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Estimation of indigestible neutral detergent fibre in forages from cell wall components

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Submitted in partial fulfilment of the requirements for the degree

MSc (Agric) Animal Science: Animal Nutrition

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Pretoria

July 2018

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DECLARATION

I declare that this dissertation for the MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not been submitted by me for a degree at any other University.

Ronel Claassen

SIGNATURE: 

DATE: July 2018

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ACKNOWLEDGEMENTS

A special thanks to Dr E. Raffrenato for his assistance during the trial period in the laboratory as well as all the advice given during this study. Dr Raffrenato`s input in the statistical analysis of the data is much appreciated. Thank you for the support and effort put in for the duration of this study.

I would like to express my sincere gratitude to my promoter Prof L.J Erasmus for his assistance, advice and patience during the study.

I would like to thank the Department of Animal and Wildlife Sciences, University of Pretoria for the funding of this project.

Thank you to my family and friends for all the support. Thank you for believing in me.

To my significant other, thank you for all the support and love given every day. Words can not describe the love I have for you and may you watch over me and our children from heaven.

Lastly, but most important, thank you to my heavenly Father for giving me the ability and the opportunity to complete this degree.

“Die Here sal jou altyddeur lei; selfs in dor streke sal Hy jou behoeftes voorsien. Hy sal jou sterk maak. Jy sal wees soos n tuin met volop water soos n fontein waarvan die water nie opdroog nie.”

Jesaja 58: 11

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SUMMARY

Estimation of indigestible neutral detergent fibre in forages from cell wall components

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Degree MSc (Agric) Animal Science: Animal Nutrition

Ruminants can utilize forage fibre material due to an unique adaptation of the stomach that is called the selective retention mechanism. The fibre cell wall is composed mostly of cellulose, hemicellulose and lignin. The first three components are measured as neutral detergent fibre (NDF). The NDF is either slowly digestible or indigestible (iNDF). Even if indigestible iNDF is unavailable to microbes, it is a critical component affecting the nutritional value of feeds. Organic matter digestibility is influenced by iNDF, therefore iNDF will influence the amount of energy available to the ruminant. Feed intake is also influenced by iNDF content which can be estimated by several methods. These methods are time consuming, expensive and not all laboratories have the necessary equipment in order to implement these methods. There is an urgent need to develop a cost effective method that can accurately predict iNDF and could be easily implemented in feed analysis laboratories. The aim of this trial was to develop accurate and precise prediction equations for the estimation of

iNDF across selected groups of forages. One hundred and two milled grain and forage samples were received from Afgri (Pty) Ltd, including oats, sorghum, lucerne, ryegrass and *Eragrostis curvula* hay. Samples were analysed in duplicate for various chemical components as well as incubated in-vitro for 240h to estimate iNDF. A simple ANOVA was used for comparison between the different groups, as well as simple linear regression analysis and stepwise multiple linear regression. Akaike`s information criterion and R^2 values were used to evaluate the models and to establish the best fit models. Indigestible NDF was predicted by generating power functions. The independent variables included NDF, acid detergent fibre(ADF), acid detergent lignin (ADL), ADF/NDF, ADL/NDF, iNDF, iNDF/NDF, hemicellulose, cellulose, hemicellulose/NDF and cellulose/NDF. Significant differences were found within groups for the different variables used in the regressions. The R^2 values for simple linear regression analysis for all the groups combined ("All") ranged between 0.03 and 0.60. The R^2 values for the individual feedstuff within the groups ranged from 0.64 to 0.99. Therefore individual species had higher iNDF prediction accuracy than the combined groups. As can be seen from the data when specific groups are considered, the value 2.4 is not appropriate. The R^2 values for the multiple linear regression analysis for the combined groups of forages in dry matter ("All") was 0.75 where the R^2 values for the specific groups ranged between 0.73 and 0.98. The R^2 values for the multiple linear regression analysis for the combined groups of forages in NDF ("All") was 0.72 where the R^2 values for the specific groups ranged between 0.21 and 0.98. It was concluded that it is possible to accurately estimate iNDF from prediction equations. Indigestible NDF can be predicted when only one variable is taken into account. The most accurate results can be obtained from using simple linear regression analysis within specific species.

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADL	Acid detergent lignin
CNCPS	Cornell Net Carbohydrate and Protein System
CPM	Cornell-Penn-Miner
DM	Dry matter
DMI	Dry matter intake
dNDF	Digestible neutral detergent fibre
iNDF	Indigestible neutral detergent fibre
IVDMD	*In vitro* dry matter digestibility
KL	Klason lignin
N	Nitrogen
NE	Net energy
NDFkd	Rate of NDF degradation
NFC	Non-fibre carbohydrate
NIRS	Near Infrared Reflectance Spectroscopy
NRC	National Research Council
OM	Organic matter
pdNDF	Potentially digestible neutral detergent fibre
SD	Standard deviation
SEM	Standard error of the mean
RSE	Standard error of regression
TDOMD	Total diet organic matter digestibility

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CHAPTER 1

1. INTRODUCTION AND MOTIVATION

Ruminants can utilize fibrous forage material, because their forestomachs utilise ruminal microbial degradation for the breakdown and fermentation of feed particles and furthermore the rumen has a selective retention mechanism that retains fibre particles for increased degradation efficiency (Krizsan and Huhtanen. 2012). Fibre is the organic portion of the feed that is the most difficult to digest and has a gut filling effect that in turn will have an influence on feed intake; a high fibre diet will generally result in lower intakes due to longer retention times (Mertens, 2002).

