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Developing a procedure to measure grinding energy of forages as a predictor of forage fragility.

Elfriede Prinsloo

Submitted in partial fulfilment of the requirements for the degree

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

In the Faculty of Natural & Agricultural Sciences

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CONTENTS

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DECLARATION

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

Elfriede Prinsloo

SIGNATURE: ……

DATE: August 2014

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“Nie met mag en krag sal jy slaag nie, maar deur my Gees, sê die Here die Almagtige”

Sagaria 4:6

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SUMMARY

Developing a procedure to measure grinding energy of forages as a predictor of forage fragility

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The structural organization of plant organs and tissues determine the intake potential through the ease of forage particle breakdown, the nature of the particles produced as well as the rate of passage from the rumen. The cell wall content of forages influences the amount of energy required for chewing, and accounts for a considerable proportion of the total energy requirement. In the past, neutral detergent fibre (NDF) has been used as the only feed characteristic to predict the filling effects of forages, but there is substantial evidence that NDF alone is inadequate to make these predictions. Forage fragility is defined as the relative rate at which the particle size of forages are reduced during processes such as chewing or milling, and forage fragility might be related to lignin concentration and digestibility, as well as to anatomical differences among plant species. The physical characteristics of feedstuffs are not measured regularly, and these physical characteristics in relation to their nutritional properties should be taken into account for more precise feed formulation. Through the measurement of grinding energy, the possibility exists to predict forage fragility as related to the chemical composition of forages, which could lead to improved predictions of animal chewing activity and energy usage during the process of chewing. In order to investigate the possibility of developing a model for the prediction of forage fragility, twenty eight different forage samples were collected from 11

different locations. Samples included legumes, C3- and C4- grasses. Dried samples were analysed for various chemical components, as well as 24-hour *in vitro* NDF digestibility (ivNDFd) and rate of NDF degradation (NDFkd). Dried samples were pre-cut with a knife mill, fitted with a 2 cm screen, after which particle size distribution for each sample was determined using a Retsch Sieve shaker. Ten g duplicate samples were milled with a laboratory hammer mill and an ultra-centrifugal mill, both fitted with a 1 mm screen, for the measurement of grinding energy. 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 2 cm samples were milled with the knife mill again, fitted with a 1 mm screen, after which particle size distribution was determined again to analyse change in particle distribution for each forage sample. The results of this study indicated that dry matter, nitrogen, *iv*NDFd, NDFkd and initial particle size (IPS) can all be associated with increased forage fragility, as there was a decrease in energy usage during grinding with an increase in any of the aforementioned components. The acid detergent fibre (ADF), NDF, total phenols (TP), non-tannic phenols (NTP), as well as the % change in particle size can all be associated with decreased forage fragility, as there was an increase in energy usage during grinding with an increase in any one of these components. 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 inconclusive in this study. Literature on energy requirement for milling operations of forages is inadequate. Grinding energy is related to the stem mechanical properties (such as maximum cutting force and stem shear strength), and physical properties (such as stem diameter, DM density and moisture content). The use of grinding energy has the potential be a practical and useful measure to predict forage fragility, however, the relative contribution of factors such as original particle size, shape, surface area, morphology and many other factors toward the fragility of forages is difficult to predict. More research is needed on the prediction of forage fragility before it can be incorporated as a meaningful input into nutritional models such as NRC, CNCPS and AMTS.

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADL	Acid detergent lignin
AMTS	Agricultural Modelling and Training Systems
BW	Body weight
CF	Crude fibre
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
CPM	Cornell-Penn-Miner
DM	Dry matter
DMI	Dry matter intake
dNDF	Digestible neutral detergent fibre
eNDF	Effective neutral detergent fibre
Esc	Specific energy consumption
FPS	Final particle size
IPS	Initial particle size
ME	Metabolisable energy
N	Nitrogen
NDF	Neutral detergent fibre
*iv*NDFd	24-hour *in vitro* NDF digestibility
NDFkd	Rate of NDF digestion
NDSC	Neutral detergent soluble carbohydrates
NFC	Non-fibre carbohydrates
NPN	Non- protein nitrogen
NRC	National Research Council

NSC	Non-structural carbohydrate
NTP	Non-tannic phenols
OM	Organic matter
Pef	Physical effectiveness factor
peNDF	Physical effective neutral detergent fibre
PSPS	Penn State Forage Particle Size Separator
RUP	Rumen undegradable protein
RVI	Roughage value index
RVU	Roughage value unit
SD	Standard deviation
SEM	Standard error of the mean
SER	Standard error of regression
TMR	Total mixed ration
TP	Total phenols
TT	Total tannins
VFA	Volatile fatty acids

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UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

LIST OF TABLES

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

LIST OF FIGURES

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

CHAPTER 1

1. GENERAL INTRODUCTION & MOTIVATION

Fibre represents an important constituent of animal feeds, and represents the portion of feeds that is bulky and difficult to digest (Mertens, 2002a). The rate of digestion of fibre is influenced greatly by the source of fibre (Chesworth, 1992). The character and nutritional value of roughage is determined by two factors: the proportion of cell wall and the grade of lignification thereof (Van Soest, 1982). Because the cell wall represents the most indigestible portion of forages, the digestibility and composition of the cell wall may comprise the factors most limiting to animal production on high-forage diets (Van Soest, 1994), as cited by Casler & Jung (2006).

Chewing activity is a response that reflects the chemical and physical properties of feeds (NDF, particle size, intrinsic fragility and moisture), and chewing activity is also a function of the type, size or age and DMI of the animal (Mertens, 1997). With decreasing quality of roughages, energy requirement for eating and ruminating can increase from 10% to 30% of the metabolisable energy (ME) contained in the feedstuff (Susenbeth *et al.*, 1998). The energy required for the activity associated with eating and ruminating can account for one-third of the ME in low-quality roughages, which leads to a significant reduction of the proportion of ME available for maintenance and production (Susenbeth *et al.*, 1998). Because chewing activity/kg DM is not a constant feed characteristic, it is difficult to use chewing activity as a direct input in diet formulation (Mertens, 2002c). The capacity of ruminants to reduce feed particle size mechanically can be a factor limiting intake (Bosch *et al.*, 1992), as cited by Susenbeth *et al.* (1998). The fact that energy needed for chewing reduces the amount of ME available for production, is mainly due to the lower efficiency of utilization of ME of roughages as compared to other feedstuffs (Orskov & MacLeod, 1990), as cited by Susenbeth *et al.* (1998).

Many reviews on digestion kinetics of cell wall carbohydrates have been published, addressing the problems associated with the estimation of kinetic parameters (Raffrenato *et al*., 2009). Simulation with the CNCPS model can demonstrate the importance of rate and extent of NDF digestion on OM and NDF digestibility (Tylutki *et al*., 2008). Results from such simulation models clearly demonstrate profound effects of the parameters as described by Tylutki *et al.* (2008) on digestibility and therefore on the supply of energy and microbial protein (Raffrenato *et al*., 2009)

The physical effective neutral detergent fibre (peNDF) system has been widely used in the CPM-Dairy (Conrnell, Penn, Miner) and CNCPS (Cornell Net Carbohydrate and Protein System) ration formulation models to predict the effect of forage particle size on cow chewing response and rumen pH

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UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

(Grant, 2010). The method proposed by Mertens (1997) for the estimation of peNDF was based on the assumption that the fragility or ease of particle size reduction during chewing is similar among different sources of NDF and explains all the variation elicited in chewing response (Grant, 2010). However, this assumption is not always correct, since forages of similar particle length and different forages or diets with similar peNDF values may elicit substantially different chewing rates per kilogram of DM. This potential variation in chewing responses has important implications for nutritional models that incorporates peNDF values and assumes that every unit of peNDF is equal, regardless of the source of NDF (Grant & Cotanch, 2005). Feeds that are equal in digestibility but have different NDF concentration will promote different intakes (MacDonald *et al.*, 2002). The differences observed in chewing responses might 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 (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.

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) reported that there is a positive relationship between NDF digestibility and forage fragility. 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 digestibility of the forage cell wall may be predictive of forage fragility (Grant, 2010). There is a need for better understanding of the factors relating to particle fragility (Allen, 1996).

The purpose of this study was to investigate the possibility of developing and proposing a procedure to predict forage fragility through the measurement of grinding energy of various forage samples, and by relating the chemical composition and the digestibility of the forages to the energy usage values obtained during grinding. Relationships between chemical composition and forage fragility can lead to improved predictions of animal chewing activity, as well as determining which chemical fractions are mainly responsible for differences in forage fragility, and therefore lead to differences elicited in chewing responses and differences in energy available to the animal for maintenance and production. Differences in fragility may then contribute to the prediction of intake for forages of different quality. This would provide valuable information to predict productivity of the animals more accurately.

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CHAPTER 2

2. LITERATURE REVIEW

2.1 INTRODUCTION

Roughages are described as edible but bulky, coarse plant material, which have 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). Roughages are a cheap and readily available source of energy for ruminants, and have a very important role in ruminant nutrition, as green and leguminous roughages constitute up to two-thirds of the total feed requirement for ruminants (Animal Husbandry, Booklet nr. 33). In addition to other nutrients, ruminants also have a requirement for fibre, and roughages are responsible for establishing the biphasic nature of the rumen, to stimulate chewing and influence the rate of passage (Lammers *et al.*, 1996). Plant fibre is a biological unit, rather than a chemical entity, and is found in the cell walls of plants (Van Soest, 1982). Plant cell walls have a complicated chemical composition consisting of lignin, cellulose, hemi-cellulose, pectin, and a quantity of protein, lignified nitrogen compounds, waxes, cutine and mineral components (Dickenson *et al.*, 2007). Cell walls comprise 20% - 80% of forage dry weight (Wilson, 1994). Lignin is made up of a group of compounds, and contains a considerable amount of nitrogen (crude protein) which is not accessible to animals (Dickenson *et al.*, 2007). The character and nutritional value of roughage is determined by two factors: the proportion of cell wall and the grade of lignification thereof (Van Soest, 1982). There is a difference in the properties of lignin between different grasses, and up to now, there are no specific chemical analyses for these qualities and therefore it cannot be accurately determined in the laboratory (Dickenson *et al.*, 2007). Developments such as secondary wall thickening in certain types of cells, deposition of lignin and cross- linking lignin to cell wall polysaccharides are most often the characteristics of cells which limit the readily available energy to ruminants (Wilson, 1994).

The feeding value of forages is related to the quality of the forage (Horrock & Vallentine, 1999). The intake potential and energy availability in forage crops are limited by the cell wall concentration and digestibility of the forage (Jung & Allen, 1995). The structural tissues in leaves and stems of forages give rise to the large fibre particles in the rumen which are resistant to particle size reduction through mastication and digestion, and there is a negative correlation between these structural tissues and forage intake (Wilson, 1994). Adequate particle length of forages is necessary for proper ruminal function, and reduced particle size

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YUNIBESITHI YA PRETORIA

has decreased the time spent chewing and has tended to decrease ruminal pH (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 longer time in the rumen to achieve this critical size, then the feed will generally have a low intake and result in low animal performance (Wilson, 1994).

Fibre effectiveness is a nutritional concept that can only be measured truly by animal response (Mertens, 2002c). Balch (1971) proposed that chewing activity (eating + ruminating time) per unit of DM could be an indication of the physical properties of the feed, which the author then called the fibrousness characteristic. Sudweeks *et al.* (1979, 1981) standardized the procedure under which chewing activity was measured and defined a roughage value index (RVI) for a variety of feeds as the minutes of total chewing time per kilogram of DM. Norgaard (1986) used chewing activity measured under standardized conditions to assess the physical structure of feeds for dairy cows. Norgaard (1986) based the system on the type of feed (physical structure group) and particle size (degree of chopping or grinding). Sauvant *et al*. (1990) defined the fibrosity index of a feed as the minutes of chewing activity per kg of DM. Sauvant *et al*. (1990) observed that the fibrosity index was highly correlated with the crude fibre concentration and the level of feed intake.

NDF consists mainly of lignin, cellulose and hemicellulose, and can be used as a measurement of the plant cell wall content (MacDonald, 2002). 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, 2002). It is known that different sources of NDF of similar particle size may elicit variable chewing responses (Grant, 2010). The consequence of this relationship is that foods which are equal in digestibility, but different in NDF content, will promote different intakes (MacDonald, 2002), as well as differences in energy needed for consumption of the forage and reduction of the forage particle sizes to achieve the critical particle size. According to Beauchemin (1991), the potential exists to formulate diets for cattle based on NDF, which represents the slowly digestible cell wall fraction of feeds, however, NDF has been used in the past to predict the filling effects of forages, but there is substantial evidence that NDF alone is not adequate to make these predictions (Allen, 1996). The filling effects of forages are influenced by the initial particle size, particle fragility, and the rate and extent of NDF digestion (Allen, 1996).

The general term “size reduction” includes cutting, crushing, grinding and milling (Henderson & Perry, 1976). 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). The term “milling” is used relative to the reduction of grain into meal

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or flour. Milling is an over-all process including size reduction, hulling, scarifying, polishing, sorting, mixing, and in some instances, chemical reactions (Henderson & Perry, 1976).

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). 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 attempts have been made in the past to establish relationships and models between the chemical and physical characteristics of forages and their potential intake by ruminants, as well as their effect on the level of production, however, results still remain inconclusive.

2.2 ROUGHAGE – IMPORTANCE IN RUMINANT DIETS

It is well known that ruminants require sufficient amounts of fibre of adequate particle length for optimum rumen function (Yang *et al.*, 2001a). The term roughages include all cultivated pastures; natural grazing veld, crop residues, silage and hays, and all of these have characteristically high fibre concentration, with their digestibility lower than 60% and a crude fibre concentration of more than 18% (Van Soest, 1982). The nutritional value of some of these roughage sources tend to decrease with maturation (Dickenson *et al.*, 2007). The crude protein value of roughages can vary as much as 25% in young, succulent, well fertilized pastures, to less than 4% in poor winter pasture and wheat straw (Van Soest, 1982). Voluntary intake of forages is a critical factor determining animal performance, and there is a negative correlation between cell wall concentration and intake in ruminants consuming a high- forage diet (Jung & Allen, 1995). Cell walls affect intake by contributing to ruminal fill (Jung & Allen, 1995).

According to Yang *et al.* (2001a), many attempts have been made to describe the fibre requirements of dairy cows, but it is difficult since adequate long fibre has not been clearly defined. NDF is used as an indication of the cell wall content or fibre fraction of feeds. Cell walls represent the most indigestible portion of forages, and the composition and digestibility of the cell wall may comprise the factors most limiting to livestock production where diets contain high amounts of fibre (Casler & Jung, 2006). Lowered effective fibre levels in the diet result in simultaneous- and linked reactions occurring, which ultimately results in lowered ruminal fermentation (Mertens, 2002c). Less effective fibre in the diet leads to lowered chewing activity by the animal, and less chewing results in less salivary buffer secretion into the rumen (Mertens, 2002c). Decreased salivary buffer production as well as increased volatile fatty acid (VFA)

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UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

concentrations result in a lowered pH (Mertens, 2002c). The rumen microbial populations change due to the lowered rumen pH, and there is a shift in the end products of fermentation as the acetate to proprionate ratio is reduced (Mertens, 2002c). This change in acetate to proprionate ratio is associated with milk fat depression and the shunting of nutrients towards fattening (Mertens, 2002c).

However, it is not only the lowered salivary buffer secretions that cause a drop in the pH. There are changes which occur in the ration when fibre is decreased, and results in a higher VFA production (Mertens, 2002c). The concentrations of crude protein (CP), ether extract (EE) and ash remains relatively stable in dairy rations (Mertens, 2000). As the fibre concentration in the ration decrease, the concentrations of non-fibre carbohydrates (NFC) or neutral detergent soluble carbohydrates (NDSC) increase (Mertens, 2002c). 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 buffers, result in a decrease in rumen pH, as most of the carbohydrates of NFC are completely and rapidly digested (Mertens, 2002c). Therefore, the interaction between peNDF and NFC may affect the requirements for effective fibre (Mertens, 2002c). Although both NDSC and NFC play a role in milk fat and ruminal pH, research suggests that it is the lack of effective fibre that is most often responsible for milk fat depression and borderline acidosis (Mertens, 2000). The NRC (1989) recommends a minimum of 25% to 28% fibre, which is measured as NDF, of which 75% of the total dietary NDF is supplied by forages. This approach of the NRC however does not take the difference in particle sizes of various forages or the contribution of fibre from concentrates into account (Yang *et al.*, 2001a). Marten (1985), as cited by Sheaffer *et al.* (1998) stated that forage quality is best evaluated by measuring dairy performance, but that we describe forage quality in terms of nutritive value, intake potential and anti- nutritional components. According to Van Soest (1982), there are a number of factors that influence the quality of forages and fibrous feedstuffs. As plants age, its nutritive value generally declines with maturity, and these changes are due to altered chemical composition which involves increased lignification and decreased proportion of leaves to stems (Van Soest, 1982).

According to Sheaffer *et al*. (1998), plant maturity is the most important factor that affects forage quality, and the changes in quality is 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). However, Van Soest (1982) stated that there are exceptions to this generalization, since not all leaves are more digestible than stems. In the case of maize for example, the nutritive value does not decline with age and maturity, but the nutritive value of stover may decline (Van

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Soest, 1982). In the case of grasses, leaves have a structural function, while the stems function as a storage organ, which can lead to stems having a higher nutritive value than the leaves (Van Soest, 1982). The quality of stems is influenced by the diameter and whether it is hollow or filled with a pith (Van Soest, 1982). 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 (Van Soest, 1982). Individual forages vary in the degree to which they decline in nutritive value with age, as the maturity of the plant and the environment differs between different forages (Van Soest, 1982).

There is very little variation in the nutritional value of immature grasses, but this change as soon as grasses begin to mature (Van Soest, 1982). Morphological variation between different forages are caused by the environment, species and time of year, and has big influences on the proportion of leaves and stems and therefore the quality of forage available to livestock (Sheaffer *et al*. 1998). During the maturation process of plants, there is an increase in a group of compounds which are known as lignin (Chesworth, 1992). 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 (Chesworth, 1992). Lignin is not considered to have any nutritional value, but instead, can have detrimental effects on the digestion of other feed components (Chesworth, 1992).

2.3 INFLUENCE OF CARBON PATHWAY ON DIGESTIBILITY AND COMPOSITION OF FORAGES (TROPICAL VS. TEMPERATE SPECIES)

There are two basic types of photosynthetic pathways that occur in plants (Kellems & Church, 2010). Grasses have photosynthetic pathways that are either C3 - species (C3- Calvin cycle pathway), which is a carbon-fixing pathway most efficient in cool growing seasons (temperate and high-altitude environments), or C4-species (C4- dicarboxylic acid pathway), which is a carbon-fixing pathway most efficient in warm tropical growing seasons (Milton, 2004). These differences in climatic growing conditions lead to anatomical differences between the C3-species and C4-species grasses (Milton, 2004). The difference between the two pathways is the intermediate compound that is formed (Kellems & Church, 2010). C4- grasses use nitrogen more efficiently than C3- grasses, and most of South Africa's grass species are of the C4-species, whereas some of the annual invasive alien species as well as most invasive perennial species are C3-species (Milton, 2004). In warm tropical climates, grasses mature more quickly, and its protein and phosphorus concentrations decrease to very low levels, while its fibre concentration increases (MacDonald *et al.*, 2002). Caswell *et al.* (1973) presented a hypothesis with some preliminary support evidence that in general, plants which possess the C4- dicarboxylic acid pathway of carbon fixation are a

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UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

poorer quality food source for herbivores than plants which possess the C3- Calvin cycle pathway, and added that herbivores tend to avoid C4- species where possible.

Tropical grasses (C4) generally have a lower digestibility than temperate grasses (C3), because its leaves have a higher vascular bundle concentration, and hence a higher lignin concentration, as well as a high concentration of dense masses of cells that resist invasion by microbes (MacDonald *et al*., 2002). In C4 - tropical grass leaves, it is the cell types with thickened secondary walls, such as the vascular bundles, sclerenchyma strands; epidermis and parenchyma bundle sheaths which contribute to low nutritional value (Wilson, 1993) as cited by Wilson (1994). These cell types form solid, multicellular blocks of cells which makes up the large particles in the rumen which requires rumination for breakdown, and are usually poorly digested due to lignification and difficulty of microbial accessibility to cell wall surfaces (Wilson, 1994). In temperate grasses, the main storage carbohydrate is fructan, which is the most abundant soluble carbohydrate, and mainly occurs in the stem (MacDonald *et al.*, 2002). Tropical- and subtropical grasses store carbohydrates mainly as starch, and is found mainly in the leaves (MacDonald *et al.*, 2002). Wilson (1994) reported that more than 50% of the reserve carbohydrates and protein of C4 - leaves are contained in specialized bundle sheath cells, and because these cells are thick and digested slowly, these nutrients are not available to ruminal microbes. Within C4- species, there is an additional barrier in the form of a thin, suberized layer within the outer section of the bundle sheath, which prevents microbial access to the inner secondary wall and the cell contents until cells are broken by chewing (Wilson, 1994). These specialised bundle sheath cells do not occur in C3- species (Wilson, 1994).

According to Kellems & Church (2010), grasses adapted to temperate climatic regions (C3-type), store more carbohydrates than C4-type grasses. Wolfson & Tainton (2000) reported that there are clear anatomical and ultrastructural differences between the C3- and C4- plants. Grasses that have the C4-type photosynthetic pathway, generally have lower protein concentration than C3-type grasses, and a larger portion of the carbohydrates in C4-type grasses are structural carbohydrates (cellulose and hemicellulose), causing C4-type grasses to have lower digestibility values and feeding values than C3-type grasses (Kellems & Church, 2010). French (1957), as cited by Wilson & Hacker (1987) observed that tropical grasses generally have a low digestibility which the author associated with the high fibre and lignin content of the grasses. Wilson *et al.* (1983) reported that C3- grasses had a high dry matter digestibility and low cell wall content, whereas C4- grasses had a low dry matter digestibility and a high cell wall content. 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.

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UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

2.4 FACTORS INFLUENCING NUTRITIONAL VALUE OF FORAGES

Many factors have been reported to influence the nutritional value of forages. Feeding value is related to the quality of the forage (Horrocks & Vallentine, 1999).

Plant species, plant anatomy and morphology

There are well known species and cultivar differences in forage quality of grasses (Collins & Casler, 1990, as cited by Cheeke, 1991). Legumes are often associated with higher daily animal response as a result of rapid digestion of consumed dry matter, a higher density of the rumen liquor, and a lower retention time in the animal (Horrocks & Vallentine, 1999). Grasses again may be favoured in a mixture with legumes or planted alone to meet more stressful environments, enhanced stand longevity and reducing agronomic or plant- animal management requirements (Horrocks & Vallentine, 1999). Tropical grasses have a lower nutritional value than temperate grasses (Cheeke, 1991). In temperate areas, grasses tend to grow and mature at a relatively slow rate, and can therefore be utilised at an early growth stage while its nutritive value is still high (MacDonald *et al.*, 2002). In tropical areas, grasses mature more rapidly, and its protein and phosphorus concentration fall to very low levels with increasing fibre levels (MacDonald *et al.*, 2002). The growth habit of pasture species also influences the value of the herbage (MacDonald *et al.*, 2002). Most tropical grasses exhibit continuous stem elongation and flowering throughout the growing season, which leads to a low leaf: stem ratio (Cheeke, 1991). This leads to lowered palatability as well as digestibility of the grass (Cheeke, 1991).

Leaves make up the most nutritional and digestible parts of plants, and there is a positive association between leafiness in pasture plants and forage quality (Cheeke, 1991; MacDonald *et al.*, 2002). Generally, leaves have a higher protein and energy concentrations, lower fibre and higher digestibility than stems (Cheeke, 1991). The anatomy of the leaves of tropical grasses differ from that of temperate grasses, as tropical grasses have more vascular bundles and thick-walled bundle sheaths, and therefore more lignin, and the mesophyll cells in the central tissue of the leaf is more densely packed than those in temperate grasses (MacDonald *et al.*, 2002).

Stage of plant development

The stage of plant maturity is a very important factor influencing the nutritional value of forages. As plants grow, there is an increased need for fibrous tissue to maintain their structure, and this causes the main structural carbohydrates (cellulose and hemicellulose) and lignin to increase, with a decrease in protein concentration (MacDonald *et al.*, 2002). This leads to the inverse relationship that exists between protein and fibre contents of a given species, although this relationship is upset with the application of nitrogenous

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fertilisers (MacDonald *et al.*, 2002). As forages mature, its nutrient value starts declining, and forages become less able to meet the requirements of livestock and the need for supplemental feeding increase (Horrocks & Vallentine, 1999). Protein, phosphorus and vitamin A (in the form of carotene) follow similar patterns throughout the growth cycle, being high when plants are immature and declining when the plant reaches maturity (Horrocks & Vallentine, 1999). The digestibility of CP also declines with increasing maturity of the plant, and the calcium levels tend to drop only slightly from immaturity to maturity, as calcium levels are influenced much more by the calcium levels in the soil than the stage of maturity of the plant (Horrocks & Vallentine, 1999). The total ash contents also decreases with increasing plant maturity (MacDonald *et al.*, 2002). The basic determinant of digestibility of forages is plant anatomy (MacDonald *et al.*, 2002). The leaf to stem ratio also declines with increasing maturity, along with more rapid decline in the stems than in the leaves (Horrocks & Vallentine, 1999).

Soil factors and fertilizers

The type of soil has a great impact on especially the mineral content of the pasture (MacDonald *et al.*, 2002). When there is a deficiency of a mineral in the soil, plants will limit their growth as well as reduce the concentration of the element in its tissues (MacDonald *et al.*, 2002).

