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The association between grinding energy and *in vitro* NDF digestion kinetics in forages

By

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Submitted in partial fulfilment of the requirements for the degree

***Magister Scientiae Agriculturae* - MSc. (Agric) Animal Science (Animal Nutrition)**

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DECLARATION

I, **Claudia Anelich**, declare that the dissertation: **"The association between grinding energy and *in vitro* NDF digestion kinetics in forages"**, which I hereby submit for the degree MSc. (Agric) Animal Science (Animal Nutrition) at the University of Pretoria, is my own work and that all the sources that I used or quoted have been indicated with complete reference and acknowledgements. This dissertation has not previously been submitted by me for a degree at this or any other tertiary institution

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CLAUDIA ANELICH **DATE**

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SUMMARY

The association between grinding energy and *in vitro* NDF digestion kinetics in forages

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Through the measurement of grinding energy, the possibility exists to predict forage fragility as related to the chemical composition of forages. It is also possible to predict a potential relationship between forage fragility and 240 hour *in vitro* neutral detergent fibre digestibility (uNDF240). These results could lead to improved predictions of particle size reduction, animal chewing activity and energy usage during the process of chewing.

Physically effective fibre (peNDF) is a key component of many nutritional models used to predict the effect of forage particle size on cow chewing response. Chewing activity is a response which reflects the chemical and physical properties of feeds, including intrinsic fragility. Forage fragility, or the ease of particle size reduction during chewing, has been said to be similar among different sources of NDF, when attempting to estimate peNDF. However, different NDF sources with similar particle sizes can elicit

variable chewing responses and this variation has serious implications for nutritional models which use peNDF values. This variation has led to numerous inaccuracies in the system; therefore factors affecting peNDF particularly forage fragility, need to be better understood as forage fragility may be closely linked to NDF digestibility. Therefore, in this study, a possible association between forage fragility and short term or long term *in vitro* NDF digestibility (*iv*NDFd) was investigated.

In order to investigate the possibility of predicting an association between forage fragility and *in vitro* NDF digestion, a total of 35 forage samples from three forage species were collected from 25 different locations. Forage species included commonly used fibre sources in ruminant nutrition in South Africa, namely *Medicago sativa*, Maize silage and *Eragrostis curvula.*

The forage samples were analysed for numerous chemical components, as well as 6-, 12-, 18-, 24-, 36-, 48-, 72-, 96-, 120-, 240-h *iv*NDFd and rate of NDF digestion (kd). The 240-h *iv*NDFd was used to estimate indigestible NDF (iNDF). Particle size distributions were measured for all forage samples. Dried samples were pre-cut with a knife mill fitted with a 2 cm screen, after which particle size distributions were determined for each sample using a Retsch sieve shaker. For the measurement of grinding energy, 10 g duplicates of the 2 cm milled samples were milled with an ultra-centrifugal mill, fitted with a 1 mm screen. During the grinding process, energy usage of the specific mill was measured using a data logger with corresponding computer software and energy transducer. Energy measurements were reported as J/g sample on dry matter (DM) basis. The 1 mm samples were then used for determining particle size distribution again, in order to analyse change in particle distribution for each forage sample.

The results of this study showed, according to the final models, that initial particle size (IPS), final particle size (FPS), cellulose and undigested NDF at 6 hours digestion (uNDF6) explain most of the variation in forage fragility. All of these variables can be associated with a decrease in forage fragility, due to an increase in energy usage during grinding with an increase in any of the aforementioned components. Upon adding species as a variable that could influence forage fragility, it could be seen that an interaction between *M. sativa* and FPS can be associated with a decrease in forage fragility, whereas an interaction between maize silage and FPS can be associated with an increase in forage fragility, due to a decrease in energy usage during grinding with an increase in this interaction. From the simple associations and correlations, it was evident that kd can be associated with increased forage fragility, as there was a decrease in energy usage during grinding with an increase in this parameter. Further

correlations and/or linear associations indicate that NDF, acid detergent fibre (ADF), uNDF18, uNDF24, uNDF36 and uNDF48 can possibly be associated with a decrease in forage fragility, due to an increase in energy usage during grinding with an increase in any one of these variables. It would be expected that acid detergent lignin (ADL) is also associated with decreased forage fragility; however, this can only be assumed as the results for the effect of lignin on forage fragility are non-significant in this study.

The use of grinding energy has the potential to be a practical and useful measure to predict forage fragility. However, the relative contribution of physical factors such as original particle size, particle shape, surface area, morphology and a multitude of chemical factors toward the fragility of forages is difficult to predict. Additional research is needed on the prediction of forage fragility and the possible relationship between forage fragility and NDF digestion and which factors influence this concept, before it can be incorporated as a meaningful and accurate input into nutritional models such as the National Research Council (NRC) and the Cornell Net Carbohydrate and Protein System (CNCPS).

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADL	Acid detergent lignin
CF	Crude fibre
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
CPM	Cornell-Penn-Miner
CPS	Critical particle size
DM	Dry matter
DMI	Dry matter intake
eNDF	Effective neutral detergent fibre
FPS	Final particle size
GI	Gastrointestinal
iNDF	Indigestible NDF
IPS	Initial particle size
Kd	Rate of NDF digestion
Kp	Rate of passage
ME	Metabolisable energy
MRT	Mean retention time
N	Nitrogen
NDF	Neutral detergent fibre

NDFd	NDF digestibility
*iv*NDFd	*In vitro* NDF digestibility
NDSC	Neutral detergent soluble carbohydrate
NFC	Non-fibre carbohydrate
NRC	National Research Council
NSC	Non-structural carbohydrate
OM	Organic matter
pdNDF	Potentially digestible NDF
Pef	Physical effectiveness factor
Pef_i	Pef prior to ball milling
Pef_{BM}	Pef after ball milling
peNDF	Physically effective NDF
RVI	Forage value index
RVU	Forage value unit
SER	Standard error of regression
TMR	Total mixed ration
uNDF	Undigested NDF
VFA	Volatile fatty acids

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CHAPTER 1

1. GENERAL INTRODUCTION AND MOTIVATION

Fibre represents an important component of ruminant animal feeds, and is considered to represent the portion of feed that is bulky and difficult to digest (Mertens, 2002b). In particular, the rate of fibre digestion is greatly influenced by the source of fibre (Chesworth, 1992). It is well known that the nutritional value of fibre (or forage) is determined by the proportion of cell wall and the degree of lignification thereof (Van Soest, 1982). According to Van Soest (1994), the cell wall represents the most indigestible portion of the forages, and therefore the digestibility and composition of the cell wall may be the factors most limiting to animal production on high-forage diets.

Many reviews on digestion kinetics of cell wall carbohydrates have been presented and published, addressing the problems associated with the estimation of kinetic parameters (Raffrenato *et al*., 2009). The importance of the rate and extent of NDF digestion on organic matter (OM) and NDF digestibility has been demonstrated by simulation with the CNCPS model (Tylutki *et al.*, 2008). These simulation results clearly demonstrate the profound effects of these kinetic parameters on digestibility and therefore on the supply of both energy and microbial protein (Raffrenato *et al.*, 2009). The accurate estimation of forage digestibility is a prerequisite for the formulation of diets, economic evaluation of forages and the prediction of animal response (Raffrenato *et al*., 2009).

Numerous nutritional models that are used today in the dairy industry require physically effective NDF (peNDF) as a key input for the model to predict lactational response (Grant & Cotanch, 2005). The peNDF system has been widely used in the Cornell-Penn-Miner (CPM) -Dairy and CNCPS ration formulation models to predict the effect of forage particle size on cow chewing response and rumen pH (Grant, 2010). Chewing activity is a response that reflects the chemical and physical properties of feeds such as NDF, particle size, intrinsic fragility and moisture. Chewing activity is also a function of the type, size or age and dry matter intake (DMI) of the animal (Mertens, 1997). Because chewing activity/kg DM is not a constant feed characteristic, it is difficult to use this response as a direct input in diet formulations (Mertens, 2002a).

The method proposed by Mertens (1997) for the estimation of peNDF assumed that the fragility or ease of particle size reduction during chewing is similar among different sources of NDF. While it is known that different sources of NDF of similar particle size may elicit variable chewing responses (Grant, 2010), the potential variation in chewing responses elicited from different forages with similar peNDF values has serious important implications for nutritional models which incorporate peNDF values and which assume that every unit of peNDF is equal regardless of the source (Grant & Cotanch, 2005). These differences observed in chewing responses may be attributed to differences in forage fragility or stem brittleness (Grant, 2010). It is difficult to predict fibre adequacy from feedstuff nutrient composition tables, as there is a lack of sufficient characterisation and a standardized validated evaluation system, including recommendations for physical characteristics of the fibre in the diets of ruminants (Zebeli *et al.*, 2007). Allen & Oba (1996) stated that although there are limited data available on the relationship between forage maturity and/- or chemical composition and the rate of particle size reduction, it is clear that differences in fragility exists among forages, and these differences must be accounted for in models predicting flow of digesta from the reticulo-rumen. MacDonald *et al.* (2011) and Grant (2010) both stated that feed of the same digestibility but different NDF content will have different intakes and therefore different chewing responses. What has also been disproven in certain cases is that the ease of particle size reduction during chewing is similar among different sources of NDF (Mertens, 1997). Therefore, to make the assumption with the peNDF system that all the peNDF values are equal regardless of the NDF source cannot be true. This leads to numerous inaccuracies in the system and it is for this reason that the system and factors affecting the peNDF system needs to be further researched, so that formulation models can be used to formulate diets more accurately. This will have large economic benefits as formulating rations will become more efficient, and as a result cow performance will also increase.

Forage fragility is defined as the relative rate at which the particle size of forages is reduced during processes such as chewing or some laboratory simulation of chewing action (Grant, 2010). Grant (2010) stated that there is a positive relationship between forage fragility and NDF digestibility. Forage fragility might be related to the lignin content and the digestibility, as well as to anatomical differences among plant species such as cell wall thickness, and therefore, the rate of digestibility of the forage cell wall may be predictive of forage fragility (Grant, 2010).

The purpose of this study was to investigate the possible association between forage fragility and short term or long term *in vitro* NDF digestibility (*iv*NDFd). The energy required to grind various samples of commonly used forages in ruminant nutrition in South Africa, was measured. In addition, the chemical composition of the forage samples was determined and related to the NDF digestibility (NDFd) at different time points during *in vitro* digestion over 240 hours (10 days). Both the chemical composition and *iv*NDFd of the forage samples were then related to the energy measurement values obtained from the grinding of the samples. Understanding the relationship between chemical composition and forage fragility, as well as the factors most affecting forage fragility, can lead to improved accuracy of nutritional models and predictions of animal performance.

CHAPTER 2

2. LITERATURE REVIEW

2.1 INTRODUCTION

Animal performance is the product of dietary nutrient and energy concentration, intake, digestibility and metabolism (Raffrenato *et al.*, 2009). There is some variation in digestible dry matter or energy intake among animals and feeds. Between 60% and 90% of this variation is related to differences in intake, whereas 10% to 40% of the variation is a result of differences in digestibility (Mertens, 1994). In order to accurately formulate diets and predict animal response, forage digestibility needs to be accurately estimated.

Forages are described as edible but bulky, coarse plant material, which have a high fibre concentration and a low digestible nutrient content (Horrocks & Vallentine, 1999). Forages contain high amounts of cell wall content, of which the nutritive value is generally significantly lower than that of the cell contents, although many types of forages can still be relatively high in digestible energy (70%) and total protein (25%) (Horrocks & Vallentine, 1999).

Plant fibre is a biological unit, rather than a chemical entity, and is found in the cell wall of plants (Van Soest, 1982). Plant cell walls have a complicated chemical composition consisting of lignin, cellulose, hemicellulose, pectin, and smaller amounts of protein, lignified nitrogen compounds, waxes, cutine and mineral components (Dickenson *et al.*, 2007). Cell walls can comprise between 20% and 80% of forage dry weight (Wilson, 1994). Plant fibre is a vital component of the ruminant's diet and is an analytical product which has nutritional characteristics that describe those forage components which have low solubility in specific solvent systems and are relatively less digestible than starch. In some cases, such as mature grasses, the cell wall and fibre concentrations of forages are very similar, whereas for legumes the fibre estimates are routinely lower than the cell wall concentration (Theander and Aman, 1980). This discrepancy is due to the solubility in neutral detergent solution of pectins, which are present in high concentrations in legume cell walls (Van Soest, 1982; Theander & Westerlund, 1993).

Neutral detergent fibre consists mainly of lignin, cellulose and hemicellulose, and can be used as a measurement of the plant cell wall content (MacDonald *et al.*, 2011). It represents the slowly digestible cell wall fraction of feeds, and according to Beauchemin (1991), the potential exists to formulate diets for cattle based on NDF. The NDF content is the primary chemical component that determines the rate of digestion of a food, resulting in a negative relationship between the rate of digestion and the NDF concentration of foods (MacDonald *et al.*, 2011). However, although NDF has been used in the past to predict the filling effects of forages, there is substantial evidence that NDF alone is not adequate to make these predictions (Allen & Oba, 1996). The filling effects of forages are influenced by the IPS, particle fragility, and the rate and extent of NDF digestion (Allen & Oba, 1996).

Figure 2.1 below gives a visual illustration of the carbohydrate fractions of plant fibre.

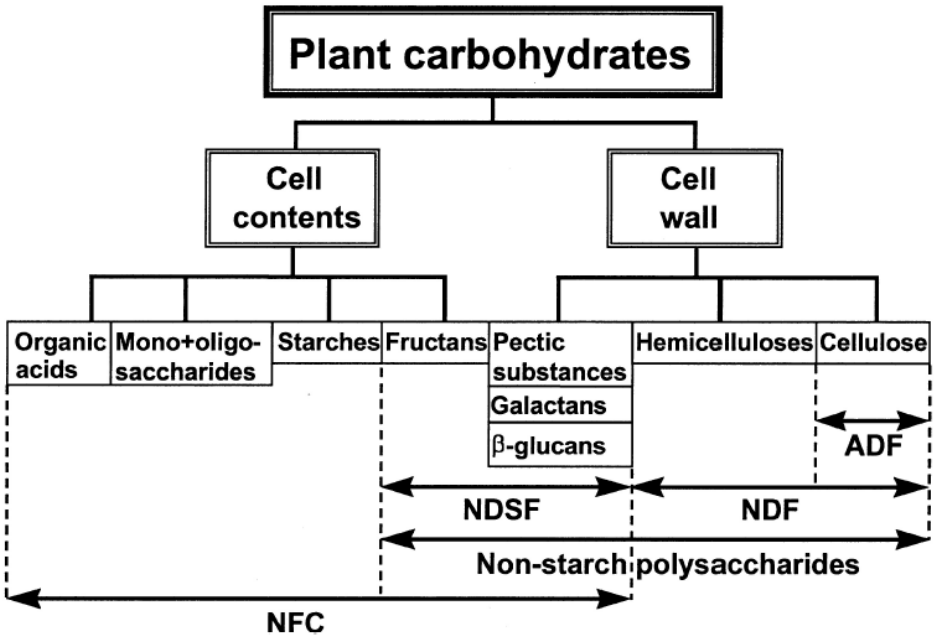

Figure 2.1 Plant carbohydrate fractions. ADF = acid detergent fibre, *β*-glucans = (1 ➔3) (1 ➔4)-*β*-D-glucans, NDF = neutral detergent fibre, NDSF = neutral detergent-soluble fibre (includes all non-starch polysaccharides not present in NDF), NFC = on-NDF carbohydrates.

The character and nutritional value of forage is determined by two factors, namely the proportion of cell wall and the grade of lignification thereof (Van Soest, 1982). Early work on digestion kinetics of forage fibre has indicated that lignin is negatively correlated with the extent of NDF digestion but not related to the rate of fibre digestion (Smith *et al.*, 1972). However, more recent work has in fact shown a negative correlation of lignin concentration with kd (Cherney *et al.*, 1986; Thorstensson *et al.*, 1992). Therefore, the feeding value of forages is related to the quality of the forage (Horrock & Vallentine, 1999), and the intake potential and energy availability in forage crops are limited by the cell wall concentration and digestibility of the forage (Jung & Allen, 1995).

Adequate particle length of forages is necessary for proper ruminal function, and reduced particle size has decreased the time spent chewing and has tended to decrease ruminal pH, potentially resulting in ruminal acidosis (Lammers *et al.*, 1996). Fibre particles must be reduced to smaller than the critical size (to pass through a 1 mm screen) to pass readily from the rumen (Wilson, 1994). If fibre particles need a longer time in the rumen to achieve this critical size, then the feed will generally cause a low intake and result in lower animal performance (Wilson, 1994).

The relationship between feed intake, concentrate inclusion, ruminal degradation processes and animal performance is very complex, and this leads to difficulties in the quantitative characterization of the effects of forage particle size (Zebeli *et al.*, 2007). Many studies have been done in the past to investigate the effects of forage particle size on feed intake, chewing activity, ruminal digestive processes and performance in high-producing dairy cows, however, the results obtained from these studies have been inconclusive (Zebeli *et al.*, 2007).

2.2 THE IMPORTANCE OF FORAGE IN RUMINANT DIETS

Modern agriculture utilises large quantities of concentrate feeds in dairy and beef production systems. However, fibrous forages remain the primary source of nutrients in extensive ruminant production systems (Jung & Allen, 1995; Yang *et al.*, 2001a; Jung, 2012). Ruminants require sufficient amounts of fibre of adequate particle length as it establishes the biphasic nature of the rumen and therefore stimulates chewing activity (Lammers *et al.*, 1996; Mertens, 1997), which is vital for maintenance of rumen function and optimisation of milk yield (Oba & Allen, 1999; Yang *et al.*, 2001a).

Ruminants can utilise fibrous forage material due to two unique occurrences in the forestomach, namely, their forestomachs make use of microbial degradation and secondly, ruminants have a selective retention mechanism that retains fibre particles for increased digestion efficiency (Krizsan & Huhtanen, 2012). Thus, forages can be referred to as functional feeds (Zinn & Ware, 2007) and are defined as the slowly digestible or indigestible fraction of feed that occupies space in the gastrointestinal tract (Mertens, 1997). The term forage or roughage includes all cultivated pastures, natural grazing veld, crop residues, silage and hays, and all of these have characteristically high fibre concentration, with digestibilities lower than 60% and a crude fibre concentration of more than 18% (Van Soest, 1982). The crude protein (CP) value of forages can vary as much as from 25% (DM basis) in young, well fertilized pastures, to less than 4% (DM basis) in poor winter pastures and wheat straw (Van Soest, 1982).

Fibre has been related to the filling properties of feeds because it is fermented and passes from the reticulorumen more slowly than the non-fibre components. So while high inclusion rates of forage in ruminant diets is a primary goal, desired levels of productivity cannot be achieved due to a main limiting factor, namely intake (Jung & Allen, 1995). Lowering the inclusion rates of fibre in the diet is a challenge because lowered effective fibre levels results in simultaneous and linked reactions occurring, which ultimately results in lowered ruminal fermentation. Less effective fibre in the diet leads to lowered chewing activity by the animal, and in turn results in less salivary buffer secretion into the rumen (Mertens, 2002a). Decreased salivary buffer production as well as increased volatile fatty acid (VFA) concentrations result in a lowered ruminal pH. Ultimately, the lower rumen pH causes microbial populations to change and this causes a shift in the end products of fermentation as the acetate to propionate ratio is reduced. This change in acetate to propionate ratio is associated with milk fat depression and the shunting of nutrients towards fattening (Mertens, 2002a).

It is important to realise that it is not only the lowered salivary buffer secretion that causes a drop in ruminal pH, but also the change in the ratio and production of VFA when dietary fibre levels are decreased (Mertens, 2002a). As the fibre concentration in the ration decreases, the concentrations of non-fibre carbohydrates (NFC) or neutral detergent soluble carbohydrates (NDSC) increase, which can ultimately lower rumen pH (Mertens, 2002a). The NFC includes starch, sugars, β-glucans, fructans, pectins and organic acids (Mertens, 2000). Organic acids are not readily fermented and do not contribute to microbial protein production (Mertens, 2000). When slowly fermenting NDF is replaced with rapidly

fermenting NFC, more VFA are produced in the rumen which will, together with the lowered secretion of salivary buffer, result in a decrease in the rumen pH, as most of the carbohydrates of NFC are completely and rapidly digested (Mertens, 2002a). Therefore, it has been suggested that the interaction between peNDF and NFC may affect the requirements for effective fibre (Mertens, 2002a). However, although both NDSC and NFC play a role in milk fat percentage and ruminal pH, research suggests that it is the lack of effective fibre that is most often responsible for a milk fat depression and ruminal acidosis (Mertens, 2000).

The cell wall fraction is the largest component of forages and it is this fraction that has been implicated as the main control mechanism of intake. A common low digestibility and high concentration of plant cell wall in forage slows down the rate of passage and thus feed intake, and this ultimately limits the energy availability in high forage diets (Galyean & Goetsch, 1993; Buxton & Redfearn, 1997; Jung, 2012). A reduction in the cell wall fraction of plants would result in higher intakes, and increased digestibility of the cell wall fraction would improve energy availability and in turn animal performance (Jung & Allen, 1995).

As the genetic potential for growth and lactation of ruminant livestock improves, the ability of contemporary forage species to meet the energy requirements of the animal will reduce even further, unless the energy availability from the forages is increased (Galyean & Goetsch, 1993).

Although forage is a limiting factor of intake and thus digestibility, there are a number of reasons, both animal and non-animal related as to why forage levels should remain at higher inclusions in ruminant diets: it was mentioned earlier that lowered fibre levels can cause a decrease in animal performance such as milk fat depression and ruminal acidosis. Other important reasons are that production costs for forage crops are lower than for grain crops, perennial forage crops are more ‘environmentally friendly’ as they reduce soil erosion and there is less water pollution associated with lower levels of fertilization and pesticides required for these crops (Jung & Allen, 1995). All of these factors make the use of forage crops highly desirable in ruminant diets.

2.3 CELL WALL STRUCTURE AND ANATOMICAL DIFFERENCES

The plant cell wall is a complex biological structure that is composed mainly of NDF (cellulose, hemicellulose and lignin) as shown in Figure 2.1. Cell wall growth occurs in two phases: in the first phase, (primary cell wall growth), the plant cell increases in size through wall elongation. Pectins, xylans, cellulose and hemicellulose are deposited during this phase. The second phase then follows (cell wall thickening) where lignin is deposited, beginning in the middle lamella and the original primary wall and then proceeding towards the centre of the plant cell into the secondary wall (Bacic *et al.*, 1988; Terashima *et al.*, 1993). The result of this pattern of lignin deposition is that the primary wall and middle lamella are the most lignified regions. This can explain why rumen microorganisms degrade plant cell walls from the lumen outwards and also why the middle lamella and primary wall regions are never fully digested (Terashima *et al.*, 1993; Jung & Allen, 1995).

Lignin does not contain any sugar units and therefore is not a carbohydrate, but is made up of large mats of chemical molecules known as phenols. Lignin is not considered to have any nutritional value, but rather, can have detrimental effects on the digestion of other feed components (Chesworth, 1992). However, lignin remains an important component of the plant as it provides mechanical strength to the plant for support and rigidity of the cell walls. Evidence shows that lignin and other cell wall constituents provide resistance to the plant from diseases, insects, cold temperatures and other abiotic and biotic stressors (Buxton & Redfearn, 1997).

Chemical analysis of forage cell walls shows that lignin is covalently bound to the hemicellulose fraction and this suggests that hemicellulose digestion may be inhibited or restricted due to the physical protection by lignin (Dekker, 1976). Cellulose is the predominant component of plant fibre and appears to be completely available to rumen micro-organisms, although at differing rates (Akin, 1986; Zinn & Ware, 2007). This is due to cellulose containing cellulose microfibrils which are bound in a matrix of particularly hemicellulose and lignin (Wilson 1993; Jung, 2012). This matrix structure suggests that cellulose digestion can be limited by the hemicellulose – lignin encasement and complete digestion can only occur when the cellulose fraction is isolated from the wall (Zinn & Ware, 2007; Jung, 2012). Thus, it is evident that lignin is the major cell wall component that limits digestion of forages in the rumen (Jung

& Deetz, 1993; Buxton & Redfearn, 1997). This negative relationship between lignin and cell wall digestibility has been recognised for many years, dating back to the 1950s (Jung & Allen, 1995), and it is important to understand that lignin impacts cell wall digestibility and not digestion of non-cell wall constituents (Jung, 2012).

A plant's nutritive value generally declines with maturity and these changes are due to an altered chemical composition which involves increased lignification and decreased proportion of leaves to stems (Van Soest, 1982). Sheaffer *et al*. (1998) stated that plant maturity is the most important factor that affects forage quality, and the changes in quality are associated with increases in lignin and cell wall deposition, and decreases in mineral content, CP, and digestible cell content such as starch (Aman & Lindgren, 1983), as cited by Sheaffer *et al.* (1998). This is evident from data published by Jung & Vogel (1986) showing ranges of values for chemical composition and digestibility of forages (temperate and tropical grasses) used in their study, as is shown in Table 2.1 on the following page. The data shows that as the plant matures, CP decreases, but overall cell wall content increases, with special attention to the cellulose and lignin composition increase and digestibility decrease.

Table 2.1 Range of values for chemical composition and digestibility of forages used in the study given from earliest to latest stage of physiological maturity (adapted from Jung & Vogel, 1986)

Forage species	Esti-mate[b]	Component (%)[a]					
		DM	CP	CW	HC	CEL	LIG
C_3 grasses							
Orchardgrass	Comp		27.2–10.0	43.1–60.8	55.7–42.9	40.4–52.3	8.1– 6.6
	Dig	84.6–65.1		85.9–54.9	90.9–61.8	87.6–57.2	74.7– 31.8
Smooth bromegrass	Comp		18.8– 6.5	38.2–64.5	46.6–41.9	49.0–49.8	3.9 7.6
	Dig	81.7–51.0		78.9–38.1	88.6–46.8	78.6–37.5	40.7– (–5.1)
C_4 grasses							
Switchgrass	Comp		17.9– 3.2	57.7–74.3	54.8–41.5	41.9–50.6	3.5– 8.1
	Dig	75.4–35.6		79.3–23.8	82.8–30.7	79.9–24.2	13.1– (–5.7)
Indiangrass	Comp		12.6– 5.1	69.7–75.9	50.4–43.1	45.0–49.8	3.6– 6.7
	Dig	65.3–41.9		68.1–37.3	72.6–41.2	67.6–36.6	38.7– 21.6
Big bluestem	Comp		11.4– 5.3	67.4–72.9	47.4–44.7	47.6–48.8	4.5– 6.3
	Dig	57.0–38.2		55.6–30.0	60.1–34.1	56.3–30.0	0 – (–1.9)
Caucasian bluestem	Comp		14.8– 4.4	62.0–77.1	50.2–41.6	46.3–50.4	3.0– 8.2
	Dig	62.8–32.9		65.0–27.2	80.6–42.6	59.3–20.6	–24.5–(–18.3)
Sand bluestem	Comp		13.7– 4.4	70.4–78.1	51.7–42.2	42.9–48.4	4.1– 8.5
	Dig	60.2–34.5		61.9–28.8	69.4–37.6	58.1–24.4	17.7– 1.6
Big × sand bluestem	Comp		15.2– 5.6	70.6–78.3	50.4–43.2	43.7–46.5	7.6– 9.1
	Dig	67.2–42.4		72.4–38.0	78.3–44.2	73.3–36.8	22.0– 1.1

[a]Forage components are: dry matter (DM), crude protein (CP), cell wall (CW), hemicellulose (HC), cellulose (CEL) and lignin (LIG); the composition data for CP and CW are expressed as a percentage of DM, whereas HC, CEL and LIG are expressed as a percentage of CW.

