding increases the *in vitro* digestibility. This increased *in vitro* digestibility results from the decreased particle size due to grinding which causes an increase in the surface area of the forage to which the microbes can adhere (Mertens & Ely, 1982; Weakly *et al.,* 1983). However, in the current study, the estimated rate of digestion for the three fractions (150 μm, 125 μm and 106 μm) did not differ significantly from each other for all three forages types, even though one might have expected differences in the rate of digestion as the 106 μm sieve would retain a lager variety of small particles than the 125 μm sieve. The 150 μm sieve would remove more small particles than the other two sieves. In a study done by Udén (1992), the author noted that particle size did not greatly affect the rate of digestion, but more the lag time, with the 'undegradable NDF' fraction hardly being affected. Although they did not remove fine particles by sieving, these findings appear to be in accordance with the findings in the current study. Conversely, Robles *et al.* (1980) observed that particles size has an effect on the rate of digestion.

4.4. Conclusion

Results from this study showed that that fine particle removal by sieving greatly affects DM disappearance, NDF disappearance and digestion rates. Dry matter and NDF disappearances were significantly higher for the un-sieved samples of all three forage types at all incubation times, which indicates a significant over-estimation of the soluble and readily digestible forage fractions. The estimated digestion rates and the predicted digested proportions were also significantly higher for the un-sieved fractions compared to the sieved fractions. For both DM and NDF disappearances, it appeared that there were no large differences between the different sieve mesh sizes (150 μm, 125 μm and 106 μm). However, from the results of the NDF disappearance, it was noted for lucerne hay that for 6 and 48 hours of incubation, differences between the samples sieved through a 150 and 106 μm were recorded. This would suggest that fine particles that can affect washing out losses were still present in the 106 μm sieved samples. Nevertheless, for lucerne hay, the 150 μm fraction did not differ from the 125 μm and the 125 μm fraction did not differ from the 106 μm fraction for both 6 and 48 hours of incubation.

It is thus evident that, when fine particles are not removed from forage samples prior to *in sacco* trials, neutral detergent degradability values can be significantly over-estimated. Therefore, it would be recommended to sieve forages samples through a 150 μm size sieve prior to *in sacco* trials. By applying this method of standardisation, more accurate estimates of digestion rates and predicted digested proportions can be made allowing feed formulation models to be more accurate and enabling the ruminant nutritionist to formulate the best diet possible in order to reach the full genetic potential of ruminants.

4.5. References

ANKOM, 2005. Method for determining acid detergent lignin in beakers, ANKOM Technology, 08/05.

Cruywagen, C.W., Bunge, G.A. & Goosen, L., 2003. The effect of milling on physical material lost through dacron bags of 53 micron pore size. J. Anim. Sci. 81 (suppl. 1)/ J. Dairy Sci. 86 (Suppl. 1):287.

Dehority, B.A. & Johnson, R.R., 1961. Effect of particle size upon the *in vitro* cellulose digestibility of forages by rumen bacteria. J. Dairy Sci. 44(12): 2242 - 2249.

Goering, H.K. & Van Soest, P.J., 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications). Agricultural Handbook Number 379. ARS-USDA, Washington, D.C., USA.

Holecheck, J.L., Vavra, M. & Pieper, R.D., 1982. Methods for determining the nutritive quality of range ruminant diets: a Review. J. Anim. Sci. 54: 363 - 376.

Kamalak, A., Canbolat, O., Gurbuz, Y. & Ozay, O., 2005. Comparison of *in vitro* gas production technique with *in situ* nylon bag technique to estimate dry matter degradation. Czech J. Anim. Sci. 50 (2): 60 - 67.

Karabulut, A., Ozgur Ozkan, C., Kamalak, A. & Canbolat, O., 2006. Comparison of the nutritive value of a native Turkish forages, tumbleweed hay (*Gundelia tournefortii L.*), wheat straw and alfalfa hay using *in situ* and *in vitro* measurements with sheep. Arch. Latinoam. Prod. Anim. 14 (3): 78 - 83.

Kitessa, S., Flinn, P.C. & Irish, G.G., 1999. Comparison of methods used to predict the *in vivo* digestibility of feeds in ruminants. Aust. J. Agric. Res. 50: 825 - 841.

Mertens, D.R. & Ely, L.O., 1982. Relationship of rate and extent of digestion to forage utilization-a dynamic model evaluation. J. Anim. Sci. 54: 895 - 905.

National Research Council, 2001. Nutrient requirements of dairy cattle. (7th Rev. ed.) National Academy. Press, Washington, D.C., USA.

Ørskov, E.R., Hovell, F.D.DeB. & Mould, F., 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5(3): 195 - 231.

Poppi, D.P., France, J. & McLennan, S.R., 2000. Intake, passage and digestibility. In: Feeding systems and feed evaluation models. Eds. Theodorou, M.K. & France, J., CAB International Publishing, Oxon, U.K. pp. 35 - 52.

Robles, A.Y., Belyea, R.L., Martz, F.A. & Weiss, M.F., 1980. Effect of particle size upon digestible cell wall and rate of *in vitro* digestion of alfalfa and orchardgrass forages. J. Anim. Sci. 51: 783 - 790.