Soil acidity is another important factor that can influence the uptake of trace elements by plants (MacDonald *et al.*, 2002). The application of fertilisers has a great effect on the mineral status of plants, and it is also known that the application of nitrogenous fertilisers increase the leaf area as well as the rate of photosynthesis in plants (MacDonald *et al.*, 2002). A consequence of this is increased levels of protein, amide and nitrate levels in the plants (MacDonald *et al.*, 2002). The application of nitrogenous fertilisers also depresses the water-soluble carbohydrate concentration of temperate grasses, which can lead to an adverse effect on fermentation if the crop is preserved as silage (MacDonald *et al.*, 2002). Fertilisers may affect the nutritive value of sward indirectly by altering the botanical composition (MacDonald *et al.*, 2002). Increasing the nitrogen levels of soil may have minimal effect on levels of N in grass-legume stands unless the legume component is substantially reduced (Horrocks & Vallentine, 1999). The application of nitrogenous fertiliser to enhance the soil N levels will not alter the cellulose or CF content, lignification or digestible energy unless the stage of growth at which the stand is utilised is changed (Horrocks & Vallentine, 1999).

Environmental and climatic factors (Weather)

Climate and season may influence the nutritive value of pasture (MacDonald *et al.*, 2002). The concentration of sugars and fructans can be influenced greatly by the amount of sunshine received by the plant (MacDonald *et al.*, 2002). Rainfall and temperature are both related to the soil moisture content, and affect the forage quality markedly (Horrock & Vallentine, 1999). High environmental temperatures, or

temperature increase above the optimum for a specific plant species, lead to increased lignification of both the leaves and stems, and reduces forage digestibility (Cheeke, 1991, Horrocks & Vallentine, 1999). In cloudy days, the soluble carbohydrate content of plants will be lower than in sunny days (MacDonald *et al.*, 2002).

Day length has a similar effect on forages as high temperatures. When days are short and nights long, total photosynthesis of plants is reduced (Cheeke, 1991). In spring, grass is generally of a higher nutritional value than autumn grass, because of the higher soluble carbohydrate and amino acid content (Cheeke, 1991). Rainfall can affect the mineral composition of forages, and during droughts, certain minerals such as calcium tend to accumulate in the plant (MacDonald *et al.*, 2002).

Grazing and harvesting systems

In continuous grazing systems, where animals are kept on the same area of pasture throughout the year, the ideal stocking rate will allow a perfect balance between new plant growth and it's harvesting by animals, where the animals are supplied by young nutritious herbage on a continuous basis (MacDonald *et al.*, 2002). However, the rate of plant regrowth usually exceeds the rate of harvesting, leading to the accumulation and maturation of herbage, reducing the nutritive value of the material (MacDonald *et al.*, 2002). If grazing pressure is too high, pasture plants are so denuded of foliage that its root reserves are depleted and they fail to regrow (MacDonald *et al.*, 2002). Both under- and over-grazing of pastures may lead to changes in its botanical composition and the nutritive value of the pasture (MacDonald *et al.*, 2002).

Diseases and insect damage

Diseases and insects that prey on forage plants can cause severe reduction in the quality and quantity of harvested forage (Horrocks & Vallentine, 1999). The nutritive value is often reduced greatly, specifically protein and carotene, and the palatability falls sharply (Horrocks & Vallentine, 1999).

2.5 PARTICLE SIZE AND CHEWING ACTIVITY

Changing the particle size of roughages has two main effects on digestion (Chesworth, 1992). Firstly, it decreases the time associated with decreasing individual particle sizes by rumen degradation so that particles can pass through the reticulo-omasal orifice, and secondly, it increases the overall surface area of particles available for microbial attack (Chesworth, 1992). Poppi *et al.* (1980) reported that feed particles retained on a 1.18 mm sieve during the wet sieving technique, had a high resistance to passage from the rumen, which led to the assumption made by Poppi *et al.* (1980) that feed passing through the 1.18 mm screen would not stimulate any chewing, and therefore the physical effectiveness factor (pef) of the feed

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YUNIBESITHI YA PRETORIA

would be equal to the proportion of DM retained on sieves with apertures larger than 1.18 mm, using vertical shaking (Mertens, 2002c). However, Oshita *et al.* (2004) presented evidence that the critical size for escape from the rumen of non-lactating dairy cows was larger than the 1.18 mm sieving fraction and closer to the size of particles retained on a 3.35 mm sieve. This means that although the peNDF system has been firmly established using the 1.18 mm sieve for the measurement of pef, the critical size for cattle might be closer to a fraction of particles that would be retained on a 3.35 mm screen (Grant & Cotanch, 2005). Weimer *et al.* (2009) reported that only particles less than 2 mm in size can readily pass from the rumen through the omasum which acts as a filtering device. Zebeli *et al.* (2007) stated 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 screen from 37.5% to 42.0%, and also led to an increase in rumination time by 100 minutes/day. There was also an increase in the consistency of the ruminal mat (Zebeli *et al.*, 2007). Zebeli *et al.* (2007) added however, 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.

The time spent masticating and the intensity of the mastication can be used quantitatively to rank feeds according to their biological fibrousness (Norgaard *et al.*, 2011). The biological fibrousness of feeds is closely related to the stimulation of salivation, the frequency of rumen motility and the formation of a stable flowing layer system in the reticulo-rumen (Norgaard *et al.*, 2011). Mertens (1997) defined chewing activity as a response reflecting the chemical and physical properties of feeds (NDF, particle size, intrinsic fragility and moisture), and stated that chewing activity is also a function of the type, size or age, and DMI of the animal. Chewing activity is influenced by breed, size, age, DMI, fibre concentration, feed particle size, and to some extent also by the method of measurement of the chewing activity (Mertens, 2002c). Because chewing activity/kg DM is not a constant feed characteristic, it is difficult to use chewing activity directly to formulate rations (Mertens, 2002c). Susenbeth *et al.* (1998) reported that additional heat is produced during chewing which can be solely attributed to eating activity. They also reported that eating increased heat production by 0.28 KJ/second or 0.25 KJ/chew, and when relating heat production to BW or metabolic BW, heat production was increased by 27 J/(min.kg BW) or 133 J/(min.kg $BW^{0.75}$) respectively (Susenbeth *et al.*, 1998). With an increase in NDF concentration of the diet, there is an increase in chewing per kilogram NDF (Mertens, 2002c). Therefore, chewing activity will be stimulated more when mature, high fibre forage is fed in comparison to a young, low fibre forage (Mertens, 2002c). 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, 2002c). 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.

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Both the physical and chemical characteristics of dairy rations are important, and therefore physically effective fibre attempts to take both physical and chemical properties of fibre, which influence the chewing activity, into account (Mertens, 2002c). Chewing activity is important for the stimulation of secretion of salivary buffers to control rumen pH, and is also an important indicator of the physical environment of the rumen which promotes optimal rumen fermentation (Mertens, 2002c). Mertens (2002c) reported that recent 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 (2002c) 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 the NDF that is retained on each of the sieves by the amount of chewing it should stimulate, but more research is needed to relate chewing activity to particle size, before the weighing factors for the particles on each sieve can be determined.

Mertens (1986) observed that chewing activity was related to NDF content, as well as particle size of a feed. Because the term roughage implies both a feed texture and a fibre value, Mertens (1986) proposed a roughage value unit (RVU) system based on the NDF content of feeds, to measure the effectiveness of different feeds to stimulate chewing activity. 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, 2002c). The RVU system were 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 roughage value adjustment factor (0-1) which was based on effectiveness, chewing activity and particle size (Mertens, 2002c). Mertens (2002c) 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 roughage 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, 2002c). In Table 2.1, 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.

Chewing activity is normally expressed as minutes per day or per kilogram of DM or NDF (Yang *et al*., 2001b). Yang *et al.* (2001b) found that chewing time was increased with high forage: concentrate ratio diets, because of the increased eating and ruminating times, but that it was decreased when expressed per kilogram of NDF intake from forage. Yang *et al.* (2001b) stated that forage particle length or grain processing did not have such a big influence on chewing activity as the forage: concentrate ratio. It was

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reported that there was a positive correlation between chewing activity and the proportion of long forage particles in the diet but not to particle length of the diets, and Yang *et al.* (2001b) added that the influence of feed particle size on the particle size distribution in different sites of the digestive tract was minimal (Yang *et al.* 2001b). Yang *et al.* (2001b) stated that there was no significant relationship between chewing activities and ruminal pH or fractional passage rate of rumen contents.

Table 2.1 Physiological effects on dairy cows with varying forage and fibre proportions in the rations (Adapted from Mertens, 2000)

Variable	% long grass hay in the diet					
	100	**80**	**60**	**40**	**20**	**0**
% NDF	70	59	48	36	25	14
% peNDF	70	57	44	32	18	6
Chewing time (min/d)	1080	1040	970	820	520	320
Saliva 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
Ruminal 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

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. In the study done by Beauchemin (1991), the results indicated that the effect of the ruminant was highly significant for the chewing activities measured during the study, as the results indicated a high degree of variation among animals of similar ages within a herd.

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2.6 PHYSICAL EFFECTIVE FIBRE AND NDF

Neutral detergent fibre (NDF) is the residue remaining after extraction when boiling forages in a neutral solution, and comprises of cellulose, hemicellulose, lignin and ash (Chaves *et al.*, 2002; MacDonald *et al.*, 2002). The neutral detergent fibre fraction is regarded as a measure of cell wall material, or the fibre fraction of feeds (Chaves *et al.*, 2002).

The concept of physical effective fibre was introduced by Mertens in 1997, which relates the physical characteristics of feeds to the rumen pH through the measurement of particle length or chewing activity (Yang *et al.*, 2001a). Mertens (1997) defined peNDF as the fraction of NDF that stimulated chewing and contributed to a ruminal digesta mat, and proposed a standard laboratory method for the measuring of peNDF. The method 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 the 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).

Mertens (1997) also proposed another method which involves the multiplication of the proportion of particles (not the proportion of NDF) retained on the 1.18 mm sieve by the NDF content of the sample to obtain peNDF (Grant & Cotanch, 2005). In Table 2.2, peNDF values for various feeds are presented, which were estimated using the method that was proposed by Mertens in 1997 (Mertens, 2002c). The primary limitation to laboratory measurement of peNDF is that methods for measuring particle size have not been standardized (Mertens, 2000).

Mertens (1997) based his method on 3 assumptions: 1) the NDF is uniformly distributed across all particle size fractions, 2) chewing activity elicited is similar for all particles retained on the 1.18 mm sieve, and 3) the fragility (ease of particle size reduction during chewing) is similar among sources of NDF (Grant & Cotanch, 2005). The second assumption made by Mertens could be addressed further by using additional sieves to characterize the particle size distributions and the relation to chewing and other responses to each size fraction more clearly (Grant & Cotanch, 2005). The third assumption however, needs further investigation (Grant & Cotanch, 2005). Mertens (1997) plotted ruminal pH against dietary peNDF, proposing that the peNDF concept and concluding that to ensure a ruminal pH of 6.0, the NDF in the diet should be maintained at or above 21%, which has since been the reference value for dietary peNDF (Cotanch *et al.*, 2007). An important aspect is to determine what the critical particle size for passage from the rumen is, and also then which fraction of particles will remain in the rumen for stimulation of chewing activity (Grant & Cotanch, 2005).

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

Feed	pef[a]	DM retained on > 1.18 mm	NDF	peNDF[b]
Standard	1.0	1.0	100	100.0
Grass hay, long	1.0	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	0.40	0.18	46	8.3
Maize, ground	0.40	0.48	9	4.3
Soya bean meal	0.40	0.23	14	3.2
Soya bean hulls	0.40	0.03	67	2.0
Rice mill feed	0.40	0.005	56	0.3

[a]Physical effectiveness factors (pef) based on chewing activity

[b]peNDF = DM retained on > 1.18 mm screen x NDF

Note: Vertical shaking motion was used to separate particles

The physical effective neutral detergent fibre (peNDF) 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). The peNDF is therefore related to the fibrousness characteristic as proposed by Balch (1971), the roughage value index as proposed by Sudweeks *et al.* (1979, 1981), the physical structure as proposed by Norgaard (1986), and the fibrosity index as proposed by Sauvant *et al*. (1990), as all of these are related to chewing activity (Mertens, 2002c). However, peNDF differs from these concepts as it is a feed characteristic which is measured on a fixed scale (0 to 1) and reference value (long grass hay containing 100% NDF), and not simply a biological response, measured as chewing minutes/ DM which varies with the conditions under which it was measured (Mertens, 2002c).

The peNDF concept was originally defined from an analytical perspective as the proportion of the original sample NDF found in particles that were left over on a 1.18 mm sieve when a sample was sieved using vertical shaking method (Cotanch & Grant, 2006). The peNDF provides a more consistent measure of effective fibre than chewing activity, as effective fibre is independent of differences between animals, and is based on two fundamental properties of feed, namely fibre and particle size (Mertens, 2002c).

Variations caused by animals and experimental differences are minimized because pef are fractions in which the animal effects in the numerator and denominator cancel out (pef = [min. of chewing/kg NDF in test feed] / [min. of chewing/kg NDF in long grass hay]) (Mertens, 2002c). Therefore, pef is a proportional change in the chewing response that is expected, and should be relatively constant among ruminants (Mertens, 2002c).

Grant & Cotanch (2005) reported that many nutritional models used in the dairy industry today require peNDF as a key input for the model to predict lactational response. Both the CPM-Dairy and CNCPS (Cornell Net Carbohydrate and Protein System) ration formulation models incorporate peNDF to predict the effect of forage particle size on cow chewing response and rumen 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, which makes 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. Reid e*t al.* (1988) stated that there is a significant effect of forage class (legumes, C3- or C4- grasses) on the slope and intercept for regressions of DMI on NDF for both cattle and sheep, which indicates that the filling effect of NDF varies with different forages.

2.7 RELATIONSHIP BETWEEN CHEWING ACTIVITY AND NDF CONTENT

MacDonald *et al.* (2002) stated that feed intake is more closely related to the rate of digestion than to digestibility *per se*, although these two measures are related to one another. This means that foods which are digested more rapidly are highly digestible, leading to high intakes by animals (MacDonald *et al.*, 2002). MacDonald *et al.* (2002) reported that the primary chemical component in foods which determines the rate of digestion of the food is neutral detergent fibre, which is a measure of the cell wall content. This leads to the negative relationship between NDF content of foods and the rate of digestion (MacDonald *et al.*, 2002).

Animals ruminate in proportion to the cell wall content of their diet (Van Soest, 1982). Cell wall characteristics associated to the anatomical structure of plants influence the ease of breakdown (comminution), through the processes of chewing and digestion, and also the size and shape of the resulting particles, which in turn influences the rate of passage from the rumen (Wilson, 1994). Van Soest (1982) defined comminution as the physical breakdown of particulate matter involving reduction in size and volume. Dairy rations should generally contain at least 25% NDF, and a large proportion of dietary NDF should be supplied through forages (NRC, 1989). The concentration of NDF in the diet is closely related to chewing activity, and is a determinant of rumen pH of dairy cows (Allen, 1997), which is why dairy rations

are often formulated to a specific NDF content (Oba & Allen, 1999). However, there is a wide variation in NDF content of forages, depending on the species, maturity and growing environment (Oba & Allen, 1999).

The digestibility of NDF is an important parameter of forage quality, as it varies widely in its degradability in the rumen, thereby affecting animal performance (Allen & Oba, 1996). Beauchemin (1991) reported that the chewing activity increased from 767 min/day to 796 min/day and 853 min/day as the fibre concentration was increased from 31% to 34% and 37%. The author added that there was a linear decrease in rumination time adjusted for fibre intake from 59.0 min/kg NDF to 54.2 min/kg NDF as there was as increase in fibre intake, and the rumination time was higher for early bloom hay than for mid bloom hay (57.3 min/kg NDF vs. 55.5 min/kg NDF) (Beauchemin, 1991). These results therefore suggest that chewing activity can be determined from the NDF content of the diet (Yang *et al.* 2001b). It is reported that chewing time per kilogram of NDF fraction intake for either ruminating or eating time was less for high NDF diets than for low NDF diets (Beauchemin, 1991). It has therefore been concluded that although there was a positive correlation between total chewing time and NDF fraction intake, the chewing time per unit of NDF fraction intake might decrease as the dietary NDF content increases (De Boever *et al.*, 1990). This suggests that the efficiency with which forage stimulates chewing activity increases when the forage is present in small amounts in the diet (Yang *et al.*, 2001b).

Tables 2.3 and 2.4 represent the results from the study done by Beauchemin (1991) relating NDF concentration of the diet to the number of chews and chewing activity. Grant (1997), as cited by Yang *et al.* (2001b) proposed that cows may feature an adaptive mechanism during which rumination is less efficient (less chews per kilogram of NDF fraction intake) when fed low forage diets. Oba & Allen (2000) stated that eating time either per day or per kilogram of DMI was greater for cows when fed high NDF diets compared to low NDF diets.

Table 2.3 Effects of dietary NDF concentration and hay quality on daily chewing activities (Adapted from Beauchemin, 1991)

	NDF					
	31%		34%		37%	
Chewing activity	Early	Mid	Early	Mid	Early	Mid
min/day						
Eating	367	311	381	361	447	387
Ruminating	402	434	423	427	443	429
Total chewing	769	765	804	788	889	816
min/kg DM						
Eating	16.2	14.8	17.4	16.1	21.0	17.1
Ruminating	17.8	19.5	19.3	19.0	21.0	18.9
Chewing	33.9	34.3	36.7	35.1	41.9	36.0
min/kg NDF						
Eating	51.5	46.5	52.3	46.1	58.4	45.7
Ruminating	56.6	61.4	57.7	54.5	57.7	50.7
Chewing	108.1	107.9	109.9	100.6	116.2	96.4
min/kg hay NDF						
Eating	114.6	124.7	85.6	85.0	72.4	68.8
Ruminating	126.0	164.5	93.1	100.4	71.4	76.2
Chewing	240.5	289.2	178.8	185.4	143.8	145.0

Early = Harvested in early bloom stages

Mid = Harvested in midbloom stage of maturity

Generally, as forage matures, there is a decrease in leaf to stem ratio, and an increase in the lignification of the cell walls (Beauchemin, 1991). This leads to an increase in the NDF concentration, and a decrease in the NDF digestibility, which can lead to a decrease in milk production (Beauchemin, 1991). The increase of concentrate in the diet therefore acts to compensate for decreased milk production due to the negative effect of lowered forage quality (Beauchemin, 1991). According to Beauchemin (1991), the effects of differences in forage qualities due to different maturities can be minimized by formulating diets for specific NDF concentrations.

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Table 2.4 Effects of dietary NDF concentration and hay quality on number of chews (Adapted from Beauchemin, 1991)

	NDF					
	31%		34%		37%	
Variable	Early	Mid	Early	Mid	Early	Mid
Chews/day (x 10^3)						
Eating	25.72	22.70	27.83	24.96	33.86	27.21
Ruminating	25.03	27.53	26.36	26.79	28.19	27.07
Chews/g DM						
Eating	1.13	1.01	1.26	1.11	1.58	1.20
Ruminating	1.10	1.23	1.20	1.19	1.33	1.19
Chews/kg NDF						
Eating	3.61	3.16	3.79	3.18	4.42	3.22
Ruminating	3.52	3.87	3.57	3.40	3.67	3.20
Chews/kg hay						
Eating	8.02	8.46	6.17	5.86	5.48	4.84
Ruminating	7.85	10.37	5.75	6.28	4.55	4.80

Early = Harvested in early bloom stages

Mid = Harvested in midbloom stage of maturity

2.8 FORAGE FRAGILITY AND PARTICLE SIZE REDUCTION

In general, forage fragility is defined as the relative rate at which the particle size of a forage is reduced during chewing or some laboratory simulation of chewing action (Grant, 2010). The rate of particle size reduction is largely dependent on rumination rate, chewing efficiency and cell wall fragility (Allen & Mertens, 1988). The fragility of particles is probably related to the intrinsic characteristics of the cell wall, as well as to weakening of the cell wall during microbial fermentation (Allen & Mertens, 1988). The differences in forage fragility affect the rate of particle size breakdown and retention time in the reticulo-rumen (Poppi *et al.*, 1981; McLeod & Minson, 1988), as cited by Allen (1996). 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 DM intake are not always consistent. The peNDF system is based on the assumption that forage particle size explains all the variation elicited in chewing response (Mertens, 1997). However, this assumption is not always correct, since forages of similar particle length may elicit substantially different chewing times per kilogram of DM, and these differences in chewing responses might be attributed to differences in forage fragility or stem brittleness (Grant, 2010).

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Fibre content and the amount and nature of lignification are the characteristics responsible for affecting particle size breakdown during ingestive mastication and rumination, which influence the suitability of particles for ruminal exit (Minson, 1990). Mechanical energy is needed for the breakdown of materials and particle size reduction, and also to overcome the friction between moving parts in the machine (Ghorbani *et al.*, 2010). Although the rate of digestion and intake can be related to the concentration of cell wall in the feed, the physical form of the cell walls also affect intake (MacDonald *et al.*, 1995). The mechanical grinding of roughages partially destroys the structural organization of cell walls, leading to acceleration of the breakdown of these cell walls in the rumen and therefore increasing feed intake (MacDonald *et al.*, 1995). The fragility of the particles of a forage influences the filling effect in the rumen, as well as the rate and extent of NDF digestion (Allen, 1996). Differences in fragility of forage particles can affect the rate of particle size breakdown, as well as retention time in the reticulo-rumen (Poppi *et al.*, 1981; McLeod & Minson, 1988), as cited by Allen (1996), which will in turn affect intake by the animal.

Grant (2010) measured forage fragility as the rate of particle size reduction of a feed when exposed to ball milling, and stated that forage fragility can lead to improved predictions of chewing responses when in combination with measurements of particle sizes. There have been several suggestions reported for the measurement of forage particle fragility (Grant, 2010). Troelson & Bigsby (1964) suggested a mechanism to specifically examine forage particle size distribution after artificial mastication. More recently, two methods used more commonly 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). Casler *et al.* (1996) used a ball mill to grind forages and developed a particle size reduction index based on the findings of the study done by the authors. The percentage of particles that passed through a 1 mm screen upon milling was considered to be an index of forage fragility (Casler *et al*, 1996). According to Troelson & Campbell (1968), particle size and shape highly relates to the feed intake, digestion and metabolic products of ruminants. There is little data available reporting 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 (Allen, 1996). There is a need for greater understanding of the factors relating to particle fragility (Allen, 1996).

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2.9 INFLUENCE OF FORAGE FRAGILITY ON DIGESTIBILITY OF FORAGES

The influence of cell wall characteristics on nutritional value of forages, specifically with regards to low forage digestibility and the difficulty of particle breakdown has clearly become more evident (Wilson, 1994). Growth environment significantly influences the content and digestibility of cell walls in forage (Wilson, 1982), as cited by Wilson (1994). Akin (1986), as cited by Allen & Mertens (1988) observed that plant tissues differ in rates of disappearance, which suggested that tissue morphology also affects the accessibility and rate of digestion. The digestibility of grasses is influenced greatly by the ratio of leaves: stems, and as plants mature, there is an increase in the proportion of stems and a decrease in the proportion of leaves (MacDonald *et al.*, 2002). Allen & Mertens (1988) reported that digestibility is directly related to the fraction of fibre that is potentially digestible. The relationship between NDF content of feeds and its digestibility can be described very clearly when comparing legumes and grasses. At an equal digestibility, legumes contain less cell wall than grasses and consumed in quantities about twenty percent higher than grasses (MacDonald *et al.*, 2002). Another difference between legumes and grasses is that the lignification in legumes is generally restricted to the vascular bundles, whereas in grasses the lignification is more widely distributed, resulting in a greater inhibitory effect on digestion of grasses (MacDonald *et al.*, 2002).

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). Tropical plants more specifically produce fibres that are broken down very slowly in the rumen (Chesworth, 1992). Various attempts have been made in the past to correlate the digestibility of forage plants with their chemical composition, especially with regard to the cell wall residues (crude fibre, normal acid fibre, lignocellulose), or the more clearly defined cell wall contents such as lignin and cellulose (Chenost, 1966). Many reviews on digestion kinetics of cell wall carbohydrates have been written, addressing the problems associated with the estimation of kinetic parameters (Raffrenato *et al*., 2009). Simulation with the CNCPS model can demonstrate the importance of rate and extent of NDF digestion on OM and NDF digestibility (Tylutki *et al*., 2008). Results from the simulation models clearly demonstrate profound effects of the parameters as described by Tylutki *et al.* (2008) on digestibility and therefore on the supply of energy and microbial protein (Raffrenato *et al*., 2009).

Grant (2010) reported that there is a positive relationship between NDF digestibility and forage fragility. 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. Therefore, the digestibility of the forage cell wall may be predictive of forage fragility (Grant, 2010). In Table 2.5, the digestibility and fragility values for different grass hays used during a study by Grant (2010) are illustrated.

Table 2.5 Digestibility and fragility values obtained from grass hays used during chewing study (Adapted from Grant, 2010)

	Hay A	Hay B	Hay C	Hay D
24-h IVNDFD[1], % of NDF	31.4	43.7	54.8	47.3
120-h IVNDFD, % of NDF	49.3	60.2	74.1	65.4
Indigestible NDF, %	50.7	39.8	25.9	34.6
Fragility, %	46.2	30.0	80.7	63.9

[1]In vitro neutral detergent fibre digestibility

Figure 2.1 illustrates the results of the change in forage fragility versus the 24- hour *in vitro* NDF digestibility for a range of forages, as reported by Grant (2010). The results from the study indicate that there is a trend for forage fragility to increase linearly as the *iv*NDFd (%) increases (Grant, 2010). According to Grant (2010), the potential exists to combine a "fragility factor", which is related to NDF digestibility, 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.