[b]Composition (Comp), digestibility (Dig)

However, Van Soest (1982) also stated that there are exceptions to this generalization, since not all leaves are more digestible than stems. In the case of grasses, leaves have a structural function, while the stem functions as a storage organ, which can lead to stems having a higher overall nutritive value than the leaves. The quality of stems is influenced by the diameter and whether it is hollow or filled with a pith. Larger stems might be more digestible, because the lignification is distributed more thinly (Van Soest, 1982). The pith is usually much less lignified than the cortex, and therefore hollow stems tend to be less digestible. The degree to which individual forages decline in nutritive value may vary with age, as the maturity of the plant and the environment differs between different forages (Van Soest, 1982).

It is evident that there are anatomical differences between legumes and grasses with regards to lignin content and digestibility. Legume leaves contain less cell wall content than that of grass leaves, and the increase in cell wall concentration seen with maturity of grass leaves is not observed in legume leaves (Wilman *et al.*, 1977; Wilman & Altimimi, 1984). This lower concentration of cell wall and therefore lignin in legume leaves makes legumes leaves more digestible. The cell wall concentration of stems for all forages is higher compared to the leaf cell wall concentration, and this cell wall content in stems increases with plant maturity (Griffin & Jung, 1983; Jung & Vogel, 1992). This especially affects the digestibility of mature legume plants because as the plants mature, the ratio of leaf to stem decreases, hence the plants will have increased concentrations of lignin therefore lowering the digestibility of such legume plants (Jung & Allen, 1995; Buxton & Redfearn, 1997; Jung, 2012).

It is noticeable that the effect of lignin on fibre digestibility appears to be greater in grasses than in legumes, and there also seems to be a difference in the properties of lignin between different grasses. Generally, it has been seen that lignin accounts for 40 – 60% of the variation in cell wall digestion (Jung, 2012). Some studies have also indicated no significant relationship between lignin concentration and cell wall digestibility, but these studies involved samples of whole forages, mainly at a single stage of maturity and which were analyzed independently for the leaf and stem fractions (Jung & Allen, 1995; Jung, 2012). Figure 2.2 illustrates both the effect of lignin on cell wall digestibility, as well as the non-significant relationship when looking at a specific maturity target.

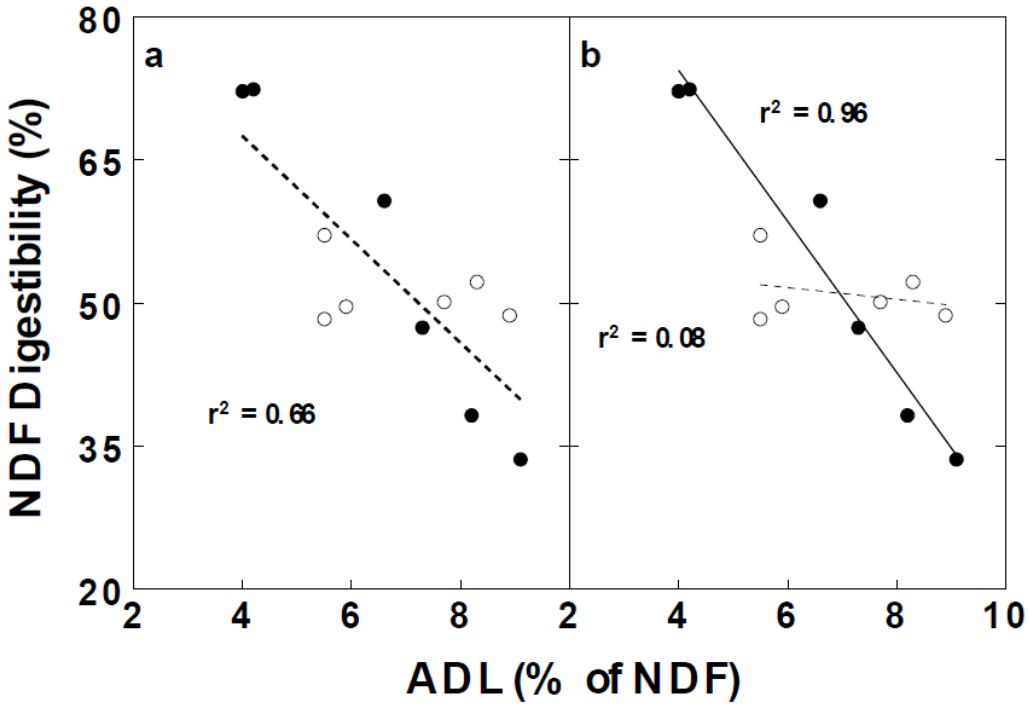

Figure 2.2 Relationships between acid detergent lignin (ADL) concentration and *in vitro* rumen neutral detergent fibre (NDF) digestibility of two grass species harvested at three maturity stages (●) and six genetic lines of one grass species harvested at a single maturity stage (○) when examined across all samples (a) and by individual sample set (b) (Adapted from Jung, 2012)

A challenge is that there are no specific chemical analyses for cell wall components and therefore it cannot be accurately determined in the laboratory (Dickenson *et al.*, 2007). There is an analytical method of determining lignin in forages, known as ADL, but this often severely underestimates the lignin content of grasses (Jung *et al.*, 1993; Hatfield *et al.*, 1994). Another method known as Klason Lignin, which is the residue remaining after two stage sulphuric acid hydrolysis that is commonly used to determine the neutral sugar components of cell wall polysaccharides (Theander & Westerlund, 1986, as cited by NRC, 2001). Differences in the ADL and Klason lignin methods account for the difference in lignin values as measured by these two methods (Lowry *et al.*, 1994, as cited by NRC, 2001). Klason lignin values are typically two to four times greater for grasses than the sulphuric ADL estimates, and 30% higher for legumes, according to Jung *et al.* (1997), as cited by NRC (2001). Hatfield *et al.* (1994) concluded that

the Klason lignin method was a more accurate estimate of plant cell wall lignin content than is ADL. Other literature suggests that an acid soluble lignin fraction (which is ADF) is lost in the Acid Detergent Lignin step of the ADL procedure, thereby resulting in underestimations of lignin content by the ADL method (Lowry *et al.*, 1994, as cited by NRC, 2001). However, Raffrenato & Van Amburgh (2010) have described a dynamic relationship between ADL and iNDF (obtained after 240 hours of *in vitro* fermentation of substrate), and they have suggested that with forage group-specific ranges according to the ADL/NDF ratio of a specific forage, plant lignin content could be predicted more accurately.

It is clear that the most important factor affecting plant composition is the plant maturation process, whereby an increase in maturity of the plant is coupled with an increase in stem and overall lignification (Jung, 2012). Because of the decrease in leaf to stem ratio as forages mature, and the strong effect of maturity on lignin concentration in stems, it makes sense that strong negative correlations have been found from samples at various stages of maturity (Van Soest, 1982; Jung & Allen, 1995).

2.4 THE INFLUENCE OF TROPICAL VS TEMPERATE FORAGES (C_3- AND C_4-PATHWAYS) ON DIGESTIBILITY

Grasses can be separated into two photosynthetic pathways: C_3-grasses (C_3-Calvin cycle) which grow in temperate climates with cool temperatures and high altitudes, and C_4-grasses (C_4-Calvin cycle) which grow in tropical environments (Akin, 1986; Nelson & Moser, 1994; Milton, 2004). These differences between climatic growing conditions lead to anatomical differences between the C_3- and C_4-species (Milton, 2004). Nitrogen is used more efficiently by C_4-grasses than C_3-grasses, and most of South Africa's grass species are of the C_4-species, whereas some of the annual invasive alien species as well as most invasive perennial species follow the C_3-pathway (Milton, 2004).

In warm tropical climates, grasses mature more quickly, and the protein and phosphorus concentrations decrease to very low levels, while the fibre concentration increases (MacDonald *et al.*, 2011). Legumes are similar to C_3-grasses as legumes follow only the C_3-photosynthetic pathway, but can be separated into warm- and cool-season types based on their adaptation to temperature. Temperature has

a large impact on forage quality such that when cell wall material is deposited at lower temperatures, it is less lignified and therefore higher in digestibility when compared to lignification at higher temperatures (Nelson & Moser, 1994).

Temperate grasses (C_3) have a higher ratio of mesophyll to vascular tissue (phloem and xylem) than tropical grasses (C_4). In the leaves of legumes and temperate grasses, the mesophyll cells are more loosely arranged, or packed, than in tropical grasses, and the percentage of intercellular airspaces in temperate grasses and legumes is much higher than in tropical grasses (Wilson, 1993). It is believed that this loose arrangement allows for more rapid penetration of microbes into the leaf and therefore quicker digestion of the leaf material by the animal (Hanna *et al.*, 1973).

Another anatomical characteristic of leaves that affects digestibility is the parenchyma bundle sheath (Weston, 2002). In temperate grass species, the parenchyma bundle sheath is a distinct structure whose cell wall appears to be as easily degraded as the wall of the mesophyll tissue. On the other hand, the parenchyma bundle sheath in many tropical grasses is a rigid, thick-walled, weakly lignified structure that appears to be resistant to degradation by rumen microorganisms and is slowly or only partially degraded (Akin, 1986). This makes the rate of digestion of tropical grasses slower than for temperate grasses due to the longer retention time in the rumen, to allow for adequate degradation of the NDF fraction and digestion of non-cell wall constituents (Mertens & Loften, 1980).

In temperate grasses, the main storage carbohydrate is fructan, which is the most abundant soluble carbohydrate and is found predominantly in the stem. Tropical- and subtropical grasses store carbohydrates as starch, and is found mainly in the leaves (MacDonald *et al.*, 2011). Wilson (1994) reported that more than 50% of the reserve carbohydrates and protein of tropical grass leaves are contained in the specialized bundle sheath cells, and because these cells are thick and digested slowly, these nutrients are not readily available to ruminal microbes. Within C_4-species, there is an additional barrier in the form of a thin, suberized layer within the outer section of the bundle sheath. This layer is waxy and prevents microbial access to the inner secondary wall and the cell contents until cell walls are broken or comprimised by chewing (Wilson, 1994). This also explains why tropical grasses have a lower nutritive value than temperate grasses (Wilson, 1993). Minson & McLeod (1970), as cited by Wilson &

Hacker (1987) observed that the mean digestibility of tropical grasses is about 13 percentage units lower than that of temperate grasses.

Legumes have a high rate of cell wall degradation due to the fact that they follow the C_3-photosynthetic pathway, similar to temperate grasses. In addition, lignin deposition is more localised and there is a greater proportion of core than of noncore lignin (Jung & Deetz, 1993). Legumes have a more rapid digestion rate of potentially digestible NDF (pdNDF) than grasses, but grasses have a larger portion of NDF that is potentially digestible (Buxton & Redfearn, 1997).

Further anatomical differences between grasses and legumes exist, influencing the extent of degradation. Leaves of grasses have parallel venation, with each leaf containing several significant vascular bundles. During ingestive chewing, the grass leaves are mainly broken down longitudinally into long, narrow particles. These particles require significant chewing during rumination to allow the release of the non-cell wall constituents. Legume leaves have reticulate venation and a single, large central vascular bundle and a network of fine bundles which are easily degradable. Chewing during ingestion breaks down the leaves into small, 'blocky' particles which makes microbial attachment easy and therefore allows for easier digestion and release of non-cell wall constituents (Wilson, 1993; Weston, 2002; Jung, 2012).

What is interesting to note here is that Caswell *et al.* (1973) presented a hypothesis with some supporting data that, in general, plants which possess the C_4-photosynthetic pathway are of poorer quality than plants which follow the C_3-pathway. The authors also stated that herbivores tended to avoid the C_4-species where possible.

2.5 DIGESTION IN THE RUMINANT: MEAN RETENTION TIME (MRT) AND FACTORS INFLUENCING DIGESTION IN THE RUMEN

Plant cell wall material is resistant to the animal's digestive system, as most animals lack the enzymes capable of degrading cellulose, hemicellulose and lignin. The major organisms that attack these substances via fermentation are bacteria and fungi, normally found in the rumen of herbivores (Van Soest,

1994). In addition to this, the use of the more slowly fermenting substrates is limited by the retention time of feed in the digestive tract and most importantly, the adaptation and evolution of the herbivore's digestive tract (Van Soest, 1994). The advantage that ruminant animals have over monogastric animals is that they have evolved large fermentation chambers in their stomachs, affording them the superior ability to utilise plant cell wall constituents through microbial degradation and fermentation in a largely anaerobic environment (Van Soest, 1994). According to Van Soest (1994), cellulose and hemicellulose (cellulosic carbohydrates) may average approximately 50% of the Metabolisable energy (ME) consumed by ruminants. Lignin is not included in this ME estimation due to the unhydrolyzable linkages that make up lignin, giving this polyphenol the term, a condensed substance. Simple phenolic substances can be utilised by anaerobic organisms, but it seems that condensed substances are limited to aerobic organisms. Therefore, lignin degradation in the rumen will be limited and as a result, lignin sets a limit on the maximum degradability of the plant cell wall (Terashima *et al.,* 1993; Van Soest, 1994).

The cellulosic carbohydrates are insoluble, therefore only fungi and bacteria can degrade these substances (Leschine, 1995), for example, cellulose is predominantly degraded by a slow process involving cellulolytic bacteria in the rumen (Weimer, 1992). The evolution of the ruminant digestive system allows for substantially longer mean retention time (MRT) of fibre particles in the reticulorumen, to allow the cellulose breakdown to occur, thus improving the utilization of the cell wall components. However, a consequence of this increased MRT is a possible restriction on feed intake due to the fill effect (Lechner-Doll *et al.*, 1991; Faverdin *et al.*, 1995).

The MRT is defined as the ratio of the amount of any component of digesta in a specific segment of the gastrointestinal (GI) tract to the flow of that digesta component through or from that segment (Faichney, 1993). Rate of passage is a measure of the time during which a portion of digesta is exposed to the processes of chewing and rumination i.e. digestion and absorption in a segment of the GI tract (Faichney, 1993). This rate of passage is measured as MRT and is a function of feed intake but is also influenced by the physical and chemical characteristics of the diet, animal factors and climatic or environmental factors (Faichney, 1986). Physical and chemical characteristics of the diet are collectively represented by NDF which can be fractioned into effective NDF (eNDF) and peNDF and these will be discussed later (Mertens, 1997; Zinn & Ware, 2007). Animal factors include breed, sex, genotype, growth, age, parity, stage of lactation, milk yield, pregnancy, diseases and body condition (Ingvartsen,

1994). As an example, during peak lactation, energy requirements are high, therefore more concentrates will be included in the diet, so rate of passage will increase and MRT will decrease (due to less time for digestion and absorption) compared with a high forage diet (Kadzere *et al.,* 2002; MacDonald *et al.*, 2011). Climatic and environmental factors include temperature, humidity, food additives, frequency of feeding, space allowance and photoperiod (Ingvartsen, 1994). An example of the impact of climatic factors is the effect of temperature. The thermoneutral zone of dairy cattle is between 15˚C and 25˚C (for beef cattle the thermoneutral zone is between 20˚C and 33˚C); at temperatures below the thermoneutral zone, intake is increased, therefore rate of passage is increased and as such MRT decreases, and vice versa (Baumgard & Rhoads, 2009; MacDonald *et al.*, 2011).

Retention time of fibre in the stomach is influenced by numerous factors, in particular IPS, rate of particle size reduction, IPS density and rate of digestion (Zinn & Ware, 2007).

Initial particle size

Initial fibre particle size is dependent on feed processing, for example, pelleted feed will have a larger substrate surface area for microbial attachment and will lead to a faster rate of digestion with decreased MRT, but could potentially mean that the fibrous component of the diet may not be fully digested due to the cell wall components (Welch, 1986; Faichney, 1986; MacDonald *et al.,* 2011). Conversely, forages fed in the longer form may be retained longer in the rumen due to slower microbial attachment, which is a result of less attachment points for microbiota and therefore increased MRT and more complete fibre digestion (Owens & Goetsch, 1986).

Rate of particle size reduction

Rate of particle size reduction is influenced by chewing and rumination, with rumination being the most important activity (Weston, 2002; Zinn & Ware, 2007). Microbial fermentation has little effect on reducing the length of feed particles, but it assists with size reduction during rumination by weakening the particle's cell walls (Murphy & Kennedy, 1993). The mechanical grinding of forages partially destroys the structural organization of cell walls, leading to accelerated breakdown of these cell walls in the rumen (MacDonald *et al.,* 2011). Chewing during eating (ingestive chewing) reduces large particles, releases soluble nutrients from fresh forages for fermentation and also exposes the inner structures of forage material for attachment by microbes in the reticulorumen. Chewing during rumination (ruminative

chewing) is a process known as chewing of the 'cud' which is essentially regurgitated digesta. This process serves two purposes: it damages the regurgitated digesta cell walls thus further exposing the inner plant structures for microbial attack, and it reduces the particle size of refractory material (Ulyatt *et al.*, 1986).

The reticulorumen undergoes strong contractions to mix the digesta and theoretically, it is possible that the movement created by the mixing can reduce the size of fibre particles that have been weakened by chewing and microbial attack. However, chewing during eating and rumination are the predominant means of reducing particle sizes of long forage for exit out of the reticulorumen (Ulyatt *et al.*, 1986). Although chewing as a whole is important for reducing particle size, Ulyatt *et al.* (1986) pointed out that chewing during rumination in particular, reduces larger quantities of forage DM (27-39% hay dietary DM) compared to chewing during eating (12-15% forage DM).

Particle density

Particle size density influences particle passage from the reticulorumen to the omasum through the reticulo-omasal orifice (Van Soest, 1982; Lechner-Doll *et al.*, 1991; Clauss *et al.*, 2011). Particle density is also related to particle size: particle density influences sorting in the reticulum, whereas particle size influences particle retention in the fibrous mat of stratified rumen contents i.e. the 'filter-bed' effect (biphasic nature of the rumen) (Clauss *et al.*, 2011).

The "filter-bed" effect

The 'filter-bed' effect is the formation of a fibrous rumen mat or raft towards the dorsal rumen. This mat is made up of large undigested forage particles of low density. These particles are also buoyant due to fermentation gasses being trapped within the plant NDF structure. The particles are subjected to rumination and chewing causing them to increase in density due to the release of the fermentation gasses by breakdown of the particles' NDF structure by microbes. Their increased density and reduced size allows them to move downwards towards the more liquid ventral rumen and reticulum where the particles escape to the omasum by propulsive movements of the reticulorumen (Faichney, 1986; Lechner-Doll *et al.*, 1991; Weston, 2002; Schulze *et al.*, 2014). Stem particles are more likely than leaf particles to be incorporated in the rumen mat due to the stem's structure i.e. higher degree of lignification compared with leaves (Kennedy, 2005). Interestingly, Rinne *et al.* (2002) stated that the clearance from the reticulorumen

of digestible cell wall particles occurs at a slower rate than for indigestible cell wall particles. Therefore, estimated passage rate of dietary cell wall components is greatest for lignin and least for hemicellulose, being the most digestible part of the NDF structure (Egan & Doyle, 1985). This seems to be a consequence of differential sorting within the reticulorumen of particles that have undergone differing degrees of digestion and in addition, that have differing chemical and physical properties (Kennedy, 2005).

Critical particle size

Faichney (1993) mentioned the possibility of a concept of critical particle size (CPS), where the probability of a particle leaving the reticulorumen decreases exponentially with increased particle size (Poppi *et al.*, 1980; Ulyatt *et al.*, 1986). This concept implies that in order for particles to leave the rumen via the reticulo-omasal orifice, particles must be reduced to a CPS. The CPS represents the threshold between the two basic pools existing in the rumen, namely the rumen mat containing particles too large and buoyant to leave and the more liquid pool containing particles small enough to leave the rumen (Lechner-Doll *et al.*, 1991). The CPS of particles has been specified empirically in terms of the pore size of a sieve that will allow particles small enough to leave through the reticulo-omasal orifice, but will retain larger particles. The CPS of forage particles for cattle and sheep has been suggested by many and seems to be within the range of 1 to 2 mm, averaging at approximately 1.18 mm, depending on the type of forage fed along with any processing (Poppi *et al.*, 1980; Zinn & Ware, 2007). However, in some cases, particles as large as 5 cm in length have been seen to pass out of the rumen into the omasum (Welch, 1986).

This leads to the definitions of digestion and digestibility, which are two commonly misused terms in nutrition. Digestion refers to the extent of degradation. The extent of ruminal fibre digestion is a function of rate of digestion (k_d) and rate of passage (k_p). Digestibility is a qualitative measurement, referring to the susceptibility to degradation (Zinn & Ware, 2007). It is important to point out some important connections such as digestibility being directly proportional to the digestible fraction of fibre and the rate of fibre digestion, but inversely proportional to the rate of escape of particles out of the rumen (Allen & Mertens, 1988).

The main concern with respect to fibre digestion is the effect on energy intake and therefore animal performance. The rumen has an upper limit on its physical capacity and as the rate of fibre digestion in the rumen decreases, the amount of slowly digestible organic matter increases and MRT increases, whereas feed intake decreases (Zinn & Ware, 2007). As already discussed, a primary factor influencing the rate of fibre digestion in the rumen is the digestibility of the forage source i.e. the NDF structure of the plant. This will be affected by stage of maturity, post-harvest processing and method of preservation (Zinn & Ware, 2007).

The main limiting factor, however, is not digestibility *per se*, but rather MRT and passage rate, along with rate of particle size reduction particularly in diets where fibre inclusions are high (Zinn & Ware, 2007). A good example of this is evident in the tabular values of the NRC (1996). Early bloom lucerne and fresh Bermuda grass (Couch grass) have total digestible nutrient values of 60% and 64%, respectively. Digestion of NDF from lucerne hay is 45%, whereas NDF digestion of Bermuda grass is higher at 65%. From these values it appears that Bermuda grass seems to be more digestible and would therefore appear to be the better choice. However, although the fibre components of lucerne are less digestible, the physical characteristics make it more brittle and therefore less chewing is required and there is faster digestion due to an increased surface area for attack by rumen microbes. This, compared to Bermuda grass which takes almost twice as long to pass through the rumen as lucerne, shows that while the energy value of Bermuda grass may be higher than lucerne, its feeding value may not (Zinn & Ware, 2007).

2.6 NDF AND THE EFFECTIVENESS OF FIBRE

Total dietary fibre can be determined using the collective term NDF which measures total fibre and determines differences between grasses and legumes, warm and cool season grasses and forages and concentrates (Mertens, 1997; Mertens, 2002a). The NDF fraction of forage consists of cellulose, hemicellulose and lignin. This fibre fraction determines the rate of digestion of a feed resulting in a negative relationship between the rate of digestion and the NDF content of feeds, illustrating that NDF represents the indigestible or slowly digestible fraction of forage (Beauchemin, 1991; Mertens, 2002b;

MacDonald *et al.*, 2011). As a result, NDF has been related to feed intake, feed density, chewing activity, digestibility and rate of digestion, as well as depression of digestibility with high levels of intake (Mertens, 1997).

In dairy rations, when most of the fibre comes from long or coarsely chopped forage, NDF can be used directly to formulate rations with minimum forage, as would be the requirements with dairy cows in peak lactation. However, when finely chopped forage sources are used, NDF is less effective for formulating rations (Mertens, 1997; Mertens, 2002a; Mertens, 2002c). This is because forages are high in fibre and this implies a physical texture, whereby the coarse fibre stimulates chewing activity and influences the biphasic nature of the rumen. If the fibre is finely chopped or ground, the 'forage value' is eliminated and chewing time is reduced, hence minimising rumination time. Analysis of rations has led to the general conclusion that it is the effectiveness of fibre that needs further research as this factor is the primary cause of problems in diets with low fibre inclusions (Mertens, 1997; Mertens. 2002c).

More recent research suggests that NDF can be separated in an attempt to better explain and understand the concept of the effectiveness of fibre in maintaining milk fat production and animal health, as indicated below.

Indigestible NDF (iNDF)

Indigestible NDF is the fraction of NDF that is completely indigestible due to it being unavailable to the rumen microorganisms. This portion of NDF can only be cleared from the digestive tract by passage. The commonly used method to estimate iNDF is long term *in vitro* digestion of forage in rumen fluid over 240 hours. The residue of NDF remaining after 240 hours of incubation is the undigested NDF (uNDF240) and is often used as an estimate of iNDF (Comb, 2016). The digestibility of the remaining fibre is known as the pdNDF and this portion of NDF can be cleared from the digestive tract by both passage and by microbial digestion. The pdNDF determines the availability of NDF (Jančík *et al.*, 2008; Raffrenato & Erasmus, 2013), thus forage digestibility is constrained by both iNDF and the rate of digestion of pdNDF (Van Soest, 1994). Estimation of iNDF is important for accurate and precise predictions of energy values and microbial protein synthesis from digested NDF in the rumen. Indigestible NDF has also been characterised as the most important factor affecting the digestibility of the total diet OM (Nousiainen et al., 2004). Indigestible NDF has a predictable digestibility, can be used for

the estimation of the pdNDF fraction and plays an important role in contributing to the rumen load. Determination of iNDF should be included in all basic feedstuff analysis due to its predictable digestibility, therefore it can be used to estimate pdNDF as: NDF – iNDF (Ellis *et al.*, 1999; Comb, 2016).