Smith, L.W., Goering, H.K., Waldo, D.R. & Gordon, C.H., 1970. *In vitro* digestion rate of forage cell wall components. J. Dairy Sci. 54: 71 - 76.

Statistica 9, 2010. StatSoft Inc., USA.

Udén, P., 1992. The influence of leaf and stem particle size *in vitro* and of sample size *in sacco* on neutral detergent fibre fermentation kinetics. Anim. Feed Sci. Technol. 37: 85 - 97.

Van Soest, P.J. & Robertson, J.B., 1985. Analysis of forages and fibrous feeds. A laboratory manual for animal science 613. Cornell University, Ithaca, New York, USA.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583 - 3587.

Van Soest, P.J., Amburgh, M.E., Robertson, J.B. & Knaus, W.F., 2005. Validation of the 2.4 times lignin factor for ultimate extent of NDF digestion, and curve peeling rate of fermentation curves into pools. Proc. Cornell Nutr. Conf. Feed Manuf., East Syracuse, NY. Cornell University, Ithaca, NY. Pp. 139 - 149.
Available at: http://nutritionmodels.tamu.edu/papers/VanSoestetalCNC2005139.pdf

(Accessed 25 November 2010)

Vanzant, E.S., Cochran, R.C. & Titgemeyer, E.C., 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76: 2717 - 2729.

Weakly, D.C., Stern, M.D. & Satter, L.D., 1983. Factors affecting disappearance of feedstuffs from bags suspended in the rumen. J. Anim. Sci. 56: 493 - 507.

Willman, D., Foulkes, G.R. & Given, D. (1996). A comparison of four methods of estimating the rate and extent of cell wall degradation in grass silages. Anim. Feed Sci. Technol. 63: 99 - 109.

CHAPTER 5

General conclusion

The modern consumer desires animal products that are low in fat and that contain more protein. Furthermore, due to the rapidly growing world population there is a huge demand for an increase in animal protein production. Dairy cattle farmers are thus forced to increase the productivity of their cows and the quality of their animal products in order to remain compatible in the market. With an increase in the genetic potential for milk production of a dairy herd, the nutritional management becomes the most essential tool in order to gain maximal production and profitability of a herd.

Today, modern predictive models, such as CPM Dairy and AMTS.cattle are indispensible when formulating ruminant diets that insure increased energy intake of the ruminant and subsequent increased production without causing health ailments related to high energy diets. These predictive models rely on accurate quantitative information obtained from *in situ* and *in vitro in sacco* methods which are also used to simulate the interactions in the rumen in order to study the functionality of the rumen itself and to test the quality of forages and feeds.

Research has shown that extremely large variation of methods and results exist within and between laboratories and standardization of methods is thus regarded as essential. The accepted method for *in sacco* trials (NRC, 2001) requires that the feed samples are ground through a 2 mm screen. However, when milling forage samples through a 1 or 2 mm screen, the samples contain particles that are so small that they are able to pass through the pores of the dacron bags. This would lead to an over-estimation of the soluble fraction of the forage samples, which is a common source of variation of digestibility data.

For the purpose of this thesis a study was devised that removed these fine particles by sieving samples through different mesh size sieves to test the hypothesis that the removal of particles <150 μm would decrease the estimated DM and NDF disappearance values obtained from *in sacco* trials. The first study confirmed that between 11 and 20% of the sample can be classified as 'fine material' which is able to wash out of dacron bags, and therefore can lead to an overestimation of the soluble fraction. Furthermore, it was shown that when these fine particles

were removed from the sample by sieving through a sieve with either a pore size of 106 μm, 125 μm, or 150 μm, the chemical composition of the fractions that resulted for lucerne hay did not differ from the un-sieved original sample. For oat hay, the results for all three fractions for NDF and CP differed significantly from the original un-sieved sample. The three sieved fractions of oat hay nonetheless did not differ from each other. Wheat straw showed no difference between the three factions from the original un-sieved sample for NDF, but conversely for CP the fractions differed significantly from the original samples. However, the fractions did not differ from each other. The crude protein results of oat hay and wheat straw suggested that similar studies with protein sources could be of value for standardizing *in vitro* digestibility procedures.

In the second study, it was shown that fine particle removal by sieving greatly affected DM disappearance, NDF disappearance and digestion rates. Dry matter and NDF disappearances were significantly higher for the un-sieved samples of all three forage types at all incubation times. This would suggest a significant over-estimation of the soluble and readily digestible forage fractions due to fine particle washout losses. The estimated digestion rates and the predicted digested proportions were also significantly higher for the un-sieved fractions compared to the sieved fractions. It further appeared that there were no differences among sieve mesh sizes (150 μm, 125 μm and 106 μm) in terms of effect on DM and NDF disappearances, although it appeared that the 150 μm generally resulted in the lowest washout losses.

It would thus be recommended to sieve forage samples through a 150 μm size sieve prior to *in sacco* trials as this would reduce over-estimation due to fine particle washout. Subsequently more accurate estimates of digestion rates and predicted digested proportions could be made, allowing feed formulation models to be even more accurate and enabling the ruminant nutritionist to formulate the best diet possible in order to increase the energy intake and the productivity of ruminants.

5.1. References

National Research Council, 2001. Nutrient requirements of dairy cattle. (7th Rev. ed.) National Academy. Press, Washington, D.C., USA.