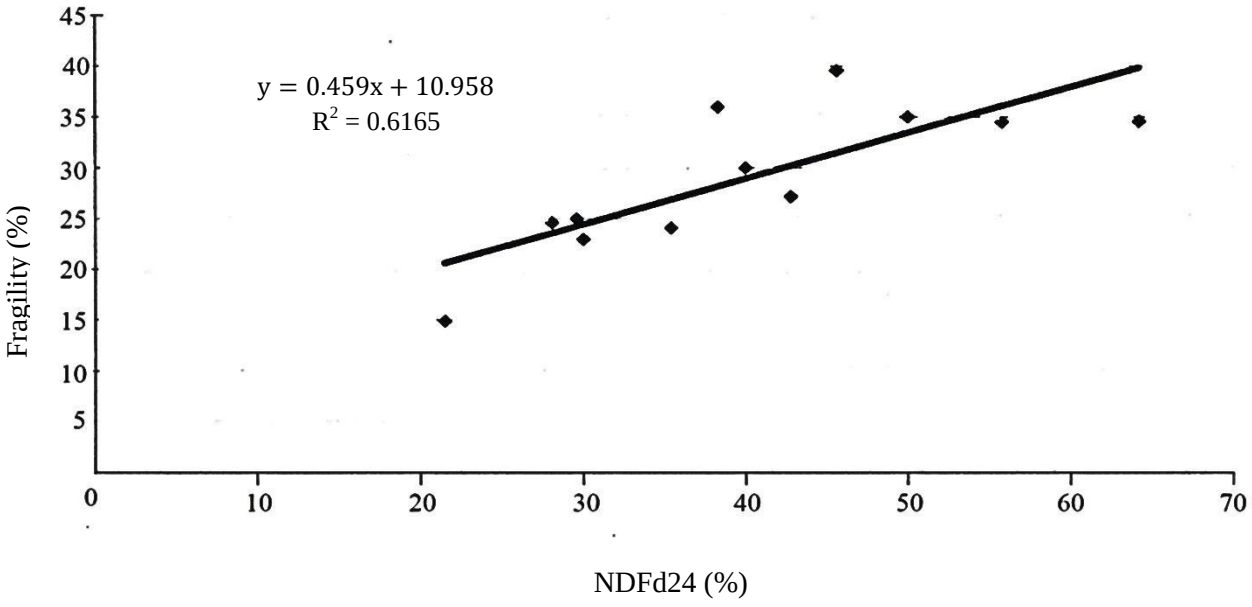

Figure 2.1 Relationship between the 24-hour *in vitro* NDF digestibility of various forages and the fragility of the forages as measured by change in physical effectiveness factor following ball milling (Adapted from Grant, 2010)

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2.10 ENERGY COST ASSOCIATED WITH EATING FOR RUMINANTS

The amount of energy required for chewing accounts for a considerable proportion of the total energy requirement, and the amount of energy required for chewing reduces the amount of ME available for production (Susenbeth *et al.*, 1998). It can therefore impact the level of productivity to a great extent, since the ME in roughages is utilized with a much lower efficiency than other feedstuffs (Susenbeth *et al.*, 1998). The energy cost of eating varies greatly between different feeds, especially when related to the amount of feed ingested (Susenbeth *et al.*, 1998). More time is spent eating and ruminating grass than legumes (Moseley & Dellow, 1985) as cited by Galyean & Goetsch (1993). As the quality of roughages decrease, the requirement of energy for eating and ruminating can increase from 10% to 30% of the ME provided to the animal in the feedstuff (Susenbeth *et al.*, 1998). The efficiency of ME utilization declines with increasing maturity of forages, because of the decreased forage N concentration and the increased amount of indigestible residue passing through the gut (Minson, 1990). The capacity of ruminants to reduce feed particle size mechanically can be a factor limiting intake (Bosch *et al.*, 1992) as cited by Susenbeth *et al.* (1998). When intake levels are normal, most cows will typically ruminate 10-13 hours per day (Weimer *et al.*, 2009). In cattle, approximately all mastication occurs during rumination, rather than during eating (Weimer *et al.*, 2009). Grazing cattle will normally take ~ 3 x 10^4 bites per day during eating (Weimer *et al.*, 2009). Mertens (1997) reported that cows receiving long hay devoted approximately 100-200 min/kg fibre intake per day to total chewing (eating and ruminating), or approximately 50-150 min/kg DM per day. Cows that received mixed rations with grains, concentrates and forages chewed substantially less (Mertens, 1997). Weimer *et al*. (2009) stated that the large expenditure of time on physical pre-treatment (total chewing) does not relate to a large expenditure of energy, since almost all mastication in cattle occurs during rumination rather than eating.

Susenbeth *et al.* (1998) measured energy cost of eating in steers, and found a value of 133 J/min/kg body weight$^{0.75}$, which was similar than the value found by other researchers, namely 124 J/min/kg body weight$^{0.75}$. Susenbeth *et al.* (1998) also measured rumination energies and reported a much lower energy cost to the animal (36 J/min/kg bodyweight$^{0.75}$) than for eating. It has been reported that for steers fed high quality grass hay, 18 kJ/MJ of the metabolizable energy intake was spent on rumination (Susenbeth *et al.*, 1998). Aikman *et al*. (2008) measured eating and rumination times in dairy cattle and reported that for high-producing cows that was fed 88% forage during the dry period, the energy for rumination per unit of ME intake was similar to that reported for steers as reported by Susenbeth *et al.* (1998). Lactating cows fed 62% forage devoted energy to rumination that was approximately equal to 1% of ME intake (Susenbeth *et al.*, 2008). Based on the values as reported by Susenbeth *et al.* (1998), energy spent on rumination compares favourably with the mechanical energy required to grind forages (Weimer *et al.*, 2009).

2.11 MEASUREMENT OF FORAGE FRAGILITY AND DIRECT MECHANICAL ENERGY

Comminution is defined as the breaking, chopping or grinding of larger objects into smaller particles (Yancey *et al.*, 2013). Bovine rumination involves the tearing or shearing of leaves and stems, specifically longitudinally, to increase the surface area available for microbial attack (Weimer *et al*., 2009). Grinding energy is described by Yancey *et al.* (2013) as the actual energy (work) going into the grinding process per unit quantity of processed material, including drive chain inefficiencies, electrical power factor losses and friction. The chemical composition of forage has a major influence on the physical and milling properties of cell wall (Van Soest, 1982). Laredo & Minson (1973) reported that the energy needed for grinding 1g of tropical grass stems with 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.

According to Cotanch *et al.* (2007), there has recently been the development of a ball milling method to assess the fragility of a wide range of forages. The method consisted briefly of milling dried samples with a ball mill loaded with ceramic balls, which is suggested to mimic the grinding action of the molar teeth of the animal, after which the forage samples were sieved using the standard method for the measurement of pef prior to ball milling (pef_1), and again following ball milling (pef_{RM}) (Cotanch *et al.*, 2007). The fragility is then determined as the change in pef value (the proportion of particles ≥ 1.18 mm sieve as determined by dry vertical sieving) of the ball-milled forage from the original sample using the following equation:

$$(pef_1 - pef_{RM})/pef_1 \times 100\% \qquad \text{(Equation 2.1)}$$

A fragility value of 100% would indicate that the forage is highly fragile (brittle), and the particle size was completely reduced to less than 1.18 mm. A fragility value of 0% would indicate a very hard forage, and that there was no reduction of particle size upon ball milling, with $pef_1 = pef_{RM}$ (Cotanch *et al.*, 2007).

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 (Arthur *et al.*, 1982). Arthur *et al.* (1982) stated that in general, the specific energy requirement tended to decrease with an increase in grinding rate. During the experiments done by Arthur *et al.* (1982), two tub-grinders (W.H.O. unit and Medallion unit), as well as

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a Ro-Tap test sieve system to determine particle size distribution. The authors reported that between rice straw, wheat straw and maize stover, corn stover had the highest heating value (17.24 MJ/kg), and rice straw the lowest (16.63 MJ/kg) as determined by a bomb calorimeter (Arthur *et al.*, 1982).

Bitra *et al.* (2009a) defined total specific energy (MJ/Mg) as size reduction energy to operate the hammer mill plus that imparted to biomass, and effective specific energy as energy imparted to biomass. In the study done by Bitra *et al.* (2009a), mechanical energy was measured during size reduction using a hammer mill for switchgrass, wheat straw and maize stover. Bitra *et al.* (2009a) 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 characterized 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 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.*, 2009a).

Mani *et al*. (2004) reported that energy requirement showed a rapid increase with a decrease in particle size. The authors observed that switchgrass required the highest effective specific energy to grind with a hammer mill of 99 MJ/Mg, while maize stover had a lower effective specific energy requirement of 40 MJ/Mg, both using a 3.2 mm screen (Mani *et al.*, 2004). Mani *et al.* (2004) reported that for the grinding of switchgrass, the specific energy usage was 93 - 245 KJ/kg of DM, depending on the extent of grinding (screen size) and the moisture content of the sample (Mani *et al.*, 2004). Mani *et al*. (2004) estimated mechanical energy indirectly by using a Wattmeter to monitor the electric motor.

Hammer mills have been given merit because of its 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 produces 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).

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Lopo (2002) reported that the ratio of particle size distribution of the material before and after grounding, moisture content, bulk and particle densities, feed rate of the material and machine variables determine the energy requirement of grinding the biomass. Himmel *et al.* (1985) reported that total specific energy for size reduction of wheat straw using a hammer mill with a 1.6 mm screen was twice that for a 3.2 mm screen. During the study done by Himmel *et al.* (1985), electric power was measured indirectly using a wattmeter, correcting for power factors, but not accounting for motor efficiency.

Fang *et al.* (2000) examined the energy requirements for the milling of wheat with a roller mill, and reported that kernel hardness had the most significant effect on energy and power requirements, and 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).

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.

In the past, research was aimed at measuring energy indirectly. Balk (1964) used a wattmeter to relate hammer mill total specific energy with the moisture content and feed rate of coastal Bermuda grass. Balk (1964) reported that the moisture content and grind size influenced the specific energy. Samson *et al.* (2000) reported that the total specific energy requirement of switchgrass hammer milling was 162 MJ/mg with the use of a 5.6 mm screen. Jannasch *et al.*(2001) reported energy usage of 14.9, 55.9 and 74.5 KWh/tonne for course chopping, fine grinding and pelleting on a wet basis, respectively. It was reported that total specific energy for hammer mill grinding of maize increased from 17 to 46 MJ/mg when hammer tip speed was increased from 54 to 86 m/s for a 6.4mm thick hammer (Agriculture Canada, 1971). Arthur *et al.* (1982) reported that total specific grinding energy required decreased from 2696 to 1181 MJ/mg with an increase in screen size from 12.7 to 50.8 mm for wheat straw bales using a tub grinder, and that the grinding rate increased from 0.137 to 0.267 mg/min with an increase in the screen size from 12.7 to 50.8 mm.

It is reported that high speed hammer mills fitted with smaller diameter rotors are efficient at grinding fine and hard material; however, as the material moves around the mill parallel to the screen surface at high tip speeds, the openings are only partially effective (Von Bargen *et al.*, 1981). At slower speeds, the material impinges on the screen at a greater angle, causing greater amounts of coarser feed to pass through (Von

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Bargen *et al.*, 1981). The operation speeds of the hammer mill seem to be critical to find the appropriate effective specific energy demand for biomass size reduction (Bitra *et al.*, 2009a).

The nominal biomass particle sizes produced during hammer mill grinding depend on various operating factors of the mill itself (Bitra *et al.*, 2009a). Himmel *et al.* (1985) observed that the retention of ground wheat straw on a 60 mesh screen decreased when the hammer mill screen size was decreased from 3.2 to 1.6 mm, which indicated the skewing of the particle size distribution curve to finer sizes. Yang *et al.* (1996) used the data from alfalfa hay grinded by a hammer mill, and fitted the particle size distribution data to a log-normal distribution curve with a median size of 238 µm and a standard deviation of 166 µm. Yang *et al.* (1996) reported that the size of the alfalfa grind that was used during a study done by the authors, varied with the size of the screen that was used in the hammer mill. Yang *et al.* (1996) added that it also changes with other factors such as moisture content, type of blade assembly, screen wear and hammer rotational speed. Mani *et al.* (2004) determined sieve based particle size distribution of hammer milled wheat and barley straws, corn stover, and switchgrass. The authors reported a positive skewness in distribution for various hammer mill screens for the particle size distribution of corn stover (Mani *et al.*, 2004).

Sieves have a long history and acceptance in various industries and provide a standardized format for the measurement of particle sizes, even with published values of offset (Bitra *et al.*, 2009a). Bitra *et al.* (2009a) 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 corn 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).

2.12 MATHEMATICAL MODELS PREVIOUSLY USED TO EXPRESS SPECIFIC ENERGY REQUIREMENT DURING PARTICLE SIZE REDUCTION PROCESSES

There are many different theories and models previously used to quantify the energy usage during the process of grinding, with each process characterising size reduction in a different way (Naimi, 2008). Walker *et al.* (1937), and Earle & Earle (1983), as cited by Naimi (2008) suggested the following equation:

$$dE = -K_{L^n}^{dL} \qquad \text{(Equation 2.2; Naimi, 2008)}$$

Where: dE = the differential energy required

L = the particle size

K = constants

n = constant. The value of n depends upon three theories on particle breakage: the Rittinger theory, the Kick theory and the Bond theory (Naimi, 2008).

The Rittinger theory:

In 1867, the Rittinger theory was introduced (Bond, 1961) as cited by Naimi (2008). The hypothesis of this theory is that the work done for grinding and crushing is directly proportional to the new surface area produced (Naimi, 2008). Based on this theory, the surface area of the feed and product has to be calculated (Naimi, 2008). The theory assumes that the energy input is completely transferred to the surface area of the ground particle (Naimi, 2008). Equation 2.3 and 2.4 illustrate the Rittinger theory as published by Naimi (2008) and Ghorbani *et al.* (2010). The Rittinger theory made the assumption that the size reduction is essentially a shearing procedure and consequently, the energy requirement is proportional to the new surface created, which in turn are proportional to the square of a common linear dimension, so the value of *n* in Equation 2.2 would be 2 (Henderson & Perry, 1976).

$$E = C_R\ (L_2|L_1)$$ (Equation 2.3; Ghorbani *et al.*, 2010)

or: $$\Delta E = C_R(\frac{1}{L_p} - \frac{1}{L_f})$$ (Equation 2.4; Naimi, 2008)

Where: C_R = the Rittinger constant

L_1 = the initial screen opening size in mm

L_2 = the final screen opening size in mm

L_p = product particle size

L_f = feed particle size

The Kick theory:

Kick's theory was introduced in 1885 (Bond, 1961) as cited by Naimi (2008). In this theory, the assumption is made that the energy required to reduce particles of the initial size L_f, a size change of dL is directly proportional to the size reduction ratio dL/L (Naimi, 2008), or put in other words, the energy requirement is a function of a common dimension of the material, so the value of *n* in Equation 2.2 would be 1 (Henderson & Perry, 1976; Naimi, 2008). Equation 2.5 and 2.6 illustrate Kick's theory as published by Naimi (2008) and Ghorbani *et al.* (2010).

$$E = C_K \left({}^{1}/_{L_2} - {}^{1}/_{L_1}\right)$$ (Equation 2.5; Ghorbani *et al.*, 2010)

or: $$\Delta E = C_K ln \frac{L_f}{L_p}$$ (Equation 2.6; Naimi, 2008)

Where: C_K = the Kick's constant

L_1 = the initial screen opening size in mm

L_2 = the final screen opening size in mm

L_p = product particle size

L_f = feed particle size

The Bond theory:

In 1952, Bond introduced the third theory (Naimi, 2008). Bond's theory stated that the work input is proportional to the new crack tip length produced in particle (cracks first appears on the surface then penetrates in the volume), and is equal to the work represented by the product minus that represented by the feed (Naimi, 2008). Bond made the assumption that the value of *n* in Equation 2.2 is equal to ${}^{3}/_{2}$ (Henderson & Perry, 1976). The following Equations illustrate Bond's theory as published by Naimi (2008) and Ghorbani *et al.* (2010):

$$E = C_B \left(\frac{10}{\sqrt{L_2}} - \frac{10}{\sqrt{L_1}}\right)$$ (Equation 2.7; Ghorbani *et al.*, 2010)

$$W = \frac{10Wi}{\sqrt{L_{p,80\%}}} - \frac{10Wi}{\sqrt{L_{f,80\%}}}$$ (Equation 2.8; Naimi, 2008)

For practical calculations, the sieve size through which 80% of the experimental sample could pass (measured in microns), was selected as the criterion of particle size (Bond, 1961), as cited by Naimi (2008).

Where: C_B = the Bond constant

L_1 = the initial screen opening size in mm

L_2 = the final screen opening size in mm

$L_{p80\%}$ = diameter of sieve in microns through which 80% of product passes through

$L_{f80\%}$ = diameter of sieve in microns through which 80% of feed passes through

W = work input in kilowatt hours per short ton (U.S. ton, 2000 pounds = 907.185 kg)

W*i* = work index

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The work index is a parameter showing the resistance of the material to grinding (Naimi, 2008). It is defined as the kWh per short ton required for reducing the material from theoretically infinite feed size to 80% passing through 100 microns (Bond, 1961), as cited by Naimi (2008). If a material is homogenous for size reduction, the work index of that material will remain constant through all size reduction stages (Naimi, 2008).

Ghorbani *et al.* (2010) estimated the performance of hammer mills by measuring the energy requirement and the particle size distribution of the ground product (Ghorbani *et al.*, 2010). During the research done by Ghorbani *et al.* (2010), specific energy consumption (E_{sc}) was expressed as illustrated in Equation 2.9.

$$E_{sc} = \frac{\text{Net input electric energy (KJ)}}{\text{Weight of chopped alfalfa (kg)}} \qquad \text{(Equation 2.9)}$$

Ghorbani *et al.* (2010) determined the particle size distribution of the alfalfa chops, and thereafter they expressed the energy necessary for size reduction of the particles using Equation 2.10:

$$E = C \int_1^2 \frac{dL}{L^n} \qquad \text{(Equation 2.10)}$$

Where E is the specific energy consumption ($KJ.kg^{-1}$), dL is the differential size, L is the screen opening size (mm) and C and n are constants (Ghorbani *et al.*, 2010). The results from the study done by Ghorbani *et al.* (2010) are presented in Table 2.6.

Table 2.6 Specific energy requirement for grinding alfalfa chops (Adapted from Ghorbani *et al.*, 2010)

Sieve opening size (mm)	Geometric mean of chopped sample (mm)	Screen opening size of hammer mill (mm)	Geometric mean of ground sample (mm)	Average E_{sc} ($KJ.kg^{-1}$)
18	1.96	1.68	0.317	30.51^{a}+ (1.83)
		2.38	0.336	16.71^{d} ± (0.56)
		3.36	0.402	12.36^{e} ± (0.99)
		4.76	0.422	6.96^{h} ± (0.56)
15	1.68	1.68	0.317	25.89^{b} ± (0.55)
		2.38	0.336	14.01^{e} ± (0.97)
		3.36	0.402	10.78^{f} ± (0.22)
		4.76	0.422	6.67^{hi} ± (0.62)
12	1.53	1.68	0.317	20.61^{c} ± (0.42)
		2.38	0.336	10.63^{f} ± (1.15)
		3.36	0.402	8.73^{g} ± (0.42)
		4.76	0.422	5.65^{i} + (0.71)

Numbers in the parentheses are standard deviations; in final column, means with different letters are statistically different at 5% probability level.

Ghorbani *et al.* (2010) used the three models of Rittinger, Kick and Bond as described by Bond (1952, 1961), as cited by Naimi (2008), to estimate parameters from the specific energy requirement data obtained from the experiment done by the authors. Ghorbani *et al.* (2010) determined the accuracy of the fitted models by calculating the percentage errors of the predicted specific energy requirement with Equation 2.11.

$$\text{Relative error} = \frac{(\text{predicted}_i - \text{experimental}_i)}{\text{experimental}_i} \times 100 \qquad \text{(Equation 2.11)}$$

2.13 HYPOTHESIS

The hypothesis for this experiment is:

H_0: The procedure proposed was not suitable for predicting fragility across different forage species.
H_1: The procedure proposed was suitable predicting fragility across different forage species.

H_0: Differences in grinding energy requirement of forages cannot be related to specific chemical fractions.
H_1: Differences in grinding energy requirement of forages can be related to specific chemical fractions.

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. The forage samples were collected from regions in and around Gauteng and Kwazulu-Natal. 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. During the chemical analyses, *in vitro* NDF digestibility was determined using rumen fluid collected from 2 rumen cannulated cows at the University of Pretoria Experimental Farm. The second phase involved the measurement of forage fragility, where an ultra-centrifugal mill (Retsch, Model ZM 200, Retsch GmbH, Haan, Germany), from the University of Pretoria was used, as well as a hammer mill (Perten, Model 3100, Perten instruments, Hägersten, Sweden), from Afgri Labworld (Isando, Gauteng). 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 28 different forage samples were collected from 11 different locations (Table 3.1). After collection, all samples were sun-/air dried, after which sample preparation started. Samples were sun-/air dried to decrease particle shrinkage which takes place when forages are oven-dried.

Table 3.1 Summary of forage samples collected for study

Type of forage	Scientific Name	Carbon fixation pathway	Number of samples
Annual ryegrass	*Lolium multiflorum*	C_3	2
Blue buffalo grass/Foxtail buffalo grass	*Cenchrus ciliaris*	C_4	1
Cocksfoot	*Dactylis glomerata*	C_3	1
Guinea grass/Bushveld buffalo grass	*Panicum maximum*	C_4	2
Lucerne	*Medicago sativa*	Legume (C_3)	6
Maize stover	*Zea mays*	C_4	3
Perennial ryegrass	*Lolium perenne*	C_3	1
Rhodes grass	*Chloris gayana*	C_4	1
Smuts finger grass	*Digitaria eriantha* subsp. *eriantha*	C_4	4
Star grass	*Cynodon nlemfuensis*	C_4	1
Tall fescue	*Festuca arundinacea*	C_3	1
Weeping love grass	*Eragrostis curvula*	C_4	5
	TOTAL SAMPLES		28

3.2.2 Chemical analysis

A representative sample of each of the forages was milled using a Retsch ultra-centrifugal mill, fitted with a 1 mm screen, and analysed in duplicate for dry matter (DM), organic matter (OM), ash, starch, nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), 24-h *in vitro* NDF digestibility (*iv*NDFd), rate of NDF digestion (NDFkd) and plant secondary metabolites, such as total phenols (TP), non-tannic phenols (NTP) and total tannins (TT), using standard laboratory procedures.

3.2.2.1 Dry matter, organic matter & ash

All forage samples were analysed for DM according to AOAC (2000) procedure 934.01. Porcelain crucibles were placed into a 100 °C oven for approximately an hour to dry completely, after which the crucibles were allowed to cool in a desiccator and empty weights of the crucibles were recorded. Approximately 2 g of each milled sample was weighed into a porcelain crucible and placed into a 100 °C oven for 24 hours. The following day (after drying), the crucibles with dried samples were placed in a desiccator for 30 minutes to cool down and weighed immediately afterwards. Weights of individual empty porcelain crucibles were deducted from the weights of crucibles with test sample to determine the weight loss of the samples during drying. The DM of the sample was calculated as follows and values multiplied by 100 to be expressed as a percentage:

% Moisture = [(weight loss on drying (g)) / (weight of test sample (g)] x 100

% Dry matter = 100 - % Moisture

Organic matter concentration was calculated as the difference between DM and ash concentrations. Ash determination of samples was done in accordance to AOAC (2000) procedure 942.05. The ash concentration was determined by placing the dried sample from the DM analysis into a 550 °C muffle furnace for four hours. The porcelain crucibles were allowed to cool down in the oven for two hours after which it was placed in a desiccator. The crucible with the test sample ash was weighed, and the ash concentration of the sample was calculated by the difference of the crucible containing the ash with the dry sample, from the DM analysis, divided by the mass of the sample. Values were multiplied by 100 and expressed as percentage.

3.2.2.2 Starch

Samples were analysed in duplicate for starch concentration as described by the methods of MacRae & Armstrong (1968), Faichney & White (1983), and AOAC (1984) procedure 996.11. 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 30 ml distilled water was added to each tube. Tubes were then mixed carefully and placed in the autoclave for 2 hours at 120 °C. Samples were removed from the autoclave after it cooled down 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 them 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. Tubes were rinsed thoroughly with warm distilled water, adding to the volume in the 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 a Whatman no.2 filter paper, collecting the filtrate in sample bottles.

A 1 ml sample solution was diluted to 50 ml distilled water, after which 0.5 ml sample solution was added with 0.5 ml of the standard glucose solutions (5%, 10%, 25% and 50%) into 10 ml test tubes. Precisely 2.5 ml colour reagent was added into the test tubes, after which the tubes were placed in a dark room for 30 minutes. After 30 minutes, the reaction was stopped by adding 1 ml 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 as well as the DM concentration of the sample. The starch concentration was calculated as follows:

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

$$\% \text{ Starch} = (\text{Absorbance} - c) / x * 0.912 * 0.5 / \text{sample mass}$$

3.2.2.3 Crude protein

The crude protein 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. The method is as follows: approximately 0.2 g of each sample was weighed into a sample holder (referred to as boats). The boat is first placed in the purge region of the combustion tube where it is repeatedly washed with oxygen to remove all traces of atmospheric contamination. The purged sample is then pushed into the "hot zone" of the furnace. The furnace temperature and pure oxygen environment cause the sample to combust. The combustion process converts any elemental nitrogen into N_2 and NO_x gasses. The resulting combustion gasses are swept through the inner combustion tube and over the sample. The combustion products are then returned through the hot zone between the inner and outer combustion tubes. Upon exiting the furnace the combustion gasses pass through a furnace filter tube, thermoelectric cooler, anhydrone tube, a particle filter and then collected in a ballast tank. The NO_x gasses are reduced to N_2, and these gasses are passed over Lecosorb and Anhydrone where CO_2 and water are removed respectively. The N_2 remains in the helium flow and is analysed as it passes through a thermal conductivity cell. The nitrogen concentration is quantitated from the thermal conductivity cell signal and corrected for ballast temperature, pressure and sample mass. The final product is displayed as percent nitrogen, which can be multiplied by the factor 6.25 for conversion to crude protein percentage.