Effective NDF (eNDF)

Effective NDF is associated with milk fat percentage and rumen pH and refers to the ability of a feed to replace forage so that the percentage of fat in milk is effectively maintained (Mertens, 1997; Mertens, 2002a). The eNDF reflects the physical as well as the chemical properties of fibre including IPS, density and fragility, or the ease of particle size reduction through chewing and digestion (Zinn & Ware, 2007).

Physically Effective NDF (peNDF)

Physically effective NDF is a concept that was introduced by Mertens in 1997 and relates the physical characteristics of feeds to the rumen pH, through the measurement of particle length or chewing activity (Yang *et al.*, 2001a; Beauchemin & Yang, 2005). Mertens (1997) defined peNDF as the fraction of NDF that stimulates chewing and contributes to a ruminal digesta mat. The animal response associated with peNDF is chewing activity (the sum of eating and ruminating time), and the peNDF of a feed is the product of its NDF concentration and its physical effectiveness factor (pef). The pef varies from 0, when NDF is not effective in stimulating chewing activity, to 1, when NDF promotes maximum chewing activity (Mertens, 1997; Mertens, 2002c). The pef is related to fibre concentration, particle size and reduction in particle size (fragility). Therefore, peNDF is related to the stratification of ruminal contents, which is a crucial factor in the selective retention of large particles in the rumen, the stimulation of rumination and ruminal motility, and the dynamics of ruminal fermentation and passage (Mertens, 2002a).

It has been estimated that the minimum peNDF intake should be 20% of the ration DM (Yang *et al.*, 2001a). Several physical feed factors influence pef but particle size measurement is the only factor mutual to all effective fibre systems (Yansari *et al.*, 2004). Because of the above relationship, Mertens (1997) proposed that the pef of individual feeds could be measured based on chewing activity, with the primary physical influential factor being particle size (Grant & Cotanch, 2005). However, chewing time

requires animal experimentation, so an alternative approach was needed. Mertens (1997) then proposed the standardised laboratory method for measuring peNDF, where the particles that are retained on a 1.18 mm sieve is effectively the pef value of a feed, which also relates to the CPS theory referred to earlier (Poppi *et al.,* 1980; Mertens, 1997; Yang *et al.,* 2001a). The author also proposed a standard laboratory method for measuring peNDF which involves the combination of chemical and physical laboratory methods to estimate peNDF (Mertens, 1997). The NDF content of the feed would be determined chemically, after which the NDF content would be determined directly with the use of dry sieving (Mertens, 1997). The proportion of DM retained on a 1.18 mm sieve would be measured using vertical shaking, and peNDF calculated by subtracting the amount of NDF from the total sample NDF (Mertens, 1997). A similar, yet alternative approach was also suggested by Mertens (1997), whereby the proportion of particles (not the proportion of NDF) retained on a 1.18 mm sieve is multiplied by the NDF content of the sample to obtain peNDF. This method is based on three assumptions, namely that: 1) the NDF is distributed evenly across all particle size fractions; 2) chewing activity elicited is similar for all particles retained on a 1.18 mm sieve; and 3) the fragility, or ease of particle size reduction during chewing is similar among NDF sources.

The first assumption may be valid for some forage types, however, the second assumption could be further researched by the possible inclusion of additional sieves, for example a 1.18 mm sieve as well as a 3.35 mm sieve. This may help to better characterize the particle size distribution and relate chewing response to each size fraction better. The third assumption needs to be further studied. One way of going about this is the possible use of grinding energy as a measure of fragility differences in forages with the same particle size. The combination of size fractionation plus a simple measure of fragility would potentially offer an improvement to the peNDF system (Mertens, 1997; Grant & Cotanch, 2005).

Grinding energy, otherwise known as comminution energy, is the energy required to grind a sample through a mill. Comminution has been defined as the breaking, grinding or chopping of larger particles into smaller ones, much like the process of chewing (Grant, 2010; Yancey *et al.*, 2013). A main reason why grinding energy is a possible measure of forage fragility is due to the chemical composition of forage having a major influence on the physical and milling properties of the plant cell wall (Van Soest, 1982), which is the case with chewing of forage by the cow.

Although the peNDF system has been established using a 1.18 mm sieve, what is interesting to note is that some researchers have found that a larger CPS may be more appropriate for cattle (Grant & Cotanch, 2005). Oshita *et al.* (2004) presented data suggesting that the CPS for non-lactating dairy cows was closer to the fraction retained on a 3.35 mm sieve, which seems plausible as it was mentioned earlier that Welch (1986) found particles as large as 5 cm leaving the rumen. In Table 2.2, peNDF values of various feed ingredients are presented, which were estimated using the method suggested by Mertens in 1997 (Mertens, 2002a).

Table 2.2 Estimating the physically effective NDF of feeds using chemical (NDF) and physical (DM retention) measurements in the laboratory (Adapted from Mertens, 2002a)

Feed	pef[a, b]	DM retained on >1.18 mm sieve	NDF[c]	peNDF[d, e]
Standard	1.00	1.00	100	100
Grass hay, long	1.00	0.98	65	63.7
Legume hay, long	0.95	0.92	50	46.0
Legume silage, coarse chop	0.85	0.82	50	41.0
Legume silage, fine chop	0.70	0.67	50	33.5
Maize silage	0.85	0.81	51	41.5
Brewers grain	0.4	0.18	46	8.3
Maize, ground	0.4	0.48	9	4.3
Soybean meal	0.4	0.23	14	3.2
Soybean hulls	0.4	0.03	67	2.0
Rice mill feed	0.4	0.005	56	0.3

[a] Physical effectiveness factor

[b] pef based on chewing activity

[c] Neutral detergent fibre

[d] Physically effective NDF

[e] DM retained on >1.18 mm sieve x NDF = peNDF

The peNDF standardised system was developed with the objective of predicting chewing response accurately, based on the measurement of forage or feed particle sizes and the NDF content (Grant, 2010). It therefore closely resembles older indices such as the forage value index (RVI) proposed by Sudweeks *et al.* (1981), the fibrosity index proposed by Sauvant *et al.* (1990) and the physical structure as proposed by Norgaard (1986). Although similar, the peNDF system differs from these indices in that it is based on the NDF content as a reference value (long grass hay containing 100% NDF) and relative effectiveness of NDF in promoting chewing (pef: fixed scale of 0 to 1), and not simply a biological response, measured as minutes of chewing/kg DM which varies with the conditions under which it was measured (Mertens, 2002a).

The overall concept of peNDF is based on the hypothesis that the fibre in a long feed particle (larger than 1cm) promotes chewing and salivary secretion, which assists in neutralising acid production during ruminal digestion of feeds. The fibre that promotes chewing is considered to be effective because it remains in the rumen longer (Yansari *et al.*, 2004; Beauchemin & Yang, 2005). Salivary buffer secretion is an important factor in maintaining ruminal pH and therefore peNDF is a critical factor in animal health and milk fat depression through its relationship with buffer secretion and rumen pH (Mertens, 2002c).

Because peNDF relates only to the physical aspects of forage, it is a more restricted term than eNDF. The peNDF will always be less than the NDF, whereas eNDF can be less than or greater than the NDF concentration of a feed (Mertens, 2002c). The reasoning behind this is as follows: the animal response associated with eNDF is a depression in milk fat percentage. The effectiveness of NDF in maintaining milk fat production can vary from less than 0, when a feed depresses milk fat percentage, to greater than 1 when a feed maintains milk fat percentage more effectively than it maintains chewing activity. The base for measuring effectiveness of fibre is the NDF concentration in the feed, however, because values can be greater than 1, this suggests that there are other factors in feeds which stimulate milk fat production and influence the eNDF value. It has also been suggested that milk fat depression may not be the best indicator of rumen fermentation, and therefore eNDF may be a less sensitive indicator than peNDF of the effectiveness of fibre in preventing intake depressions and acidosis (Mertens, 1997; Mertens, 2002a). The eNDF includes the effects of peNDF, therefore it is expected that the eNDF value should be larger than the peNDF value for most feeds (Mertens, 1997).

Mertens (1997) summarised a large data set, showing that a positive correlation exists between peNDF measured by dry sieving (using a vertical shaker), ruminal pH and milk fat percentage in dairy cattle. From the data set it can be seen that the minimum requirement for peNDF in lactating dairy cows is approximately 21 – 23% of ration DM to maintain ruminal pH above 6.0 and milk fat above 3.4% for Holstein cows. An illustration of the relationship between ruminal pH and peNDF is shown in Figure 2.3 below.

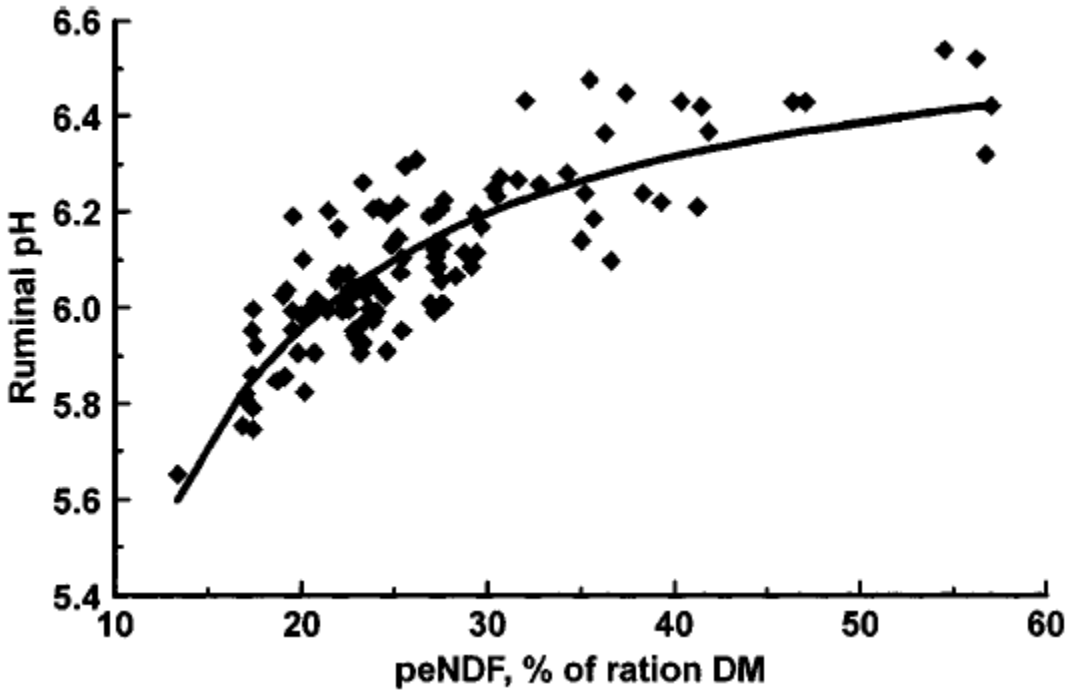

Figure 2.3 Relationship of observed ruminal pH adjusted for citation effects (♦) to physically effective neutral detergent fibre (peNDF) (Adapted from Mertens, 1997)

Overall, the peNDF measurement provides a more consistent measure of effective fibre than chewing activity because it is independent of animal differences and is based on two fundamental properties of feeds: fibre and particle size. Variations due to animal and experimental differences are minimized because pef are fractions in which the animal effects in the numerator and denominator cancel (pef = [minutes of chewing per kg of NDF in the test feed] / [minutes of chewing per kg of NDF in long

grass hay]). Thus, pef is a proportional change in expected chewing response that should be relatively consistent among ruminants (Mertens, 2002a).

Since 1997, many nutritional models used in the dairy industry require peNDF as a key input value for the model to predict lactational response (Grant & Cotanch, 2005). The peNDF system has become widely used in both the CNCPS and CPM-Dairy ration formulation models to predict the effect of forage particle size on cow chewing response and ruminal pH (Grant, 2010). The potential variation in chewing responses elicited from different forages or diets with similar peNDF values has great implications for nutritional models that incorporate peNDF values, as these models make the assumption that every unit of peNDF is equal, regardless of the source (Grant & Cotanch, 2005). Oba & Allen (2000) stated that although there is a positive relationship between forage NDF in the diet and ruminal pH, forage NDF varies in physical effectiveness by particle size.

An important note to make here is the incorporation of rate of digestion, or k_d, into the models. The k_d is in constant competition with passage rate in order to maintain rumen balance (including pH). Unfortunately the models currently only use book values for k_d with the result that the models underestimate rates of digestion for high quality fibre feedstuffs. The potential consequence is the inclusion of more concentrate which could lead to rumen imbalances. Fibre and lignin levels increase with maturity, while rate of digestion decreases, and these differences are not reflected in the current CNCPS model.

2.7 THE RELATIONSHIP BETWEEN CHEWING ACTIVITY AND NDF

Chewing activity has been defined as a response reflecting the chemical and physical properties of feeds such as NDF, particle size, intrinsic fragility and moisture (Mertens, 1997). Chewing activity is influenced by breed, size, age, dry matter intake (DMI), fibre concentration, feed particle size, and to some extent also by the method of measurement of the chewing activity (Mertens, 2002c). Some of the variation in chewing activity due to animal size or feed intake level can be minimised by the unit-less pef ratios. Consequently, peNDF values should be constant for a feed and are generally additive in feed

formulation systems. What is unclear is how chewing activity can be used to assign values to feed in a unified, quantitative system (Mertens, 1997).

An illustration of the peNDF system and the use of particle size to accurately determine chewing response was presented by Grant (2010). Two grass hays with similar pef but different NDF digestibilities (NDFd) and fragility values were analysed. The more digestible and fragile grass hay elicited 28 minutes/day less chewing time than the less digestible and less fragile grass hay. The peNDF system would have predicted similar chewing responses for the forages/kg NDF based on their similar pef values. An important question to ask here is whether the 30 minutes/day not predicted by the particle size will result in a biological difference in cow performance. By considering values for saliva production and associated buffer delivery to the rumen during chewing, it can be seen that based solely on particle size, the peNDF system does an adequate job of predicting chewing response and associated saliva and buffer flow to the rumen of the cows fed the two grass hays in question (Grant *et al.*, 1990; Grant, 2010). However, there is potential for improvement in prediction of chewing responses, particularly for forages that differ more in NDF and digestibility (Grant, 2010). Other comparisons between grass hays with similar NDFd but considerably different fragility values shows that NDFd may be more important than fragility *per se* when measuring chewing response of similarly sized forage particles (Grant, 2010).

Mertens (1986) proposed a forage value unit (RVU) system, based on the NDF content of feeds, to measure the effectiveness of different feeds to stimulate chewing activity, because the term forage implies both a feed texture and a fibre value. Although the system was related to chewing activity and effective fibre, the system differed from these concepts as the RVU were constant feed characteristics (Mertens, 2002a). The RVU system was based on a clearly defined standard, using a hypothetical long grass hay containing 100% NDF as the standard, and the RVU values would be directly proportional to the NDF concentration of the feed, multiplied by a forage value adjustment factor (0 – 1) which was based on effectiveness, chewing activity and particle size (Mertens, 2002a). Mertens (2002a) standardized the effectiveness values of various researchers in 1992, so that all values would be based on a common scale, using long grass hay as a reference to develop forage value adjustment factors that can be multiplied with the NDF content to obtain RVU values for feeds. The system was conceptually based on chewing activity, but the adjustment factors were based on estimates from effectiveness in maintaining milk fat percentage

(Mertens, 2002a). In Table 2.3, the results of a study done by Mertens (2000) are given, where the effects of differing fibre proportions in the diet on various physiological responses is illustrated.

Table 2.3 Physiological effects on dairy cows with varying forage and fibre proportions in the rations (Adapted from Mertens, 2000)

	% Long grass hay in the diet					
Variable	**100**	**80**	**60**	**40**	**20**	**0**
% NDF[a]	70	59	48	36	25	14
% peNDF[b]	70	57	44	32	18	6
Chewing time (min/d)	1080	1040	970	820	520	320
Salivary secretion (L/d)	200	196	189	174	143	123
Salivary bicarbonate (kg/d)	2.5	2.4	2.3	2.2	1.8	1.5
Rumen pH	6.8	6.7	6.5	6.2	5.8	5.0
Ruminal VFA (mM)	85	95	105	115	125	135
Ruminal acetate (molar %)	70	66	61	55	48	40
Ruminal propionate (molar %)	15	18	22	27	33	40
A:P ratio	4.7	3.7	2.8	2.0	1.4	1.0
Milk fat %	3.7	3.6	3.5	3.4	3.0	1.0

[a] Neutral detergent fibre

[b] Physically effective NDF

A study reported by Zebeli *et al.* (2007) showed that increasing the forage particle size in diets containing high amounts of concentrate, led to an increase in the proportion of DM retained on a 1.18 mm sieve from 37.5% to 42.0%, and also led to an increase in rumination time by 100 minutes/day. The study also showed an increase in the consistency of the ruminal mat. However, Zebeli *et al.* (2007) added that particle breakdown in the rumen, short term rumen pH, fibrolytic capacity of the digesta as well as the proportion of mat in the rumen decreased. Mertens (1997) reported that grass and *M. sativa* hays resulted in a range of 111-152 minutes of chewing/kg NDF, while oat straw required 200 minutes of chewing/kg NDF. With an increase in NDF concentration of the diet, there is an increase in chewing time/kg NDF as can be seen in a study by Mertens (2000). Therefore, chewing activity will be stimulated more when

mature, high fibre forage is fed in comparison to a young, low fibre forage (Mertens, 2002a). For every 10% increase in NDF above 40% for *M. sativa* and 55% for grasses, there can be a decrease of 0.5% in the peNDF requirement (Mertens, 2002a). According to Weimer *et al.* (2009), the ability of ruminants to reduce the particle size of ingested feed decreases when forages become highly lignified, and the fermentation rate begins to decline as well.

Mertens (2002a) reported that results from studies investigating animal response to varied dietary peNDF concentrations, as measured by the various particle separator techniques, have been inconclusive, since there appears to be differences in the ability of the various on-farm techniques to measure pef values that resemble the pef values measured with the use of the dry sieving technique. Mertens (2002a) also stated that the limitation that not all particles larger than 1.18 mm will result in the same amount of chewing, can be overcome by weighing off the NDF that is retained on each of the sieves by the amount of chewing it should stimulate. However, more research is needed to relate chewing activity to particle size before the weighing factors for the particles on each sieve can be determined.

According to Yang *et al.* (2001b) forage particle size and NDF content of the diets were more reliable indicators of chewing activity than the NDF concentration of the forage. Yang *et al.* (2001b) stated that eating or ruminating time increased with increasing NDF concentration in the diet, and added that increasing the forage content of the diet is more effective in stimulating chewing activity than altering the forage particle lengths in the diet, since the forage particle length only affects the eating activity and not the ruminating activity.

2.8 THE RELATIONSHIP BETWEEN FORAGE FRAGILITY, PARTICLE SIZE REDUCTION AND DIGESTIBILITY

The differences in chewing response not explained by particle size might be attributed to differences in forage fragility or related characteristics such as stem brittleness (Grant, 2010). Grant (2010) defined forage fragility as the relative rate at which forage is reduced in particle size during

chewing or some laboratory simulation of chewing action, such as ball milling, where the balls are said to mimic the grinding action of the molar teeth.

The rate of particle size reduction is largely dependent on rumination rate, chewing efficiency and cell wall fragility (Mertens, 1988). The fragility of particles is probably related to the intrinsic characteristics of the cell wall such as lignin content and anatomical differences, as well as to weakening of the cell wall during microbial fermentation (Allen & Mertens, 1988; Van Soest, 1994). As a result, cell wall digestibility may be predictive of forage fragility (Grant, 2010). In Table 2.4, the digestibility and fragility values for various grass hays used during a simulated chewing study conducted by Grant (2010) are illustrated. As can be seen, Hay C has the highest fragility percentage, along with the lowest iNDF percentage, showing a likely relationship between lignin and fragility of particles.

Table 2.4 Digestibility and fragility of grass hays used in a simulated chewing study (Adapted from Grant, 2010)

Item	**Hay A**	**Hay B**	**Hay C**	**Hay D**
24-hr ivNDFd, % NDF[a]	31.4	43.7	54.8	47.3
120-hr ivNDFd, %NDF	49.3	60.2	74.1	65.4
iNDF%	50.7	39.8	25.9	34.6
Fragility, %	46.2	30.0	80.7	63.9

[a] *in vitro* neutral detergent fibre digestibility

Based on the suggestion that cell wall digestibility may be predictive of forage fragility, Grant (2010) stated that there is the potential to combine a "fragility factor", related to NDFd, with the derived pef values gained through sieving, to arrive at a superior value to predict cow chewing response. Grant (2010) concluded that NDFd and fragility are related, and this relationship can be used to improve predictions of chewing response to peNDF when forage NDF sources differ in chewing response. Yansari *et al.* (2004) stated that essentially, fragility or rate of particle size reduction will determine digestibility of a feed in the rumen. If the particle size reduction is rapid, then particles will escape the rumen at a faster rate, therefore decreasing digestibility of NDF.

In Figure 2.4 is illustrated the results of the change in forage fragility, or change in pef value, versus the 24 -h *iv*NDFd for a range of forages as reported by Grant (2010). The results from the study indicate a trend for forage fragility to increase linearly as the *iv*NDFd (%) increases (Grant, 2010). The R^2 value shows that the 24-h *iv*NDFd explains 60% of the variation seen in forage fragility (Grant, 2010).

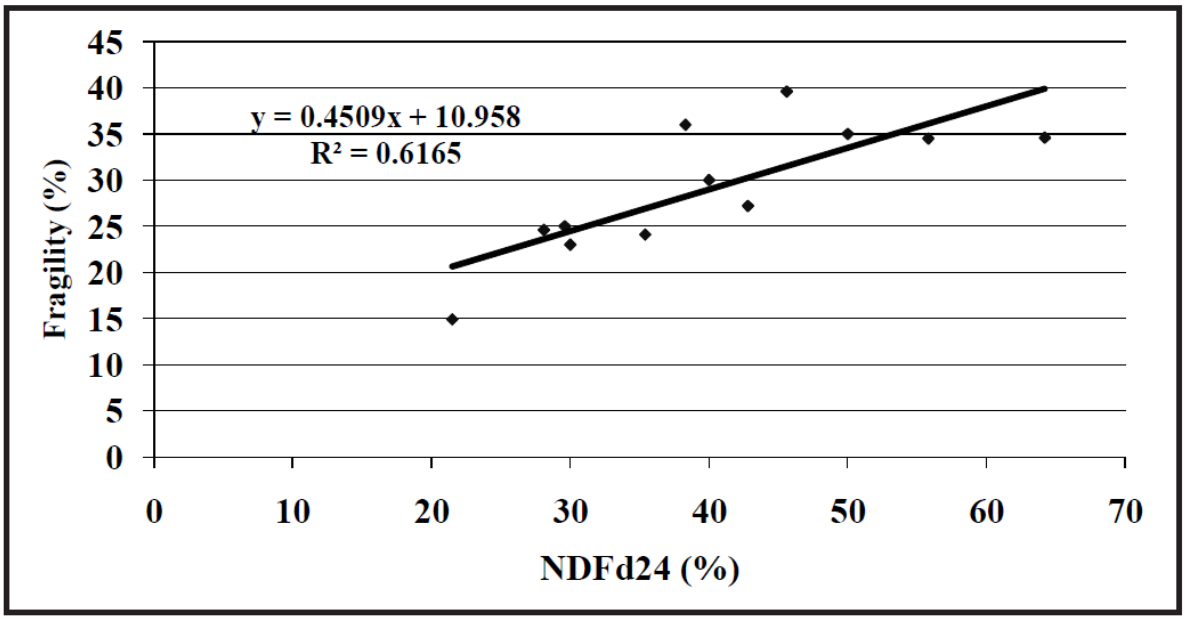

Figure 2.4 Relationship of the 24-hour *in vitro* NDF digestibility of various forages with the fragility of the forages as measured by change in physical effectiveness factor following ball milling (Adapted from Grant, 2010)

The differences in forage fragility will affect the rate of particle size breakdown and retention time in the reticulo-rumen as discussed under section 2.5 (Poppi *et al.*, 1980). Romney & Gill (2000) stated that the resistance to comminution (reduction in particle size) is positively related to fibre content, but that relationships measured with the use of NDF and DMI are not always consistent. The peNDF system is based on the assumption that forage particle size explains all the variation elicited in chewing response. This assumption however, is not always correct since forages of similar particle length may elicit substantially different chewing times/kg NDF (Grant, 2010). This difference in chewing response is a recognised limitation in the peNDF system. The consequence of this relationship is that foods which are

equal in digestibility but different in NDF content will promote different intakes (MacDonald *et al.*, 2011).

There is little data available explaining the relationship between forage maturity and/ or chemical components, and the rate of particle size reduction, although it is clear that there are differences in the fragility among forages, and these effects should be accounted for in models that aim to predict the flow of digesta from the reticulo-rumen accurately. There is a need for greater understanding of the factors relating to particle fragility (Allen & Oba, 1996).

2.9 MEASUREMENT OF FORAGE FRAGILITY

The general term "size reduction" includes cutting, crushing, grinding and milling. Mechanical means are used for size reduction, without a change in the chemical properties of the material, and uniformity in size and shape of the particles of the end product is desired, but seldom obtained (Henderson & Perry, 1976). There have been several suggestions reported for the measurement of forage particle fragility, including Troelsen & Bigsby (1964) who suggested a mechanism to specifically examine forage particle size distribution after artificial mastication. However, more recently, two methods are generally used. These include comminution energy, which is the energy required to grind a sample through a mill, and shear force, which measures the force needed for a blade to pass through the forage stem using a Warner-Bratzler or similar machine, commonly used in meat science laboratories (Grant, 2010). The most common mechanical preprocessing technologies focus on size reduction and include hammer and knife milling or grinding, chipping, shredding and ball roller milling (Yancey *et al.*, 2013).

Grinding energy is described by Yancey *et al.* (2013) as the actual energy or work going into the grinding process per unit quantity of processed material, including drive chain inefficiencies, electrical power factor losses and friction. Yancey *et al.* (2013) also defined comminution as the breaking, grinding or chopping of larger particles into smaller ones. The chemical composition of forage has a major influence on the physical and milling properties of the plant cell wall (Van Soest, 1982). Laredo & Minson (1973) reported that the energy needed for grinding 1g of tropical grass stems using a laboratory

mill was overall substantially more than the energy needed for grinding tropical grass leaves, and stated that there is a significant correlation between voluntary intake and grinding energy.