3.2.2.4 Neutral detergent fibre

The NDF concentration of the samples was determined using the method as described by Mertens (2002a) which is the AOAC official method 2002.04. This method is based on using refluxing in beakers. Approximately 0.5 g of each test sample was weighed and transferred 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 that were covered with round cold-water 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 an hour, after which beakers were taken off one-by-one. Samples and solutions were poured into 50 ml fritted-disk Gooch crucibles, which were placed on a vacuum filter unit. Another 250 µl α-amylase was added to each crucible. Samples were rinsed repeatedly with boiling distilled water while on the vacuum filter unit to remove all the neutral detergent solution, followed by rinsing with acetone. Samples were then placed in a 105 °C oven to dry overnight. The following day, residual NDF samples were weighed using the hot weighing procedure (Goering & Van Soest, 1970), and were then placed in the muffle furnace for 4 hours at 550 °C. After 4 hours, muffle furnace was switched off and samples left over night to cool down, and the following day the remaining ash of the samples were 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 four small 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 four 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). The NDF concentration 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 concentration of the samples was determined using the method as described by Raffrenato & Van Amburgh (2011), where approximately 1 g of each test sample was weighed and transferred into a 500 ml Berzelius unspouted glass beaker, and 100 ml acid detergent 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 an 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 acid detergent solution, followed by rinsing with acetone. 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, muffle furnace was switched off and samples left over night to cool down, and the following day the remaining ash of the samples were weighed, again using the hot weighing method. The acid detergent fibre concentration 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 concentration, the process was exactly the same as for ADF determination up to 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% sulphuric acid. Glass rods were used to mix the dried sample with the sulphuric acid. Because the sulphuric acid slowly filters 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 thoroughly and repeatedly with boiling water to remove any traces of acid, followed by rinsing with acetone. 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 four hours at 550 °C to be ashed. After four 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 four small 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 four 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).

The ADL concentration 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 24-h *in vitro* neutral detergent fibre digestibility

The 24-h *in vitro* was done in accordance to the method proposed by Goering & Van Soest (1970). Two rumen cannulated Holstein cows from the University of Pretoria experimental farm were used to harvest rumen fluid for the *in vitro* analysis. The cows remained on its standard TMR-based diet with no extra roughage 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 two blank samples were included. Blank bottles consisted only of rumen fluid and the medium described by 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 24 hours under constant CO_2 positive pressure. After the 24 hour period, samples were poured into 500 ml Berzelius glass beakers, after which 50 ml NDF solution as well as 0.5 g anhydrous sodium sulphite was added. NDF was determined as previously described in section 3.2.2.4.

3.2.2.7 Rate of NDF degradation

After the 24-hour *in vitro* NDF digestibility was determined, the rate of NDF digestion was calculated. Figure 3.1 represents an illustration of an example of the calculations done during the determination of the rate of NDF digestion. The rate of NDF digestion was calculated according to the method as described by Van Soest *et al*. (2005). The rate of NDF digestion was calculated as % NDF per hour.

Table 3.2 NDF pool size calculations with pool specific rates (Adapted from Van Soest *et al.*, 2005)

Feed name	*L. multiflorum* 1				
NDF, %DM	41.38				
lignin, %DM	6.76				
hour	NDF digestibility	A	Unitized A	lag,(h)	kd, (%/ h)
0	0.00	0.6079	1.0000		
24	38.80		0.3618	3.00	4.84
Unavailable NDF, %NDF	0.3921				
Unavailable NDF, %DM	16.224				
k1 pool NDF kd, %/h	8.59	Equation 6			
k2 pool NDF kd, %/h	0.31	Equation 7			
Normalized P1	74.74	Equation 8			
NDF pool 1, % NDF	45.44				
NDF pool 2, % NDF	15.36				
Unavailable NDF, % NDF	39.21				

3.2.2.8 Secondary plant metabolites

The analysis of secondary plant metabolites was done according to the methods as described by the laboratory manual for the FAO/IAEA (2000). The analysis was divided into two parts, with the first part involving extractions and the second part involves three sections. During part one, extractions were done as follows: Approximately 100 mg of each test sample was weighed accurately in duplicate and placed into 15 ml tubes. The tubes were then filled with 5 ml of 700:300 v/v aqueous acetone, after which the tubes were vortexed and placed in an ultrasonic water bath for 20 minutes at room temperature. After the 20 minutes, tubes were centrifuged for 10 minutes at 3000-xg at 4 °C. After centrifuging, the watery substance was poured off into separate tubes, and tubes were again filled with 5 ml 700:300 v/v aqueous acetone and process repeated as described above. The watery substance was added to the supernatant from the first pouring off.

Part two includes section one: measuring total phenols based on the Folin-Ciocalteu method, section two: measuring non-tannic phenols based on the PVPP method, and section three: measuring total tannins based on the buthanol-HCL-iron method.

Section one: Total phenols

A standard calibration curve was prepared as follows: twelve empty tubes were filled in duplicate with 0, 0.01, 0.02, 0.03, 0.05, and 0.1 ml supernatant. Tubes were filled up to 0.5 ml with distilled water, after which 0.25 ml folin reagent as well as 1.25 ml of a 20% sodium carbonate solution was added to each tube. All tubes were then vortexed, after which absorbance was recorded with the spectrophotometer set to 725 nm. The amount of total phenols was calculated as tannic acid equivalent from the standard calibration curve and expressed on a DM basis.

Section two: non-tannic phenols

Section two involved the removal of tannin-containing extract, which was done using Polyvinyl-polypyrolidone (PVPP), which binds tannins. Exactly 100 mg PVPP was weighed into duplicate test tubes, after which 1 ml distilled water was added. 1 ml of tannic containing extract was added to the tubes and vortexed. The tubes were then placed at 4 °C for 15 minutes, and vortexed again, after which the tubes were placed in the centrifuge at 3000-g for 10 minutes. The supernatant was collected and absorbance recorded with the spectrophotometer set to 725 nm. Absorbance values were once again used to calculate the non-tannic phenol values from the standard calibration curve.

Total tannins (Condensed tannins)

Total tannins were determined by pipetting 0.25 ml extract into test tubes, and 0.25 ml acetone was added to each tube, after which 50 µl ferric reagent was added. Exactly 1.5 ml butanol-HCl was added to each tube and then vortexed. After this, tubes were placed in a 100 °C water bath for 60 minutes. Tubes were left to cool down and absorbance recorded with the spectrophotometer set to 550 nm. If there were condensed tannins present in the extract, the extract in the tubes would turn pink in colour. Condensed tannins were calculated as leucocyanidin equivalent according to the following formula:

(Absorbance at 550nm x 78.26 x Dilution factor) / (% DM)

Where the dilution factor = 0.5-ml / (volume of extract taken)

3.3 PHASE 2: FORAGE FRAGILITY MEASUREMENT

All dried forage samples were pre-cut with a knife mill (Retsch GmbH, Model SM 100, Haan, Germany), fitted with a 2 cm screen, in an attempt to minimize variability across forages in terms of intial particle size. A knife mill was chosen as it has been reported to chop forages uniformly and effectively under various crop and machine conditions (Tabil *et al.*, 2011). Figure 3.1 illustrates the knife mill that was used during this study.

Figure 3.1 Illustration of Retsch knife mill used during experiment

3.3.1 Particle size distribution

Initial particle size (IPS) was defined as the particle size after pre-cutting with the 2 cm screen, and final particle size (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 distribution, the following sieves were included on the sieve shaker: 25 mm, 10 mm, 7.1 mm, 5 mm, 2.5 mm, 1 mm, 500 µm, 250 µm, and a base. 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 sieve shaker by descending mesh size. Samples were placed on the sieve shaker

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for 4 minutes, at a 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 after the samples were ground by the knife mill (Retsch GmbH, Model SM 100, Haan, Germany), this time fitted with a 1 mm screen. The following sieves were included on the sieve shaker: 7.1 mm, 5 mm, 2.5 mm, 1 mm, 500 µm, 250 µm, 100 µm, 50 µm, and the base. A volume between 250 ml and 500 ml, depending of 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 sieves used during this study is given in Figure 3.2.

Figure 3.2 Example of Retsch sieve shaker and individual sieve used during this 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 3.3. After recording of all weights the nominal geometric mean particle size for each sample was determined.

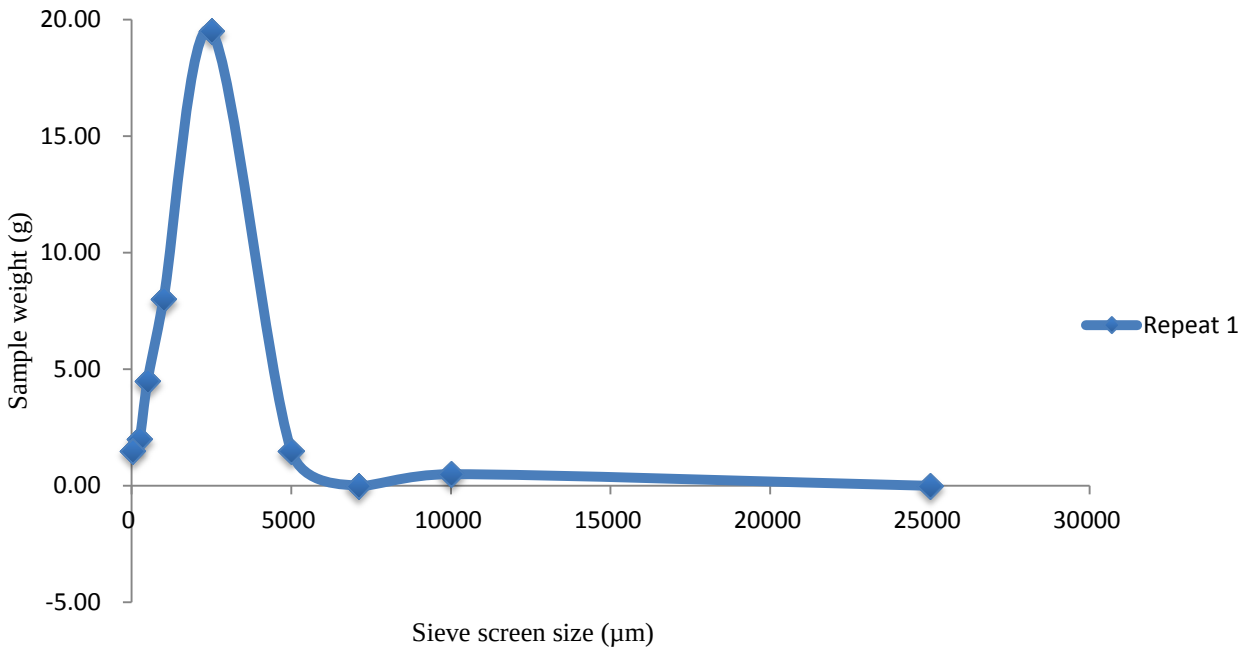

Figure 3.3 Illustration of example of individual sample particle size distribution curve of *L. multiflorum*

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:

(Geometrical mean particle size (µm) after 2 cm milling / Geometrical mean particle size (µm) after 1 mm milling) /

Geometrical mean 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), and a hammer mill (Perten, Model 3100, Perten instruments, Hägersten, Sweden). Different mills were used during the measurement of forage fragility to make comparison between grinding energy values obtained for the different forage samples from different mills possible. For both the ultra-centrifugal mill and the hammer mill; 10 g duplicate samples were grinded with each of the above mentioned mills, fitted with a 1 mm screen. In Figures 3.4 and 3.5, the Retsch ultra-centrifugal and Perten laboratory hammer mill that were used during this experiment are illustrated.

Figure 3.4 Illustration of Retsch ultra-centrifugal mill used during this experiment

Figure 3.5 Illustration of Perten laboratory hammer mill used during this experiment

During the grinding process, energy usage of the specific 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). Mills were switched on and when energy usage stabilized, forage samples were poured into the feeder of each mill as consistently as possible. Energy measurements were reported as J/g DM sample.

Computer software: Trendreader 2 version 2.39

Trendreader 2 is a 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: Smartreader Plus 3

The smartreader 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 used during this experiment.

Figure 3.6 Illustration of Smart Reader Plus data logger 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 directly proportional to kW/kVA for demand or kWh/kVAh for consumption (http://www.acrsystems.com). In Figure 3.7, the energy transductor (power bullet) used during the measurement of grinding energy is illustrated.

Figure 3.7 Illustration of energy transductor used during this experiment

Figure 3.8 illustrates the direct energy measurement during grinding in watts/second. From this graph, the accumulative integrals of the direct energy measurement data was 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 /1g sample on DM basis, and the average value between the two replicates reported.

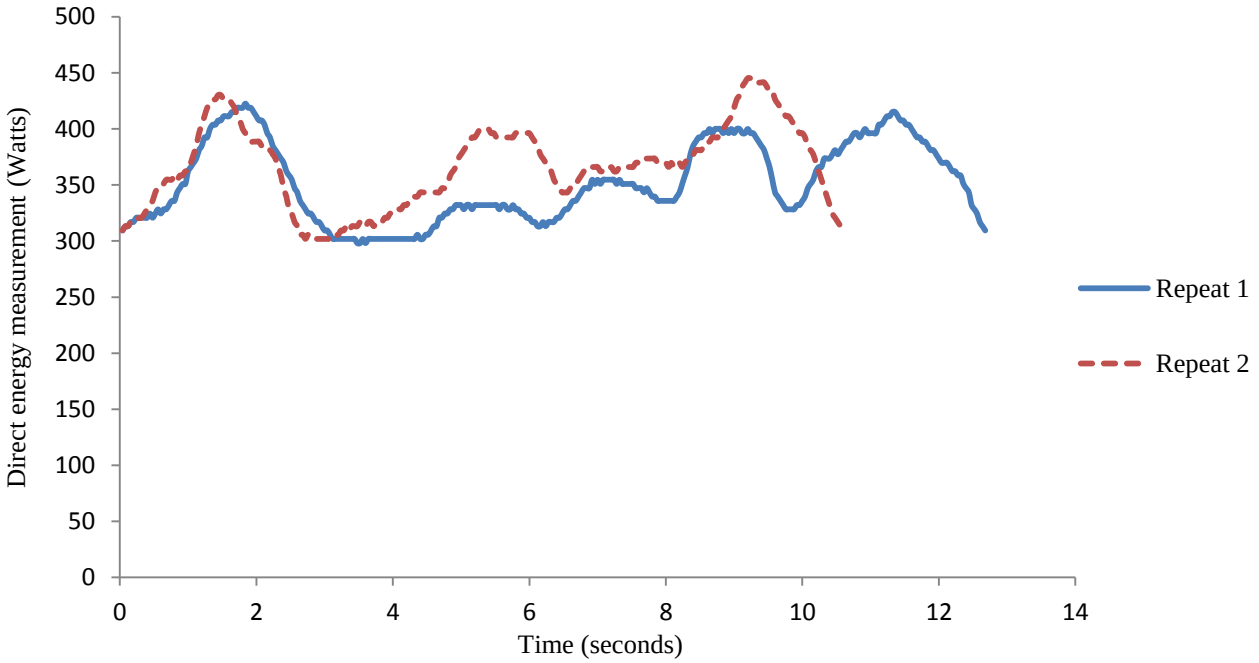

Figure 3.8 Example of direct energy measurement curve obtained during grinding of *E. curvula* sample 3

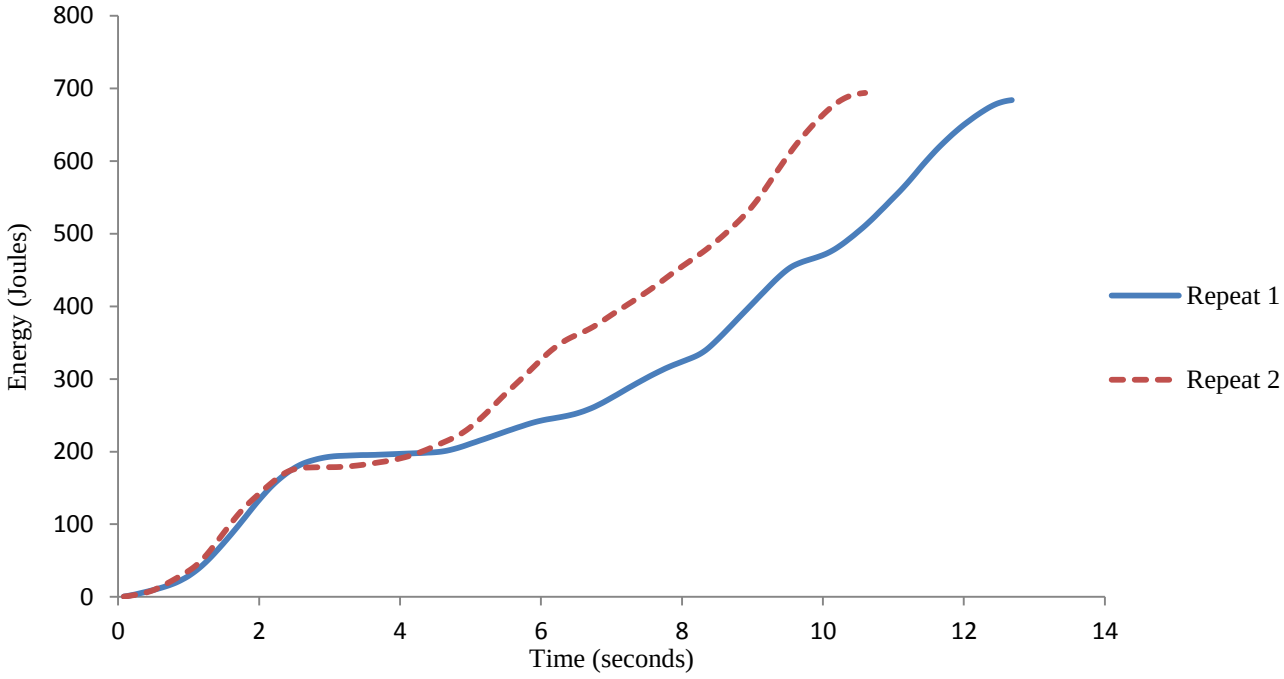

Figure 3.9 Example of accumulative integrals of direct energy measurement curves obtained during grinding of *E. curvula* sample 3

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3.4 STATISTICAL ANALYSIS

All data analysis was done using the statistical program GenStat® (Payne *et al.*, 2012). The t-test for two dependent samples (n= 28) was performed on the energy values obtained between the Retsch ultra-centrifugal mill and the Perten laboratory hammer mill to determine whether there was a significant difference between the energy values obtained from the two mills.

Tests for linearity and possible non-linearity between the energy values obtained from both the Retsch ultra-centrifugal mill and the Perten laboratory hammer mill and the chemical components measured was done. Testing for linearity between the *y*- and different *x*-variables indicated that only initial particle size was non-linear and the quadratic term was included in the modelling.

Pearson's correlation coefficients, which indicate the measure of the linear relationship between two random variables, were used to test for co-linearity between chemical components. Correlations falling within the range of $-1 < r < 1$ indicates strong relationships between the two variables.

Multiple regression analysis was used to best predict observed grinding energy measurements of the mills from a set of explanatory *x*- variables (Draper & Smith, 1981). All possible subset selection was applied to the energy measurement values obtained from both mills as response variables with explanatory variables (DM, OM, N, NDF, ADF, ADL, *iv*NDFd, NDFkd, TP, NTP, IPS, and % change in particle size) using the RSEARH procedure of GenStat® (Payne *et al.*, 2012). The best subset model for C4-grasses for each of the mills, as well as the best subset model for all the samples in the study for both mills (legumes, C3- and C4-grasses) were fitted. The stopping rule (criterion) to select the best subset model was that the adjusted R^2 value should increase by 1%. A significance level of $P \leq 0.05$ was used during the statistical analysis of this experiment. Because there were only a few C3- grass and legume samples in this study, the best subset model for each of these separate groups could not be determined.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 CHEMICAL COMPOSITION OF FORAGES

Results of the analysis of DM, OM, ash, starch, and N for each sample are presented in Table 4.1.

Table 4.1 DM, OM, ash, starch and N concentration of forage samples used during this experiment (DM basis)

	Analysis (g/100 g DM)				
Roughage sample	**DM**	**OM**	**Ash**	**Starch**	**N**
Medicago sativa 1	90.3	91.3	8.67	0.77	3.44
Medicago sativa 2	90.2	89.9	10.1	1.27	3.37
Medicago sativa 3	90.4	90.4	9.61	2.12	3.01
Medicago sativa 4	90.9	88.8	11.2	1.41	2.79
Medicago sativa 5	89.1	88.6	11.4	2.70	3.28
Medicago sativa 6	90.0	92.3	7.71	1.42	2.76
Lolium multiflorum 1	90.1	87.6	12.4	1.27	2.18
Lolium multiflorum 2	91.9	90.1	9.89	2.23	2.57
Lolium perenne 1	92.0	87.4	12.6	1.05	2.70
Dactylis glomerata 1	92.7	89.5	10.5	2.65	2.48
Festuca arundinacea 1	91.8	90.1	9.91	2.58	2.47
Cenchrus ciliaris 1	91.5	92.3	7.70	0.56	0.72
Panicum maximum 1	92.1	90.7	9.30	1.54	1.21
Panicum maximum 2	90.7	92.2	7.76	1.01	1.13
Eragrostis curvula 1	91.5	96.0	3.97	1.55	0.90
Eragrostis curvula 2	93.3	96.3	3.68	1.76	1.02
Eragrostis curvula 3	93.1	95.9	4.11	2.30	1.02
Eragrostis curvula 4	93.1	95.5	4.52	1.28	1.31
Eragrostis curvula 5	90.8	87.9	12.1	1.71	1.34
Maize stover 1	92.5	94.1	5.95	1.19	0.90
Maize stover 2	93.4	95.0	4.98	8.04	0.52
Maize stover 3	94.0	94.6	5.40	0.88	0.53
Chloris gayana 1	91.1	89.1	11.0	0.86	1.09
Digitaria eriantha 1	92.0	95.5	4.54	1.30	0.79
Digitaria eriantha 2	92.5	93.0	7.04	0.75	1.90
Digitaria eriantha 3	91.1	93.1	6.86	1.06	1.08
Digitaria eriantha 4	91.6	92.9	7.10	0.36	1.19
Cynodon nlemfuensis 1	91.6	90.9	9.11	1.25	1.02

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

DM consists of organic matter as well as the ash (minerals) component. From Table 4.1 it is observed that all of the forages used in this study had a DM concentration ranging between 89.1 g/100g as the lowest and 94.0 g/100g as the highest. MacDonald *et al.* (2002) stated that the DM composition of pasture grass is variable. It has been stated that the DM composition of grass is dependent on the relative proportions of cell wall constituents and cell contents (MacDonald *et al.*, 2002). When comparing the DM concentration of all the samples as presented in Table 4.1, it is evident that maize stover had the highest numerical DM concentration of all the samples. The *M. sativa* samples, *L. multiflorum* sample 1 and *P. maximum* sample 2 had the lowest numerical DM concentration between all of the forage samples. Horrocks & Vallentine (1999) reported a DM value of 83% - 89% for *L. multiflorum*, which is lower than the DM value for the *L. multiflorum* used in this study. MacDonald *et al.* (2002) reported values ranging between 165 g/kg – 338 g/kg DM and OM digestibility between 0.59 g/kg – 0.80 g/kg over a period of 4 months for *L. perenne*. A large proportion of the OM of forages consists out of cell walls (35% - 80%) which provides the structural integrity to the plants (Romney & Gill, 2000). The OM concentration is the difference between the DM concentration and the ash, and the OM concentration of the samples varied between 87.4 g/100g as the lowest and 96.3 g/100g as the highest.

The ash content represents the inorganic constituents (minerals) of feed, and the mineral content of grass is variable (MacDonald *et al.*, 2002). There is a decrease in the numerical total ash content with increasing maturity (MacDonald *et al.*, 2002). The species of plants, stage of maturity, type of soil, climate and seasonal conditions are important factors determining the mineral status of forages (MacDonald *et al.*, 2002). The mineral content of plants is further influenced by fertiliser application (MacDonald *et al.*, 2002). Legumes have a higher calcium and magnesium concentration than grasses, and tropical forages contain less calcium than temperate species (Cheeke, 1991). MacDonald *et al.* (1995) reported a value of 100 g/kg ash on DM basis for young grass. It is evident from the results in Table 4.1 that the ash concentration of the *M. sativa* samples tend to be numerically higher than most of the grass species. According to MacDonald *et al.* (2002), legumes tend to be richer in the major minerals and certain trace minerals than grasses. The results from Table 4.1 clearly indicate that the C3 – type forages had a numerically higher ash concentration than the C4 –type forages, except *E. curvula* 5 and *C. gayana* which also had a high numerical ash concentration. Some of the C3-grasses (*L. multiflorum*, *L. perenne* and *D. glomerata*) also had a high ash concentration. The high ash concentration seen from Table 4.1 for some of the C3- and C4-species, may be attributed to many factors. It could be that the soil in which these grasses were established was highly fertile, that the plants were still very young, or the application of fertiliser. MacDonald *et al.* (2002) reported 108 g/kg ash as the proximate composition for an Italian ryegrass sample on a DM basis. MacDonald *et al.* (2002) reported values ranging between 68 g/kg- 88 g/kg ash over a period of 4 months for *L. perenne*. Mertens (2002b) reported an ash value of 8.9% - 9.1% for *M. sativa*, 7.1% - 8.5% for C3-grasses, 6.9% - 7.3% for C4

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

grasses and 7.0% for maize stover. Mani *et al.* (2006) reported a value of 7.46% ash for maize stover and 5.49% for switchgrass (C4-grass). Reverdin (2000) reported values of 7.5% ash for maize stover and 10.8-11.1% for *M. sativa*. These values are in agreement with the values obtained in this study.

The starch concentration of the roughages used in this study was very low as illustrated in Table 4.1, with no values equal to or above 3 g/100g on DM basis, with the lowest value being 0.36 g/100g for *D. eriantha* sample 4. Maize stover sample 2 however had 8.04 g/100g starch, the highest value for the forage samples used in this study. The starch concentration of the legume species did not differ from that of the non-legume species. Mertens (2002b) reported a starch value of 2.0 -2.6 g/100g for *M. sativa*, 1.6 -2.1 g/100g for C3-grasses, 2.3 -2.6 g/100g for C4-grasses and 12.8 g/100g for maize stover. These values support the starch composition of the forage samples used in this study, except for the starch of maize stover, which was higher than the values in this study.

Protein is the major nitrogen-containing compound, and according to MacDonald *et al.* (1995), the greatest portion of protein is present as enzymes in plants, of which the concentration decrease with maturity. The N in forages consists of proteins and various non-protein N compounds, such as amino acids, amides, nitrates and ammonia (Cheeke, 1991). Leaves have a high quality protein (good amino acid balance) (Cheeke, 1991), and therefore forages with higher leaf: stem ratio will have a higher protein value. Bailey (1962), as cited by Minson (1990) stated that chewing increases the rate of protein degradation in the rumen. 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). There is more variation in the N concentration of the forage samples as indicated in Table 4.1, with the C3–type forages clearly having a higher numerical N concentration than the C4 –type forages, and *M. sativa* having the highest numerical N concentration (3.44 g/100g) of all the forage samples, and maize stover having the lowest N concentration (0.52 g/100g) between all the forage samples. MacDonald *et al.* (2002) stated that in general, tropical grasses tend to be lower in protein concentration than temperate species, which supports the lower N values for C4-grasses than the N values for C3-grasses in this study. It is also clear that legumes had a higher numerical N concentration than non-legume species. Chesworth (1992) stated that the N concentration of grass decreases as the plant matures. Waghorn *et al.* (1989) reported that the N concentration of ryegrass was lower than the N concentration of *M. sativa*, which is in agreement to the values obtained for ryegrass and *M. sativa*. Cheeke (1991) 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. Minson (1990) reported that temperate grasses generally contain more CP than tropical grasses with mean concentrations of 129 g/kg and 100 g/kg DM respectively, as illustrated in Figure 4.1.

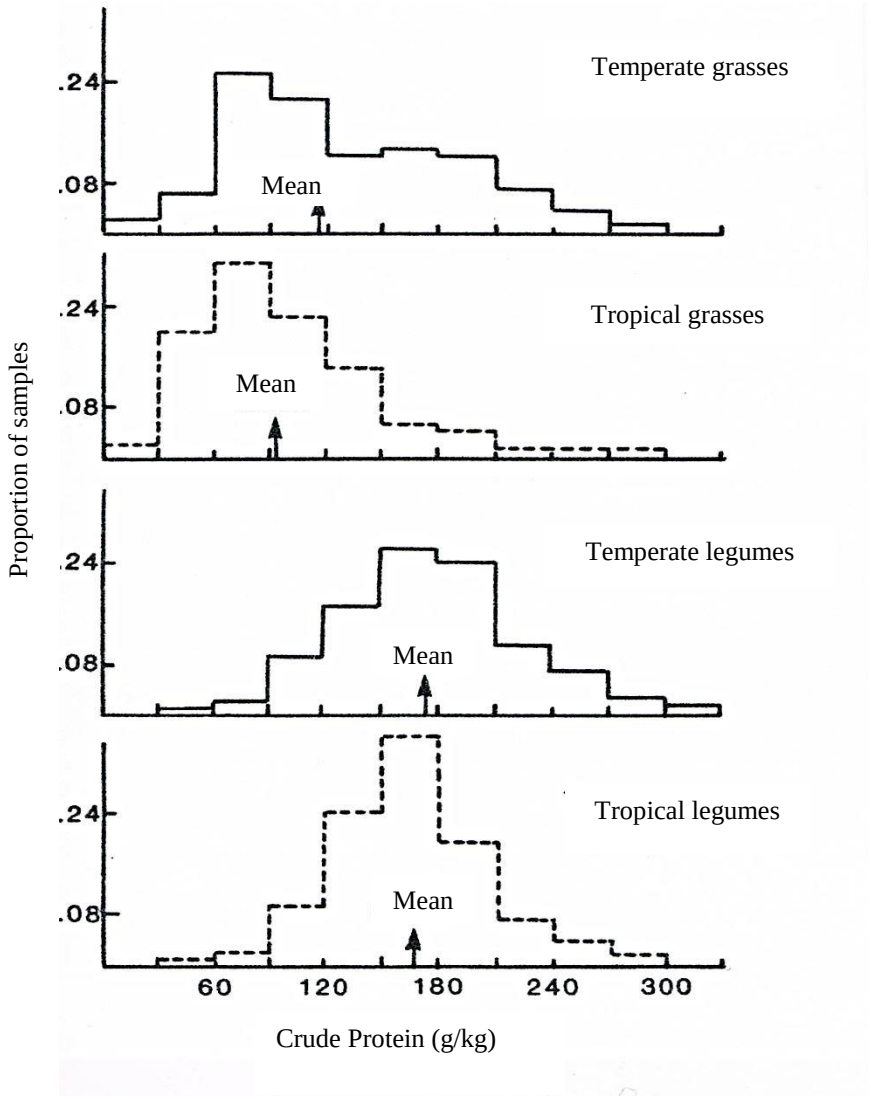

Figure 4.1 Crude protein concentration in temperate and tropical grasses and legumes (world data) (Adapted from Minson, 1990)

The results on the chemical analysis reporting ADF, NDF and ADL are presented in Table 4.2.

Table 4.2 The acid detergent fibre, neutral detergent fibre and acid detergent lignin concentrations of the experimental forage samples (DM basis)

	Analysis (g/100 g DM)		
Roughage sample	**ADF**	**NDF**	**ADL**
Medicago sativa 1	33.0	41.4	6.76
Medicago sativa 2	35.7	40.8	5.89
Medicago sativa 3	36.4	42.4	6.91
Medicago sativa 4	37.2	43.3	7.87
Medicago sativa 5	40.7	47.4	10.4
Medicago sativa 6	44.0	51.0	8.74
Lolium multiflorum 1	26.2	44.4	1.47
Lolium multiflorum 2	31.9	58.3	4.98
Lolium perenne 1	32.6	57.1	2.73
Dactylis glomerata 1	33.1	62.5	3.75
Festuca arundinacea 1	28.2	52.3	2.26
Cenchrus ciliaris 1	50.1	78.9	6.42
Panicum maximum 1	39.6	72.8	3.90
Panicum maximum 2	44.3	76.0	5.51
Eragrostis curvula 1	46.4	81.2	4.36
Eragrostis curvula 2	40.3	81.9	5.82
Eragrostis curvula 3	42.9	77.3	6.49
Eragrostis curvula 4	43.6	77.0	5.42
Eragrostis curvula 5	36.3	61.7	4.01
Maize stover 1	50.7	83.8	5.73
Maize stover 2	46.1	75.2	4.31
Maize stover 3	61.9	80.1	10.6
Chloris gayana 1	43.8	72.6	5.33
Digitaria eriantha 1	45.1	79.8	4.77
Digitaria eriantha 2	39.6	68.3	4.69
Digitaria eriantha 3	44.2	76.0	5.39
Digitaria eriantha 4	44.7	72.7	5.43
Cynodon nlemfluensis 1	43.3	76.6	6.17

The ADF consists of cellulose, lignin, lignified N and silica, and according to MacDonald *et al.* (2002) there is a strong statistical correlation between the ADF content of forages and the extent to which it is digested. The ADF might be the most important analyses, as it is negatively correlated with forage digestibility (Horrocks & Vallentine, 1999). Lignin and silica, which occurs in the ADF fraction, are structural inhibitors to the digestion of other associated nutrients and are classified as anti-nutritional factors (Horrocks & Vallentine, 1999). Mayland (1986), as cited by Horrocks & Vallentine (1999) reported that grasses can contain silica concentrations of up to 10% on DM basis. The numerical ADF results varied between C3- and C4- species and according to the results in Table 4.2, maize stover had the highest numerical ADF concentration (61.9 g/100g) and *L. multiflorum* and *F. arundinacea* had the lowest numerical ADF concentration (26.2 g/100g -31.9 g/100g). From the results obtained it would seem that in general, the C3-grasses have a lower ADF concentration than *M. sativa* and the C4-grasses. MacDonald *et al.* (2002) reported values ranging between 22.7 g/100g - 34.7 g/100g ADF over a period of 4 months for *L. perenne*. There was a decrease in all components except ADF and lignin which increased over the period of 4 months (MacDonald *et al.*, 2002). Horrocks & Vallentine (1999) reported a value of 31.0% - 37.0% ADF for *M. sativa*, and a value of 35.0% - 42.0% ADF for *L. multiflorum*, which is in agreement with the results of this study. MacDonald *et al.* (2002) reported that the ADF concentration may range from between 20.0% to 45.0% in mature grass species. Holland *et al.* (1990), as cited by Horrocks & Vallentine (1999) reported a value of 28.0% - 41.0% ADF for *M. sativa* which agrees with the values obtained in this study, and 43.0% ADF for maize stover which is slightly lower than the values obtained in this study. Mertens (2002b) reported an ADF value of 36.0% - 40.0% for *M. sativa*, 30.0% - 36.8% for C3-grasses, 32.9% - 38.7% for C4-grasses and 42.0% for maize stover. Reverdin (2000) reported values of 43.7% ADF for maize stover, and 36.5% - 37.0% for *M. sativa*. These values support the ADF values obtained from chemical analysis in this study.

The NDF includes lignin, cellulose and hemicellulose, and is regarded as a measure of the cell wall material of plants (MacDonald *et al.*, 2002). Although NDF is commonly used as an estimate for the cell wall fraction in forages, the NDF residue does not retain the pectin fraction of plant cell walls (Jung & Lamb, 2003). Grasses have very low pectin concentration in their cell walls, and therefore NDF concentration is a good indication of cell wall concentration in grasses, however, pectin levels in the cell walls of legumes are much higher, and therefore NDF can severely underestimate the cell wall concentration in legumes (Jung & Lamb, 2004). From a nutritional point this is not a major problem, since pectin is rapidly and relatively completely digested in the rumen (Hatfield & Weimer, 1995), as cited by Jung & Lamb (2003). According to Table 4.2, maize stover and *E. curvula* sample 1 and 2 had the highest numerical NDF concentration of all the forage samples (83.75 g/100g). The numerical NDF concentration of the C4-grasses was much higher than the numerical NDF concentration of *M. sativa* and C3-grasses. It would also seem that C3-grasses have

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YUNIBESITHI YA PRETORIA

higher numerical NDF concentration than *M. sativa*. Horrocks & Vallentine (1999) reported a value of 42.0% - 50.0% NDF for *M. sativa*, which is in agreement with the results of this study. Holland *et al.* (1990), as cited by Horrocks & Vallentine (1999) reported a value of 38.0% - 53.0% NDF for *M. sativa*, which also supports the values obtained in this study, and 68.0% NDF for maize stover, which is lower than the values obtained in this study. Julier *et al.* (1999) reported a value of 39.67% NDF in *M. sativa* plants. Mertens (2002b) reported a NDF value of 47.5% - 52.1% for *M. sativa*, 55.5% - 65.3% for C3-grasses, 68.9% - 73.3% for C4-grasses and 70.0% for maize stover. Reverdin (2000) reported values of 77.6% NDF for maize stover, and 52.5% - 55.3% for *M. sativa*. All these values are in agreement with the NDF analysis results reported in this study.

There was variation in the numerical ADL results between the *M. sativa*, C3- and C4- grasses as illustrated in Table 4.2. Lignin concentration of forages typically ranges between 5% - 20%, 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). It can be seen that all the samples had numerical ADL concentration below 10 g/100g, except maize stover sample 3 and *M. sativa* sample 5 which had an ADL concentration slightly above 10 g/100g. Jung *et al.* (1997) reported that there are negative correlations between the lignin concentration and DM as well as between lignin concentration and fibre digestibility. Macdonald *et al.* (2002) reported 5.2 g/100g lignin as the proximate composition for an Italian ryegrass sample on DM basis. MacDonald *et al.* (2002) reported values ranging between 1.6% – 4.9% lignin over a period of 4 months for *L. perenne*. According to MacDonald *et al.* (2002), the most important factor influencing the composition and nutritive value of pasture is stage of growth. With increasing maturation, there is an increase in structural carbohydrates such as cellulose and hemicellulose, an increase in lignin, and a decrease in protein content, leading to the inverse relationship between the protein and fibre contents in a given species, although this relationship is upset by the application of nitrogenous fertilisers (MacDonald *et al.*, 2002). Lignin is a major anti-nutritional component in grasses (Chaves *et al.*, 2002), and the negative effect of lignin in grasses is greater than in legumes because of the localized deposition of lignin in legumes and a greater proportion of core than noncore lignin (Buxton & Fritz, 1985; Jung, 1989), as cited by Gaylean & Goetsch (1993). The localization of lignin in legumes limits the physical restriction of lignin on cell wall digestion (Moore & Cherney, 1986), as cited by Gaylean & Goetsch (1993). The lower proportion of noncore lignin in legumes than in grasses might be associated with less inhibitory effects on microbial activity (Fukushima *et al.*, 1991), as cited by Gaylean & Goetsch (1993). Lignin is not only mostly indigestible, but the presence of lignin also inhibits the availability of associated cellulose and hemicellulose (Horrocks & Vallentine, 1999). According to Chaves *et al.* (2002) lignin is more prevalent in stems of grasses than in leaves, and lignin is absent from the leaves of legumes. Chaves *et al.* (2002) added that there is less cross linkages between lignin and hemicellulose in the stems of legumes than in grasses, and therefore

the lignin concentration does not have such a negative effect on the nutritional value of legumes as in grasses (Chaves *et al.*, 2002). The high numerical ADL concentration of *M. sativa* sample 5 and maize stover sample 3 indicates high concentrations of core lignin within these samples, which could mean that these samples were more mature than the other samples, however, these maize stover samples consisted mostly of stems, which explains the high concentration of lignin measured during analysis.

Horrocks & Vallentine (1999) reported a value of 8% - 10% ADL for *M. sativa*, and 3% - 9% for *L. multiflorum*, which is in agreement with the results of this study. Mani *et al.* (2006) reported values of 3.12% ADL for maize stover, and 7.43% for switchgrass – a typical C4-grass, and these values support the results for ADL concentration of the forages used in this study. Reverdin (2000) reported values of 3.6% ADL for maize stover, and 10.8% - 11.1% for *M. sativa*. These values support the observations made in this study. There is no dispute about the negative effects of cell wall lignification on nutritional value of forages (Jung & Allen, 1995). There is some question though as to how much of the variation in forage digestibility can be explained by lignin concentration alone (Jung & Allen, 1995). It is clear that multiple components influence the digestibility of forages.

Neutral detergent fibre digestibility is an important parameter of forage quality (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. Casler & Jung (2006) reported that differences among species in total fibre, 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 were the most limiting factors to 24-hour *in vitro* digestibility, with NDF being the most important factor (Casler & Jung, 2006). According to Casler (2001), as cited by Casler & Jung (2006), *in vitro* NDF digestibility can be increased by decreasing lignin concentration or cross-linking between lignin and cell wall carbohydrates. According to the results in Table 4.3, maize stover sample 1 and 3 had the lowest *in vitro* NDF digestibility (9.23%), while the C3-grasses had a higher *in vitro* NDF digestibility (50.3% - 63.3%). The slow rate of NDF digestion of maize stover sample 1 and 3 (1.97%/hour and 1.55%/hour) correlates with the low NDF digestibility of maize stover, and also supports the negative correlation as reported by Jung *et al.* (1997), between the high ADL concentration of maize stover 3 and the low NDF digestibility. The rate of NDF digestion of *M. sativa* was numerically lower than that of the C3- and C4- grasses. It is important to note that increased 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) stated that lignin concentration is a key factor limiting *in vitro* NDF digestibility in *M. sativa*, and also reported that there is a negative correlation between lignin concentration and the rate of NDF digestion Jung & Allen (1995). Jung & Lamb (2003) reported that

cell wall concentration was consistently negatively correlated with both 16 hour - and 96 hour *in vitro* NDF digestibility. Julier *et al.* (1999) reported a value of 31.9% for the NDF digestibility 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 (1973) reported a positive relationship between *in vitro* rate of NDF digestibility and voluntary DM intake, and the correlation for grasses was higher than for legumes.

Table 4.3 Chemical analysis, calculations and ratios of cell wall components

	Chemical analysis			
Roughage	***iv*NDFd (%)**	**NDFkd (%NDF/hour)**	**ADF/NDF***	**ADL/NDF****
Medicago sativa 1	38.8	0.56	79.7	16.3
Medicago sativa 2	39.2	3.10	87.4	14.4
Medicago sativa 3	33.1	1.11	85.7	16.3
Medicago sativa 4	30.6	1.72	85.9	18.2
Medicago sativa 5	26.8	1.81	85.8	21.9
Medicago sativa 6	33.5	2.60	86.4	17.2
Lolium multiflorum 1	63.3	4.84	58.9	3.30
Lolium multiflorum 2	50.3	4.36	54.7	8.53
Lolium perenne 1	55.6	2.09	57.1	4.79
Dactylis glomerata 1	62.2	4.02	52.9	6.01
Festuca arundinacea 1	54.5	2.07	63.6	4.33
Cenchrus ciliaris 1	28.3	3.72	63.6	8.15
Panicum maximum 1	42.6	3.74	54.3	5.36
Panicum maximum 2	22.9	3.94	58.3	7.25
Eragrostis curvula 1	26.5	5.54	57.2	5.37
Eragrostis curvula 2	26.3	4.77	49.2	7.11
Eragrostis curvula 3	33.6	4.71	55.4	8.39
Eragrostis curvula 4	28.1	4.46	56.6	7.03
Eragrostis curvula 5	33.3	6.17	59.0	6.51
Maize stover 1	9.23	1.97	88.6	6.85
Maize stover 2	41.2	2.39	66.1	5.73
Maize stover 3	14.3	1.55	77.3	13.20
Chloris gayana 1	21.6	3.00	60.4	7.35
Digitaria eriantha 1	23.8	2.47	56.6	5.98
Digitaria eriantha 2	29.7	3.20	58.0	6.87
Digitaria eriantha 3	38.7	1.55	58.2	7.10
Digitaria eriantha 4	33.2	1.50	61.5	7.46
Cynodon nlemfuensis 1	21.8	1.45	56.5	8.06

*$\left({}^{\mathrm{ADF}}/_{\mathrm{NDF}}\right) \times 100$

**$\left({}^{\mathrm{ADL}}/_{\mathrm{NDF}}\right) \times 100$

The expression of ADF on NDF basis gives a good indication of the proportion ADF contained within NDF. According to the results presented in Table 4.3, 88.6 g/100g of the NDF in maize stover sample 1 is ADF. *M. sativa* had high numerical amounts of ADF on a NDF basis. There is no distinct difference between the ADF concentration of the C3-grasses and the C4-grasses when expressed on NDF basis. The expression of ADL on NDF basis gives an indication of the percentage ADL within NDF. As illustrated in the results in Table 4.3, maize stover sample 3 had a high ADL concentration on NDF basis, and *M. sativa* had a higher ADL concentration on NDF basis than the C3- and C4-grasses. There is no distinct difference between the ADL concentration of the C3-grasses and the C4-grasses when expressed on NDF basis.

In Table 4.4 the calculated lignin, hemicellulose and cellulose concentrations of the experimental forage samples are presented. The fibre fraction should play a major role in energy evaluation of forages, as it is more variable than the other chemical components such as the cell contents. Cellulose is a fibrous, tough, water-insoluble substance, occurs in the cell walls of plants, particularly in the stalks, stems, trunks and woody portions of the plant (Nelson & Cox, 2005), as cited by Tabil *et al.* (2011). Cellulose can comprise 40% - 60% of the dry weight of plant material (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.*, 2002). It was observed by Dehority & Johnson (1961) that the amount of cellulose digested appeared to be greater for grasses than for legumes, when they had equal amounts of lignin. This led to the suggestion made by Tomlin *et al.* (1965) that there is the existence of some basic difference between grasses and legumes in the quantity of cellulose digested per given quantity of lignin. Hemicellulose is a polysaccharide related to cellulose and accounts for about 20% - 40% of the biomass in most plants (Tabil *et al.*, 2011). Hemicellulose gives structural integrity to the plant, and is soluble in strong alkali solutions (Tabil *et al.*, 2011). The spaces in the cell wall between cellulose and hemicellulose are filled with lignin (Tabil *et al.*, 2011). Waghorn *et al.* (1989) reported that the hemicellulose and cellulose concentration of ryegrass was higher than that of *M. sativa*. Mani *et al.* (2006) reported values of 31.32% cellulose for maize stover, and 44.34% for switchgrass (a typical C4-grass), as well as hemicellulose values of 21.1% for maize stover and 30.0% for switchgrass. These values are in agreement with the results obtained in our study. 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*.

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Table 4.4 Lignin, hemicellulose and cellulose concentrations (g/100g) of the experimental forage samples on DM basis

Roughage	Lignin	Hemicellulose	Cellulose
Medicago sativa 1	6.76	8.39	26.2
Medicago sativa 2	5.89	5.15	29.8
Medicago sativa 3	6.91	6.08	29.5
Medicago sativa 4	7.87	6.08	29.3
Medicago sativa 5	10.4	6.74	30.3
Medicago sativa 6	8.74	6.96	35.3
Lolium multiflorum 1	1.47	18.3	24.7
Lolium multiflorum 2	4.98	26.5	26.9
Lolium perenne 1	2.73	24.5	29.9
Dactylis glomerata 1	3.75	29.5	29.3
Festuca arundinacea 1	2.26	24.1	25.9
Cenchrus ciliaris 1	6.42	28.7	43.7
Panicum maximum 1	3.90	33.3	35.7
Panicum maximum 2	5.51	31.7	38.8
Eragrostis curvula 1	4.36	34.7	42.1
Eragrostis curvula 2	5.82	41.7	34.4
Eragrostis curvula 3	6.49	34.5	36.4
Eragrostis curvula 4	5.42	33.4	38.2
Eragrostis curvula 5	4.01	25.3	32.3
Maize stover 1	5.73	33.0	45.0
Maize stover 2	4.31	29.1	41.8
Maize stover 3	10.6	18.2	51.3
Chloris gayana 1	5.33	28.8	38.5
Digitaria eriantha 1	4.77	34.6	40.4
Digitaria eriantha 2	4.69	28.7	34.9
Digitaria eriantha 3	5.39	31.8	38.8
Digitaria eriantha 4	5.43	28.0	39.3
Cynodon nlemfuensis 1	6.17	33.3	37.1

Table 4.5 presents the results from the analysis of secondary plant metabolites of the forage samples, which included total phenols, non-tannic phenols as well as total tannins. Secondary plant metabolites are a diverse group of molecules which is involved in the adaptation of plants to its environment, but are not part of the primary biochemical pathways of cell growth and reproduction in plants (Reed *et al.*, 2000).

Secondary metabolites are not generally present in grasses in large quantities. Tannins form a group of less complex phenolic compounds, and are known to occur in high concentrations in trees and shrubs. Tannins, like lignin, can have negative effects on the digestion of other dietary components (Chesworth, 1992).