Cotanch *et al.* (2007) stated that a ball milling method has been recently developed to assess the fragility of a wide range of forages. Forage samples are dried and then placed in a ball mill loaded with ceramic balls, as it has been suggested that this process mimics the grinding action of the molar teeth of an animal. The samples are then sieved using the standard method for measuring of pef prior to ball milling (**pef_i**) and again after ball milling (**pef_{BM}**). Fragility can then be determined as the change in pef value (the proportion of particles retained on a ≥1.18 mm sieve as determined by dry vertical sieving) of the ball-milled forage from the original sample, as follows:

$$(pef_i - pef_{BM}) / pef_i \times 100$$

A fragility value of 100% (highly fragile forage) would equate to complete reduction of particle size to less than 1.18 mm. A fragility value of 0, very tough forage, would reflect no reduction in particle size upon ball milling, $pef_i = pef_{BM}$ (Cotanch *et al.*, 2007).

Chenost (1966) conducted a study to assess the degree of fibrousness of hays by measuring the electrical energy required to pulverize the hay. Chenost (1966) reported that the fibrousness index exhibited very close relationships with the digestibility and acceptability of the hays, and stated that measurement of the fibrousness index can be very useful in determining the feeding value.

Bitra *et al.* (2009) and Mani *et al* .(2004) stated that the energy demand for grinding depends on the initial particle size, moisture content, material properties, mass feed rate and machine variables, and added that the performance of a grinding device is often measured in terms of energy requirement, geometric mean diameter, and the resulting particle size distribution. The performance of a machine for reducing the size of material is characterised by the capacity, the power required per unit of material reduced, the size and shape of the product before and after reduction, and the range in size and shape of the resultant product (Henderson & Perry, 1976). Yu *et al.* (2003) stated that the capacity of a specific grinder is dependent on the power rating of the grinder, the speed, grain, fineness and moisture content of the resulting particles. Most studies previously done on the measurement of comminution energy reported

total specific energy (Bitra *et al.*, 2009). Lopo (2002), as cited by Tabil *et al.* (2011) reported that the ratio of particle size distribution of the material before and after grinding, moisture content, bulk and particle densities, feed rate of the material and machine variables determine the energy requirement of grinding the biomass.

In a study done by Yancey *et al.* (2009), grinding energy and particle size were compared at varying moisture levels (10 – 25% in 5% increments) for maize stover, switchgrass and wheat straw. The grinding energy for maize stover and switchgrass showed a steep increase in energy as moisture content increased. The moisture content in the straw had less of an effect on the grinding energy needed to break down this forage. Mani *et al.* (2004) also reported the negative effect of moisture on grinding energy for straw, maize stover and switchgrass. Operating speed, moisture content and initial particle size appear to be crucial in minimizing effective specific energy requirements for forage size reduction (Yancey *et al.*, 2009).

Fang *et al.* (2000) examined the energy requirements for the milling of wheat using a roller mill, and reported that kernel hardness had the most significant effect on energy and power requirements. It was also reported that kernel weight and size had significant negative effects on specific energy where heavier and larger kernels were more efficient than smaller kernels in energy utilization. The authors added that moisture content had a negative effect on energy and power requirements (Fang *et al.*, 2000), which supports the similar statement made by Mani *et al.* (2004) and Yancey *et al.* (2009).

Arthur *et al.* (1982) defined specific energy requirement as the fuel energy input to the engine per unit mass of ground material (based on the wet mass of the material). The authors reported that the specific energy requirements were the greatest with the smaller screens, and the results indicated that for a specific given screen hole size, rice straw required nearly twice as much energy per unit mass as did wheat straw, and maize stover required slightly more energy than wheat straw. Numerous authors have stated that the total specific energy for a knife mill and tub grinder has been observed to have a negative correlation with screen size and mass feed rate (Arthur *et al.*, 1982; Cadoche & Lopez, 1989; Bitra *et al.*, 2009). However, Arthur *et al.* (1982) stated that grinding rate increased with an increase in screen size. The author also mentioned that in general, the specific energy requirement tended to decrease with an increase in grinding rate.

It appears, from the literature, that a hammer mill is more commonly used to grind forages. Little research exists for the measurement of grinding energy of forages using a knife mill. According to Bitra *et al.* (2009) a chopper, knife cutter or knife mill is often used for coarse size reduction (>50mm) of stalk, straw and grass feed. Knife mills reportedly worked successfully for shredding forages under various crop and machine conditions (Cadoche & López, 1989). Bitra *et al.* (2009) reported that the total specific energy (including energy to operate the knife mill) for agricultural biomass chopping increases with knife mill speed.

Hammer mills, however, have been given merit because of their ability to finely grind a wider variety of materials than other machines (Scholten & McEllhiney, 1985). Hammer mills are also relatively cheap, easy to operate and produce a range of particle sizes needed for the densification of ground material (Scholten & McEllhiney, 1985). Particle sizes are reduced by hammer mills through shear and impact actions (Ghorbani *et al.*, 2010). Particle size distribution can be used to evaluate performance of a hammer mill (Yang *et al.*, 1996).

Sieves have a long history and acceptance in various industries and provide a standardized format for the measurement of particle sizes (Bitra *et al.*, 2009). Womac *et al.* (2007) concluded that screen size has a significant effect on particle size distribution. Bitra *et al.* (2009) stated that in actual practice, the measured geometric mean diameter of biomass particle sizes using sieve analysis is less than the actual size of the particles. Womac *et al.* (2007) reported that the geometric mean dimensions of actual biomass particles varied from 5 times for particle length to 0.3 times for particle width for knife-milled switchgrass, wheat straw, and maize stover when the authors compared the results obtained to the sieve results for the geometric mean length computed by the American Society of Agricultural and Biological Engineers (ASABE).

2.10 HYPOTHESES

The hypotheses tested in this study were:

H_0: Forage fragility is not associated with short or long term NDF digestibility.

H_1: Forage fragility is associated with short or long term NDF digestibility.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 INTRODUCTION

This study was conducted at the University of Pretoria and consisted of two phases. The first phase involved the collection of forage samples from different locations where the forages were exposed to different climates, soil types and treatments such as fertilisation. The forage samples were collected from regions in and around Gauteng, Kwazulu-Natal and the Northern Cape. After collection, all samples were analysed for various chemical components. Chemical analyses were done at Nutrilab, Department of Animal and Wildlife Sciences, University of Pretoria. *In vitro* NDF digestibility was determined using rumen fluid collected from two rumen-cannulated cows at the University of Pretoria Experimental Farm. The cows used for the experiment were fed a diet with composition as per Appendix A. The second phase involved the measurement of forage fragility, where a knife mill (Retsch GmbH, Model SM 100, Haan, Germany) and an ultra-centrifugal mill (Retsch, Model ZM 200, Retsch GmbH, Haan, Germany) were used. During the third phase, the possible association between chemical analyses and grinding energy was investigated by means of simple and stepwise regressions. The trial was approved by the Animal Ethics Committee of the University of Pretoria (EC087-13).

3.2 PHASE 1: COLLECTION OF FORAGE SAMPLES AND CHEMICAL ANALYSES

3.2.1 Collection of forage samples

A total of 35 different forage samples were collected from 25 different locations, as shown in Table 3.1. The maize silage samples were collected according to the University of Nebraska Lincoln's hand probe method. After collection, the maize silage samples were dried at 55°C for 48 hours. All other samples were sun-/air-dried, after which sample preparation started.

Table 3.1 Summary of forage samples collected for the study from various regions in South Africa

Common name	Scientific Name	Number of samples	Carbon Fixation Pathway
Lucerne	*Medicago sativa*	13	C_3 (Legume)
Maize silage	*Zea Mays*	9	C_4
Weeping love grass	*Eragrostis curvula*	13	C_4
	TOTAL SAMPLES	35	

3.2.2 Chemical analysis

A representative forage sample was milled with a Retsch ultra-centrifugal mill, fitted with a 1 mm screen and analysed in duplicate for dry matter (DM), ash, nitrogen (N) which is presented as crude protein (CP), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), 6-, 12-, 18-, 24-, 36-, 48-, 72-, 96-, 120-, 240-h *iv*NDFd and kd, using standard laboratory procedures. The 240-h *iv*NDFd was used to estimate indigestible NDF (iNDF). The rates of NDF digestion were calculated using the undigested NDF residues and were calculated as %NDF per hour.

3.2.2.1 Dry matter and ash

All forage samples were analysed for DM according to AOAC (2000) procedure 934.01. Ash determination of samples was done in accordance to AOAC (2000) procedure 942.05.

3.2.2.2 Nitrogen

The nitrogen of the samples was determined using the Dumas method of nitrogen combustion as according to AOAC (2000) procedure 968.06, using a Leco Trumac Nitrogen determinator apparatus. These values were presented as crude protein (CP) values using the following calculation:

$$CP = N \times 6.25$$

3.2.2.3 Starch

Samples were analysed in duplicate for starch content as described by the methods of MacRae & Armstrong (1968), Faichney & White (1983), and AOAC (1984). The method entails the gelatinisation of all the starch in the test samples by autoclaving, followed by the enzymatic hydrolysis of starch to glucose, and lastly the determination of the glucose content by the glucose oxidase method. Approximately 0.5 g of each milled test sample was weighed accurately into large glass test tubes (40 mm x 200 mm), after which exactly 25 ml 80% ethanol was added to each test tube. The test tubes were then placed in a water bath at a temperature of between 80°C to 90°C for exactly 30 minutes. Once the test tubes were removed and allowed to cool, they were centrifuged at 2000rpm for 10 minutes. The liquid in each test tube was then suctioned off and the process repeated from the point of adding the 80% ethanol to centrifuging and sucking off the excess liquid. The test tubes were then placed in an oven at 55°C to dry overnight.

The following day, exactly 30 ml distilled water was added to each test tube. The test tubes were then stirred carefully and placed in the autoclave at 120°C for 2 hours. After the autoclave had cooled down, samples were removed and placed in a 55°C water bath, after which toluene, acetate buffer and amyloglucosidase solution were added according to the method. Tubes were incubated in the 55°C water bath for 24 hours, mixing the tubes carefully every 30 minutes for the first 2 hours. The following day, the test tubes were removed, and contents poured into a clean 100 ml volumetric flask using a funnel. Each test tube were rinsed out thoroughly with warm distilled water, adding to the volume in each volumetric flask. After the solution cooled down, the volume was made up to the 100 ml mark. Flasks were then shaken vigorously and contents filtered through Whatman no.2 filter paper, collecting the filtrate in sample bottles.

A 1 ml sample solution was diluted to 50 ml using distilled water, after which 0.5 ml sample solution was added with 0.5 ml of the standard glucose solutions (5%, 20%, 30%, 40% and 50%) into 10 ml test tubes. Precisely 2.5 ml colour reagent was added into each test tube, after which the tubes were placed in a dark room for exactly 30 minutes. After 30 minutes, the reaction was stopped by adding 1 ml 50% sulphuric acid into each test tube. A spectrophotometer was used to determine absorbance at 540 nm. A standard curve was drawn for the glucose values with % glucose on the x-axis and the absorbance

reading on the y-axis and read off the sample values as % glucose. The values obtained were multiplied by the factor 0.912 in order to convert it to % starch in the sample tested. This final value was corrected for differences in the mass of the sample used (over or below 0.5 g), any additional dilutions that were made and the DM content of the sample. The starch content was calculated as follows:

$$\gamma = mx + c \text{ (Standard curve)}$$

% Starch = (Absorbance – c) / x * 0.912 * 0.5 / sample mass

3.2.2.4 Neutral detergent fibre

The NDF of the samples was determined using the method as described by Mertens (2002b) which is the AOAC official method 2002.04. Some changes have been made to this procedure, and due to NDF being a crucial component of this study, a full description of the procedure used to determine NDF is given below.

This method is based on using refluxing in beakers. Approximately 0.5 g of each test sample was weighed into a 500 ml Berzelius unspouted glass beaker, and 0.5 g anhydrous sodium sulphite added, after which 100 ml neutral detergent solution was added. Beakers were placed on a refluxing–apparatus and were covered with condensers to minimize evaporation. As soon as the solution started boiling, 250 µl α-amylase (Thermamyl, Ankom, NY – USA) was added. Samples were left on the refluxing-apparatus to boil for 1 hour, after which beakers were taken off one-by-one. Samples were poured into 50 ml fritted-disk Gooch crucibles, which were placed on a vacuum filter unit. Samples were rinsed repeatedly with boiling distilled water while on the vacuum filter unit to remove all the neutral detergent solution. The vacuum filter was then turned off and Gooch crucibles were filled with boiling distilled water, after which another 250 µl α-amylase was added to Gooch crucibles and left for approximately 1 minute. The vacuum filter was then turned on again and samples rinsed with boiling distilled water, followed by rinsing with acetone twice. Samples were then placed in a 105°C oven to dry overnight. The following day, remaining samples were weighed using the hot weighing procedure (Goering & Van Soest, 1970), and then crucibles with dried remaining sample were placed in the muffle furnace at 550°C for 4 hours. After

4 hours, the muffle furnace was switched off and samples left over night to cool down. The following day the remaining ash of the samples was weighed, again using the hot weighing method.

The hot weighing of the remaining samples during NDF determination was done according to the method as described by Goering & Van Soest (1970), where the balance was warmed up by placing 4 small, empty glass beakers which had been in the 105°C oven on the balance plate after one another. A small beaker was taken directly out of the oven and placed on the balance until the balance stabilized at the lowest weight, and then taken off and replaced by another warm beaker from the oven and process repeated. After the balance was warmed up with 4 different warm beakers, Gooch crucibles were taken one-by-one directly from the oven and placed on the warm balance, and weights recorded (weight at which balance stabilizes). NDF was calculated as follows:

$$\% \text{ NDFom (DM basis)} = 100 (Wf - Wa)/(S*DM)$$

Where: NDFom is ash-free NDF obtained with the use of amylase

DM is (g oven-dried matter weight/g air-dried or wet test portion weight)

S is as-is test portion weight (g)

Wa is crucible weights after ashing (g)

Wf is dried crucible weights after refluxing (g)

3.2.2.5 Acid detergent fibre and acid detergent lignin

The ADF of the samples was determined using the method as described by Raffrenato & Van Amburgh (2011), where approximately 1g of each test sample was weighed into a 500 ml Berzelius unspouted glass beaker, and 100 ml ADF-solution was added. Glass beakers were then placed on a refluxing–apparatus that were covered with round cold-water condensers to minimize evaporation, and left on the refluxing-apparatus to boil for 1 hour, after which beakers were taken off one-by-one and poured into fritted-disk Gooch crucibles, which were placed on a vacuum filter. Samples were rinsed with boiling distilled water repeatedly while on the vacuum filter to remove all the ADF solution, followed by rinsing with acetone twice. Samples were then placed in a 105 °C oven to dry overnight. The following day, remaining samples were weighed using hot weighing, and then crucibles with dried

remaining sample were placed in the muffle furnace for 4 hours at 550°C. After 4 hours, the muffle furnace was switched off and samples left over night to cool down. The following day the remaining ash of the samples were weighed, again using the hot weighing method. ADF was calculated as follows:

$$\% \text{ ADFom (DM basis)} = 100 (Wf - Wa)/(S*DM)$$

Where: ADFom is ash-free ADF

DM is (g oven-dried matter weight/g air-dried or wet test portion weight)

S is as-is test portion weight (g)

Wa is crucible weights after ashing (g)

Wf is dried crucible weights after refluxing (g)

For the determination of ADL, the process was exactly the same as for ADF determination up to and including the point where the dried remaining ADF samples were hot weighed back, before being ashed. After recording the dried ADF weights, the fritted-disk Gooch crucibles were placed in a glass Pyrex tray, and each Gooch crucible filled halfway with 72% aqueous sulphuric acid. Glass rods were used to mix the dried sample with the sulphuric acid. Because the sulphuric acid slowly filtered through the crucibles, more acid had to be added. After 3 hours (mixing and adding acid every hour), crucibles were taken out and placed on the vacuum filter. Samples were washed very thoroughly and repeatedly with boiling distilled water to remove any traces of acid, followed by rinsing with acetone twice. Crucibles were once again placed in the 105°C oven over night. The following day, dried samples were hot weighed after which crucibles were placed in the muffle furnace for 4 hours at 550°C to be ashed. After 4 hours, the muffle furnace was switched off and samples left over night to cool down, and the following day remaining ash of samples were weighed, again using the hot weighing technique.

The hot weighing of the remaining samples during ADF and ADL determination was done according to the method as described by Goering & Van Soest (1970), where the balance was warmed up by placing 4 small, empty glass beakers which had been in the 105°C oven on the balance plate after one another. A small beaker was taken directly out of the oven and placed on the balance until the balance stabilized at the lowest weight, and then taken off and replaced by another warm beaker from the oven and process repeated. After the balance was warmed up with 4 different warm beakers, Gooch crucibles

were taken directly from the oven and placed on the warm balance, and weights recorded (weight at which balance stabilizes).

ADL was then calculated as follows:

$$\% \text{ ADLom (DM basis)} = 100 (Wf - Wa)/(S*DM)$$

Where: ADLom is ash-free ADL

DM is (g oven-dried matter weight/g air-dried or wet test portion weight)

S is as-is test portion weight (g)

Wa is crucible weights after ashing (g)

Wf is dried crucible weights after ADF determination (g)

3.2.2.6 6-120-h ivNDFd

The *in vitro* digestions were done in accordance with the method proposed by Goering & Van Soest (1970). Two rumen cannulated Holstein cows from the University of Pretoria Experimental Farm were used as donors of rumen fluid for the *in vitro* analyses. The cows remained on a standard TMR-based diet with no extra forage fed separately, as the TMR already included chopped *E. curvula* hay and *M. sativa*. Approximately 0.5 g of each test sample was weighed accurately in duplicate and transferred into 100 ml Schott *in vitro* bottles, and 2 blank samples were included for each time point. Blank bottles consisted only of rumen fluid and the medium described in the procedure. The *in vitro* bottles were filled with 10 ml rumen fluid and 40 ml medium, after which bottles were incubated in the *in vitro* water bath at a temperature of 39°C for the specific time period under constant CO_2 positive pressure. After the time period, samples were removed from the water bath and placed in an ice bath to stop any fermentation instantly. Samples were then poured into 500 ml Berzelius unspouted glass beakers, after which 50 ml NDF solution as well as 0.5 g anhydrous sodium sulphite was added. Neutral detergent fibre (NDF) was then determined in accordance with the method described above in section 3.2.2.4.

For the 12-, 18-, 24-, 36-, 48-, 72-, 96- and 120-h *iv*NDFd, the 100 ml Schott *in vitro* bottles with sample needed to be swirled gently once a day. The water bath also needed to be re-filled daily with pre-warmed distilled water and the CO_2 level of the tank checked.

3.2.2.7 240-h ivNDFd

The 240-h *in vitro* was done in accordance to the method proposed by Raffrenato & Van Amburgh (2010). Two rumen cannulated Holstein cows from the University of Pretoria Experimental Farm were used as donors of rumen fluid for the *in vitro* analyses. The cows remained on a standard TMR-based diet with no extra forage fed separately, as the TMR already included chopped *E. curvula* hay and *M. sativa*. Approximately 0.75 g of each test sample was weighed accurately in duplicate and transferred into 100 ml Schott *in vitro* bottles, and 2 blank samples were included. Blank bottles consisted only of rumen fluid and the medium described in the procedure. The *in vitro* bottles were filled with 10 ml rumen fluid and 40 ml medium, after which bottles were incubated in the *in vitro* water bath at a temperature of 39°C for 5 days under constant CO_2 positive pressure. After 5 days, samples needed to be re-inoculated with 10 ml rumen fluid and 40 ml medium. At the end of the 10 days (240 hours), samples were poured into 500 ml Berzelius unspouted glass beakers, after which 100 ml NDF solution as well as 0.5 g anhydrous sodium sulphite was added. Neutral detergent fibre (NDF) was then determined in accordance with the method described above in section 3.2.2.4.

For the 240-h *iv*NDFd, the 100 ml Schott *in vitro* bottles with sample needed to be swirled gently once a day. The water bath also needed to be re-filled daily with 39 °C distilled water and the CO_2 level of the tank checked.

3.2.2.8 Rate of NDF digestion (kd)

The rate of NDF digestion was calculated as %NDF per hour and is illustrated in Figure 3.1.

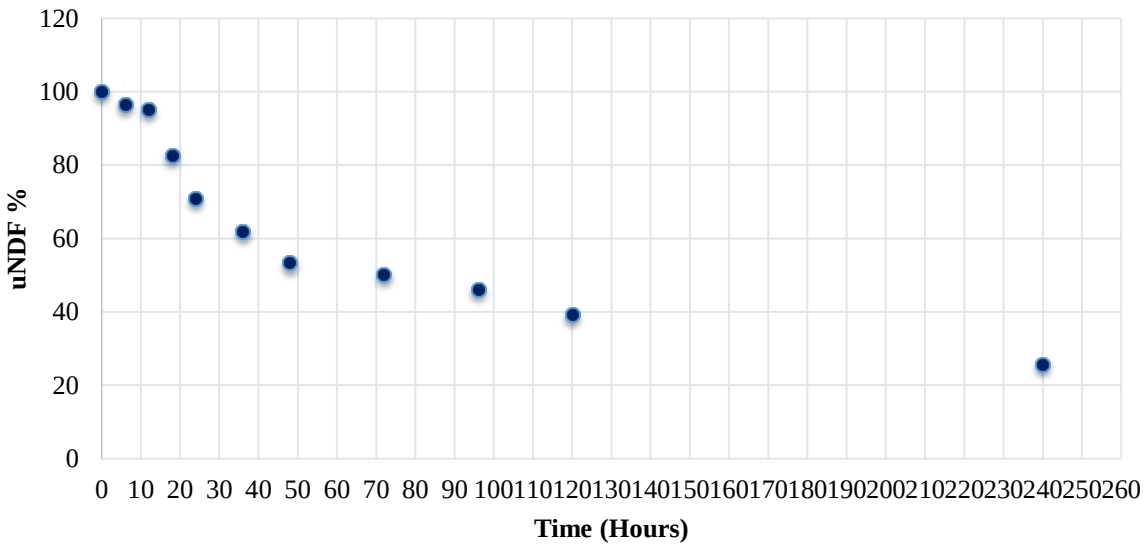

Figure 3.1 Example of illustration of uNDF being reflected over time, in order to estimate kd, for *Eragrostis curvula*

Kd was then calculated as follows:

$$Y = a + be^{(-kd\ (hour - lag))}$$

Where: y is uNDF

A = constant

B = coefficient

Kd = rate of digestion of forage

Lag = the time it takes before digestion begins

3.3 PHASE 2: FORAGE FRAGILITY MEASUREMENT

All dried forage samples were pre-cut using a knife mill (Retsch GmbH, Model SM 100, Haan, Germany), fitted with a 2 cm screen, in an attempt to minimize variability across forages of starting particle size. A knife mill was chosen as it has been reported to chop forages effectively and uniformly

under various crop and machine conditions (Cadoche & López, 1989; Tabil *et al.*, 2011). In Figure 3.2 the knife mill that was used during this study is illustrated.

Figure 3.2 Illustration of Retsch knife mill used during experiment

3.3.1 Particle size distribution

Initial particle size was defined as the particle size after pre-cutting with the 2 cm screen, and FPS was defined as the particle size after samples were ground through a 1 mm screen. Particle size distributions were determined using a sieve shaker (Retsch GmbH, Model AS 200, Haan, Germany). For IPS distributions, the following sieves were included: 25 mm, 10 mm, 7.1 mm, 5 mm, 2.5 mm, 1 mm, 500 µm, 250 µm, and a base pan. A constant volume of 800 ml per sample was placed on the top sieve (800 ml is the approximate volume it took to fill half of the base sieve with sample). Sieves were stacked on the shaker in descending mesh size. Samples were placed on the sieve shaker for 4 minutes, at constant vibration amplitude of 50. Empty sieve weights were taken before sieving, and after-sieving weights of sieves with sample were recorded again.

After energy required for grinding was measured, FPS distribution was determined for the 1 mm ground samples. The sieve shaker (Retsch GmbH, Model AS 200, Haan, Germany) was fitted the following sieves: 7.1 mm, 5 mm, 2.5 mm, 1 mm, 500 µm, 250 µm, 100 µm, 50 µm, and the base pan. A volume between 250 ml and 500 ml, depending on the amount of sample available, was used and samples were placed on the sieve shaker for 4 minutes, at a constant vibration amplitude of 50. An example of the Retsch sieve shaker and a sieve used during this experiment is illustrated in Figure 3.3.

Figure 3.3 Example of Retsch sieve shaker and individual sieve used during the study

The weights for all of the test samples were used to draw graphs indicating the general particle size distribution trend of each sample as illustrated in Figure 3.4. After recording all the weights, the nominal geometric mean particle size for each sample was determined.

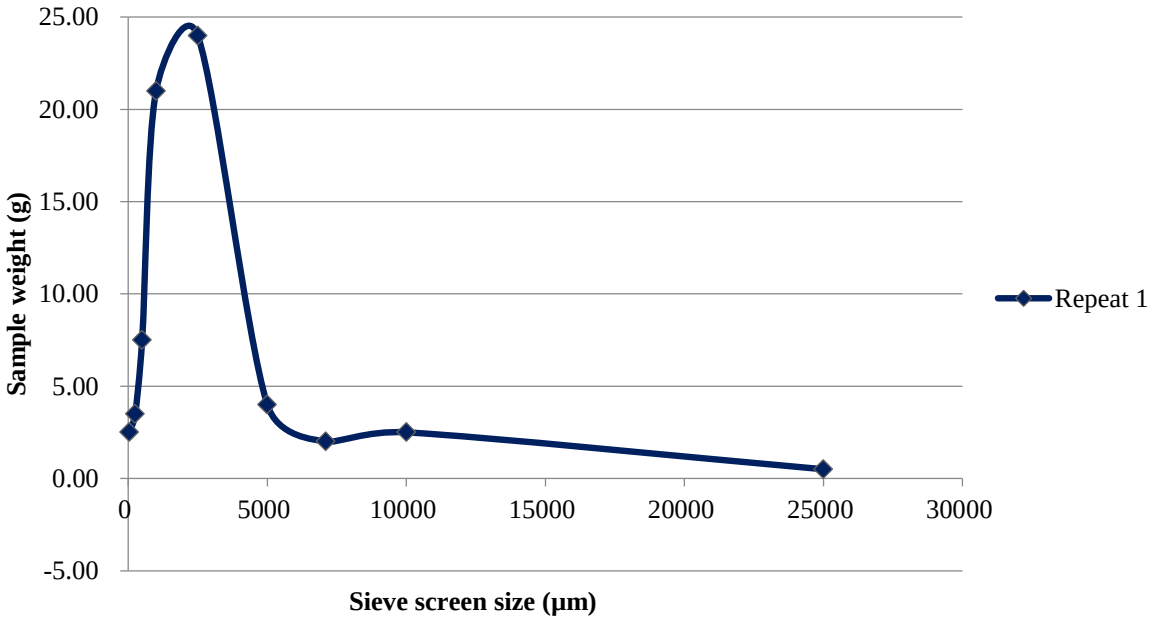

Figure 3.4 Example of illustration of sample particle size distribution curve for *Medicago sativa*

3.3.2 Change in particle size

After the calculation of the geometric mean particle size for each test sample, the percentage change in particle size for each forage sample was calculated as follows:

(Average particle size (µm) after 2-cm milling / Average particle size (µm) after 1-mm milling) /

Average particle size (µm) after 2-cm milling * 100

3.3.3 Direct energy measurement

Direct energy measurements were done using an ultra-centrifugal mill (Retsch GmbH, Model ZM 200, Haan, Germany). For the ultra-centrifugal mill, 10 g duplicate samples were passed through the mill which was fitted with a 1 mm screen. In Figure 3.5 an example of the Retsch ultra-centrifugal mill is shown.