Table 4.5 Secondary plant metabolites as analysed in legumes, C3- and C4- grasses (DM basis)

Roughage	Total phenols (g/kg)	Non-tannic phenols (g/kg)	Total tannins (g/kg)
Medicago sativa 1	4.37	2.61	1.72
Medicago sativa 2	5.31	1.09	4.22
Medicago sativa 3	4.91	0.32	4.59
Medicago sativa 4	3.68	1.54	2.14
Medicago sativa 5	2.91	0.20	2.71
Medicago sativa 6	3.17	0.12	3.05
Lolium multiflorum 1	5.78	1.39	4.39
Lolium multiflorum 2	7.79	1.07	6.72
Lolium perenne 1	7.96	1.09	6.87
Dactylis glomerata 1	9.35	0.91	8.44
Festuca arundinacea 1	9.06	1.63	7.43
Cenchrus ciliaris 1	3.70	0.20	3.50
Panicum maximum 1	5.80	0.07	5.73
Panicum maximum 2	4.35	0.26	4.09
Eragrostis curvula 1	3.13	0.28	2.85
Eragrostis curvula 2	5.71	0.22	5.49
Eragrostis curvula 3	3.58	0.16	3.42
Eragrostis curvula 4	3.95	0.18	3.76
Eragrostis curvula 5	4.34	0.00	4.38
Maize stover 1	4.27	0.17	4.09
Maize stover 2	3.11	0.84	2.28
Maize stover 3	3.94	1.33	2.61
Chloris gayana 1	2.14	0.08	2.05
Digitaria eriantha 1	3.96	0.00	3.96
Digitaria eriantha 2	13.5	0.21	13.3
Digitaria eriantha 3	9.01	0.53	8.47
Digitaria eriantha 4	8.05	0.20	7.85
Cynodon nlemfuensis 1	2.42	0.33	2.09

The non-tannic phenols include all other secondary metabolites formed in the plant such as fructosans, pectins and hemicellulose (Chesworth, 1992). The numerical total phenol concentration of most of the samples was below 10 g/kg, except *D. eriantha* sample 2, as illustrated in Table 4.5. There is variation within the samples and no distinct differences in the total phenol concentration of the different groups of forages are observed. However, the total phenol concentration of some samples (*L. multiflorum* sample 2, *L. perenne*, *D. glomerata*, *F. arundinacea* and *D. eriantha* sample 3and 4) were distinctly higher than the other experimental forage samples. The numerical non-tannic phenol concentration of all the samples was very low, with all samples having a non-tannic phenol concentration below 5 g/kg. No distinct differences between the *M. sativa*, C3- and C4-grasses are observed. Tannins may modify the digestibility of dietary protein and structural carbohydrates in forages (Mueller- Harvey & McAllan, 1992), as cited by Cherney (2000). The total tannin concentration of all the samples was below 10 g/kg, except *D. eriantha* sample 2, which had a total tannin concentration of 13.3 g/kg. No distinct differences between the total tannin concentrations of *M. sativa*, C3- and C4-grasses can be seen. According to Cheeke (1991), forages that contain tannins, especially legumes, have a high level of protected protein and may be superior protein sources as compared to forages with a high concentration of readily degradable protein.

4.2 LABORATORY ANALYSIS OF FORAGE FRAGILITY

4.2.1 Particle size distribution

Minson (1990) stated that voluntary intake is higher for legumes than for grasses, and higher for temperate than for tropical grasses. Legumes have a lower resistance (higher fragility) to reduction in particle size during chewing and rechewing, which can be due to a lower concentration of cell wall constituents (Horrocks & Vallentine, 1999). Animals consume leafy legumes and grasses in greater quantities than its counterparts with higher stem concentration as a result of greater particle size reduction from mastication and more rapid passage out of the reticulo-rumen (Horrocks & Vallentine, 1999). According to Womac *et al.* (2007), particle size distribution is influenced by operating speed, mass input rate and screen size, apart from the physical characteristics of the forages which influence particle size distribution. Particle size reduction is a critical process determining digesta volume, rates of passage, and digestion of the food particles, and these in turn determine the rate of forage intake by animals (Ellis *et al.*, 1987), as cited by Horrocks & Vallentine (1999). According to Yang *et al.* (2001a), the reduction of particle size of the forage used in a study conducted by the authors, had no effect on intake. It is important to remember that although sieve aperture size is most often used to indicate particle size, particles are able to pass through the sieve lengthwise, and can therefore be longer than the aperture size (Waghorn *et al.*, 1989).

In Table 4.6 the average particle size distribution as well as the percentage change in particle size for each of the forage samples analysed during this study is presented. The results indicate that maize stover sample 1 had the largest geometrical mean particle size after milling with the 2 cm screen (3250.6 µm). It is observed in Table 4.6 that *E. curvula* sample 2, *M. sativa* sample 1 and 3, as well as *D. eriantha* sample 1 had the smallest average particle size after milling with the 2 cm screen, measuring 884.8 µm, 948.2 µm, 907.2 µm and 978.5 µm respectively. All of the other forage samples had an average particle size ranging between 1441.5 µm – 2879.2 µm. A few of the forages showed less change in particle size between 2 cm and 1 mm milling than most of the samples. These include *E. curvula* sample 4 which had a 68.6% change in particle size, *M. sativa* sample 1 (57.3%), *M. sativa* sample 3 (50.0%), and *D. eriantha* sample 1 which had a change in particle size of 59.31%. All of the other forage samples had a change in particle size ranging between 71.8% and 84.2%.

Regarding the 1 mm particle size distribution, it was found that *E. curvula* sample 2 and *D. eriantha* sample 2 had the smallest average particle size after milling with the 1 mm screen (249.4 µm and 242.5 µm respectively), and *D. eriantha* sample 3 had the largest average particle size (753.9 µm). All of the other forage samples ranged between 346.4 µm and 582.0 µm. Mani *et al.* (2006) reported a geometric mean particle size for maize stover of 0.262 mm (262 µm) when a 1.6 mm (1600 µm) screen was fitted to the hammer mill, and 0.193 mm (193 µm) when a 0.8 mm (800 µm) screen was used. The authors also reported a geometric mean particle size for switchgrass (C4-grass) of 0.283 mm (283µm) when a 1.6 mm (1600 µm) screen was fitted to the hammer mill, and 0.253 mm (253 µm) when a 0.8 mm (800 µm) screen was used (Mani *et al.*, 2006). These values for geometric mean particle size are smaller than the particle sizes obtained in this study, however, the moisture content of the maize stover used by Mani *et al.* (2006) was 6.22% and for switchgrass was 8.00%, and the average moisture content for the maize stover samples used in this study was 6.67% and for the C4-grasses was 8.14%. 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. However, there are many other factors influencing the particle sizes such as feeding rate of samples, and mill operating speed and power. Other chemical components have a definite influence on the resistance to particle breakdown, and will therefore influence the final particle size.

Table 4.6 Particle size distribution and % change in particle size of forages after milling through a 2 cm and 1 mm screen

Average particle size distribution			
Roughage	**IPS (µm)**	**FPS (µm)**	**% change in particle size**
Medicago sativa 1	948.2	404.7	57.3
Medicago sativa 2	1947.0	435.7	77.6
Medicago sativa 3	907.2	453.4	50.0
Medicago sativa 4	1517.6	418.7	72.4
Medicago sativa 5	2167.9	423.0	80.5
Medicago sativa 6	2096.4	577.7	72.5
Lolium multiflorum 1	1745.9	364.2	79.1
Lolium multiflorum 2	2031.2	348.4	82.9
Lolium perenne 1	1441.5	319.2	77.9
Dactylis glomerata 1	2187.3	346.4	84.2
Festuca arundinacea 1	2079.5	372.7	82.1
Cenchrus ciliaris 1	2379.5	421.0	82.3
Panicum maximum 1	2203.7	418.1	81.0
Panicum maximum 2	2346.6	466.6	80.1
Eragrostis curvula 1	2109.5	581.9	72.4
Eragrostis curvula 2	884.7	249.4	71.8
Eragrostis curvula 3	1877.2	468.6	75.0
Eragrostis curvula 4	1556.7	488.7	68.6
Eragrostis curvula 5	1929.1	444.6	77.0
Maize stover 1	3250.6	525.5	83.8
Maize stover 2	2443.6	465.6	81.0
Maize stover 3	2509.0	401.8	84.0
Chloris gayana 1	2499.3	533.7	78.7
Digitaria eriantha 1	978.5	398.1	59.3
Digitaria eriantha 2	1008.0	242.5	75.9
Digitaria eriantha 3	2879.2	753.9	73.8
Digitaria eriantha 4	2681.9	439.1	83.6
Cynodon nlemfuensis 1	1508.7	411.9	72.7

In Figure 4.2 the average particle size distributions of all the samples of initial particle size (after milling with 2 cm screen) and final particle size (after milling with 1 mm screen) are presented. Mani *et al.* (2004) reported geometric mean particle sizes of 0.41 mm and 0.46 mm respectively for maize stover and switchgrass after milling through a 3.2 mm screen. As illustrated in Figure 4.2, maize stover sample 3 had the largest average particle size after milling with the 2 cm screen, and *E. curvula* sample 2 as well as *D. eriantha* sample 2 had the smallest average particle size after milling with the 1 mm screen.

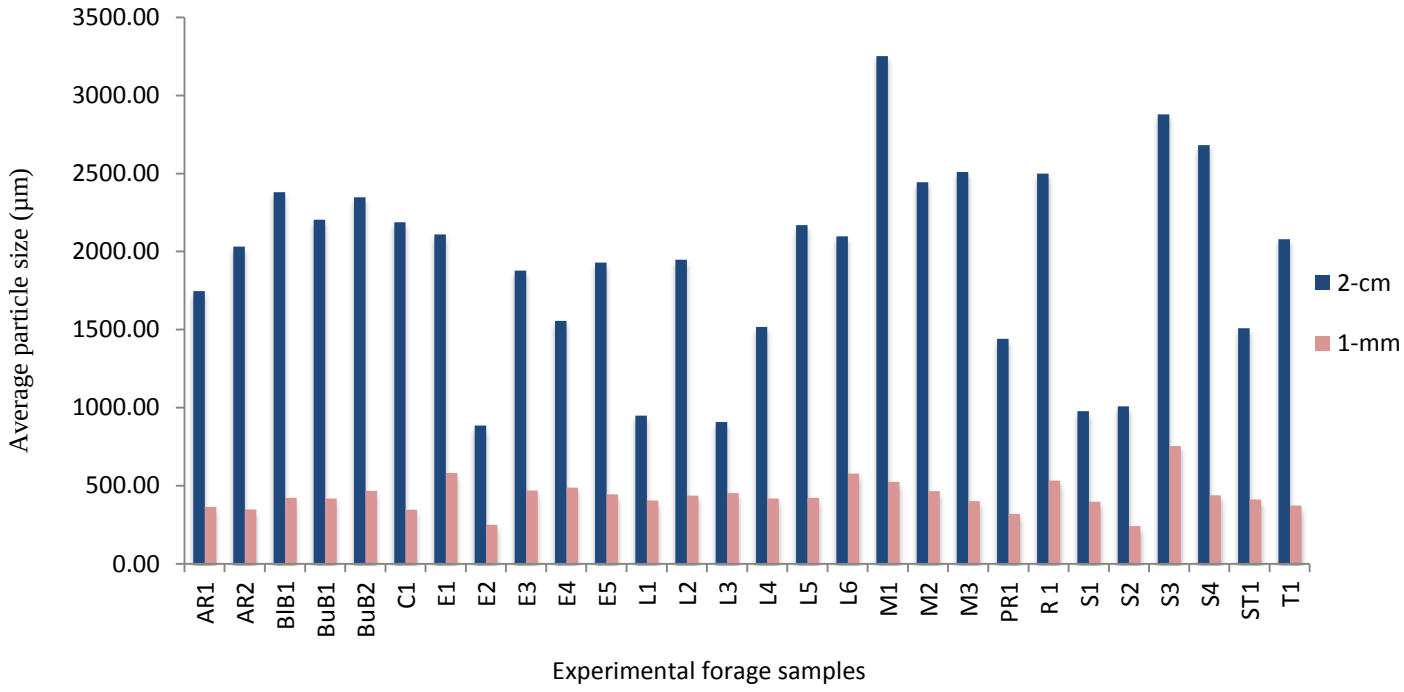

Figure 4.2 Average particle size distributions of forage samples after milling through a 2 cm and 1 mm screen

4.2.2 Direct energy measurements

According to Lopo (2002), energy required for grinding biomass depends on the particle size distribution, moisture content, bulk and particle densities, feed rate of the material and machine variables. 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 (Yu *et al.*, 2006). With an increase in fibre concentration as forages mature, there is an associated increase in the quantity of energy required to grind dried samples through a 1 mm screen, and therefore, the maturity of the forage has a big influence on the energy required for grinding (Chenost, 1966). In Table 4.7, the direct energy as measured during

grinding of the forage samples with a Retsch ultra-centrifugal mill and a Perten laboratory hammer mill, both fitted with a 1 mm screen, is presented. Energy measurements are reported in joules/g sample (DM basis).

In Table 4.7 it is observed that during milling with the Retsch ultra-centrifugal mill, *L. multiflorum* sample 1 and 2 (41.9 J/g and 43.1 J/g respectively), as well as *M. sativa* sample 1, 3 and 4 (34.9 J/g, 42.8 J/g and 48.0 J/g respectively) required the least energy of all the forages during grinding through a 1 mm screen. Maize stover sample 1 and 2 required the most energy during grinding (200.6 J/g and 216.8 J/g respectively). The energy requirements for grinding for all other samples ranged between 53.3 J/g and 169.4 J/g.

With the grinding of the forage samples using the Perten laboratory hammer mill, *M. sativa* sample 1and 3 required the least energy during grinding of all the samples (112.2 J/g and 139.6 J/g respectively), while maize stover required the most energy of all the forages to be milled through the 1 mm screen (347.8 J/g - 356.7 J/g). The other samples had energy requirement values ranging between 160.1 J/g and 334.0 J/g. The samples requiring the least and most energy during grinding is similar in the two mills used. Laredo & Minson (1973) reported a value of 337 J/g for energy expenditure of tropical grass (C4 grasses) stems during grinding, and 201 J/g for tropical grass leaves. These values are in agreement with the values observed in this study.

As illustrated in Table 4.7, the energy measurements during grinding of the experimental forage samples with the Retsch ultra-centrifugal mill was much lower than that of the Perten laboratory hammer mill during grinding. Unfortunately, the particle size distribution was measured only once and not after milling with each of the mills.

Mani *et al.* (2004) reported that maize stover had much lower energy requirement for grinding than switchgrass, and stated that it was expected that maize stover would have a low grinding energy requirement due to its low fibre concentration and the presence of more spongy vascular tissues in the stem. Bitra *et al.* (2009b) also reported lower effective specific energy for maize stover than for switchgrass. This however, is contradictory to the findings in this study, as maize stover had very high grinding energy requirement values during grinding with both mills. Weston (1985) reported comminution values of 31 kJ/kg DM for high quality grass, 64 kJ/kg DM for medium quality grass and 117 kJ/kg DM for low quality grass. The author measured comminution energy as the energy to reduce particles to a size which can readily pass through a 1 mm screen (Weston, 1985). Weston (1985) stated that less energy is required to grind legumes that grasses, even though legumes have a higher concentration of lignin that grasses. This could be due to the localized deposition of lignin in legumes (core lignin), as well as the fact that legumes do not have lignin deposition in their leaves, whereas grasses have lignin in both stem and leave fractions.

Table 4.7 Direct energy measurements of experimental forages using two different types of mills

Direct energy measurements (joules/g sample on DM basis)		
Roughage	**Retsch ultra-centrifugal mill**	**Perten laboratory hammer mill**
Medicago sativa 1	35.0	112.2
Medicago sativa 2	64.6	182.4
Medicago sativa 3	42.8	139.6
Medicago sativa 4	48.0	160.1
Medicago sativa 5	53.3	215.1
Medicago sativa 6	75.1	193.3
Lolium multiflorum 1	41.9	169.1
Lolium multiflorum 2	43.1	214.8
Lolium perenne 1	70.9	185.3
Dactylis glomerata 1	75.8	226.8
Festuca arundinacea 1	56.6	183.4
Cenchrus ciliaris 1	106.8	277.1
Panicum maximum 1	65.6	271.4
Panicum maximum 2	91.5	333.3
Eragrostis curvula 1	139.4	318.2
Eragrostis curvula 2	65.4	299.3
Eragrostis curvula 3	64.1	240.0
Eragrostis curvula 4	73.5	210.0
Eragrostis curvula 5	67.5	197.9
Maize stover 1	200.6	347.8
Maize stover 2	216.8	356.7
Maize stover 3	138.8	322.0
Chloris gayana 1	88.1	178.3
Digitaria eriantha 1	68.3	214.8
Digitaria eriantha 2	74.0	206.9
Digitaria eriantha 3	169.4	325.7
Digitaria eriantha 4	115.2	334.0
Cynodon nlemfuensis 1	78.6	217.4

In Figure 4.3, the grinding energy values obtained across samples for both mills are plotted. The hammer mill is widely used in the forage industry, because hammer mills are cheap, easy to operate and produce a wide range of particles (Mani *et al.*, 2004). Hammer mills reduce the particle size of materials through shear and impact action (Mani *et al.*, 2004). The energy usage during grinding depends on the initial particle size, moisture content of the sample, the properties of the forage being ground, the feed rate into the mill and the machine variables (Mani *et al.*, 2004). According to Mani e*t al.* (2004), the performance of the mill is measured in terms of energy usage and the geometric mean particle diameter and particle size distribution of the ground product. Yancey *et al.* (2013) stated that operating speed, moisture content and initial particle size appear to be extremely important factors in minimizing effective specific energy requirements for biomass size reduction.

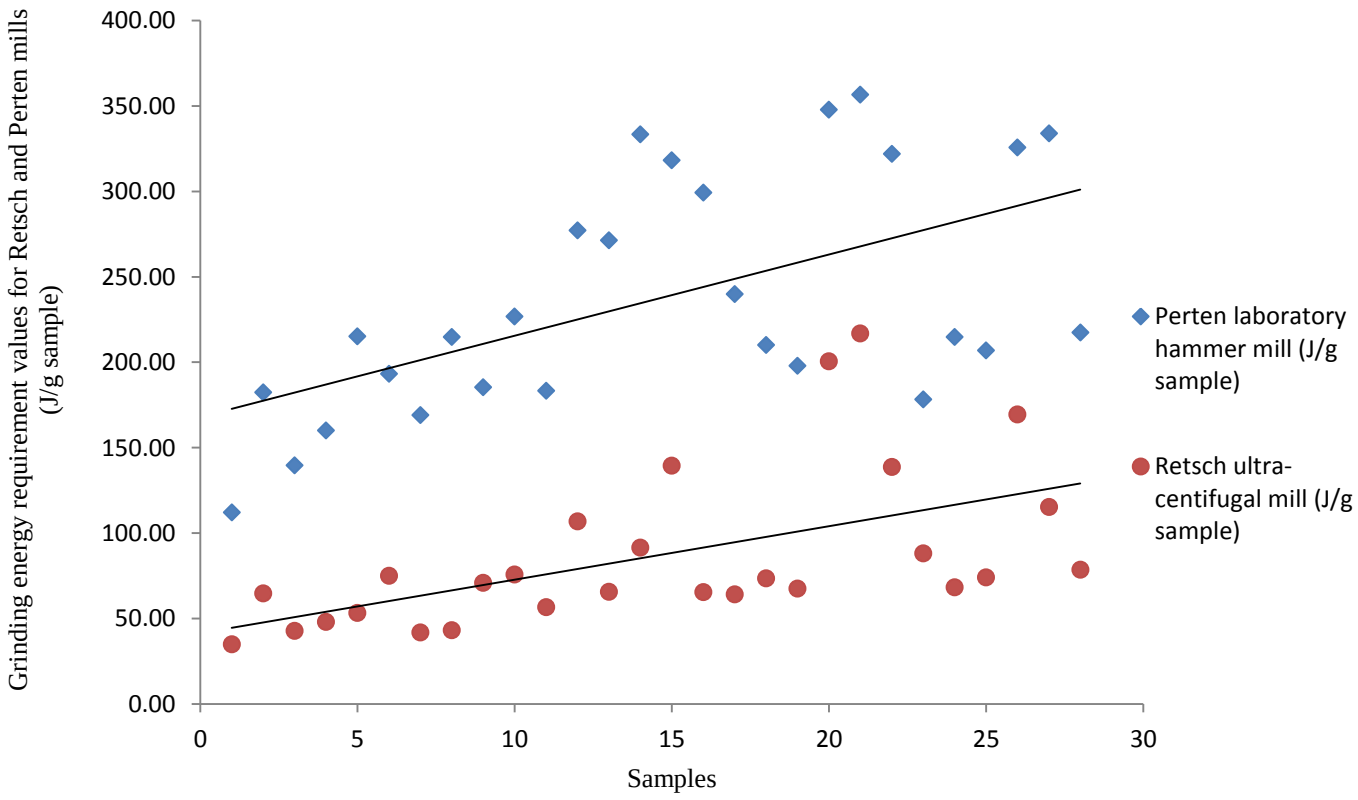

Figure 4.3 Illustration of direct energy measurement values for Retsch ultra-centrifugal mill and Perten laboratory hammer mill obtained during the grinding of the experimental forage samples (n = 28)

The mean energy requirement during grinding with the Retsch ultra-centrifugal mill was 86.8 J/g and for the Perten laboratory hammer mill was 236.9 J/g. From the t-test for two dependent variables the difference of 150.1 ± 7.8 J/g for the two mills was highly significant ($P < 0.05$). In Table 4.8, the summary of the t-test for the two mills are presented.

Table 4.8 Summary of t-test for two dependent variables during the measurement of grinding energy (n = 28)

	Mean	SD
Retsch ultra-centrifugal mill	86.8[a]	46.75
Perten laboratory hammer mill	236.9[b]	68.92

SD = Standard Deviation from the mean

[ab]Means in the same column with different superscripts differ ($P < 0.05$)

4.3 POSSIBLE ASSOCIATION BETWEEN CHEMICAL COMPOSITION AND GRINDING ENERGY

From the tests for linearity and possible non-linearity, it was clear that starch did not influence the energy required for grinding of either one of the mills significantly, and because the starch was present in the forage samples in small quantities, starch was excluded from the final models for more accurate determination of the influence of the other chemical components on grinding energy. There were no trends found between the grinding energy requirement values obtained for the legumes and C3-grasses if they were analysed together. When plotting the chemical components against the grinding energy requirement values for the C3-, C4- grasses, as well as the legumes, each of these three groups are located in separate regions of the plot area (Figure 4.4 and 4.5), and because the limited number of samples for C3-grasses and legumes, final modelling commenced on C4-grasses only.

In Table 4.9, Pearson's correlation coefficients between the grinding energy requirement of the two mills and the chemical components for C4- grasses are presented, and the chemical components with significant interactions and influences on grinding energy are indicated. Strong co- linear relationships exists between ash and OM, as well as between TT and TP, as can be seen by the correlation coefficients indicated ($-1 < r < 1$), and therefore, ash and TT were left out of the final modelling to increase accuracy of prediction from the final models. These two components were chosen to be left out since ash is included in the OM component of the forage samples, and TT is included in the TP component of the samples.

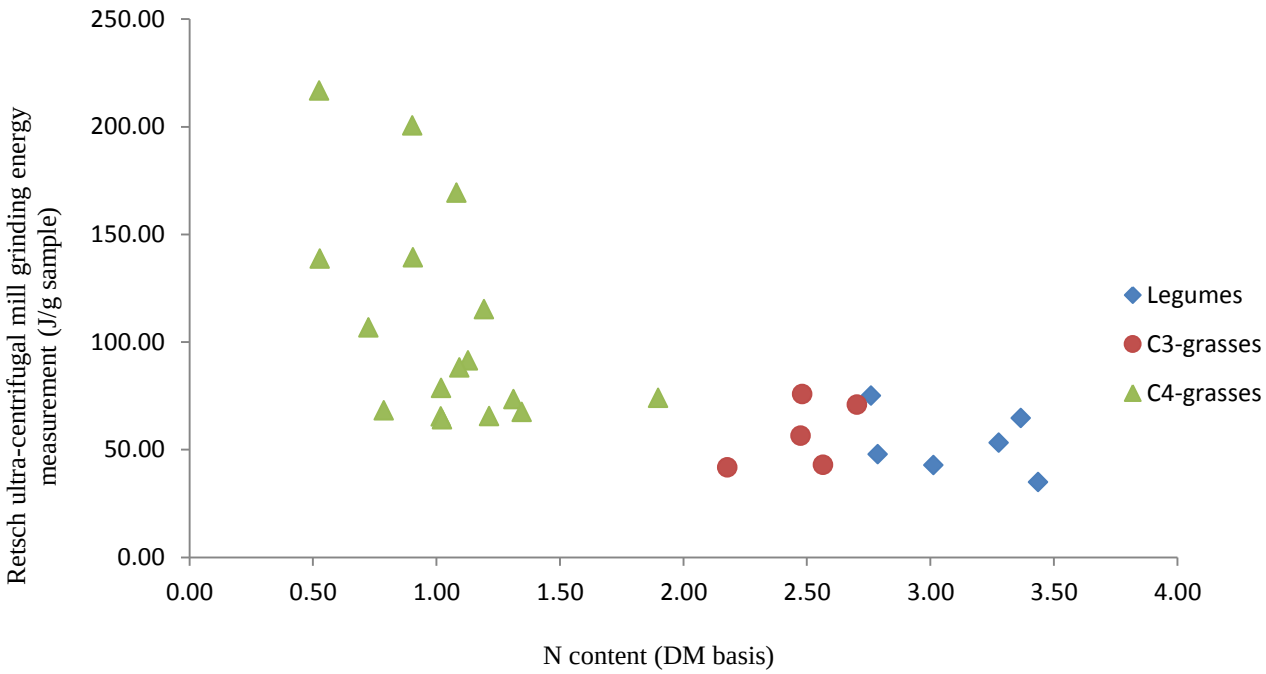

Figure 4.4 Example of the formation of separate groups by the legumes, C3- and C4 grasses when plotting the grinding energy requirement values of the Retsch ultra-centrifugal mill against the N concentration of the experimental forage samples

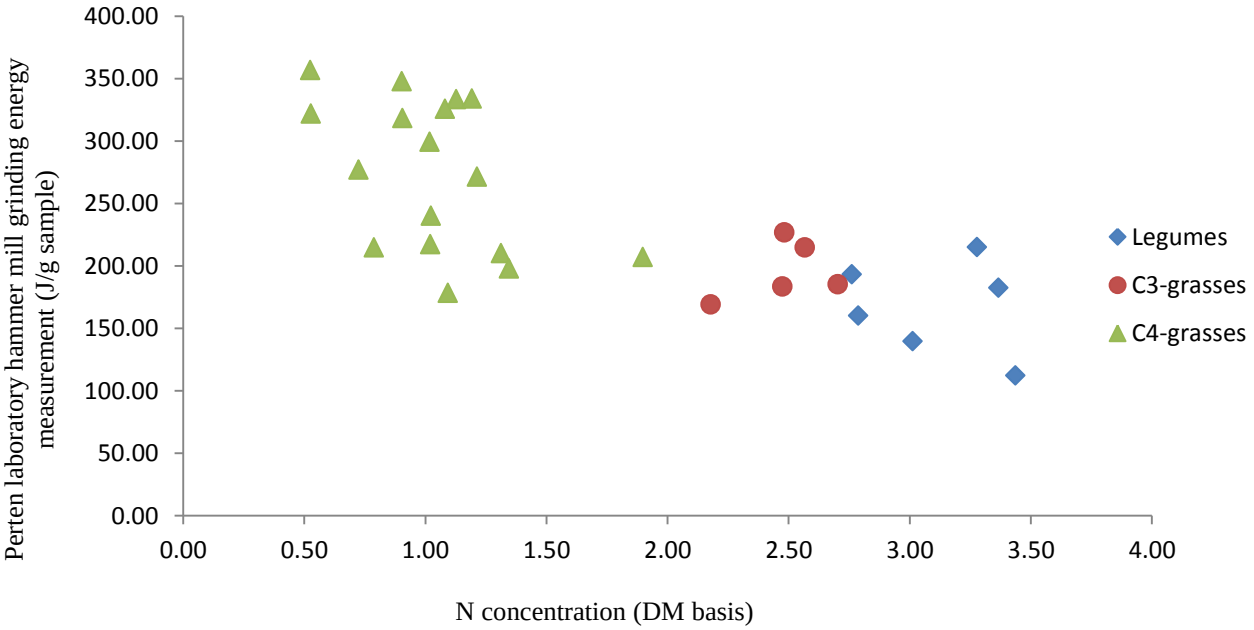

Figure 4.5 Example of the formation of separate groups by the legumes, C3- and C4 grasses when plotting the grinding energy requirement values of the Perten laboratory hammer mill against the N concentration of the experimental forage samples

Table 4.9 Pearson's correlation coefficients for Retsch – and Perten mill grinding energy requirement values with chemical components for C4-grasses (n=17)

	Perten	Retsch	DM	OM	Ash	N	ADF	NDF	ADL	*iv*NDFd	NDFkd	TP	NTP	TT	IPS	% change in particle size
Perten	-															
Retsch	0.7421***	-														
DM	0.1784	0.1683	-													
OM	0.4083	0.2439	0.6565***	-												
Ash	-0.4083	-0.2439	-0.6565***	**-1.0000*** **	-											
N	-0.4930**	-0.5211**	-0.2992	-0.3426	0.3426	-										
ADF	0.4716*	0.5248**	0.4038	0.3406	-0.3406	-0.6894***	-									
NDF	0.2184	0.1732	0.5040**	0.1602	-0.1602	-0.1472*	0.7842***	-								
ADL	0.1899	0.0991	0.4661*	0.2332	-0.2332	-0.4358*	0.8009***	0.8599***	-							
*iv*NDFd	-0.0040	-0.0831	-0.1059	-0.1033	0.1033	0.2145	-0.5601**	-0.4086	-0.5420**	-						
NDFkd	-0.2998	-0.4589*	-0.1247	-0.0154	0.0154	0.1766	-0.4877**	-0.3072	-0.3763	0.2159	-					
TP	0.0361	-0.0612	-0.0432	-0.0029	0.0029	0.6774***	-0.2865	-0.1130	-0.1777	0.3015	-0.1652	-				
NTP	0.3608	0.5791**	0.3453	0.1881	-0.1881	-0.4169*	0.0685	-0.0483	-0.1963	0.3805	-0.1644	-0.1746	-			
TT	-0.0234	-0.1245	-0.1032	-0.0375	0.0375	0.7298***	-0.3661	-0.2019	-0.2530	0.3123	-0.1092	**0.9935*** **	-0.2211	-		
IPS	0.5792**	0.7010***	-0.2034	-0.2201	0.2201	-0.3578	0.4584*	0.1703	0.1713	-0.0735	-0.3568	-0.1561	0.1483	-0.1887	-	
% change in particle size	0.4889**	0.4269*	0.0182	-0.3333	0.3333	-0.1258	0.3526	0.2909	0.2796	-0.0380	-0.2163	0.0486	0.1804	0.0083	0.7222***	-

*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001

Numbers in bold indicate co-linearity between chemical components

Perten: Perten laboratory hammer mill

Retsch: Retsch ultra-centrifugal mill

In Table 4.10, the variables with the most significant influence on the grinding energy requirement of both the Retsch ultra-centrifugal mill and the Perten laboratory hammer mill are presented, with their respective adjusted R^2, standard error of regression (SER) and probability values, for the C4- grasses.

Table 4.10 Best subset selection models to predict possible association between Retsch- and Perten grinding energy measurement values and chemical components of C4-grasses (n=17)

Retsch ultra-centrifugal mill		Perten laboratory hammer mill	
Chemical component in model	Regression coefficients (± SE)	Chemical component in model	Regression coefficients (± SE)
Intercept	238.4 (±62.3)	Intercept	1361 (± 817)
N	-49.1 (± 21.9)	DM	-44.7 (± 11.7)
*iv*NDFd	-2.412 (± 0.617)	OM	30.73 (± 5.11)
NDFkd	5.44 (± 3.70)	N	-77.2 (± 29.4)
TP	9.45 (± 2.26)	ADF	-7.27 (± 3.45)
NTP	1.707 (± 0.285)	NDF	0.2229 (± 0.0835)
IPS	0.06315 (± 0.01000)	ADL	-15.89 (± 9.11)
% change in particle size	-2.882 (± 0.981)	NDFkd	-12.71 (± 5.24)
		% change in particle size	8.85 (± 1.35)
Adjusted R^2	89.2%	Adjusted R^2	82.2%
SER	16.2	SER	25.5
Probability	<0.001	Probability	0.002

SER = standard error of regression

It is observed from the final models for the C4-grasses, as illustrated in Table 4.10, that 89.2% of the variation in energy usage during grinding with the Retsch ultra-centrifugal mill, could be explained by the N concentration, *iv*NDFd, NDFkd, the TP and NTP concentration, IPS and the % change in particle size. The regression coefficients for N concentration, *iv*NDFd, and % change in particle size are instead negative for the analysis of the Retsch mill, therefore these independent variables are associated with a decrease in grinding energy when increasing independently, while holding the other predictors. The coefficients for NDFkd, TP, NTP and IPS are instead positive, meaning with an increase in any one of these specific components/ measurements, there is an increase in energy usage during grinding with the Retsch mill.

The results for the C4-grasses, ground by the Perten laboratory hammer mill, as illustrated in Table 4.10, indicate that 82.2% of the variation observed in the grinding energy requirement could be explained by DM, OM, N, ADF, NDF, ADL, NDFkd and % change in particle size. There are negative regression coefficients between the grinding energy and the DM, N, ADF, ADL and NDFkd, indicating that these components decrease energy required for grinding processes, instead of increasing energy required for

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grinding as would be expected in general. There is a positive regression coefficient between the OM, NDF and % change in particle size and the energy required for grinding of the C4- forage samples with the Perten mill, therefore increasing energy required for grinding. In contradiction to the positive regression coefficient observed for NDFkd (5.44 ± 3.70) with the Retsch mill during grinding of the C4-grasses, NDFkd has a negative regression coefficient (-12.75 ± 5.24) for grinding the C4- grasses with the Perten mill. Another contradiction is the % change in particle size which has a negative regression coefficient of -2.882 (± 0.981) for the analysis of grinding of the C4-grasses with the Retsch mill, whereas the analysis of the Perten mill shows a positive regression coefficient of 8.85 (± 1.35).

In Table 4.11, the variables with the most significant influence on the grinding energy requirement of both the Retsch ultra-centrifugal mill and the Perten laboratory hammer mill are presented, with their respective adjusted R^2, standard error of regression (SER) and probability values, for the legumes and C3-grasses respectively.

Table 4.11 Best subset selection models to predict possible association between Retsch- and Perten grinding energy measurement values and chemical components of legumes, C3- and C4-grasses (n=28)

Retsch ultra-centrifugal mill		Perten laboratory hammer mill	
Chemical component in model	Regression coefficients (± SE)	Chemical component in model	Regression coefficients (± SE)
Intercept	-381 (± 186)	Intercept	-961 (± 328)
OM	4.15 (± 2.30)	OM	11.14 (± 3.08)
ADF	2.86 (± 1.18)	N	-18.36 (± 9.29)
ADL	-9.01 (± 2.97)	IPS^2	0.00001007 (± 0.00000350)
NDFkd	-6.64 (± 3.29)	% change in particle size	2.19 (± 1.02)
IPS^2	0.00000991 (± 0.00000231)		
Adjusted R^2	77.2%	Adjusted R^2	79.4%
SER	22.3	SER	31.3
Probability	<0.001	Probability	<0.001

SER = standard error of regression

In Table 4.11 it is observed that 77.2% of the variation in grinding energy of the pooled samples (legumes, C3- and C4- grasses), could be explained by OM, ADF, ADL, NDFkd and the quadratic term of IPS. The regression coefficients for ADL and NDFkd are negative for the analysis of the Retsch ultra-centrifugal mill, indicating that these independent variables are associated with a decrease in energy required for grinding when increasing independently while holding the other predictors, which is the opposite of what would generally be expected with regards to the effect of ADL and NDFkd on energy required during the

process of comminution. The coefficients for OM, ADF and the quadratic term of IPS are positive for the analysis of grinding energy requirement of the Retsch mill, meaning that an increase in any one of these variables are associated with an increase in energy usage during grinding. The regression coefficient for NDFkd is negative (-6.64 ± 3.29) for the analysis of grinding energy requirement of the Retsch mill during grinding of the pooled samples, which is in agreement with the negative regression coefficient (-12.71 ± 5.24) observed for the analysis of energy usage during grinding of the C4-grasses with the Perten mill, but is in contradiction with the positive coefficient observed (5.44 (± 3.70)) for the analysis of grinding energy requirement for the Retsch mill during grinding of the C4-grasses. The regression coefficient for ADL is negative (-9.01 ± 2.97) for the analysis of energy requirement of the Retsch mill during grinding of the pooled samples, which is in agreement with the negative coefficient (-15.89 ± 9.11) indicated for the analysis of energy usage during grinding of the C4- grasses by the Perten mill. The coefficient for ADF is positive (2.86 ± 1.18) during the analysis of grinding energy required for grinding of the pooled samples with the Retsch mill, which is in contradiction with the negative coefficient (-7.27 ± 3.45) for ADF during the analysis of grinding energy requirement for grinding of the C4- grasses with the Perten mill.

The results for the Perten laboratory hammer mill as illustrated in Table 4.11, indicates that 79.4% of the variation observed during grinding of all the samples could be accounted for by OM, N, the quadratic term of IPS, as well as the % change in particle size. The regression coefficient for N concentration is negative (-18.36 ± 9.29) for the analysis of energy requirement for grinding of the pooled samples with the Perten mill, indicating that N concentration is associated with a decrease in grinding energy when increasing independently. The coefficients for OM, the quadratic term of IPS and % change in particle size, are positive during grinding of the pooled samples, meaning that with an increase in any one of these specific components, there is an increase in energy required for grinding with the Perten mill. The positive coefficient (11.14 ± 3.08) observed between energy requirement during the grinding of the pooled samples with the Perten mill and OM, is in agreement with the positive coefficient observed between the grinding energy requirement of the Retsch mill during grinding of the pooled samples (4.15 ± 2.30), as well as the Perten mill during the grinding of the C4-grasses (30.73 ± 5.11). The coefficient for N-concentration is negative (-18.36 ± 9.29) during the analysis of grinding of the pooled samples with the Perten mill, which is in agreement with the negative coefficients observed for the analysis of the grinding energy requirement for the Retsch mill (-49.1 ± 21.9) and the Perten mill (-77.2 ± 29.4) during grinding of the C4-grasses. The regression coefficient for IPS is positive (0.00001007 ± 0.00000350) for the analysis of grinding energy requirement of the pooled samples with the Perten mill, which is in agreement with the positive coefficient observed during analysis of the pooled samples and the Retsch mill (0.00000991 ± 0.00000231) and the C4-grasses and the Retsch mill (0.06315 ± 0.01000). The coefficient for % change in particle size is positive

(2.19 ± 1.02) for the analysis of energy requirement during grinding of the pooled samples and the Perten mill, which is supported by the positive coefficient (8.85 ± 1.35) observed for the analysis of grinding energy requirement of the C4- grasses when ground with the Perten mill, but is in contradiction with the negative coefficient (-2.882 ± 0.981) for the analysis of grinding energy requirement of the Retsch mill during grinding of the C4- grasses.

Although modelling commenced on C4- grasses only, for comparison of model and results, pooled samples (legumes, C3- and C4- grasses) were also modelled using the RSEARCH procedure of GenStat® (Payne *et al.*, 2012).

At the end, the following models were used:

Retsch ultra-centrifugal mill model (C4-grasses):

$$y = 238.4 - 49.1\text{N} - 2.412iv\text{NDFd} + 5.44\text{NDFkd} + 9.45\text{TP} + 1.707\text{NTP} + 0.06315\text{IPS} - 2.882\%\text{ change in particle size}$$

Adjusted R^2 = 0.892; $P < 0.001$; SER = 16.2

Retsch ultra-centrifugal mill model (Pooled samples):

$$y = -381.0 + 4.15\text{OM} + 2.86\text{ADF} - 9.01\text{ADL} - 6.64\text{NDFkd} + 0.00000991\text{IPS}^2$$

Adjusted R^2 = 0.772; $P < 0.001$; SER = 22.3

Perten laboratory hammer mill model (C4-grasses):

$$y = 1361.0 - 44.7\text{DM} + 30.73\text{OM} - 77.2\text{N} - 7.27\text{ADF} + 0.2229\text{NDF} - 15.89\text{ADL} - 12.71\text{NDFkd} + 8.85\%\text{ change in particle size}$$

Adjusted R^2 = 0.822; $P = 0.002$; SER = 25.5

Perten laboratory hammer mill model (Pooled samples):

$$y = -961.0 + 11.14\text{OM} - 18.36\text{N} + 0.00001007\text{InPartSize}^2 + 2.19\%\text{ change in particle size}$$

Adjusted R^2 = 0.794; P <0.001; SER = 31.3

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The final models indicate that DM has a negative regression coefficient (-44.7 ± 11.7) for the analysis of energy required for grinding of the C4-grasses with the Perten mill. Mani *et al.* (2004) reported that moisture content had a positive correlation with specific energy usage of wheat and barley straws, maize stover and switchgrass, and Balk (1964) reported a positive correlation between the moisture content of *M. sativa* and grinding energy requirement, which indicates that increased moisture content will decrease fragility of these forages. Therefore, the assumption can be made that if moisture content is positively correlated with energy usage during grinding, DM would be negatively correlated with grinding energy requirement, which supports the observations made in this study. Ige & Finner (1976) also reported that increased moisture content reduced shearing energy in maize stover and *M. sativa*. O'Dogherty *et al.* (1995) hypothesized that dry brittle straw was weaker (more fragile) than moist tough straw.

According to the results of this study, *E. curvula* sample 1 to 4, maize stover sample 2 and smuts finger sample 1 had high OM concentrations (> 95 g/100g). The final models for prediction of forage fragility as presented in Table 4.10 and 4.11 indicated that OM has a positive regression coefficient for the analysis of grinding energy requirement. This might explain the high energy value measured during grinding of *E. curvula* sample 1 as well as maize stover sample 2 as illustrated in Table 4.7. However, the other samples which had high OM concentration did not have extreme high grinding energy requirement values, especially with the Retsch mill. It has to be assumed that this is due to the many interactions between the chemical components and other factors influencing grinding energy requirement.

N concentration showed a negative regression coefficient for the analysis of grinding energy requirement during grinding for both mills. As illustrated in Table 4.1 the N concentration of the legumes and C3-grasses tended to be higher than the N concentration of the C4-grasses. It should be taken into account that N is present in relatively small amounts in the forages (<3.44 g/100g), and therefore the overall negative correlation of N with energy usage during grinding and an increase in the fragility of the forages, will be difficult to observe, when taking into account all other factors influencing grinding energy requirements.

In Tables 4.10 and 4.11, it can be seen that there is a contradiction in the results obtained for the coefficients for ADF during the analysis of grinding energy requirement. In the model for the C4- grasses ground by the Perten mill, ADF is indicated to have a negative regression coefficient (-7.27 ± 3.45), but has a positive regression coefficient (2.89 ± 1.18) during the analysis of the pooled samples when ground with the Retsch mill. It is therefore still not clear what the exact effect of ADF is on grinding energy and therefore forage fragility is. It might be that the effect of ADF is more profound in C4-grasses than in legumes and C3-grasses, which changes the model when all samples are included. Horrocks & Vallentine (1999) reported that as ADF increases, digestibility of the forage decreases. This would implicate that the fragility should

decrease with an increase in ADF concentration, therefore leading to increased energy usage during grinding, and therefore there should theoretically be a positive correlation between ADF and grinding energy requirement.

NDF appeared only in one of the four final models fitted to the data, and had a positive regression coefficient (0.2229 ± 0.0835) for the analysis of energy usage during grinding of the C-4 grasses with the Perten mill, as presented in Table 4.10. It is difficult to draw any conclusions from the results obtained in this study regarding the effect of NDF on energy usage during grinding and therefore the exact effect 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.*, 2002), the regression coefficient should also theoretically be positive for the analysis of grinding energy requirement, indicating that an increase in NDF concentration will lead to decreased forage fragility, which supports the positive coefficient observed in this study.

As illustrated in Tables 4.10 and 4.11, ADL increases energy usage during grinding, for both models, but only when using the hammer mill. This does not explain why *L. multiflorum* sample 1 which has the lowest ADL concentration of all the samples, as illustrated in Table 4.2, has low grinding energy requirement measurements, and also does not explain why maize stover sample 3, which has the highest ADL concentration of all the samples, has a high grinding energy requirement measurements. According to the final model, ADL decreases energy required for grinding and increases forage fragility. These inconsistencies make it difficult to come to any specific conclusions regarding the effect of ADL on forage fragility, and again, it should be noted that the interactions between chemical fractions and other factors could result in this counterintuitive effect of lignin, when considering the other factors in the model. It is also interesting to note that the simple correlations between ADL only and the grinding energy of both mills were 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, a work by Rinne and collaborators (2002) has shown faster particle size reduction in more mature forages (i.e. greater fragility or brittleness of the more lignified particles). Grenet (1989) found fewer large particles (on DM basis) in boluses of cattle fed late-cut rather than early-cut ryegrass (*Lolium multiflorum*), and Wilson *et al.* (1983) observed that particle size reduction of leaves from less-digestible tropical grass (*Panicum maximum* var. *trichoglume*) declined more in size during mastication than more- digestible temperate grass (*Lolium multiflorum*) leaves. Poppi *et al.* (1981), as cited by Allen (1996) reported that 12- week regrowth's of tropical grasses were more prone to particle size reduction than 6- week regrowth's. However, we do speculate that the specific chemical and structural relationships within cell wall, between cellulose, hemicellulose and lignin, and the total amount of cell wall on DM, result in most of the grinding energy variation among forages.

Jung *et al.* (1997) reported that there is a negative correlation between lignin concentration and fibre digestibility. Lignin is a major anti-nutritional component in grasses (Chaves *et al.*, 2002). Lignin is not only mostly indigestible, but the presence of lignin also inhibits the availability of associated cellulose and hemicellulose (Horrocks & Vallentine, 1999). 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. The regression coefficient for *iv*NDFd was negative during the analysis of grinding energy requirement, therefore indicating a decrease in energy required for grinding when there is an increase in *iv*NDFd. Grant (2010) reported that the relationship between NDF digestibility and forage fragility is positive, which supports the results in this study.

The regression coefficient for NDFkd is negative in two of the final models, and positive in one of the models. It is therefore still unclear from the results obtained in this study how NDFkd influences forage fragility, although it would seem that with an increase in NDFkd, there is a decrease in grinding energy requirement and therefore, increases forage fragility. It is important to remember that NDFkd should not be viewed separately from *iv*NDFd.

In Tables 4.10 and 4.11 it can be seen that the regression coefficients for both TP and NTP are positive, indicating that an increase in any one of these components will lead to an increase in energy required for grinding. This is in agreement to the high TP concentration of *D. glomerata* sample 1 and *D. eriantha* sample 2 (10.1 g/kg and 14.6 g/kg respectively), and the high grinding energy requirement values as illustrated in Table 4.7 for these specific samples. The NTP is present in very small quantities in the forage samples (<2.89 g/kg), and although it would increase energy required for grinding since the coefficient for NTP is positive for the analysis of grinding energy requirement, the effect would be very difficult to observe in the energy measurements obtained.

It was clear from the initial models that IPS accounted for most of the variation in the energy required during the process of grinding for both mills. In Table 4.6, it is illustrated that maize stover sample 1 and smuts finger sample 3 had very high initial particle sizes (3250.6 µm and 2879.2 µm respectively). The energy usage during grinding as presented in Table 4.7 indicates that maize stover sample 1 and smuts finger sample 3 had very high energy requirement values during grinding for both mills (347.8 J/g and 325.7 J/g respectively for the Perten mill, and 200.6 J/g and 169.4 J/g respectively for the Retsch mill). This is in agreement with the final models obtained as illustrated in Table 4.10 and 4.11, which indicated a positive regression coefficient for IPS for the analysis of energy usage during grinding, thereby indicating that an increase in IPS is associated with an increase in energy required for grinding, meaning forage fragility is decreased. 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.

The regression coefficients for % change in particle size was positive in two of the models, and negative in one of the models, as illustrated in Tables 4.10 and 4.11. The results of the % change in particle size of the forage samples is illustrated in Table 4.6, and when comparing the % change in particle size to energy measurements obtained during grinding, the samples which had a high % change in particle size (83%), also had high grinding energy requirement values for both mills. The samples with low % change in particle size (50.2% – 59.31%) had relatively low grinding energy requirement values. Therefore it would seem that there is a positive correlation between the % change in particle size and energy required for grinding, thereby decreasing forage fragility. It might also be true that the samples with the smallest particle size change were the ones with initial geometrical mean size closer to the final particle size, and therefore resulting in the smallest change needed.

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CHAPTER 5

5. CONCLUSION

Forages represent an important constituent of the ruminant diet. The physical and chemical properties of forages influence their nutritional value, and chewing activity is the response reflecting the chemical and physical properties of feeds. 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. Physical effective fibre attempts to take both the physical and chemical properties of fibre, which influence the chewing activity, into account. Chewing activity is important for the stimulation of secretion of salivary buffers to control rumen pH, and is also an important indicator of the physical environment of the rumen which promotes optimal rumen fermentation. Less effective fibre levels in the diet result in simultaneous- and linked reactions occurring, which lead to lowered ruminal fermentation. Less effective fibre in the diet leads to lowered chewing activity, causing less salivary buffer secretion into the rumen, and increased VFA production due to the lowered rumen pH, leading to a change in ruminal microbial populations. The peNDF system was developed with the main objective of predicting chewing response accurately, based on the measurement of forage or feed particle sizes, as well as the NDF content, but is based on the assumption that the fragility among different sources of NDF is similar, however, this assumption is not correct. The peNDF system is widely used by many nutritional models to predict lactational response, cow chewing response and rumen pH. NDF has been used as the only feed characteristic to predict the filling effects of forages, but there is substantial evidence that NDF alone is inadequate 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.

The possibility exists to predict forage fragility from energy required for grinding as related to the chemical composition forages, but it is a concept difficult to disentangle. Inconsistencies exist in the ability to measure forage fragility in the laboratory, as indicated by the number of unexplained results as well as the amount of contradictions in the final models fitted to the data. Forage fragility cannot be attributed to one single chemical component alone, but is influenced by many factors with possible interactions among some of these factors. Numerous studies have been carried out to investigate the effect of forage particle size on feed intake, chewing activity, and ruminal processes. However, the response from most of these studies has been inconclusive due to the complexity of the interactions between many factors. Unfortunately, the low number of samples has not allowed us to test for possible interactions between the variables included. The large variability among the chemical composition and physical characteristics of the species tested during

this study, as well as the differences between the two mills used, influenced the results obtained during this study.

The results obtained from this study showed that with an increase in DM content of the forage sample, energy required for grinding decreases, indicating that DM content increases forage fragility. With an increase in OM content of the forage samples, there is an increase in grinding energy requirement, indicating that OM content decreases forage fragility. The N concentration decreases energy required for grinding of the samples, indicating that with an increase in N concentration, forage fragility increases. The exact effect of ADF on forage fragility is still unknown, as no clear conclusions could be drawn from the results of this study, as there were contradictions in the results showing the effects of ADF on grinding energy. However, based on literature, an increase in ADF content will increase energy required for grinding, and therefore ADF decreases forage fragility. According to the results of this study, NDF has a positive correlation with energy required for grinding, thereby indicating that an increase in NDF can be associated with decreased fragility of the forage. However, this effect was only observed in the C4-grasses, and the mode of action of how NDF exactly affects fragility of legumes and C3-grasses is still unknown. The result regarding ADL was inconclusive. It would be expected that an increase in ADL will increase energy requirement during grinding, indicating that ADL can be associated with decreased forage fragility, but results from previous studies have contradicted this assumption. An increase in 24-hour *in vitro* NDF digestibility is associated with a decrease in energy required for grinding of the samples, indicating that forage fragility increases with an increase in 24-hour *in vitro* NDF digestibility. An increase in the rate of NDF digestion is correlated with a decrease in energy required for grinding, thereby indicating that an increase in rate of NDF digestion can be associated with increased forage fragility. With an increase in TP and NTP, grinding energy requirement increases, indicating that the secondary metabolites TP and NTP lead to decreased forage fragility. Initial particle size has an important influence on grinding energy requirement and with an increase in initial particle size, energy required for grinding decreases, indicating increased initial particle size can be associated with increased forage fragility. An increase in the % change in particle size from initial to final particle size was correlated with increased energy required during grinding. Therefore an increase in % change in particle sizes of the forages used during this study indicated decreased fragility of the forages. If a large particle has a low fragility due to the concentration of chemical components which affects forage fragility negatively, the more energy will be needed to break that particle into smaller pieces. How these interactions and the results obtained will change with various larger and smaller particles, with different concentrations of specific chemical components and relationships, still needs to be investigated.