Figure 3.5 An illustration of the Retsch ultra-centrifugal mill used in the study

During the grinding process, energy usage of the ultra-centrifugal mill was measured using a data logger (ACR Systems Inc, Smartreader Plus 3, Surrey, Canada) with corresponding computer software (ACR Systems Inc, Trendreader 2 version 2.39, Surrey, Canada) and energy transducer (powerbullet) (ACR Systems Inc, Powerbullet PB- 133, Surrey, Canada). The mill was switched on and when energy usage stabilized, forage samples were poured into the feeder of the mill as consistently as possible. Energy measurements were reported as J/g DM sample.

Computer software: Trendreader 2 version 2.39

Trendreader 2 is an effective graphing software package developed for ACR smartreaders. The software enables logged data to be collected and analysed accurately within seconds (http://www.acrsystems.com).

Data Logger: Smart Reader Plus 3

The Smart Reader Plus 3 data logger is an 8-channel AC current, voltage and temperature logger. The applications of this data logger includes: energy usage profile (real power – kW), power consumption monitor (energy delivered – kWh), electrical load study (apparent power – kVA), and 2 or 3 phase balancing (amperage – A) (http://www.acrsystems.com). Figure 3.6 illustrates the data logger that was utilised during this study.

Figure 3.6 Illustration of the Smart Reader Plus 3 data logger that was used during the measurement of grinding energy in this experiment

Power bullet:

The power bullet is used as a power or energy transductor, designed for monitoring demand and consumption in residential, commercial and industrial applications. The power bullet is a line-powered, phase-to-phase unit with outputs compatible with the voltage or pulse inputs of most measurement systems. The transducer outputs are directly proportional to kW/kVA for demand or kWh/kVAh for consumption (http://www.acrsystems.com). In Figure 3.7 the energy transductor (powerbullet) used during the measurement of grinding energy is shown.

Figure 3.7 Illustration of the energy transductor used during measuring of the grinding energy in the study

Figure 3.8 illustrates the direct energy measurement during grinding in watts/second. From this graph, the cumulative integrals of the direct energy measurement data were calculated as illustrated in Figure 3.9, so that the energy values could be reported as joules used / 10 g sample. These values were then converted to joules / 1 g sample on a DM basis, and the average value between the two replicates reported.

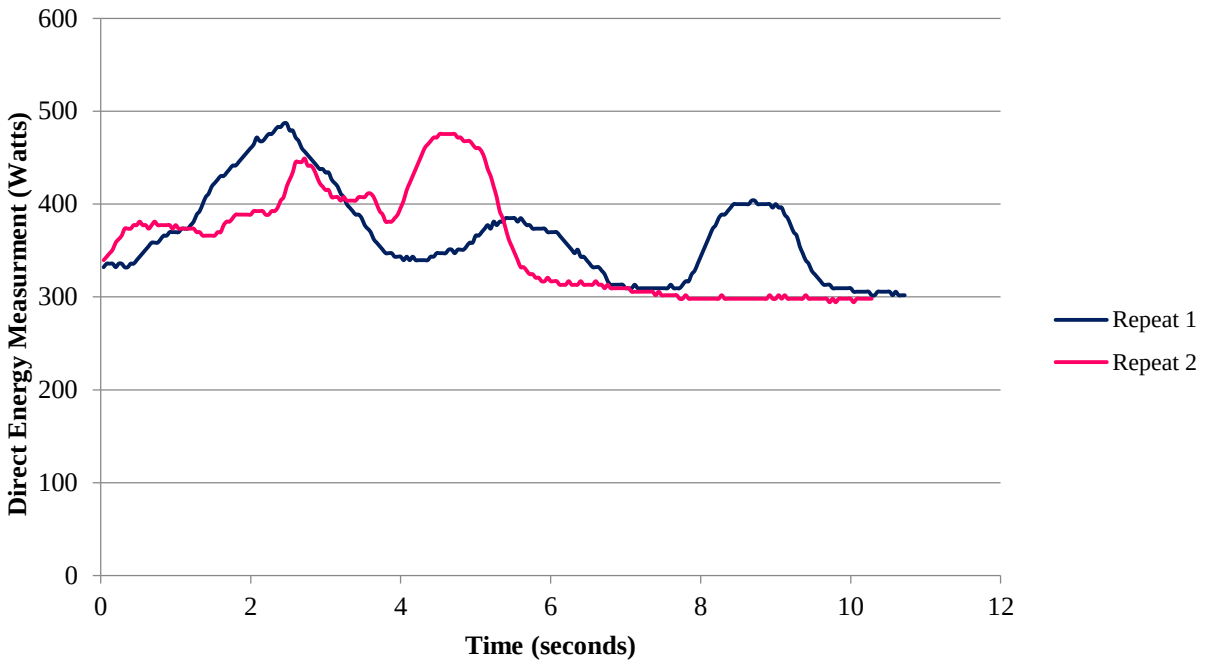

Figure 3.8 Example of direct energy measurement curve obtained during grinding of *M. sativa* in watts/second

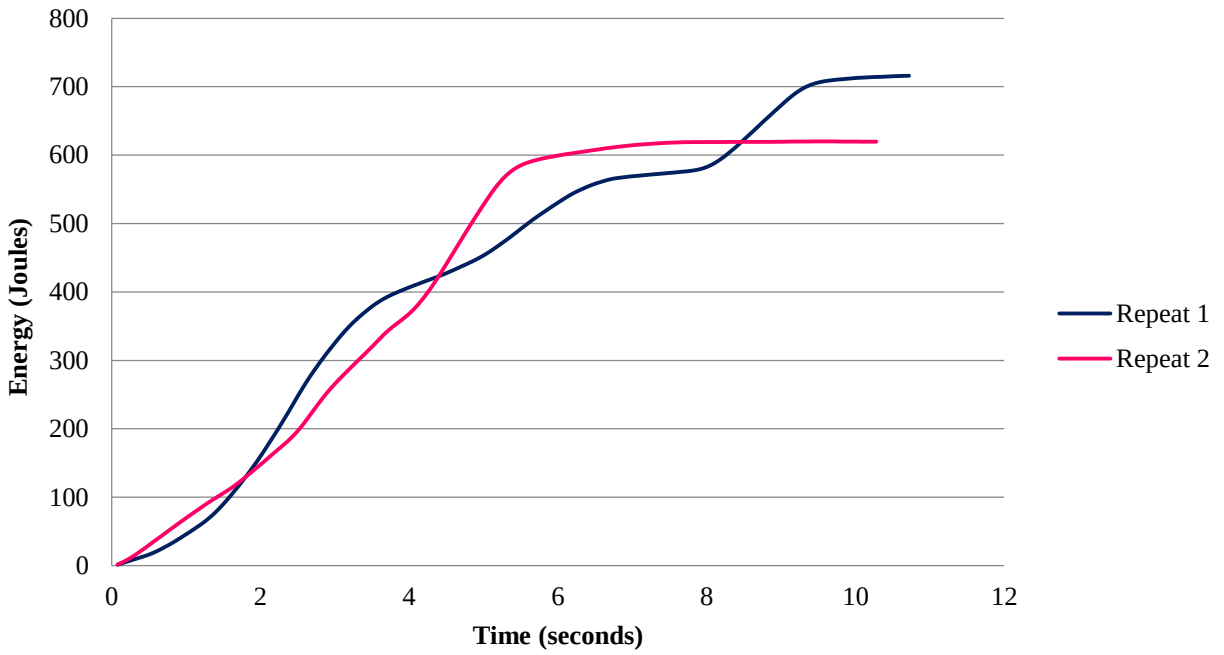

Figure 3.9 Example of cumulative integrals of direct energy measurement curves obtained during grinding of *M. sativa*

3.4 STATISTICAL ANALYSIS

All statistical analysis was done using the statistical program Genstat® (Payne *et al.*, 2012). *In vitro* NDF residues were modelled against time in hours for each sample to estimate the kd and lag time, according to the following equation:

$$Y = a + be^{(-kd\,(t-l))}$$

Where: y is uNDF

a is the constant and b is a regression coefficient

kd is the rate of NDF digestion over 240 hours

t is the time in hours

l is the lag time before *in vitro* digestion begins (in hours)

Tests for linearity, possible non-linearity and outliers between the energy values and individual chemical and physical descriptions of the forages were done in accordance with the linear regression equation:

$$Y = a + bx$$

Where: y is energy DM

a is the constant and b is the slope of the linear relationship

x is a parameter e.g. Particle size 1 mm

Testing for linearity between the *y*- and different *x*-variables showed that particle size of 1 mm was most highly correlated with energy DM. Not all the *x*-variables had a linear relationship with energy DM.

Pearson‘s correlation coefficients, which indicate the measure of the linear relationship between two random variables, were used to test for linearity between chemical components. Correlation coefficients, falling within the range of $-1 < r < 1$, indicates how strong relationships are between two variables. Close to -1 or 1 are extremely strong, while close to zero indicates no relationship.

Multiple stepwise regression analysis was used to determine which variables would most affect variation in energy on a DM and AS IS basis. Two models were run, one excluding DM, starch, CP and all uNDF measurements together, and the other model excluding DM, starch, CP and all kd estimates together. A third model was run which included both uNDf and kd measurements. The different models were run to avoid collinearity among the variables used. Finally, to test the effect of species on energy DM, stepwise regression was run on all chemical components (excluding DM, starch, CP) including species interaction.

The stopping rule (criterion) to select the best subset model was that the adjusted R^2 values should increase by 1%. In addition to this, a significance level of $P \leq 0.1$ (10%) was used to select the best model during the statistical analysis of the data from this study.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 CHEMICAL COMPOSITION AND FIBRE FRACTION OF FORAGES

The sample forages were analysed for DM, ash, nitrogen presented as CP, starch, NDF, ADF, ADL, 6-, 12-, 18-, 24-, 36-, 48-, 72-, 96-, 120- and 240-h *iv*NDFd, using standard laboratory procedures. From these, kd was calculated. The 240-h *iv*NDFd was used to estimate iNDF. The rates of NDF digestion were calculated using the undigested NDF residues and were calculated as %NDF per hour.

4.1.1 Dry matter, ash, starch and CP analyses

In Table 4.1 below, DM, ash, starch and CP values for all forage samples in this study are tabulated. An important note to make is that the maize silage values are second DM values as per the sample collection method and DM method explained in Chapter 3.

Table 4.1 Dry matter, ash, starch and crude protein concentrations of forage samples used in this study (DM basis, n = 35)

	Analysis (g / 100g DM)			
Forage sample	**DM[a]**	**Ash**	**Starch**	**CP[b]**
Medicago sativa 1	94.67	8.55	1.85	15.64
Medicago sativa 2	94.78	8.17	1.57	17.31
Medicago sativa 3	94.51	8.00	2.31	16.43
Medicago sativa 4	93.73	2.92	2.13	13.99
Medicago sativa 5	94.77	7.93	1.85	9.20
Medicago sativa 6	89.04	7.51	0.93	20.41
Medicago sativa 7	88.74	7.52	1.02	17.47

Medicago sativa 8	88.99	8.70	4.99	17.95
Medicago sativa 9	88.74	5.68	1.30	16.72
Medicago sativa 10	90.80	10.62	3.97	18.09
Medicago sativa 11	89.59	7.92	2.40	19.18
Medicago sativa 12	87.74	7.47	2.96	15.31
Medicago sativa 13	90.31	9.25	4.25	19.02
Maize silage 1	92.92	3.17	21.84	7.81
Maize silage 2	94.83	8.85	22.03	6.97
Maize silage 3	93.84	10.28	32.73	6.81
Maize silage 4	93.77	4.40	23.18	6.28
Maize silage 5	93.16	3.63	17.84	6.18
Maize silage 6	92.92	3.74	1.53	8.06
Maize silage 7	92.44	2.99	20.65	6.21
Maize silage 8	91.94	3.95	25.41	6.20
Maize silage 9	93.13	4.97	9.68	6.19
Eragrostis curvula 1	93.59	3.33	1.76	2.80
Eragrostis curvula 2	94.08	3.93	1.90	4.72
Eragrostis curvula 3	93.85	3.15	1.58	7.96
Eragrostis curvula 4	94.10	1.19	1.62	5.79
Eragrostis curvula 5	94.96	3.50	2.91	3.21
Eragrostis curvula 6	90.78	3.83	1.99	5.20
Eragrostis curvula 7	91.53	3.39	2.08	7.00
Eragrostis curvula 8	90.36	3.95	3.28	7.10
Eragrostis curvula 9	89.94	8.09	2.44	9.53
Eragrostis curvula 10	90.88	1.97	2.36	6.89

Eragrostis curvula 11	88.24	3.22	0.93	9.92
Eragrostis curvula 12	90.01	4.80	8.26	9.71
Eragrostis curvula 13	90.67	3.67	1.39	7.23

[a] Dry matter

[b] Crude protein = N x 6.25

Dry matter is made up of OM such as starch and crude protein, and the ash or mineral component of forage. From Table 4.1, it is observed that the samples used in the study had DM concentrations ranging from 87.74 g/100g as the lowest and 94.96 g/100g as the highest.

According to Table 4.1, *M. sativa* sample 12 and *E. curvula* sample 11 had the lowest numerical DM concentrations of all the forage groups of 87.74 g/1 00g and 88.24 g/100g, respectively. Smith *et al.* (1972) reported a range of between 66 – 80 g/100g soluble DM values for *M. sativa*, which is lower than the DM values obtained for *M. sativa* samples in this study. Another study performed in South Africa showed *M. sativa* DM values ranging between 86.5 – 94.4 g/100g (Scholtz *et al.*, 2009), which are similar to the DM values for *M. sativa* in this. A possible cause for a difference in values is the stage of maturity at which the forage samples were harvested (Nelson & Moser, 1994). For example, Smith *et al.* (1972) determined a DM value of 66 g/100g for *M. sativa* harvested at the pre-bloom stage, compared to 47 g/100g for *M. sativa* harvested at an early pod stage.

Soto-Navarro *et al.* (2014) noted DM concentrations for *E. curvula* ranging between 91.64 – 92.36 g/100g, which are similar to the DM concentrations for the *E. curvula* forages used in this study. Table 4.1 shows that maize silage had the highest numerical DM concentration overall, averaging at 93.22 g/100g. Mertens (2002b) reported a DM value for maize silage to be 92.3 g/100g, which is similar to the DM values determined in this study. Dry matter concentrations can vary in maize silage due to stage of maturity at which the maize was harvested, as can be seen in Table 4.2, which illustrates chemical compositions of maize silages harvested at different stages of maturity (Beever & Mould, 2000).

Table 4.2 Chemical composition of two maize silages harvested at different stages of maturity (Adapted from Beever & Mould, 2000)

	Low DM[a]	High DM
Dry Matter (g/kg fresh weight)	264-290	323-348
Starch	165-272	194-311
Crude Protein	57-119	84-108

[a] Dry matter

Ash is simply the mineral or inorganic component of the diet. The mineral content of forage depends on species, stage of growth, soil type, cultivation conditions as well as fertilizer application. Important to note is that the ash content of a forage decreases with maturity (MacDonald *et al.,* 2011). Legumes have a higher calcium and magnesium concentration than grasses, and tropical forages contain less calcium than temperate species (Cheeke, 1991). MacDonald *et al.* (2011) found that legumes tend to be richer in the major minerals and some trace minerals compared to grasses.

From Table 4.1, it is evident that the ash values for most of the *M. sativa* samples were numerically higher than the grass species (*E. curvula),* in accordance with the findings of MacDonald *et al.* (2011). Mertens (2002c) reported ash values for *M. sativa* that range from 8.9 - 9.1 g/100g and Scholtz *et al.* (2009) reported numerical ash values of between 7.25 – 29.5 g/100g for *M. sativa* which are slightly higher than the findings reported by Mertens (2002c). Ash values of 3.8 – 4.6 g/100g and 6.9 – 7.3 g/100g were documented by Mertens (2002c) for maize silage and warm season grasses such as *E. curvula,* respectively.

The results in Table 4.1 show that *M. sativa* had the highest average ash value of 7.11 g/100g DM, with *E. curvula* being the lowest at 3.69 g/100g DM. These values lie outside the range reported by Mertens (2002c). However, some of the *E. curvula* samples had high ash concentrations, in particular *E. curvula* sample 9 (8.09 g/100g DM), which could be attributed to many factors, such as; it may be that the soil in which these grasses were established was highly fertile, that the plants were still very young, or the application of fertilizer treatment (MacDonald *et al.,* 2011). Maize silage ash values for this study lie between 2.99 – 10.2 g/100g DM which is also different from the findings of Mertens (2002c). Based on the varying ranges documented, it is clear that there can be a large variation in plant composition. Some

of this variation can be attributed to legumes being richer in the major minerals and certain trace minerals than grasses, as stated by MacDonald *et al.* (2011). Furthermore, soil contamination of samples could be a contributing factor to higher ash results, as could be the case for maize silage sample 3 (10.28 g/100g). This could be the most important factor contributing to higher ash values.

The starch values, shown in Table 4.1, varied substantially between forage species. The lowest starch concentration was 0.93 g/100g, for *M. sativa* sample 6, and the highest value was 32.73 g/100g for maize silage sample 3. Mertens (2002c) reported starch values between 24.7 – 35.6 g/100g for maize silage, 2.0 – 2.6g/100g for *M. sativa* and 2.3 – 2.6 g/100g for warm season grass, such as *E. curvula*. The starch concentrations for the forages used in this study differed from the findings by Mertens (2002c), in some cases, substantially. According to MacDonald *et al.* (2011), temperate grasses and legumes main storage form of carbohydrate is fructan, whereas for tropical grasses, the primary storage carbohydrate is starch. This could explain why *M. sativa* starch levels in this study were extremely low, compared to the *E. curvula* starch levels.

True protein is the main nitrogenous compound in forage and it decreases in concentration with forage maturity. The amino acid composition of cellular protein varies little among grasses as most of this protein is present as enzymes in the plants (MacDonald *et al.*, 2011). The N in forage consists of proteins and various non-protein N compounds, such as ammonia, nitrates, amino acids and amides (Cheeke, 1991). Forage cell walls are disrupted during the process of feed consumption and fermentation, thereby exposing the CP to rumen microbes for digestion (Minson, 1990). Bailey (1962), as cited by Minson (1990), found that chewing also increases the rate of degradation of forage protein in the rumen. Leaves have a high quality protein (good amino acid balance), and the leaf blade has the highest concentration of CP. Therefore, forages with a high leaf: stem ratio will have a higher protein value (Minson, 1990; Cheeke, 1991).

The highest production of microbial CP is associated with immature, fresh, highly digestible forages, while the production of microbial CP is low when dried, mature forages are fed to ruminants (Minson, 1990). This is partly due to the decrease in N concentration with plant maturity (Minson, 1990). It has been shown that legumes generally have a higher CP concentration compared to non-legume species (Minson, 1990). This can also be seen from the results of Mertens (2002c) for CP concentrations

of 14.3 – 17.2 g/100g for *M. sativa*, 9.4 – 13.7 g/100g for *E. curvula* and 8.0 – 8.6 g/100g for maize silage. This study's results varied substantially within species as can be seen from Table 4.1, where *E. curvula* CP values varied from 2.80 g/100g to 9.92 g/100g. It is also evident that *M. sativa* had the highest numerical CP concentration of 20.41 g/100g, which is in fact lower than the highest CP level documented by Scholtz *et al.* (2009) and by Van Zyl *et al.* (2014) of 27.8g/100g and 26.7 g/100g, respectively, both for South African *M. sativa* samples.

In general, temperate grasses contain higher CP levels than tropical grasses (Minson, 1990; MacDonald *et al.*, 2011). Minson (1990) showed this in a study where mean CP concentrations of 129 g/kg for temperate grasses and 100 g/kg for tropical grasses were reported (on a DM basis). This is illustrated in Figure 4.1 below.

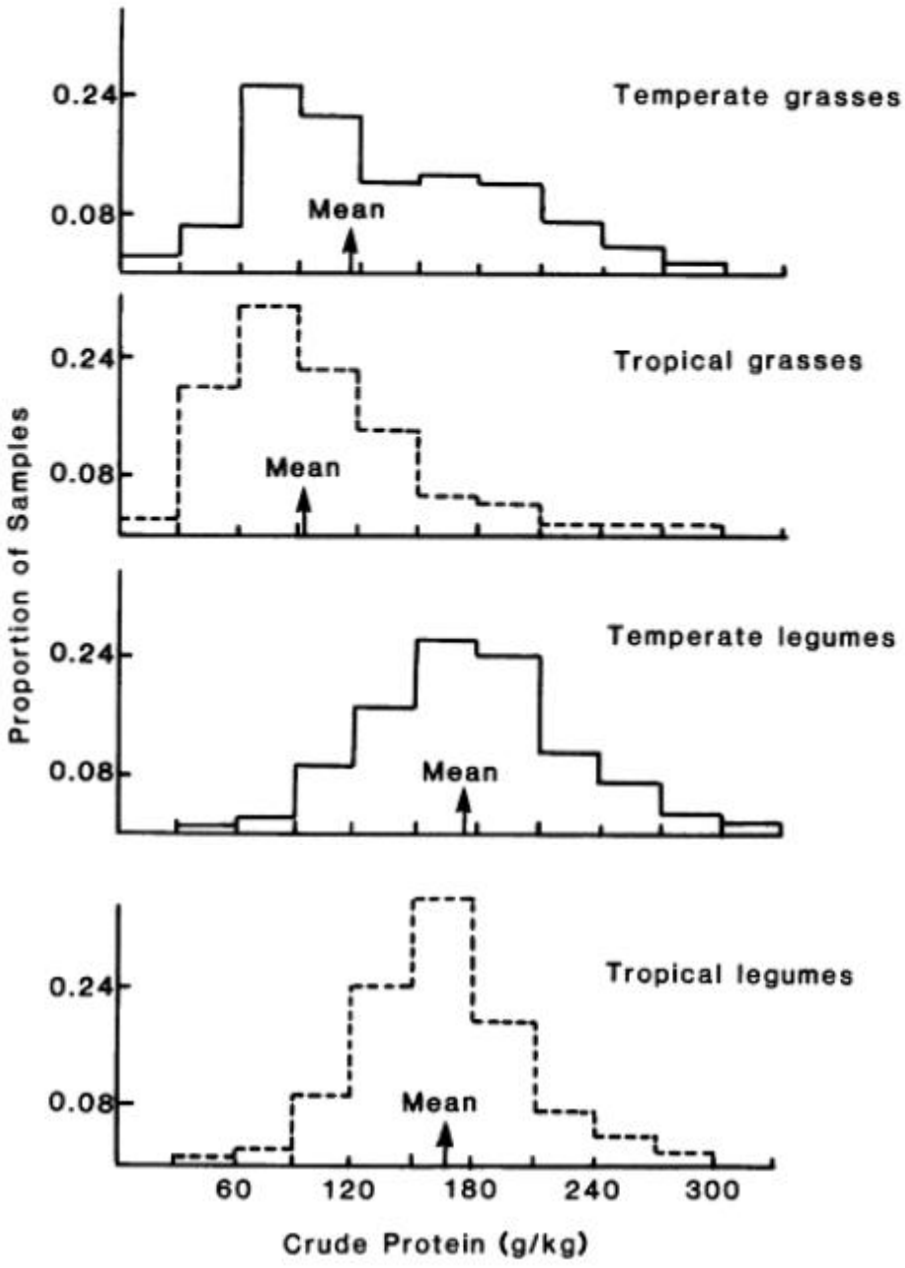

Figure 4.1 Crude protein concentration in temperate and tropical grasses and legumes (world data) (Adapted from Minson, 1990)

Figure 4.1 is supportive of the results in Table 4.1, where *E. curvula* (tropical grass) had the lowest level of CP of 2.80 g/100g. An explanation of this is given by Cheeke (1991), who stated that tropical grasses use N more efficiently than temperate grasses because of less total N in its tissues and therefore have a lower CP content than temperate grasses. The higher N content of the *M. sativa* samples is likely due to its higher leaf: stem ratio, compared to the mature, low leaf: stem ratio of *E. curvula.*

4.1.2 Results of the chemical analyses for NDF, ADF and ADL

Analyses for NDF, ADF, ADL and the ratios of cell wall components are shown below, in Table 4.3.
The ratios of cell wall components in Table 4.3 illustrate the proportion of ADF and ADL contained within NDF, respectively. In addition, these ratios of cell wall components provide additional information on the quality of the fibre samples as ADF and ADL are, in theory, part of NDF (the cell wall).