Chemical composition of forages clearly has an influence on forage fragility, but for now, only possible effects and assumptions can be made regarding the influence of chemical composition on forage fragility, since it is clear that multiple factors influence forage fragility at the same time. More research is needed to investigate the relationship between chemical components and factors influencing forage fragility for better prediction of forage fragility. It is clear that NDF alone cannot be used to accurately predict the effect of the roughage component of the diet as related to chewing activity, rumen environment, and ME available for animal production. The factors that determine the nutritional value of forages are complex. The energy required for eating and ruminating should be considered as a quantifiable component of the total energy budget instead of an indefinite consequence of feed composition or processing. There is a need for better understanding of the factors relating to particle fragility, as there is limited data on the relationship between chemical composition and the rate of particle size reduction, although it is clear that differences in fragility exist among forages and these differences need to be accounted for in models that predict intake and flow of digesta in ruminants.

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CHAPTER 6

6. CRITICAL EVALUATION

In order to develop an effective method to predict forage fragility accurately in the laboratory by measuring grinding energy, a wide range of forages used in the diets of ruminants have to be evaluated. Forage fragility could be measured more accurately when all possible factors influencing the chemical composition of the forages are kept similar for all samples, such as maturity level of the plants, environmental factors including fertiliser treatments, climate and season, soil composition, as well as cutting and drying methods, although all these factors can become very complex.

The results for forage fragility is clearly also influenced by the type of mill used during grinding of the samples and direct energy measurement. Not all mills are equally efficient in grinding forages, because the purpose for which the mills were manufactured differ, and therefore the measurement of forage fragility can be improved by comparing grinding results from a wide range of mills commonly used in laboratories.

In this study, particle size distribution was measured only once for each sample, not for every mill used. The efficiency of different mills could be determined more accurately if forage samples are ground with a 2 cm screen of each specific mill used, after which particle size distribution is determined, and then ground with a 1 mm screen of that specific mill again, after which particle size distribution is determined once again. It might also increase the accuracy of prediction of forage fragility if IPS is more homogenous, since it has a major influence on energy required for grinding. Other mills could be tested for more homogenous initial particle size, or the initial particle size could be decreased which would decrease the variability. The particles could be sieved after pre- grinding, however, that will lead to biased results, since the distribution of chemical components are not similar across different particle sizes.

In this study, too few legume and C3- grass samples were collected to determine the effects of chemical components on forage fragility as accurately as the C4- grass species. Therefore, in future studies, more legume samples (C3 and C4) as well as C3- grass species should be collected and evaluated for accurate estimations of the effects of various chemical components on the energy requirement during grinding used in the process of development of a method to estimate forage fragility in the laboratory. This study therefore only serves as a possible guideline for future modelling with equal number of replications and much greater numbers of samples of legumes as well as C3-grasses. It is also very important to remember, that the final statistical models proposed in this study, is based on the chemical composition and number of experimental forage samples used in this specific study. With more samples, and possibly more variability in chemical composition analyses values of other samples, the models might change. Therefore

once again, this study serves as a basis for future studies and the development of an accurate model to predict forage fragility in the laboratory.

Actual chewing activity measured *in vivo* will provide valuable information for comparing efficiency of laboratory predictions of forage fragility with relation to specific chemical components, and to determine which mills are the most accurate to provide measurements that correlate to the chewing activity of cows and therefore, the most accurate and useful estimation of energy used during grinding of forages.

REFERENCES

Agriculture Canada, 1971. Size reduction. Agricultural Materials Handling manual. Agriculture Canada,Ottawa,ON.

Aikman, P.C., Reynolds, C.K. & Beever, D.E., 2008. Diet digestibility, rate of passage, and eating and rumination behavior of Jersey and Holstein cows. J. Dairy Sci. 91, 1103-1114.

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

Allen, M.S., 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fibre. J. Dairy. Sci. 80, 1447-1462.

Allen, M.S. & Mertens, D.R., 1988. Evaluating constraints on fibre digestion by rumen microbes. Br. J. Nutr. 188, 261-270.

Allen, M.S. & Oba, M., 1996. Fibre digestibility of forages. Proceedings of the 57th Minnesota Nutr. Conf. Protiva Tech. Symp. Ext. Special programs, Bloomington, MN. University of Minnesota, St. Paul. pp. 151-171.

Animal Husbandry: AHS-3, Booklet nr. 33. Feeds for farm animals and poultry.

AOAC., 1984. Official methods of analysis (14th ed). Association of Official Analytical Chemists, Inc., Arlington, Virginia, USA.

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

Arthur, J.F., Kepner, R.A.,Dobie, J.B., Miller, G.E. & Parson, P.S., 1982. Tub grinder performance with crop and forest residues. Trans. ASAE 25(6), 1488-1494.

Balch, C.C., 1971. Proposal to use time spent chewing as an index of the extent to which diets for ruminants possess the physical property of fibrousness characteristics of roughages. Br. J. Nutr. 26, 383.

Balk, W.A., 1964. Energy requirements for dehydrating and pelleting coastal Bermudagrass. Trans. ASAE. 4, 349-355.

Beauchemin, K.A., 1991. Effects of dietary neutral detergent fibre concentration and alfalfa hay quality on chewing, rumen function, and milk production of dairy cows. J. Dairy Sci. 74, 3140-3151.

Bitra, V.S.P., Womac, A.R., Chevanan, N., Miu, P.I., Igathinathane, C., Sokhansanj, S. & Smith, D.R., 2009a. Direct mechanical energy measures of hammer mill comminution of switchgrass, wheat straw and corn stover and analysis of their particle size distributions. Powder Technol. 193: 32-45.

Bitra, V.S.P., Womac, A.R., Cannayen, I., Miu, P.I., Yang, Y.T. & Sokhansanj, S., 2009b. Comminution energy consumption of biomass in knife mill and its particle size characterization. ASABE paper no. 095898. St. Joseph, Michigan. ASABE.

Casler, M.D., Schneider, D.K. & Combs, D.K., 1996. Development and application of a selection criterion for particle size breakdown of smooth bromegrass leaves. Anim. Feed Sci. Technol. 61, 57-71.

Casler, M.D. & Jung, H.G., 2006. Relationships of fibre, lignin, and phenolics to in vitro fibre digestibility in three perennial grasses. Anim. Feed Sci. Technol. 125, 151-161.

Caswell, H., Reed, F., Stephenson, S.N. & Werner, P.A., 1973. Photosynthetic pathways and selective herbivory: A hypothesis. Am. Nat. 107 (956), 465-480.

Chaves, A.V., Waghorn, G.C. & Tavendale, M.H., 2002. A simplified method for lignin measurement in a range of forage species. Proc. NZGA. 64, 129-133.

Cheeke, P.R., 1991. Applied animal nutrition: feeds and feeding. Macmillan publishing company. Macmillan Inc., NY.

Chenost, M., 1966. Fibrousness of forages: its determination and its relation to feeding value. Proc. 10th Int. Grassl. Congr. July 7-16. University of Helsinki, Finland. pp. 406-411.

Cherney, D.J.R., 2000. Characterization of forages by chemical analysis. In: Forage evaluation in ruminant nutrition. Eds Givens, D.I., Owen, E., Axford, R.F.E. & Omed, H.M. CABI publishing, CAB International, Wallingford, UK. pp. 281-300.

Chesworth, J., 1992. Ruminant nutrition. The tropical agriculturalist series. Macmillan education ltd, London, in cooperation with the CTA (Technical centre for agriculture and rural cooperation), Wageningen, The Netherlands.

Cotanch, K.W. & Grant, R.J., 2006. Measuring physically effective fibre on-farm to predict cow response. Proc. Cornell. Nutr. Conf. for feed manufacturers. October 24-26. Syracuse, NY. pp. 151-160.

Cotanch, K.W., Grant, R.J., Darrah, J., Wolford, H.M. & Eguchi, T., 2007. Development of a method for measuring forage fragility. J. Dairy Sci. 90 (Suppl. 1), 563-564 (Abstr.).

De Boever, J.L., Andries, J.I., De Brabander, D.L., Cottyn, B.G. & Buysse, F.X., 1990. Chewing activity of ruminants as a measure of physical structure—a review of factors affecting it. Anim. Feed Sci. Technol. 27,281-291.

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

Dickenson, E.B., Hyam, G.F.S., Breytenbach, W.A.S., Metcalf, H.D., Basson, W.D., Williams, F.R., Scheepers, L.J., Plint, A.P., Smith, H.R.H., Smith, P.J., Van Vuuren, P.J., Viljoen, J.H., Archibald, K.P. & Els, J.M., 2007. Pasture handbook. Kejafa Knowledge Works. Maanhaarrand. pp. 21-250.

Draper, N. & Smith, H., 1981. Applied regression analysis (2nd ed). John Wiley & Sons, NY. pp. 709.

Faichney, G.J. & White, G.A., 1983. Methods for the analysis of feed eaten by ruminants. CSIRO. Australia. ADSRI- Jan 1988.

Fang, Q., Hanna, M.A., Haque, E. & Spillman, C.K., 2000. Neural network modelling of energy requirements for size reduction of wheat. Trans. ASAE. 43(4), 947-952.

FAO/IAEA., 2000. Quantification of tannins in tree foliage. A laboratory manual for the FAO/IAEA co-ordinated research project on 'Use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage. FAO/IAEA working document. IAEA, Vienna. pp. 4-6.

Galyean, M.L. & Goetsch, A.L., 1993. Utilization of forage fibre by ruminants. In: Forage cell wall structure and digestibility. Eds: Jung, H.G., Buxton, D.R., Hatfield, R.D. & Ralph, J. American Society of Agronomy Inc., Crop Science Society of America Inc., Soil Science Society of America Inc., Madison, Wisconsin, USA. pp. 33-71.

Ghorbani, Z., Masoumi, A.A. & Hemmat, A., 2010. Specific energy consumption for reducing the size of alfalfa chops using a hammer mill. Biosyst. Eng.105, 34-40.

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

Grant, R., 2010. Forage Fragility, fibre digestibility, and chewing response in dairy cattle. Tri-State Dairy Nutr. Conf. April 20-21. Fort Wayne, Ohio State University, Columbus. pp. 27-40.

Grant, R.J. & Cotanch, K.W., 2005. Physically effective fibre for dairy cows: Current perspectives. Proc. Cornell. Nutr. Conf. for feed manufacturers. October 18-20. Syracuse, NY. pp. 61-70.

Grenet, E., 1989. A comparison of the digestion and reduction in particle size of Lucerne hay (*Medicago sativa*) and Italian ryegrass hay (*Lolium italicum*) in the ovine digestive tract. Br. J. Nutr. 62, 493-507.

Henderson, S.M. & Perry, R.L., 1976. Agricultural Process Engineering (3rd ed). The Avi Publishing Company, Inc., Westport, Connecticut. pp. 131-157.

Himmel, M., Tucker, M., Baker, J., Rivard, C., Oh, K. & Grohmann, K., 1985. Comminution of biomass: hammer and knife mills. Biotechnol. Bioeng. Symp. 15, 39-58.

Holtzapple, M.T., Humpbrey, A.E. & Taylor, J.D., 1989. Energy requirements for the size reduction of poplar and aspen wood. Biotechnol. Bioeng. 33, 207-210.

Horrocks, R.D. & Vallentine, J.F., 1999. Harvested forages (1st ed). Academic Press. London. pp. 4.

Ige M.T. & Finner, M.F., 1976. Optimization of the performance of the cylinder type forage harvester cutterhead. Trans. ASAE. 19 (3), 455-460.

Jannasch, R., Quan, Y. & Samson, R., 2001. A process and energy analysis of pelletizing switchgrass. Final report, Natural Resources Canada: Alternative Energy Division.

Julier, B., Lila, M., Furstoss, V., Travers, V. & Huyghe, C., 1999. Measurement of cell-wall digestibility in Lucerne using the filter bag technique. Anim. Feed Sci. Technol. 79, 239-245.

Jung, H.G. & Allen, M.S., 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73, 2774-2790.

Jung, H.G. & Lamb, J.F.S., 2003. Identification of Lucerne stem cell wall traits related to *in vitro* neutral detergent fibre digestibility. Anim. Feed Sci. Technol. 110, 17-29.

Jung, H.G. & Lamb, J.F.S., 2004. Prediction of cell wall polysaccharide and lignin concentrations of alfalfa stems from detergent fibre analysis. Biomass. Bioenerg. 27, 365-373.

Jung, H.G., Mertens, D.R. & Payne, A.J., 1997. Correlation of acid detergent lignin and klason lignin with digestibility of forage dry matter and neutral detergent fibre. J. Dairy Sci. 80, 1622-1628.

Kellems, R.O. & Church, D.C., 2010. Livestock feeds and feeding (6th ed). Pearson education Inc., USA. pp. 115-117, 514.

Lammers, B.P., Buckmaster, D.R. & Heinrichs, A.J., 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79, 922-928.

Laredo, M.A. & Minson, D.J., 1973. The voluntary intake, digestibility, and retention time by sheep of leaf and stem fractions of five grasses. Aust. J. Agr. Res. 24, 875-888.

Lopo, P., 2002. The right grinding solution for you: roll, horizontal or vertical. Feed management 53 (3), 23-26.

MacDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 1995. Animal Nutrition (5th ed). Pearson Education Ltd., Edinburgh Gate, Harlow.

MacDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 2002. Animal Nutrition (6th ed). Pearson Prentice Hall. Harlow.

MacRae, J.C. & Armstrong, D.G., 1968. Enzyme method for determination of α-linked glucose polymers in biological materials. J. Sci. Food Agric. 19, 578-581, 1968.

Mani, S., Tabil, L.G. & Sokhansanj, S., 2004. Grinding performance and physical properties of wheat and barley straws, corn stover and switchgrass. Biomass. Bioenerg. 27, 339-352.

Mani, S., Tabil, L.G. & Sokhansanj, S., 2006. Effects of compressive force, particle size and moisture content on mechanical properties of biomass pellets from grasses. Biomass. Bioenerg. 30, 648-654.

Mertens, D.R., 1973. Applications of theoretical mathematical models to cell wall digestion and forage intake in ruminants. PhD dissertation. Cornell University, Ithaca, NY.

Mertens, D.R., 1986. Effect of physical characteristics, forage particle size and density on forage utilization. Proc. Nutr. Symp., St. Louis, MO. Am. Feed Ind. Assoc., Arlington, VA. pp. 91.

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

Mertens, D.R., 2000. Physically effective NDF and its use in formulating dairy rations. 11th Florida. Ruminant Nutr. Symp., Gainesville. January 13-14. pp. 142-160.

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Mertens, D.R., 2002a. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85, 1217-1240.

Mertens, D.R., 2002b. Measuring fibre and its effectiveness in ruminant diets. CNCPS V.5.0.34. Model development papers. Mertens 2002 PNC.

Mertens, D. R., 2002c. Physical and chemical characteristics of fibre affecting dairy cow performance. Proc. Cornell. Nutr. Conf. for feed manufacturers. Cornell University, Ithaca, NY. pp. 125–144.

Milton, S.J., 2004. Grasses as invasive alien plants in South Africa. S. Afr. J. Sci 100, 69-75.

Minson, S.J., 1990. Forage in ruminant nutrition. Ed: Cunha, T.J. Academic Press, San Diego, California, USA.

Naimi, L.J., 2008. Experiments and modelling of size reduction of switchgrass in a laboratory rotary knife mill. MSc thesis, University of British Columbia, Vancouver.

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

Norgaard, P., 1986. Physical structure of feeds for dairy cows. In: New developments and future perspectives in research on rumen function. Ed: Neimann-Sorensen, A. Commission for the European Communities, Luxemburg. pp. 85.

Norgaard, P., Nadeau, E., Randby, A. & Volden, H., 2011. Chewing index system for predicting physical structure of the diet. The Nordic feed evaluation system. Eur. Fed. Anim. Sci. 30, 127-132.

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

Oba, M. & Allen, M.S., 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fibre: 2. Chewing activities. J. Dairy Sci. 83, 1342-1349.

O'Dogherty, M.J., Huber, J.A., Dyson, J. & Marshall, C.J., 1995. A study of the physical and mechanical properties of wheat straw. J. Agric. Eng. Res. 62 (2), 133-142.

Oshita, T., K. Nonaka, S. Kume. & T. Nakui., 2004. Effects of forage type on particle size distribution of ruminal digesta and faeces of non-lactating cows fed high quality forage. Livest. Prod. Sci. 91, 107-15.

Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B. & Soutar, D.M., 2012. Introduction to GenStat® for Windows™ (15th ed). VSN International, Hemel Hempstead, Hertfordshire, UK. http://www.genstat.co.uk/

Poppi, D.P., Norton, B.W., Minson, D.J. & Hendricksen, R.E., 1980. The validity of the critical size theory for particles leaving the rumen. J. Agric. Sci. 94, 275-280.

Raffrenato, E., Fievisohn, R., Cotanch, K.W., Grant, R.J., Van Soest, P.J., Chase, L.E. & Van Amburgh, M.E., 2009. aNDF, NDFd, iNDF, ADL and kd: What have we learned? Proc. Cornell. Nutr. Conf. for feed manufacturers. October 20-22. East Syracuse, NY.

Raffrenato, E. & Van Amburgh, M.E., 2011. Technical note: Improved methodology for analyses of acid detergent fibre and acid detergent lignin. J. Dairy Sci. 94, 3613-3617.

Reed, J.D., Krueger, C., Rodriguez, G. & Hanson, J., 2000. Secondary plant compounds and forage evaluation. In: Forage evaluation in ruminant nutrition. Eds: Givens, D.I., Owen, E., Axford, R.F.E. & Omed, H.M. CABI publishing, CAB International, Wallingford, UK. pp. 433-448.

Reid, R.L., Jung, G.A. & Thayne, W.A., 1988. Relationships between nutritive quality and fibre components of cool season and warm season forages: a retrospective study. J. Anim. Sci. 66, 1275-1291.

Reverdin, S.G., 2000. Characterization of feedstuffs for ruminants using some physical parameters. Anim. Feed Sci. Technol. 86, 53-69.

Rinne, M., Huhtanen, P. & Jaakkola, S., 2002. Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. J. Anim. Sci. 80, 1986-1998.

Romney, D.L. & Gill, M., 2000. Intake of forages. In: Forage evaluation in ruminant nutrition. Eds: Givens, D.I., Owen, E., Axford, R.F.E. & Omed, H.M. CABI publishing, CAB International, Wallingford, UK. pp. 43-62.

Samson, R., Duxbury, P., Drisdale, M. & Lapointe, C., 2000. Assessment of pelletized biofuels. PERD program: Natural Resources Canada, Contract 23348-8-3145/001/SQ.

Sauvant, D., Dulphy, J.P. & Michalet-Doreau, B., 1990. The concept of fibrosity index of ruminant feeds. Prod. Anim. 3(5), 309-318.

Scholten, R.L. & McEllhiney, R.R., 1985. The effects of prebreaking in hammer mill particle size reduction. ASABE Paper No. 85, 3542. St. Joseph. MI. ASABE.

Sheaffer, C.C., Seguin, P. & Cuomo, G.J., 1998. Sward characteristics and management effects on cool-season grass forage quality. In: Grass for dairy cattle. Eds: Cherney, J.H. & Cherney, D.J.R. CABI publishing, New York, USA. pp. 73- 77.

Smith, L.W., Goering, H.K. & Gordon, C.H., 1972. Relationships of forage compositions with rates of cell wall digestion and indigestibility of cell walls. J. Dairy. Sci. 55, 1140.

Sudweeks, E.M., Law, S.E., Ely, L.O., McCullough, M E. & Sisk, L.R., 1979. Development and application of a roughage value index for formation of dairy rations. The University of Georgia, College of Agriculture Experiment Stations. Research Bulletin 238, pp. 1-21.

Sudweeks, E.M., Ely, L.O., Mertens, D.R., & Sisk, L.R. 1981. Assessing minimum amounts and form of roughages in ruminant diets: roughage value index system. J. Anim. Sci. 53, 1406 – 1407.

Susenbeth, A., Mayer, R., Koehler, B. & Neumann, O. 1998. Energy requirement for eating in cattle. J. Anim. Sci. 76, 2701- 2705.

Tabil, L., Adapa, P. & Kashaninejad, M., 2011. Biomass feedstock pre-processing – Part 1: Pre-treatment. Biofuel's engineering process technology. Ed: Dos Santos Bernardes, M.A.

Tomlin, D.C., Johnson, R.R. & Dehority, B.A., 1965. Relationship of lignification to *in vitro* cellulose digestibility of grasses and legumes. J. Anim. Sci. 24, 161-165.

Troelsen, J.E. & Bigsby, F.W. 1964. Artificial mastication – a new approach for predicting voluntary consumption by ruminants. J. Anim. Sci. 23, 1139-1142.

Troelson, J.E. & Campbell, J.B., 1968. Voluntary consumption of forage by sheep and its relation to the size and shape of particles in the digestive tract. Anim. Prod. 10, 289-296.

Tylutki, T.P,, Fox, D.G., Durbal, V.M., Tedeschi, L.O., Russell, J.B., Van Amburgh, M.E., Overton, T.R., Chase, L.E. & Pell, A.N., 2008. Cornell net carbohydrate and protein system; a model for precision feeding of dairy cattle. Anim. Feed Sci. Technol. 143, 174-202.

Van Soest, P.J., 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. J. Anim. Sci. 24, 834-843.

Van Soest, P.J., 1982. Nutritional ecology of the ruminant. O & B Books, Inc., USA. pp. 31-35.

Van Soest, P.J., Van Amburgh, M.E., Robertson, J.B. & Knaus, W.F., 2005. Validation of the 2.4 times lignin factor for ultimate extent of NDF digestion, and curve peeling rate of fermentation curves into pools. Proc. Cornell. Nutr. Conf. for feed manufacturers. East Syracuse, NY. pp. 139-149.

Von Bargen, K., Lamb, M. & Neels, D.E., 1981. Energy requirements for particle size reduction of crop residues. ASABE Paper no 81-4062. St. Joseph, Michigan. ASABE.

Waghorn, G.C., Shelton, I.D. & Thomas, V.J., 1989. Particle breakdown and rumen digestion of fresh ryegrass (*Lolium perenne* L.) and Lucerne (*Medicago sativa* L.) fed to cows during a restricted feeding period. Br. J. Nutr. 61, 409-423.

Weimer, P.J., Russell, J.B. & Muck, R.E., 2009. Lessons from the cow: what the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass. Biores. Technol. 100, 5323-5331.

Weston, R.H., 1985. The regulation of feed intake in herbage-fed ruminants. Proc. Nutr. Soc. Aust. 10, 55-62.

Wilson, J.R., 1994. Review: Cell wall characteristics in relation to forage digestion by ruminants. J. Agric. Sci. 122, 173-182.

Wilson, J.R., Brown, R.H. & Windham, W.R., 1983. Influence of leaf anatomy on the dry matter digestibility of C3, C4 and C3/C4 intermediate types of *Panicum* species. Crop Sci. 23(1), 141-146.

Wilson, J.R. & Hacker, J.B., 1987. Comparitive digestibility and anatomy of some sympatric C3 and C4 arid zone grasses. Aust. J. Agric. Res. 38 (2), 287-295.

Wolfson, M.M. & Tainton, N.M., 2000. Grasses. In: Pasture management in South Africa. Ed: Tainton, N.M., University of Natal press. Pietermaritzburg, South Africa. ISBN 0-86980-960-1.

Womac, A.R., Igathinathane, C., Bitra, P., Miu, P., Yang, T., Sokhansanj, S. & Narayan, S., 2007. Biomass pre-processing size reduction with instrumented mills. ASABE Paper no 076046. St. Joseph, Michigan. ASABE.

Yancey, N., Wright, C.T. & Westover, T.L., 2013. Optimizing hammer mill performance through screen selection and hammer design. Biofuels 4(1), 85-94.

Yang, W., Sokhansanj, S., Crerar, W.J. & Rohani, S. 1996. Size and shape related characteristics of alfalfa grind. Can. Agr. Eng. 38(3), 201-205.

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

Yang, W.Z., Beauchemin, K.A. & Rode, L.M., 2001b. Barley processing, forage: concentrate, and forage length effects on chewing and digesta passage in lactating cows. J. Dairy Sci. 84, 2709-2720.

Yu, M., Womac, A.R. & Pordesimo, L.O., 2003. Review of biomass size reduction technology. ASABE Paper no 036077. St. Joseph, Michigan. ASABE.

Yu, M., Womac, A.R., Miu, P.I., Igathinathane, C., Sokhansanj, S. & Narayan, S., 2006. Direct energy measurement systems for rotary biomass grinder – Hammermill. ASABE Paper no 066217. St. Joseph, Michigan. ASABE.

Zebeli, Q., Tafaj, M., Weber, I., Dijkstra, J., Steingass, H. & Drochner, W., 2007. Effects of varying dietary forage particle size in two concentrate levels on chewing activity, ruminal mat characteristics, and passage in dairy cows. J. Dairy Sci. 90, 1929-19.