Table 4.3 The neutral detergent fibre, acid detergent fibre, acid detergent lignin concentrations of the forage samples used in this experiment (DM basis) and ratios of cell wall components (n = 35)

	g/100g DM			g/100NDF	
Forage sample	**NDF[a]**	**ADF[b]**	**ADL[c]**	**ADF/NDF[d]**	**ADL/NDF[e]**
Medicago sativa 1	42.19	35.22	7.00	83.48	16.60
Medicago sativa 2	44.83	36.35	6.47	81.10	14.42
Medicago sativa 3	43.47	35.43	6.50	81.52	14.96
Medicago sativa 4	54.11	36.89	7.02	68.17	12.97
Medicago sativa 5	60.63	55.29	11.43	91.19	18.86
Medicago sativa 6	43.13	33.79	4.31	78.34	9.99
Medicago sativa 7	43.26	38.98	5.01	90.10	11.59
Medicago sativa 8	58.47	49.75	8.38	85.09	14.33
Medicago sativa 9	51.73	47.01	5.50	90.86	10.63
Medicago sativa 10	36.78	34.93	6.99	94.96	19.00
Medicago sativa 11	41.76	37.58	7.61	89.98	18.22
Medicago sativa 12	56.37	50.09	10.80	88.86	19.16
Medicago sativa 13	42.60	37.51	7.50	88.06	17.60
Maize silage 1	47.76	28.45	2.91	59.57	6.09
Maize silage 2	45.98	26.80	2.68	58.27	5.83
Maize silage 3	42.81	25.77	1.70	60.19	3.97
Maize silage 4	46.58	33.03	1.91	70.91	4.09
Maize silage 5	46.85	33.76	2.05	72.06	4.38
Maize silage 6	55.91	36.89	4.57	65.98	8.18
Maize silage 7	52.04	34.45	3.96	66.20	7.61
Maize silage 8	44.04	29.02	2.83	65.88	6.42

Maize silage 9	64.11	42.95	4.24	67.00	6.62
Eragrostis curvula 1	82.01	47.43	5.31	57.83	6.48
Eragrostis curvula 2	80.47	48.43	6.40	60.18	7.96
Eragrostis curvula 3	82.04	47.94	6.58	58.43	8.03
Eragrostis curvula 4	76.52	41.56	4.49	54.31	5.87
Eragrostis curvula 5	82.42	30.21	8.06	36.65	9.78
Eragrostis curvula 6	81.13	45.97	3.58	56.65	4.41
Eragrostis curvula 7	81.42	41.08	4.91	50.46	6.03
Eragrostis curvula 8	79.92	45.71	5.17	57.19	6.47
Eragrostis curvula 9	64.61	36.70	3.16	56.80	4.90
Eragrostis curvula 10	82.03	43.99	4.36	53.63	5.32
Eragrostis curvula 11	80.23	40.43	4.40	50.39	5.48
Eragrostis curvula 12	74.36	44.05	3.36	59.23	4.52
Eragrostis curvula 13	80.24	44.72	3.77	55.73	4.69

[a] Neutral detergent fibre

[b] Acid detergent fibre

[c] Acid detergent lignin

[d] $(^{ADF}/_{NDF})$ x 100

[e] $(^{ADL}/_{NDF})$ x 100

Neutral detergent fibre (NDF) consists of cellulose, hemicellulose and lignin and is regarded as a measure of the cell wall fraction in forages (Jung & Lamb, 2003; MacDonald *et al*., 2011). Pectin and glucans also form part of the cell wall of forages and these are referred to as the soluble fibre fraction as they are highly soluble in neutral detergent solution (Hall, 1998; Paulson *et al.*, 2008) and as a result, the NDF residue does not retain the pectin fraction of forage cell walls (Theander & Westerlund, 1993). From a nutritional perspective, the loss of pectin from NDF is not a major issue as pectin is rapidly and relatively completely digested in the rumen (Hatfield & Weimer, 1995). Grasses have low pectin

concentrations and therefore, even though the NDF method tends to underestimate total cell wall fraction of forages, NDF concentration is a good indicator of the cell wall concentration in grasses. Legumes, however, contain much higher concentrations of pectin and NDF concentration can severely underestimate the cell wall fraction in legumes (Theander & Westerlund, 1993; Jung & Lamb, 2003; Paulson *et al.*, 2008).

According to Table 4.3, *M. sativa* sample 10 had the lowest numerical NDF concentration of 36.78 g/100g and *E. curvula* sample 5 had the highest numerical NDF concentration of 82.42 g/100g. The *E. curvula* forage group had the overall highest numerical NDF concentrations. Soto-Navarro *et al.* (2014) reported values for *M. sativa* ranging between 30.97 – 37.23 g/100g which are substantially lower than the NDF values obtained in this study, which ranged between 36.78 – 60.63 g/100g. However, Reverdin (2000) reported values of 52.5 – 55.3 g/100g for *M. sativa*, while Scholtz *et al.* (2009) reported values ranging between 28.9 – 65.9 g/100g for NDF and Van Zyl *et al.* (2014) reported a range for NDF for South African *M. sativa* samples between 29.1 – 49.3 g/100g. The NDF numerical values obtained in this study for *M. sativa* fall more within these ranges rather than the ranges stated by Soto-Navarro *et al.* (2014). The exception is *M. sativa* sample 5 which had an NDF value of 60.63 g/100g, falling more within the range reported by Scholtz *et al.* (2009). Regarding *E. curvula*, numerical values of 80.24 – 83.46 g/100g were documented by Soto-Navarro *et al.* (2014), which supports the values obtained in this study for the same species. Neutral detergent fibre concentrations of 68.9 g/100g – 73.3 g/100g for *E. curvula* have been reported by Mertens (2002c), which is lower than the values obtained from this study. Mertens (2002a, 2002c) also reported values for NDF of 40 g/100g and 41 – 51 g/100g for maize silage, respectively. Numerical NDF concentrations of 51.0 – 53.0 g/100g were reported by Horrocks & Vallentine (1999) for maize silage. The maize silage NDF analyses of this study are in accordance with these values, with the exception of maize silage sample 9 being higher at 64.11 g/100g.

Acid detergent fibre consists of cellulose, lignin, lignified nitrogen and silica (MacDonald *et al.*, 2011). According to MacDonald *et al.* (2011), there is a good statistical correlation between ADF of forage and the digestibility of forage. The ADF may be the most important analyses, as it is negatively correlated with forage digestibility (Horrocks & Valentine, 1999). This is due to the lignin and silica being structural inhibitors to digestion of other associated nutrients and therefore, being classified as anti-nutritional factors (Horrocks & Valentine, 1999). Maryland (1986), as cited by Horrocks & Valentine

(1999), stated that grasses can contain as much as 10% silica DM basis. Although ADF recovers most, if not all the lignin and cellulose from feeds, with some contamination from other compounds, because ADF does not recover hemicellulose, it is not an accurate estimate of fibre in feeds as it was merely developed as a preparatory step to determining lignin concentration in forages (Mertens, 2002c).

As shown in Table 4.3, maize silage sample 3 had the lowest ADF concentration of 25.77 g/100g and *M. sativa* sample 5 had the highest ADF value of 55.29 g/100g. Overall, the average numerical ADF values were highest for *E. curvula* followed by *M. sativa* and lastly, with the lowest overall values for maize silage. Soto-Navarro *et al.* (2014) documented ADF levels of between 20.61 – 25.59 g/100g for *M. sativa*, and Reverdin (2000) reported values of 36.5 – 37.0 g/100g ADF for *M. sativa*. Horrocks & Valentine (1999) documented slightly higher ADF values for *M. sativa* at 38.0 – 50.0 g/100g, with Scholtz *et al.* (2009) reporting a maximum value of 47.3 g/100g for ADF for local *M. sativa*. Regarding *E. curvula*, Soto-Navarro *et al.* (2014) documented values for ADF of 43.55 – 46.25 g/100g and Mertens (2002c) reported lower values ranging between 32.9 – 38.7 g/100g. The ADF numerical values obtained in this study for *E. curvula* and *M. sativa* concur with the above mentioned results, with the exception of *M. sativa* sample 5 (55.29 g/100g), which had an ADF value higher than the cited ranges.

Acid detergent lignin (ADL) involves treatment of the ADF fraction with 72% sulphuric acid, effectively dissolving the cellulose. However, some lignin is lost in the ADL procedure at the ADF step due to some lignin, particularly for grasses, being soluble in acid at high temperatures. Because of this, grasses have a much poorer lignin recovery in ADL than observed for legumes (Hatfield *et al*., 1994). Forage hemicellulose and cellulose concentrations are estimated by the difference in the detergent system (NDF minus ADF and ADF minus ADL, respectively), thus the loss of lignin at the ADF step results in over-estimation of these cell wall polysaccharides (Theander and Westerlund, 1993). According to MacDonald *et al.* (2011), the most important factor influencing the composition and nutritive value of a pasture is the stage of growth. With increasing plant maturation, there is an increase in structural carbohydrates such as cellulose and hemicellulose, an increase in lignin, and a decrease in protein content, thus leading to the inverse relationship between the protein and fibre contents in a given species (MacDonald *et al.*, 2011). Lignin is the most indigestible component of forage and in addition to this, lignin also inhibits the availability and thus digestion of associated cellulose and hemicellulose as it is cross-linked to hemicellulose and acts as a binder for cellulose fibres (Horrocks & Vallentine, 1999; Tabil

et al., 2011). It is for this reason that there is an accepted dogma that lignin is the primary cell wall component that limits digestion of forage in the rumen (Jung & Deetz, 1993; Van Soest, 1994).

Anatomically, lignin concentration is higher in grass stems than the leaves, and lignin is absent from legume leaves (Chaves *et al.*, 2002; Paulson *et al.*, 2008). Lignin is also covalently bound to hemicellulose, and cellulose is often present as microfibrils bound in a matrix of hemicellulose and lignin (Jung, 2012). Chaves *et al.* (2002) stated that there are less cross linkages between lignin and hemicellulose in the stems of legumes than grasses, therefore, the lignin concentration does not have as large a negative effect on nutritional value of legumes as with grasses (Chaves *et al.*, 2002). Van Soest (1982) and Hatfield *et al.* (1994) stated that lignin is also a major anti-nutritional factor of grasses. The reason for this, in addition to the above, is due to legumes having a higher proportion of core/localized lignin compared to grasses, which contain high amounts of non-core lignin (Jung, 1989). This allows for easier cell wall degradation of legumes due to the limit on physical restriction by lignin on cell wall digestion (Moore & Cherney, 1986), as cited by Gaylean & Goetsch (1993). As an example, MacDonald *et al.* (2011) stated that in young pasture grass containing only 50 g lignin/kg DM, 80% of the cellulose may be digested, but in older herbage with 100 g lignin/kg, the proportion of cellulose digested may be less than 60%.

It is further shown in Table 4.3 that there was some variation among the ADL values. Lignin concentration of forages typically ranges between 5 – 20 g/100g, with values for legumes generally higher than the values for grasses (Van Soest, 1982), however, large differences exist for lignin content within plant species (Allen & Mertens, 1988). This concurs with the ADL analyses for this study. The ADL values in this study ranged between a minimum of 1.70 g/100g for maize silage sample 3 and a maximum of 11.43 g/100g for *M. sativa* sample 5. This range falls within the range stipulated by Van Soest (1982). The average numerical ADL value for *E. curvula* was 4.89 g/100g and for *M. sativa*, 7.27 g/100g, also in accordance with the statement made by Van Soest (1982) where legume lignin levels are higher than grass lignin levels. Horrocks & Vallentine (1999) reported a value of 8 – 10 g/100g ADL for *M. sativa*, whereas Reverdin (2000) reported higher values of 10.8 – 11.1 g/100g for *M. sativa*. Some of the ADL values in this study are lower than the reported ranges.

The expression of ADF on a NDF basis gives a good indication of the proportion ADF contained within NDF. According to the results in Table 4.3, *M. sativa* had the highest overall numerical amounts of ADF on an NDF basis, with the highest being 94.96 g/100g DM for *M. sativa* sample 10. It is also interesting to note here that *M. sativa* sample 5 was seen to have the highest ADF value for this experiment, and second highest value for ADF on NDF basis (91.19 g/100g DM). The expression of ADL on a NDF basis gives an indication of percentage ADL contained within NDF. As illustrated in Table 4.3, *E. curvula* sample 5 had a high ADL concentration on NDF basis, but *M. sativa* again had the highest overall numerical ADL concentration on NDF basis.

4.1.3 Hemicellulose, cellulose and lignin analyses

A further breakdown of the individual components of NDF, ADF and ADL is presented in Table 4.4. These fractions are hemicellulose, cellulose and lignin.

Table 4.4 Hemicellulose, cellulose and lignin concentrations (g/100g) of the experimental forage samples on DM basis (n = 35)

Forage sample	Hemicellulose	Cellulose	Lignin
Medicago sativa 1	6.97	28.22	7.00
Medicago sativa 2	8.47	29.89	6.47
Medicago sativa 3	8.04	28.93	6.50
Medicago sativa 4	17.23	39.87	7.02
Medicago sativa 5	5.34	43.85	11.43
Medicago sativa 6	9.34	29.48	4.31
Medicago sativa 7	4.28	33.96	5.01
Medicago sativa 8	8.72	41.37	8.38
Medicago sativa 9	4.73	41.51	5.50
Medicago sativa 10	1.85	27.94	6.99
Medicago sativa 11	4.18	29.97	7.61
Medicago sativa 12	6.28	39.29	10.80

Medicago sativa 13	5.09	30.01	7.50
Maize silage 1	19.31	25.55	2.91
Maize silage 2	19.19	24.11	2.68
Maize silage 3	17.05	24.07	1.70
Maize silage 4	13.55	31.13	1.91
Maize silage 5	13.09	31.71	2.05
Maize silage 6	19.02	32.32	4.57
Maize silage 7	17.59	30.49	3.96
Maize silage 8	15.02	26.19	2.83
Maize silage 9	21.16	38.71	4.24
Eragrostis curvula 1	34.59	42.11	5.31
Eragrostis curvula 2	32.05	42.02	6.40
Eragrostis curvula 3	34.10	41.35	6.58
Eragrostis curvula 4	34.96	37.06	4.49
Eragrostis curvula 5	52.21	22.15	8.06
Eragrostis curvula 6	35.17	42.39	3.58
Eragrostis curvula 7	40.33	36.17	4.91
Eragrostis curvula 8	34.21	40.54	5.17
Eragrostis curvula 9	27.91	33.54	3.16
Eragrostis curvula 10	38.04	39.63	4.36
Eragrostis curvula 11	39.81	36.03	4.40
Eragrostis curvula 12	30.32	40.69	3.36
Eragrostis curvula 13	35.52	40.95	3.77

Table 4.4 illustrates the calculated hemicellulose, cellulose and lignin values of the forage samples used in this study. Cellulose is a fibrous, tough, water-insoluble substance found in the cell walls of plants (Tabil *et al.*, 2011) and comprises 40 – 60% of the dry weight of plant material (Encyclopaedia Britannica, 2008; USDE, 2006, as cited by Tabil *et al.*, 2011). Cellulose is particularly found in the stems, trunks, stalks and all woody portions of the plant (Nelson & Cox, 2005). Hemicellulose is a polysaccharide related to cellulose and is present in almost all plant cell walls along with cellulose.

Hemicellulose comprises 20 – 40% of the biomass of most plants and it provides structural integrity to the cell (Tabil *et al.*, 2011).

The cellulose concentration of grass is reported to be in the range of 200 g/kg – 300 g/kg DM, and the hemicellulose concentration varies between 100 g/kg and 300 g/kg DM (MacDonald *et al.*, 2011). Lignin fills the spaces in the cell wall between cellulose and hemicellulose and it is covalently linked to hemicellulose and thereby crosslinks different plant polysaccharides, conferring mechanical strength to the cell wall and consequently to the whole plant structure (Chabannes *et al.*, 2001). Mani *et al.* (2006) reported values of 21.11 g/100g hemicellulose and 31.32 g/100g cellulose for maize stover, and 30.0 g/100g hemicellulose and 44.34 g/100g cellulose for switchgrass (a typical C_4-grass). The hemicellulose and cellulose values for maize silage and *E. curvula* in this study are similar to the findings reported by Mani *et al.* (2006), although with some variation such as the hemicellulose value for *E. curvula* sample 5 which had a hemicellulose value of 52.21 g/100g. The lignin values in Table 4.4 are the same as the ADL values in Table 4.3. It is important to note that at comparable stages of maturity, Van Soest (1965) reported that the proportion of digestibility coming from fibrous components such as cellulose and hemicellulose differs greatly between grasses and *M. sativa*. What is interesting to note from Table 4.4, is that *M. sativa* had the highest average numerical lignin values, and this supports the findings from Table 4.3, where it can be seen that *M. sativa* had the highest ADL on NDF basis, or rather percentage of ADL within NDF. In addition, hemicellulose and cellulose values for *M. sativa* from this study fall slightly outside the ranges reported by Scholtz *et al.* (2009) of 5.27 – 19.9 g/100g and 16.3 – 36.4 g/100g, respectively.

4.1.4 *In vitro* NDF digestibilities, iNDF and rates of NDF digestion

In vitro NDF digestibilities ranging from 6- to 120-h were recorded and from these, the rate of digestion was calculated as kd in % digestion per hour. In particular, the 24-h *iv*NDFd is presented in Table 4.5 as this is the most commonly used time point for NDFd commercially when evaluating forages for quality and ultimately digestibility. The 24-h time point and sometimes a 30-h time point are used since these time points are the most likely retention times for fibre for high producing cows. Furthermore, research has shown that the concentration of NDF and Klason lignin within the NDF fraction are the most limiting factors to 24-h *in vitro* digestibility, with NDF being the most important factor (Casler & Jung, 2006).

Also presented in Table 4.5 is the 240-h *iv*NDFd which is presented as undigested NDF (uNDF240) and this fraction essentially represents the iNDF fraction. The ratio of uNDF240 to ADL is shown to illustrate the degree to which ADL negatively affects indigestibility of forage.

Table 4.5 Chemical analysis of *in vitro* NDF digestion, indigestible NDF and calculations of rates of NDF digestion

Chemical Analysis				
Forage sample	**24h-ivNDFd[a] (%)**	**Kd[b] (%/hour)**	**uNDF240[c] (g/100g NDF)**	**uNDF240/ADL (DM basis)**
Medicago sativa 1	35.50	3.91	49.50	7.07
Medicago sativa 2	43.30	4.75	36.50	5.65
Medicago sativa 3	37.50	5.79	49.94	7.68
Medicago sativa 4	51.40	4.65	41.54	5.92
Medicago sativa 5	25.21	2.55	50.73	4.44
Medicago sativa 6	30.39	3.47	45.34	10.53
Medicago sativa 7	28.54	3.18	48.66	9.70
Medicago sativa 8	23.83	3.42	49.82	5.94
Medicago sativa 9	22.54	2.77	49.65	9.03
Medicago sativa 10	27.85	5.07	55.14	7.89
Medicago sativa 11	26.89	3.41	54.17	7.12
Medicago sativa 12	24.21	2.95	51.15	4.73
Medicago sativa 13	35.89	3.79	45.28	6.04
Maize silage 1	49.86	4.04	20.56	7.07
Maize silage 2	50.13	3.94	22.29	8.31
Maize silage 3	51.06	2.85	26.96	15.87
Maize silage 4	33.04	2.37	26.36	13.82
Maize silage 5	32.11	2.73	36.82	17.93
Maize silage 6	44.31	3.54	26.25	5.74
Maize silage 7	40.91	3.38	34.15	8.62

Maize silage 8	37.75	2.89	25.33	8.96
Maize silage 9	46.63	3.32	19.62	4.62
Eragrostis curvula 1	20.55	1.63	34.88	6.57
Eragrostis curvula 2	16.54	1.49	45.51	7.11
Eragrostis curvula 3	27.47	2.15	32.63	4.96
Eragrostis curvula 4	28.40	1.70	26.52	5.90
Eragrostis curvula 5	14.78	1.35	39.99	4.96
Eragrostis curvula 6	29.09	1.97	25.65	7.17
Eragrostis curvula 7	28.81	1.50	30.16	6.14
Eragrostis curvula 8	28.21	2.43	37.79	7.31
Eragrostis curvula 9	37.40	1.69	39.55	12.50
Eragrostis curvula 10	21.67	1.53	35.23	8.08
Eragrostis curvula 11	18.82	1.08	33.65	7.65
Eragrostis curvula 12	21.74	2.02	31.36	9.34
Eragrostis curvula 13	16.67	1.63	28.38	7.53

[a] *In vitro* NDF digestibility

[b] Rate of NDF digestion

[c] Undigested NDF at 240 hours (also called indigestible NDF)

Digestibility of NDF is an important parameter of forage quality, according to Oba & Allen (1999). Jung & Lamb (2003) identified lignin as the main limiting factor to *in vitro* NDF digestibility of *M. sativa*. However, according to Jung & Allen (1995), the effect of lignin on fibre digestibility is greater in grasses than in legumes. Grasses generally have higher fractions of potentially degradable fibre as well as lower rates of digestion than legumes (Smith *et al.*, 1972). Casler & Jung (2006) reported that differences in total fibre among species, which was measured as NDF, were parallel to differences in lignin concentration. It has also been reported that the concentration of NDF and Klason lignin within the NDF fraction are the most limiting factors to 24-h *in vitro* digestibility, with NDF being the most important factor (Casler & Jung, 2006). According to Casler (2001), as cited by Casler & Jung (2006), *iv*NDFd can be increased by decreasing lignin concentration or cross-linking between lignin and cell wall carbohydrates.

As illustrated in Table 4.5, *E. curvula* sample 5 had the lowest 24-h *iv*NDFd of 14.78%, while *M. sativa* sample 4 had the highest *iv*NDFd of 51.40%, at 24 hours incubation. The slow rate of NDF digestion of *E. curvula* sample 5 (1.35%/hour) correlates with the low NDF digestibility of *E. curvula* and, also supports the negative correlation reported by Jung *et al.* (1997), between lignin concentration and NDF digestibilities. *Eragrostis curvula* is a C_4-grass and therefore has a lower digestible NDF. The rates of NDF digestion for *M. sativa* and maize silage were numerically higher than for the *E. curvula* grass, which is in accordance with the statement made by Jung & Allen (1995) as well as Chaves *et al.* (2002), where lignin concentration does not have as large a negative effect on nutritional value of legumes as with grasses. It is important to note that increased plant maturity leads to a decrease in the rate of NDF digestion (Smith *et al.*, 1972), which makes the interpretation of the rate of NDF digestion values more complex. Jung & Lamb (2003) reported that cell wall concentration was consistently negatively correlated with both 16-h and 96-h *iv*NDFd. Julier *et al.* (1999) reported a value of 31.9% for the NDFd of *M. sativa*. The authors stated that a large genetic variation for digestibility of *M. sativa* has been observed, which can be explained by the variation in cell wall content (or NDF), and digestibility of the cell wall (Julier *et al.*, 1999). Mertens (1993) reported a positive relationship between *in vitro* rate of NDFd and voluntary DMI, and the correlation for grasses was higher than for legumes.

The uNDF at 240-h (uNDF240) is the NDF fraction unavailable to microbial digestion in ruminants after 240-h *in vitro* fermentation, and recently assumed to estimate iNDF (Raffrenato & Van Amburgh, 2010). Digestibility of the remaining fibre, the pdNDF (pdNDF = NDF – iNDF), determines the availability of NDF. Forage digestibility is thus constrained by iNDF and the rate of digestion of pdNDF (Van Soest, 1994). Furthermore, iNDF has been characterised as the most important factor affecting the digestibility of the total diet OM (Nousiainen *et al*., 2004). According to Ellis *et al.* (1999), determination of iNDF should be included in all basic feedstuff analysis because it has a predictable digestibility (digestion rate of zero), and can be used for the estimation of the pdNDF, and has an important role in contributing to the rumen digesta load. However, because iNDF does not contribute energy to the animal, it should not be included in the estimation of forage energy content (Traxler *et al.*, 1997). In a study by Kramer *et al.* (2012), it was shown that lignin concentration on a DM basis is the best single predictor of iNDF (DM basis) for grasses, but not for legumes. According to a study by Wilson & Kennedy (1996), lignin is mainly found in the xylem tissue of legumes, in which lignin concentrations reach levels which render the cells indigestible. In contrast, lignin concentrations in other tissues are low thus making these

cells completely digestible. Grasses contain relatively less lignin, which is distributed among all tissues except phloem, which thus explains why lower levels of indigestible fractions can limit cell wall digestion by rumen microbes (Wilson & Kennedy, 1996).

Raffrenato & Erasmus (2013) published average iNDF values of 31.97% for maize silage, 52.50% for *M. sativa* and 33.57% for C_4-species such as *E. curvula.* According to the results in Table 4.5, *M. sativa* had an overall average numerical uNDF240 value of 48.26%, maize silage 26.48% and *E. curvula* 33.95%, respectively. These values differ slightly to the values reported by Raffrenato & Erasmus (2013), with the exception of *E. curvula*, which is similar. The degree to which ADL negatively affects indigestibility can be represented by the ratio of uNDF240 to ADL (uNDF240/ADL). The results in Table 4.5 for iNDF and the impact of ADL on digestion of forages concur with the results and explanations surrounding ADL and lignin for Table 4.3. However, the uNDF240/ADL ratio values for *M. sativa* and *E. curvula* from this study are similar to the ratios reported by Raffrenato & Erasmus (2013) for *M. sativa* and typical C_4-grasses, with two outliers (*M. sativa* sample 6 with a value of 10.53 and *E. curvula* sample 9 with a value of 12.50). Following from this, the maize silage ratios for this study were higher than the ratios reported by Raffrenato & Erasmus (2013).

In Table 4.6, the estimated kd per sample obtained from a first order decay model of the uNDF time points over 240-h is represented, along with their respective standard error of regression (SER), which is a measure of the accuracy of the predictions. When comparing the kd per sample over 240 hours, the significance level used was $p \leq 0.001$.

Table 4.6 Estimated rates of NDF digestion from undigested NDF (uNDF) decay models per sample over 240 hours

		Parameter estimates[a]	
Forage sample	**Kd[b](%/hour)**	**Adjusted R^2**	**SER[c]**
Medicago sativa 1	3.9	96.6	3.17
Medicago sativa 2	4.7	95.1	4.51
Medicago sativa 3	5.8	95.0	3.58
Medicago sativa 4	4.6	97.7	2.80

Medicago sativa 5	2.6	96.7	3.00
Medicago sativa 6	3.5	95.5	3.70
Medicago sativa 7	3.1	95.0	3.76
Medicago sativa 8	3.4	96.9	3.14
Medicago sativa 9	2.3	94.7	3.53
Medicago sativa 10	5.1	87.0	4.80
Medicago sativa 11	3.4	97.4	2.78
Medicago sativa 12	2.9	98.7	1.82
Medicago sativa 13	3.8	94.3	4.73
Maize silage 1	4.0	93.6	6.71
Maize silage 2	3.9	92.6	7.62
Maize silage 3	2.3	92.2	7.26
Maize silage 4	2.4	97.7	3.83
Maize silage 5	2.7	96.9	4.02
Maize silage 6	3.5	96.5	4.84
Maize silage 7	3.4	96.7	4.55
Maize silage 8	2.9	98.2	3.82
Maize silage 9	3.3	94.0	7.31
Eragrostis curvula 1	1.6	98.8	2.40
Eragrostis curvula 2	1.5	92.9	5.49
Eragrostis curvula 3	2.2	99.3	2.00
Eragrostis curvula 4	1.7	97.5	3.70
Eragrostis curvula 5	1.4	98.0	3.04
Eragrostis curvula 6	1.9	95.60	5.29
Eragrostis curvula 7	1.5	98.30	3.27
Eragrostis curvula 8	2.4	94.20	4.97
Eragrostis curvula 9	1.7	88.10	8.00
Eragrostis curvula 10	1.5	97.80	3.28
Eragrostis curvula 11	1.0	98.50	2.65
Eragrostis curvula 12	2.0	98.70	2.82

Eragrostis curvula 13	1.6	97.80	3.83

[a]Significance level: $p \leq 0.001$

[b]Rate of NDF digestion

[c] Standard error of regression

4.2 LABORATORY ANALYSIS OF FORAGE FRAGILITY

4.2.1 Particle size distribution and energy measurements

Grasses contain higher concentrations of NDF and ADF compared to legumes (Paulson *et al.*, 2008), which supports the observation by Minson (1990) that the fibre content is greater in grasses compared with legumes, tropical compared with temperate and leaf compared with stem. Legumes are more susceptible to particle breakdown (higher fragility) compared to grasses during chewing and re-chewing (Horrocks & Vallentine, 1999), and this is readily apparent when forages are mechanically ground; when shred, grasses tend to produce long, thin pieces whereas legumes typically grind to finer and more cubical shapes. This increased susceptibility to particle size reduction of legumes is likely due to a combination of cell size of plant tissues, tissue organization, and cell wall thickening (Paulson *et al.*, 2008).

Minson (1967), as cited by Minson (1990), stated that the intake of poor quality forages can be substantially increased through grinding or pelleting as these two processes decrease the particle size of forage. Particle size reduction is a complex yet critical process as it determines digesta volume, rate of passage and digestion of food particles which all together determines the rate of forage intake (Ellis *et al.*, 1987, as cited by Horrocks & Vallentine, 1999). Animals are more likely to consume larger quantities of leafy legumes and grasses compared to its counterparts with higher stem concentration. This is as a result of higher rate of particle size reduction (fragility) from mastication and higher rate of passage through the reticulo-rumen (Horrocks & Valentine, 1999).

In Table 4.7, the results for the initial and final geometric mean particle size, after the two grinding steps (i.e. 2 cm and 1 mm screens), and the percentage (%) change in particle size for each of the forage

samples analyzed in this study are presented. The % change in particle size was calculated as the IPS and was more variable across forages than was expected. Therefore, looking only at FPS would not be representative since FPS is affected by IPS.

The *E. curvula* sample 13 had the largest average particle size after milling with the 2 cm screen (4252.0 µm). It can also be observed from Table 4.7 that *E. curvula* sample 7, along with *M. sativa* sample 6, had the smallest average particle size after milling with the 2 cm screen with values of 726.6 µm and 993.1 µm, respectively. The rest of the forage samples had mean particle size ranging between 1008.2 µm – 3771.4 µm. According to the results in Table 4.7, a few of the forage samples exhibited less change in average particle size between the 2 cm and 1 mm milling than the majority of the samples. These include *M. sativa* sample 6 which had a 60.85% change in particle size, *M. sativa* sample 12 (62.70%) and *E. curvula* sample 7 which had a 65.6% change in particle size. The rest of the forage samples had % particle size changes in the range of 69.26% and 89.43%.

When analysing the results for the FPS, it can be seen that *M. sativa* sample 7 (233.1 µm) and *E. curvula* sample 7 (249.9 µm) had the smallest mean FPS after milling with the 1 mm screen. Whereas, *E. curvula* sample 5 had the largest average FPS at 563.0 µm, after milling with the 1 mm screen. The rest of the samples measured between 272.7 µm and 540.7 µm. Although little research has been done on particle size reduction using a knife mill, Mani *et al.* (2004) reported a geometric mean FPS for switchgrass (C_4-grass) of 283 µm when a 1.6 mm screen was fitted to a hammer mill, and 253 µm when a 0.8 mm screen was used. These results could be comparable to the results for *E. curvula* in Table 4.7, as *E. curvula* is also a C_4-grass. However, the reported values by Mani *et al.* (2004) for geometric mean FPS of switchgrass are lower than the values obtained during this study. It is important to keep in mind that the moisture content of the switchgrass used by Mani *et al.* (2004) was 8.00%, whereas the average moisture content of the C_4-grass, *E. curvula*, used in this study was 8.23 % and this difference in moisture could impact the effectiveness and time needed for grinding. Although, differences in the fibre composition of the two grasses mentioned, is more likely the reason for the differences in time needed for grinding.

Yancey *et al.* (2013) reported that grinding energy for maize stover and switchgrass showed a steep increase as moisture content increased; therefore an increase in moisture content increases resistance to particle breakdown, so the higher the moisture content, the higher the energy consumption. However,

there are many other factors influencing the final particle sizes such as feeding rate of samples, and mill operating speed and power as well as other chemical components, which will have an influence on the resistance to particle breakdown (Balk, 1964). Therefore, it may be that the FPS for *E. curvula* used in this study are larger due to their higher moisture content, and in turn the higher grinding energy required to grind these particles, compared to the lower moisture of the switchgrass used in the study by Mani *et al.* (2004). In addition, the size and number of sieves used when grinding will also affect the FPS.

Table 4.7 Particle size distribution and percentage (%) change in particle size of forages after milling through a 2 cm (IPS) and 1 mm screen (FPS)

Average particle size distribution			
Forage sample	**IPS[a] (µm)**	**FPS[b] (µm)**	**% change in particle size**
Medicago sativa 1	2081.1	416.8	79.97
Medicago sativa 2	1976.3	448.5	77.30
Medicago sativa 3	2035.9	417.8	79.48
Medicago sativa 4	2011.6	399.3	80.15
Medicago sativa 5	2262.7	478.5	78.85
Medicago sativa 6	993.1	388.8	60.85
Medicago sativa 7	1920.3	233.1	87.86
Medicago sativa 8	1385.2	425.4	69.29
Medicago sativa 9	1968.8	513.4	73.92
Medicago sativa 10	1712.7	348.9	79.63
Medicago sativa 11	1860.1	410.6	77.92
Medicago sativa 12	1179.9	440.1	62.70
Medicago sativa 13	2217.1	365.9	83.50
Maize silage 1	2073.4	445.0	78.54
Maize silage 2	2113.9	369.5	82.52
Maize silage 3	1710.8	370.9	78.32
Maize silage 4	2046.2	378.8	81.49
Maize silage 5	2023.5	373.1	81.56

Maize silage 6	2430.3	333.5	86.28
Maize silage 7	1438.4	313.7	78.19
Maize silage 8	2985.5	315.6	89.43
Maize silage 9	1166.8	291.1	75.05
Eragrostis curvula 1	3310.1	424.4	87.18
Eragrostis curvula 2	3771.4	453.8	87.97
Eragrostis curvula 3	3151.6	390.5	87.61
Eragrostis curvula 4	1555.7	348.2	77.62
Eragrostis curvula 5	1831.6	563.0	69.26
Eragrostis curvula 6	1355.0	408.8	69.83
Eragrostis curvula 7	726.6	249.9	65.60
Eragrostis curvula 8	1623.6	406.7	74.95
Eragrostis curvula 9	1289.0	290.5	77.46
Eragrostis curvula 10	2532.9	333.6	86.83
Eragrostis curvula 11	1289.3	298.1	76.88
Eragrostis curvula 12	1008.2	272.7	72.95
Eragrostis curvula 13	4252.0	540.7	87.28

[a] Initial particle size

[b] Final particle size

In Figure 4.2 the geometric mean particle size distributions of all the forage samples used in this study for IPS, after milling with a 2 cm screen and FPS, after milling with a 1 mm screen are illustrated. In the graph, *M. sativa* is represented as MS, maize silage is represented as M and *E. curvula* is represented as ES. *E. curvula* sample 13 had the largest average FPS, and *E. curvula* sample 7 as well as *M. sativa* sample 6 had the smallest average FPS, as is evident from the graph.

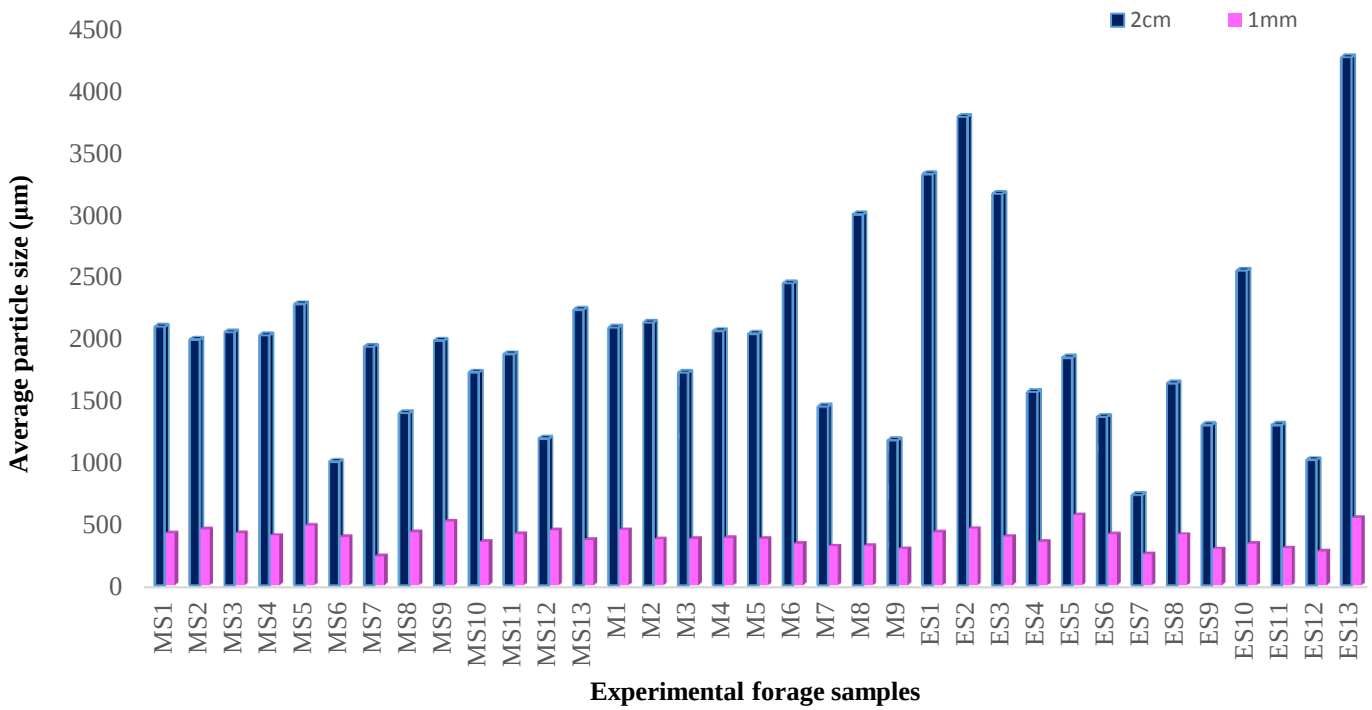

Figure 4.2 Average particle size distributions of forage samples after milling through a 2 cm (IPS) and 1 mm screen (FPS). MS = *M. sativa*, M = maize silage, ES = *E. curvula*

4.2.2 Direct Energy Measurements

According to Yu *et al.* (2006) factors such as biomass species, moisture content, particle size and shape, surface area before and after grinding, bulk density, feed rate rotor speed, machine specification, clearance setting, and cutting speed have an important influence on the processing requirements of biomass. However, the energy required for grinding biomass is influenced by and depends on the particle size distribution, moisture content, bulk and particle densities, feed rate of the material and machine variables such as screen size classification, rotary speed (rpm) and mass throughput (Womac *et al.*, 2007; Lopo, 2002, as cited by Tabil *et al.*, 2011).

According to Chenost (1966), as plants mature there is an increase in the fibre concentration, with an associated increase in the energy needed to grind the dried plant matter through a 1 mm sieve. This is

further illustrated by Laredo and Minson (1973) who stated that when leaf and stem fractions of tropical grasses were compared, the stem fraction had a higher grinding energy value than the leaf fraction even though there was no difference in dry matter digestibility.

In Table 4.8, the direct energy as measured during the grinding of the forage samples using a Retsch ultra-centrifugal mill fitted with a 1 mm screen, is presented. Energy measurements are reported in joules/g sample (DM basis). The mean energy requirements during grinding of *M. sativa*, maize silage and *E. curvula* were 113.19 J/g, 93.55 J/g and 139.7 J/g, respectively.

Table 4.8 Direct energy measurements of experimental forages using a Retsch ultra-centrifugal mill

Direct energy measurements (joules/g sample on DM basis)	
Forage sample	**Retsch ultra-centrifugal mill**
Medicago sativa 1	67.19
Medicago sativa 2	93.80
Medicago sativa 3	70.67
Medicago sativa 4	85.48
Medicago sativa 5	157.01
Medicago sativa 6	59.24
Medicago sativa 7	63.83
Medicago sativa 8	172.40
Medicago sativa 9	286.01
Medicago sativa 10	77.90
Medicago sativa 11	185.47
Medicago sativa 12	67.66
Medicago sativa 13	84.84
Maize silage 1	64.97
Maize silage 2	53.33
Maize silage 3	74.13

Maize silage 4	111.13
Maize silage 5	77.87
Maize silage 6	161.90
Maize silage 7	111.46
Maize silage 8	52.55
Maize silage 9	134.62
Eragrostis curvula 1	201.75
Eragrostis curvula 2	188.27
Eragrostis curvula 3	170.21
Eragrostis curvula 4	65.88
Eragrostis curvula 5	229.25
Eragrostis curvula 6	127.18
Eragrostis curvula 7	65.22
Eragrostis curvula 8	107.16
Eragrostis curvula 9	74.19
Eragrostis curvula 10	187.06
Eragrostis curvula 11	80.91
Eragrostis curvula 12	75.92
Eragrostis curvula 13	243.49

4.3 POSSIBLE ASSOCIATION BETWEEN CHEMICAL COMPOSITION AND GRINDING ENERGY

4.3.1 Simple correlations and linear associations

In Table 4.9, Pearson's correlation coefficients between the grinding energy requirement (as energy DM) and each independent variable is represented, and the independent variables with significant correlation coefficients between pairs of variables on grinding energy are indicated. These coefficients are

an indication of the strength of the linear relationship between pairs of variables. Pearson's correlation was used for this section as it is a measure of linear dependence between two variables.

As expected, a strong co-linear relationship exists between energy on an as is basis and energy on a DM basis, made evident by the correlation coefficient ($r = 0.999$; $p \leq 0.001$). Therefore energy on an as is basis was left out of the final model in order to increase accuracy of prediction from the final model set. This parameter was chosen to be left out due to all energy as is values being converted into DM values for uniformity as all other variables are expressed on a DM basis. The extremely high positive correlation between DM and as is energy values also shows the substantially small effect that moisture had in the grinding energy values, also because of the very small amount of moisture left in the samples. Starch, CP and DM values for each sample were also left out of the model as the aim of the model was to specifically assess the relationship between grinding energy and *in vitro* digestibility.

Table 4.9 Pearson's correlation coefficients for the grinding energy requirement values with the independent variables (n = 35)

Energy DM[a]	-									
IPS (2 cm)[b]	0.484**	-								
FPS (1 mm)[c]	0.596***	0.431**	-							
% change in PS[d]	0.156	0.786***	-0.087	-						
Kd[e]	-0.426*	-0.153	-0.033	0.016	-					
NDF[f]	0.422*	0.157	0.076	-0.040	-0.792***	-				
ADF[g]	0.558***	0.117	0.322	-0.161	-0.431**	0.631***	-			
ADL[h]	0.226	0.009	0.394*	-0.214	0.179	-0.009	0.629***	-		
ADF/NDF[i]	-0.045	-0.106	0.165	-0.089	0.631***	-0.713***	0.074	0.594***	-	
ADL/NDF[j]	-0.021	-0.075	0.261	-0.150	0.560***	-0.483**	0.217	0.856***	0.860***	-
Hemicell[k]	0.219	0.132	-0.083	0.039	-0.753***	0.902***	0.235	-0.361*	-0.934***	-0.726***
Cellulose	0.580***	0.137	0.235	-0.111	-0.583***	0.757***	0.955***	0.372*	-0.137	-0.066
uNDF6[l]	0.240	0.069	-0.231	0.067	-0.620***	0.482**	0.109	-0.268	-0.503**	-0.451**
uNDF12	0.287	-0.056	-0.090	-0.137	-0.640***	0.562***	0.243	-0.165	-0.468**	-0.370*
uNDF18	0.425*	-0.012	0.033	-0.182	-0.729***	0.618***	0.561***	0.133	-0.222	-0.143
uNDF24	0.505**	0.164	0.255	-0.139	-0.650***	0.612***	0.694***	0.362*	-0.086	0.057
uNDF36	0.415*	0.166	0.255	-0.085	-0.554***	0.526**	0.599***	0.420*	-0.042	0.153
uNDF48	0.354*	0.099	0.244	-0.135	-0.347*	0.341*	0.570***	0.512**	0.179	0.339*
uNDF72	0.274	-0.043	0.263	-0.188	-0.085	0.126	0.554***	0.676***	0.437**	0.578***
uNDF96	0.261	-0.194	0.295	-0.371*	-0.018	0.032	0.458**	0.553***	0.470**	0.522**
uNDF120	0.194	-0.080	0.244	-0.217	0.103	-0.109	0.399*	0.627***	0.593***	0.655***
uNDF240	0.137	-0.062	0.259	-0.174	0.290	-0.297	0.333	0.731***	0.748***	0.807***
	Energy DM	IPS (2 cm)	FPS (1 mm)	% change in PS	Kd	NDF	ADF	ADL	ADF/NDF	ADL/NDF

Hemicell	-											
Cellulose	0.418*	-										
uNDF6	0.543***	0.232	-									
uNDF12	0.569***	0.352*	0.557***’	-								
uNDF18	0.463**	0.619***	0.555***	0.671***	-							
uNDF24	0.381*	0.691***	0.401*	0.495**	0.881***	-						
uNDF36	0.326	0.556***	0.296	0.366*	0.769***	0.929***	-					
uNDF48	0.110	0.486**	0.212	0.295	0.709***	0.871***	0.926***	-				
uNDF72	-0.151	0.405*	0.046	0.098	0.537***	0.703***	0.790***	0.910***	-			
uNDF96	-0.214	0.337*	-0.081	0.017	0.482**	0.620***	0.677***	0.817***	0.906***	-		
uNDF120	-0.359*	0.238	-0.064	0.026	0.437**	0.565***	0.62***	0.809***	0.920***	0.911***	-	
uNDF240	-0.557***	0.120	-0.251	-0.160	0.267	0.399*	0.475**	0.689***	0.831***	0.791***	0.922***	-
	Hemicell	Cellulose	uNDF6	uNDF12	uNDF18	uNDF24	uNDF36	uNDF48	uNDF72	uNDF96	uNDF120	uNDF240

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

[a] Grinding energy on a DM basis

[b] Initial particle size by milling with 2 cm screen

[c] Final particle size by milling with 1 mm screen

[d] % change in particle size

[e] Rate of NDF digestion

[f] Neutral detergent fibre

[g] Acid detergent fibre

[h] Acid detergent lignin

[i] Expression of acid detergent lignin expressed on a neutral detergent fibre basis

[j] Expression of acid detergent lignin on a neutral detergent fibre basis

[k] Hemicellulose

[l] Undigested NDF at various time points from 6 hours to 240 hours during *in vitro* NDF digestibility

In Table 4.10, the individual variables with the strongest linear relationship to energy DM are presented, with their respective adjusted R^2 values, SER and significance levels. The adjusted R^2 value is the R^2 statistical measure of how close the data are to the fitted regression line, but in this case, which has been adjusted for the number of predictors in the model. Probability values are in place to show and/or select the best model for the data.

Table 4.10 Best fit linear models to predict possible linear association between grinding energy measurement values (J/g DM) and independent variables, individually (n = 35)

Independent variable in model	Parameter estimates				
	Intercept (± SE[a])	Slope (± SE)	Adjusted R^2	SER[b]	Significance Level
FPS[c]	-66.4 ± 44.1	0.479 ± 0.113	33.5	50.6	****
Cellulose	-89.2 ± 51.3	5.95 ± 1.45	31.7	51.3	****
ADF[d]	-73.9 ± 50.5	4.7 ± 1.24	29.0	52.2	****
uNDF24[e]	-94.9 ± 64.0	3.089 ± 0.918	23.3	54.3	***
IPS[f]	41.4 ± 25.8	0.0387 ± 0.012	21.1	55.1	***
Kd[g]	184.7 ± 26.5	-2296 ± 848	15.7	57.0	**
uNDF18	-118 ± 88.1	3.08 ± 1.14	15.6	57.0	**
NDF[h]	22 ± 37.2	1.606 ± 0.601	15.3	57.1	**
uNDF36	-34.3 ± 58.9	2.429 ± 0.926	14.7	57.3	**
uNDF48	-4.2 ± 57.1	2.15 ± 0.990	9.9	58.9	**
uNDF12	-131 ± 145	2.88 ± 1.67	5.5	60.3	*

* $p \leq 0.1$; ** $p \leq 0.05$; ***$p \leq 0.01$; **** $p \leq 0.001$

[a] Standard error of regression

[b] Standard error

[c] Final particle size

[d] Acid detergent fibre

[e] Undigested NDF at certain time points during *in vitro* NDF digestibility

[f] Initial particle size

[g] Rate of NDF digestion

[h] Neutral detergent fibre

The following equation was used:

*Energy DM = y = Intercept + parameter estimate (slope) * independent variable + error*

As can be seen from Table 4.9, strong positive correlations exist between energy DM and FPS, cellulose and ADF of 0.596, 0.580 and 0.558 respectively. This is further illustrated in Table 4.10, from which can be seen that the individual independent variables with the strongest linear relationship to grinding energy DM were FPS, cellulose and the ADF fraction of forage. These linear relationships show that FPS can account for 33.5% of the variation in grinding energy required to grind a forage sample, whereas cellulose can account for 31.7% of the grinding energy variation, and ADF at a lower value of 29%.

The values from Table 4.10 only represent associations and therefore no clear cause-effect conclusions can be made. However, it is interesting to note the energy increase needed for every unit increase in the variables. For example, for every unit increase in cellulose, there is an increased energy requirement of 5.95 J/g DM, compared to an increased energy requirement of 4.7 J/g DM for every unit increase of ADF. Furthermore, the negative slope value for kd of -2296 J/g DM implies that for every unit increase in kd, there is a large decrease in the energy required for grinding. However, the relationship between kd and grinding energy is not strong since the R^2 value is only 15.7 J/g DM and the Pearson's correlation coefficient for these two variable is -0.426 ($p \leq 0.05$), suggesting that other factors will influence grinding energy more than kd. Figure 4.3 shows a visual example of the linear relationship between grinding energy requirements and the independent variables, for example, FPS.

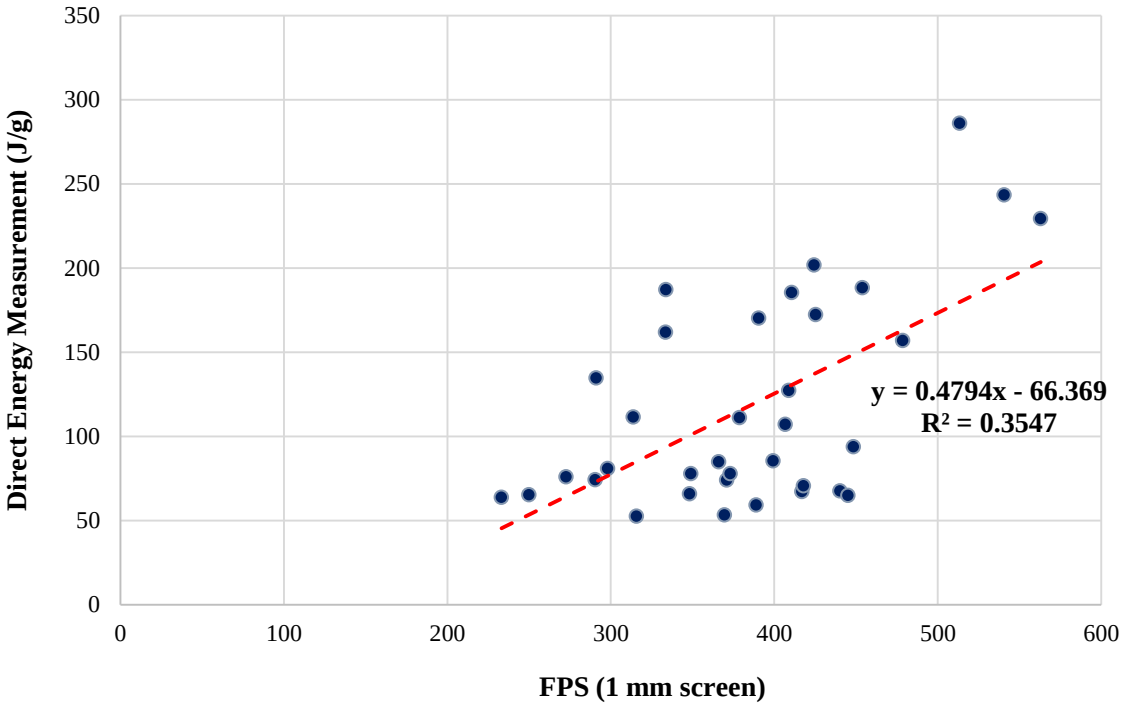

Figure 4.3 Illustration of the linear relationship between grinding energy requirements and final particle size (FPS) of the forage samples (n = 35)

4.3.2 Stepwise regressions for predicting variables affecting variation in grinding energy

Table 4.11 illustrates the stepwise regression results when determining which of the independent variables most affect the variation in energy required to grind the forage samples. The regression is shown per step to emphasize which the three main factors are that affect energy DM, with their respective adjusted R^2 values, SER, regression coefficients and significance levels.

Table 4.11 Stepwise regression, per step, to determine the best subset model to predict which independent variables most affect variation in grinding energy requirements (n = 35)

Independent variable in model per step	Adjusted R^2	SER[a]	Regression coefficients (± SE[b])	Probability
1. FPS[c]	33.5	50.6	0.479 ± 0.113	***
2. Cellulose	53.2	42.4	4.78 ± 1.24	***
3. uNDF6[d]	59.4	39.5	4.19 ± 1.74	**
4. IPS[e]	62.0	38.2	0.017 ± 0.01	*

* $p \leq 0.1$; ** $p \leq 0.05$; *** $p \leq 0.001$

[a] Standard error of regression

[b] Standard error

[c] Final particle size

[d] Undigested NDF at 6 hours of *in vitro* NDF digestion

[e] Initial particle size

Table 4.12 illustrates the stepwise regression results when determining which of the independent variables most affect the variation in grinding energy requirement, with the inclusion of species as a factor. The regression is shown per step, again to emphasize the influence of species when included in the model. Values are given with their respective adjusted R^2 values, SER and significance levels.

Table 4.12 Stepwise regression, per step, to determine the best subset model to predict which independent variables most affect variation in grinding energy requirement when species is included as an explanatory variable

Independent variable in model per step	Adjusted R^2	SER[a]	Regression coefficients (± SE[b])	Probability
FPS[c]	33.5	50.6	0.479 ± 0.113	***
Cellulose	53.2	42.4	4.78 ± 1.24	***
uNDF6[d]	59.4	39.5	4.19 ± 1.74	**
IPS[e]	62.0	38.2	0.017 ± 0.01	*
FPS.Species *M. sativa*	67.5	35.4	0.223 ± 0.217	**
FPS.Species Maize silage	67.5	35.4	-0.311 ± 0.207	**
ADL/NDF[f]	69.7	34.1	-4.80 ± 2.82	*

* $p \leq 0.1$; ** $p \leq 0.05$; *** $p \leq 0.001$

[a] Standard error of regression

[b] Standard error

[c] Final particle size

[d] Undigested NDF at 6 hours of *in vitro* NDF digestibility

[e] Initial particle size

[f] Acid detergent lignin expressed on a neutral detergent fibre basis

It is observed from the final model in Table 4.11 that a total of 62.0% of the variation in energy used for grinding forage samples with a Retsch ultra-centrifugal mill can be explained by the independent variables FPS, cellulose, uNDF6 and IPS. While only 33.5% of the variation in energy required during grinding can be explained by FPS, it is interesting to note that when cellulose was included in the model, the variation in energy required during grinding increased by almost 20% to a value of 53.2%. This clearly shows that cellulose has a significant influence on the requirement of grinding energy. The regression coefficients are positive for all four independent variables in the final model, indicating that with an increase in any one of these variables/measurements, there will be an accompanying increase in energy usage during grinding with a Retsch ultra-centrifugal mill in this study and a decline in forage fragility.

Table 4.12 shows that species was added as a factor to the model, and this increased the variation in grinding energy needed during comminution from 62.0% to 69.7%. By adding species to the model, the factors explaining this variation in grinding energy requirement now include FPS, cellulose, uNDF6, IPS, FPS×species and ADL/NDF. The interaction between FPS and species (FPS×species) increases the explained variation in energy required for grinding by 5.5%, and the ratio between ADL and NDF (ADL/NDF) in the model from Table 4.12 explains an additional 2.2% of the variation in comminution energy required. The ADL/NDF is an important ratio, as Raffrenato & Van Amburgh (2010) explain that it could result in plant lignin being predicted more accurately for a forage. The regression coefficient for ADL/NDF is negative, which means that as this variable/measurement decreases, the grinding energy requirement increases and forage fragility decreases, when keeping all the other variables constant.

From Tables 4.11 and 4.12, it is evident that cellulose has a positive regression coefficient (4.78 ± 1.24) for the analysis of grinding energy requirement, which, as explained earlier, implies that the forage fragility decreases with increasing cellulose concentration as more energy will be needed to grind a forage. According to the results from this study, *M. sativa* sample 5 had the highest cellulose concentration of 43.85 g/100g, followed by *E. curvula* sample 6 with a cellulose concentration of 42.39 g/100g. This may explain the higher energy values measured when grinding these two samples. However, other samples which did not have high cellulose concentrations, elicited higher grinding energy values compared to *M. sativa* sample 5 and *E. curvula* sample 6. It has to be assumed that this is due to the many interactions between the chemical components and other factors influencing grinding energy requirement, such as lignin encasing cellulose in the cell wall of plants (Jung, 2012). This may be an explanation for the high energy used for grinding of the forages which do not necessarily have high cellulose concentrations. Cellulose also has strong negative correlations with kd of -0.583 ($p \leq 0.001$), suggesting that as the cellulose level increases, the rate of fibre degradation of forage decreases, and therefore forage fragility declines. This is in agreement with the relationship between cellulose and grinding energy requirement as discussed above. This is also in agreement with the relationship between kd and energy needed for comminution.

There is a negative correlation between kd and energy required for grinding of -0.426 ($p \leq 0.01$) and kd has a negative linear slope of -2296 J/g DM. Both indicate that an increased rate of forage cell wall digestion (kd) will result in a decreased energy requirement for grinding due to increased forage fragility. Interestingly, there is a strong negative correlation between NDF and kd of -0.792 ($p \leq 0.001$).

Therefore it would seem that with an increase in NDF concentration, there is a decrease in kd, therefore more grinding energy will be needed due to reduced forage fragility. The results from this study concur with the correlations, as it can be observed from Tables 4.3 and 4.5 that the NDF values for *E. curvula* are the highest between the species, and kd values for the *E. curvula* forage group are the lowest, as would be expected based on the correlations above.

Based on Table 4.9's data, NDF has a medium positive correlation with energy required for grinding of 0.422 ($p \leq 0.05$), indicating that forage fragility will decrease as the NDF content of forage increases. According to the results from this study, the *E. curvula* forage group had the highest overall NDF values and corresponding energy values measured when grinding these forages. Due to NDF not forming part of the final regression model, it is difficult to draw conclusions from the results on the exact magnitude of the effect of NDF on forage fragility. However, because NDF includes lignin, cellulose and hemicellulose, and is regarded as a measure of the cell wall material of plants (MacDonald *et al.*, 2011), the regression coefficient should also theoretically be positive for the analysis of the grinding energy requirement, indicating that an increase in NDF concentration will lead to decreased forage fragility, which supports the positive correlation observed in this study. The relationship between NDF and the early digestion time points is an interesting one. Neutral detergent fibre shows strong positive correlations ($p \leq 0.001$) with uNDF12 of 0.562, uNDF18 (0.618) and uNDF24 (0.612), clearly illustrating a strong influence of NDF on the early digestion of fibre (Table 4.9).

The earliest digestion time point, uNDF6 has a positive regression coefficient of 4.19 ± 1.74 ($p \leq 0.05$) for the analysis of grinding energy requirement, showing that an increase in the uNDF6 will increase the grinding energy required, thus lowering forage fragility. Some other early time points in the 240-h NDFd cycle, such as uNDF18, uNDF24, uNDF36 and uNDF48 all showed moderate positive correlations with energy required for grinding, as is evident from Table 4.9. However, the longer time points for NDFd showed no significant correlations or associations with grinding energy requirement.

Although ADF did not form part of the final model, it is interesting to note that ADF is positively correlated with energy required for grinding (0.558, $p \leq 0.001$), as can be seen from Table 4.9. Acid detergent lignin is also linearly associated with energy DM, shown in Table 4.10. Horrocks & Vallentine (1999) reported that as ADF increases, digestibility of the forage decreases. This suggests a negative correlation between ADF and forage digestibility, and this would imply that forage fragility decreases

with an increasing ADF content. This is supported by the positive correlation found in this study between grinding energy requirement and ADF, illustrating that as ADF concentration increases, so does grinding energy required due to lower forage fragility. Further evidence to support this is the Pearson's correlation coefficient of -0.431 ($p \leq 0.01$) between ADF and kd showing that as ADF content increases, rate of NDF digestion decreases. Acid detergent fibre is less important as it does not contribute to energy estimations anymore, but the results show that it represents a value highly correlated to the fragility of the forage (with cellulose), and probably to the chewing needed by the animal.

The data shown in Table 4.9 clearly illustrates that there are strong positive correlations between ADL and uNDF72 (0.676, $p \leq 0.001$), uNDF96 (0.553, $p \leq 0.001$) , uNDF120 (0.627, $p \leq 0.001$) and uNDF240 (0.731, $p \leq 0.001$). These positive correlations between ADL and the above uNDF time points support the literature stating that lignin is the major cell wall component that limits extent of digestion of forages due to it being largely indigestible (Buxton & Redfearn, 1997; Horrocks & Valentine, 1999). Lignin is not only indigestible, but the presence of lignin also inhibits the availability of associated cellulose and hemicellulose (Horrocks & Vallentine, 1999). Jung *et al.* (1997) reported that there is a negative correlation between lignin concentration and fibre digestibility. In addition, Allen & Mertens (1988) stated that the maximal rate of fibre digestion is dependent on the intrinsic characteristics of the fibre including chemical composition and physical structure, such as lignin concentration among others. What is interesting to note here, is that ADL did not show significant correlations with the shorter digestion time points of 6, 12 and 18 hours. This suggests that lignin does not become a limiting factor for fibre digestion until later.

The simple correlation between ADL and the grinding energy of the Retsch ultra centrifugal mill was numerically positive but low and non-significant (Table 4.9). Even if it would be expected for lignin to have a positive correlation with energy requirement for grinding, and to decrease forage fragility, numerous earlier research papers have shown possible contradictions. Results from work done by Prinsloo (2014) clearly illustrate such inconsistencies and contradictions. A study conducted by Rinne *et al.* (2002) has also shown a possible contradiction to this, whereby they have documented faster particle size reduction in more mature forages (i.e. greater fragility or brittleness of the more mature and thus lignified particles). Previous research supporting this has been reported by Poppi *et al.* (1981), where it was shown that 12- week regrowth of tropical grasses were more prone to particle size reduction than the regrowth at 6- weeks. However, it is possible that the specific chemical and structural relationships within

the cell wall between cellulose, hemicellulose and lignin, and the total amount of cell wall on DM basis, can result in most of the grinding energy variation among forages.

From Tables 4.11 and 4.12, it is evident that FPS ($p \leq 0.001$) and IPS ($p \leq 0.1$) both influenced the energy required when grinding forages with a Retch mill, significantly. The regression coefficients for FPS and IPS were both positive values of 0.479 ± 0.113 and 0.017 ± 0.01, respectively. This shows that with an increase in IPS or FPS, more energy will be required to grind the forages and hence forage fragility declines. Furthermore, Pearson's correlation coefficients from Table 4.9 show a positive value of 0.484 ($p \leq 0.01$) between IPS and energy DM, thus supporting the above findings. The results for IPS are in agreement with the findings of Prinsloo (2014). According to Table 4.7, *M. sativa* sample 9 had a high IPS of 1968.8 µm and FPS of 513.4 µm, and *E. curvula* sample 13 had a high IPS of 4252.0 µm, and a FPS of 540.7 µm, compared to the other samples. Both of these forage samples had the highest direct energy measurement for grinding (Joules/g sample DM) within their respective species. Results from Table 4.8 indicate that the measurements were: 286.01 J/g for *M. sativa* sample 9 (with 73.92% change in particle size) and 243.49 J/g for *E. curvula* sample 13 (with 87.28% change in particle size). Interestingly, Arthur *et al.* (1982) reported that grinding energy requirement increased as the particle size decreased, and Holtzapple *et al.* (1989) stated that grinding energy increased greatly as the particle size is reduced. This supports the results from this study, which show that FPS has a larger influence on grinding energy requirement than IPS. Furthermore, the strong positive correlation found between IPS and % change in particle size of 0.786 ($p \leq 0.001$) from Table 4.9 suggests that increased IPS results in decreased forage fragility, due to increased energy required for grinding to FPS.

When comparing the % change in particle sizes from IPS to FPS illustrated in Table 4.7, to direct energy measurements obtained during grinding as illustrated in Table 4.8, it is evident that samples which had a high % change in particle size had high grinding energy requirements, as can be seen with *E. curvula* sample 13. The opposite can be seen for samples with low % change in particle size. However, there was variation amongst samples, for example, according to the above explanation, it would be expected that *M. sativa* sample 7 would have a high direct energy value for grinding due to the % change in particle size being large (87.86%), but the grinding energy value was only 63.83 J/g (Table 4.8). The primary objective of performing an initial grinding was to obtain a homogenous pool of particle sizes across all species before initiating the trial. Unfortunately poor homogeneity was achieved even when

using the same 1 mm sieve to sieve all the samples. It is also possible that the sieving process may have biased the quality of the particles selected.

The FPS×species interaction is significant for the species *M. sativa* and maize silage. From Table 4.12, it is noted for *M. sativa,* the regression coefficient is positive (0.223 ± 0.217) indicating that the energy required for grinding increases as the interaction between FPS and the forage species *M. sativa* increases, thus lowering forage fragility. Whereas, when the species is maize silage, the regression coefficient is negative (-0.311 ± 0.207), meaning that as this interaction between FPS and species increases, forage fragility increases as energy needed for grinding is less.

Results from this study have perhaps lead to more questions than answers, but it has emphasised the complexity of the relationship between forage fragility, particle size reduction and the various fibre fractions in feeds. It also highlights the need for more in-depth research in this field and the necessity to incorporate the concept of forage fragility into future nutritional prediction models.

CHAPTER 5

5. CONCLUSION

Ruminants require sufficient amounts of fibre of adequate particle length as it establishes the biphasic nature of the rumen, and therefore stimulates chewing activity. Animals ruminate in proportion to the cell wall content of their diet, and chewing activity is related to the NDF content as well as the particle size of the feed. Physically effective fibre attempts to take both the physical and chemical properties of fibre which influence the chewing activity, into account. Chewing activity is vital for the stimulation of the secretion of salivary buffers to control the rumen pH, and is also an important dictator of the physical environment of the rumen for optimal ruminal fermentation. Less effective fibre in the diet leads to lowered chewing activity by the animal, which decreases salivary buffer production and increases VFA concentrations, ultimately resulting in a lowered ruminal pH and potentially the development of metabolic disorders such as ruminal acidosis. Furthermore, this can cause microbial populations to change and can result in altered end products of fermentation.

The peNDF standardised system was developed with the objective of predicting chewing response accurately, based on the measurement of forage particle size and the NDF content. The peNDF measurement provides a more consistent measure of effective fibre than chewing activity due to it being independent of animal differences and is based solely on two fundamental properties of feed: fibre content and particle size. Many nutritional dairy models require peNDF as a key input value for prediction of lactational response, cow chewing response and rumen pH. The peNDF system is based on two assumptions, namely that the forage fragility (relative rate of particle size reduction during chewing or some laboratory simulation of chewing action) among different sources of NDF is similar, and that forage particle size explains all the variation elicited in chewing response. Both of these assumptions, however, are not correct, as has been proven in this present study.

Neutral detergent fibre has been used as the only feed characteristic to predict the filling effects of forages, but there is substantial evidence to show that NDF alone is not sufficient to make these predictions. The filling effect of forages varies with differences in initial particle size, particle fragility,

and the rate and extent of NDF digestion. Differences in the fragility of forages affect the particle size breakdown and retention time in the reticulo-rumen.

There is the potential to combine forage fragility and NDF digestibility as these two factors are related, in order to improve and better predict cow chewing response. The possibility exists to predict forage fragility from energy required for grinding as related to the chemical composition of forages, however this is not a simple concept and numerous inconsistencies exist in the ability to measure forage fragility in the laboratory. Some of these inconsistencies are evident in this study in some of the unexplained or contradictory results.

The results obtained from this study show that an increase in cellulose will result in a decrease in forage fragility. Due to some inconsistencies between cellulose concentration and grinding energy, it has to be mentioned that a more than likely cause for the higher grinding energies for some forage samples which did not have high cellulose levels, is the possible encasement of cellulose by lignin. It was also evident from the results that cellulose is negatively correlated with rate of degradation of forage (kd), therefore an increase in cellulose will cause a decline in kd, resulting in reduced fragility. The results from this study also show that as kd increases, so does forage fragility. As for NDF, it is expected that an increase in the NDF concentration of forage will lead to a decrease in forage fragility, however, NDF did not form part of the final models hence it is not clear how much of an effect NDF has on fragility. However, NDF does consist of cellulose, hemicellulose and lignin and it is evident from the final models that cellulose has a large negative effect on fragility, so it may be possible that NDF could have the same effect as cellulose on forage fragility due to both factors being positively correlated to fragility. However, the fact that NDF was not included in the final model demonstrates again that NDF is not a uniform entity across forages and its quality is highly variable. The earliest time point in digestion, uNDF6 was the only time point to have an impact on the variation in forage fragility, according to the final models obtained in this study. The results of the study show that an increase in the undigested NDF at 6 hours is associated with an increase in energy required for grinding, indicating that forage fragility decreases with increasing uNDF6. Strong associations or relationships between the other digestion time points and grinding energy were non-significant, making for an inconclusive result.

As with NDF, ADF did not form part of the final model, therefore the exact effect of ADF on forage fragility is unknown. Although, ADF was found to be positively correlated and linearly associated with grinding energy and therefore it may be possible that an increase in ADF content causes a decline in

fragility. The result regarding ADL was inconclusive. While it would be expected that ADL will increase energy requirement for grinding, indicating that ADL can be associated with decreased forage fragility, results from numerous previous studies have contradicted this assumption. While ADL results were non-significant for this study, it is interesting to observe the relationship between ADL and the longer and shorter time points for the NDF digestion analysis. These results support other studies findings of lignin being highly associated with the indigestible fraction of fibre. However, the results suggest that this only becomes a limiting factor during the latter stages of digestion, once the more digestible components of fibre have been digested. Initial particle size and FPS were both found to have important influences on grinding energy requirement, whereby an increase in either of these variables is associated with an increase in energy required for grinding, thus indicating that increased IPS or FPS can be associated with a decrease in the fragility of forage. There was some variation seen amongst the samples for the expected relationship between % change in particle size and grinding energy required. A possible explanation for the variation could be due to the varying concentrations of chemical components which affect fragility negatively, such as cellulose. For example, if a large particle (IPS) has a low fragility due to the concentration of chemical components which affects forage fragility negatively, more energy will be required to break down that particle into smaller particle sizes (FPS). How these specific interactions and the results obtained will change with various larger and smaller particles, with different concentrations of specific chemical components and relationships, still requires investigation.

It is evident that the chemical composition of forages has an influence on forage fragility. However, currently only possible effects and assumptions can be made as to the extent of this influence due to the simultaneous and multi-factorial influence on forage fragility. The results for ADL prove that the association between forage fragility and NDF digestibility is not a simple concept, as ADL may not have been significant as an individual component; however, its relationship with other variables has proven interesting. It can be concluded that the fragility of forage can be better explained from regression models compared to using only individual chemical components as variables.

More research is needed to investigate the relationship between chemical components and factors influencing forage fragility for better prediction of forage fragility as a parameter in nutritional models. It is clear that NDF alone cannot be used to accurately predict the effect of the forage component of the diet as related to chewing activity, rumen environment, and ME available for animal production. While this study has indicated a possible association between NDF digestibility and forage fragility, further research

is needed to better understand the specific factors relating to particle fragility, as there is some variation and limited data on the relationship between chemical composition and rate of particle size reduction. Further research is also needed to investigate the association between NDF digestibility and forage fragility and factors that could influence this association. What is clear from this study is that differences in fragility exist between and within forage groups and these differences need to be accounted for in models that predict feed intake, digestion and performance of ruminants.

CHAPTER 6

6. CRITICAL EVALUATION

Forage fragility could be measured more accurately if all possible factors affecting the chemical composition of forages are kept similar for all samples, such as maturity level of the plants and environmental factors such as fertilizer application, harvesting time, cutting and drying methods, climate and soil composition.

It is clear that the results for forage fragility are influenced by the type of mill used during grinding of the samples and direct energy measurements, and it is evident that not all mills are equally efficient in grinding forages, as the purpose for which each mill was manufactured differs. There is no extensive literature to support the effectiveness of the knife mill, as was used in this study; therefore the possible use of a wider range of mills for grinding of samples may improve the accuracy of measurement of forage fragility. Other possible mills to make use of are the ball mill and hammer mill. In addition to this, it may be useful to use maize silage in its original form (fresh or wet) as this is the form in which it is fed to dairy cows. However, the risk of using wet silage is the lack of accurate readings for grinding energy. There is the potential for a specialized mill to be used for this analysis.

For this study, a major limitation was the lack of a homogenous initial particle size. Obtaining a homogenous initial particle size, perhaps through sieving or some other method, will vastly improve the reliability of studies similar to this one.

In vitro predictions of forage fragility through NDF digestibility at different time points is a complex process and can cause large variations in the measurements. Multiple repetitions may help to improve the accuracy of these measurements. In addition, when attempting to explain forage fragility for regression equations, the presence of CP and starch may have been useful to include to compare the effects of these two components compared to cell wall fractions and uNDF or kd, even within groups.

Actual chewing activity measured *in vivo* will provide valuable information for comparing efficiency of *in vitro* predictions of forage fragility with relation to specific chemical components of

forage. This could aid in determining which mills are the most accurate for providing measurements that correlate to chewing activity of cows and therefore, the most accurate and useful estimation of energy used during grinding of forages.

Precision feeding tools such as measuring chewing activity is currently becoming more readily available and more cost effective too. These such tools should be an integral part of studies such as described in this dissertation.

The presence of CP and starch would have been useful to compare the effect of these two components compared to cell wall fractions and uNDF or kd, even within groups.

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APPENDIX A

Table A1 Total Mixed Ration composition fed to the cannulated cows used in the study

Feed component	Kg/day (as fed)
Eragrostis curvula hay	3.80
Water	20.22
M.sativa hay	7.4
Molasses meel	2.00
UP Milkmaster Anthon R7080 ELO	15.49

Table A2 Undigested NDF (uNDF) values across 240 hours *in vitro* digestion

	Chemical Analyses (%)										
Roughage sample	**uNDF 0h**	**uNDF 6h**	**uNDF 12h**	**uNDF 18h**	**uNDF 24h**	**uNDF 36h**	**uNDF 48h**	**uNDF 72h**	**uNDF 96h**	**uNDF 120h**	**uNDF 240h**
Medicago sativa 1	100	85.00	84.33	71.23	64.50	63.68	59.19	53.29	48.47	48.44	49.50
Medicago sativa 2	100	82.49	79.53	62.86	56.70	51.01	48.96	45.54	47.87	37.00	36.50
Medicago sativa 3	100	85.73	81.30	64.73	62.50	60.25	59.02	57.50	51.62	52.75	49.94
Medicago sativa 4	100	71.85	65.86	51.29	48.60	48.98	45.12	47.28	47.45	44.07	41.54
Medicago sativa 5	100	92.80	83.27	78.56	74.79	71.33	64.98	64.12	52.35	51.54	50.73
Medicago sativa 6	100	92.77	82.15	77.71	69.61	63.50	62.83	52.91	58.81	52.75	45.34
Medicago sativa 7	100	97.15	81.21	77.50	71.46	68.08	65.37	61.79	55.57	54.30	48.66
Medicago sativa 8	100	92.68	86.86	81.19	76.17	60.89	57.73	56.42	55.47	53.45	49.82
Medicago sativa 9	100	92.72	87.33	80.22	77.46	69.66	70.34	63.31	64.74	56.73	49.65

Medicago sativa 10	100	94.86	78.23	76.24	72.15	70.17	70.00	69.62	64.17	62.26	55.14
Medicago sativa 11	100	98.28	89.25	83.42	73.11	67.45	62.44	59.23	56.26	56.94	54.17
Medicago sativa 12	100	93.95	85.43	78.12	75.79	69.74	65.22	61.23	56.55	56.29	51.15
Medicago sativa 13	100	91.68	89.90	73.94	64.11	57.70	56.56	54.70	43.50	50.32	45.28
Maize silage 1	100	96.08	77.01	57.99	50.14	48.83	39.89	38.55	34.80	28.32	20.56
Maize silage 2	100	95.78	88.20	56.89	49.87	45.09	41.43	34.46	29.99	30.15	22.29
Maize silage 3	100	91.43	77.50	63.54	48.94	41.50	36.32	33.50	33.73	31.11	26.96
Maize silage 4	100	92.50	88.29	77.32	66.46	62.28	46.72	42.00	40.18	35.55	26.36
Maize silage 5	100	94.26	90.01	83.93	67.89	58.85	53.54	47.36	44.69	42.11	36.82
Maize silage 6	100	97.57	77.91	67.76	55.69	50.26	44.00	40.73	35.82	30.00	26.25
Maize silage 7	100	93.80	88.84	73.72	59.09	53.43	45.76	39.59	33.51	37.00	34.15
Maize silage 8	100	97.34	86.48	71.93	62.25	49.66	44.00	34.99	26.31	26.34	25.33
Maize silage 9	100	93.58	92.22	68.84	53.37	41.08	36.58	33.49	30.94	22.79	19.62
Eragrostis curvula 1	100	93.45	86.01	82.04	79.45	74.52	62.01	50.70	48.99	43.50	34.00
Eragrostis curvula 2	100	97.02	89.23	83.71	83.46	82.07	75.78	61.54	45.00	46.50	45.51
Eragrostis	100	95.98	83.17	78.74	72.53	65.42	56.00	48.95	39.99	39.46	32.63

curvula 3											
Eragrostis	100	91.32	80.09	76.64	71.60	68.36	55.99	46.19	46.67	33.50	26.52
curvula 4											
Eragrostis	100	98.23	92.57	89.20	85.22	81.47	67.98	61.05	53.44	44.01	39.99
curvula 5											
Eragrostis	100	96.47	95.20	82.57	70.91	61.87	53.29	50.29	46.13	39.17	25.65
curvula 6											
Eragrostis	100	98.22	97.20	86.32	79.19	75.10	63.82	50.31	45.40	38.45	30.16
curvula 7											
Eragrostis	100	96.81	90.09	83.50	71.79	61.44	59.04	57.32	53.54	50.39	37.79
curvula 8											
Eragrostis	100	97.68	91.00	84.54	62.60	57.45	56.93	46.98	44.53	42.24	39.55
curvula 9											
Eragrostis	100	98.74	96.75	84.99	78.33	74.73	64.38	57.24	52.09	48.20	35.23
curvula 10											
Eragrostis	100	99.12	97.77	87.53	81.18	77.98	71.92	62.56	55.33	49.39	33.65
curvula 11											
Eragrostis	100	99.00	89.39	83.97	78.26	62.65	57.00	47.06	41.00	38.12	31.36
curvula 12											
Eragrostis	100	98.90	94.64	85.08	83.33	69.02	62.41	44.73	43.43	40.96	28.38
curvula 13											

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