The fibre cell wall is composed of many components, including cellulose, hemicellulose and lignin. These three components are analytically known as neutral detergent fibre (NDF) which is the insoluble fibre in feed that is indigestible or slowly digestible. A knowledge of NDF digestibility in forage is critical for effective ruminant feeding, due to the variability of NDF in terms of extent of rumen degradation and its effect on animal performance (Oba and Allen, 1999). The measurement of NDF digestibility is also important, because it affects the potentially available energy from the forage (Raffrenato *et al*. 2009). The most common method to estimate NDF digestion is the *in sacco* nylon bag technique (Huhtanen *et al*. 2006).

Ellis *et al*. (1999), defines indigestible neutral detergent fibre (iNDF) as an ideal nutritional entity because it is digested at a rate of zero, meaning iNDF is unavailable to microbial fermentation even when retention time is increased to infinity (Huhtanen *et al*. 2006). The iNDF fraction describes the intrinsic properties of plant cell walls, but it is not known whether extrinsic factors such as diet composition will influence iNDF concentration (Mertens, 2002). Indigestible NDF content is therefore an important indicator of the quality of forage cell wall carbohydrates, as well as a good predictor of *in vivo* digestibility of roughages (Nousianen *et al*. 2003). According to Nousianen *et al*. (2004), iNDF is the most important factor affecting the total diet organic matter digestibility (TDOMD). The relationship between iNDF and organic matter digestibility indicates that iNDF will be useful in the nutritive value prediction of forage (Nousianen *et al*. 2003). Knowledge of the iNDF concentration of forage has also several experimental benefits: it can be used as an internal marker when measuring total-tract digestibility (Huhtanen *et al*. 1994) and can be used as a marker for evaluating passage rates in rumen evacuation studies (Krizsan and Huhtanen. 2012).

Indigestible NDF requires extended *in situ* (*in sacco*) or *in vitro* incubation periods. Kinetic models can be used to estimate iNDF, which will describe NDF disappearance over digestion time (Ellis *et al*. 2011). Estimation of iNDF is not a mathematical concept but rather a critical biological principle on which digestion kinetics and rates are based. Digestion rates are more often than not calculated without subtracting the indigestible fraction or by subtracting a fraction that was estimated by a too short fermentation period, therefore resulting in the underestimation of the indigestible fraction (Mertens, 2000). This is the main reason why longer fermentation periods are needed. If the indigestible fraction is not subtracted, the potential digestible fraction will be overestimated (Mertens, 2000). Energy is not supplied by iNDF; therefore it should not be included in the estimation of forage energy supply. Indigestible NDF is used as an input factor in feed evaluation systems such as the Norwegian feed evaluation system (Norfor) and the Cornell Net Carbohydrate and Protein System (CNCPS) (Fox *et al*. 2004, Kramer *et al*. 2012). Standard methods for iNDF determination, like the *in sacco* technique and the 240h *in vitro* fermentation procedure are time consuming, labour intensive and expensive. There is a need for developing a system for the prediction of iNDF on a routine basis to better assess forage quality. Prediction equations that are compiled from basic parameters of chemical analysis are less expensive and faster, which will be beneficial for institutions without the availability of experimental animals (Jančík *et al*. 2008).

There is renewed interest in feeding higher forage diets to dairy cows, due to high-priced maize and other feed commodities, which encourages the feeding of lower starch diets. Lower starch diets are possible by replacing starch with fermentable carbohydrates from non-fibre sources or by feeding higher forage-to-concentrate rations.

The aim of this study was to develop equations for different forage classes (legumes, grasses, silage) to estimate iNDF, as a low cost alternative to labour intensive long term *in vitro* fermentation incubations.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Introduction

There is renewed interest in feeding higher forage diets to dairy cows, due to high-priced maize and other feed commodities, which encourages the feeding of lower starch diets. Lower starch diets are possible by replacing starch with fermentable carbohydrates from non-fibre sources such as citrus pulp and soya hulls for or by feeding higher forage-to-concentrate rations. High forage diets also have the advantage of environmental benefits as well as beneficial health consequences ruminants like dairy cows and feedlot cattle. It is therefore critical to understand the importance of digestible and indigestible NDF in a diet or prediction model, since the amount in forages which are often the main ingredients of the diet, will to a great extent determine how much energy is available to the animal. There are many factors affecting iNDF as well as many methods available to predict the iNDF content of a feed.

This literature review commences with a discussion on the importance and function of fibre in the ruminant diet and thereafter cell wall development, composition and differences between different types of forages will be discussed. A discussion on cell wall development and composition is included since it affects intake and therefore animal performance. Lastly, the effect of NDF on ruminant performance, the importance and role of iNDF and the different methods available to determine iNDF content will be discussed in detail.

2.2 Importance and function of fibre in ruminant nutrition

Forages are used extensively as the primary feedstuff in ruminant livestock production systems. Grazed forages, and therefore forage fibre, provide the foundation for ruminant production systems around the world. In animal feed evaluation systems, fibre is determined as the residue remaining after extraction of feed with neutral or acid detergent solution. Ruminants have the ability to digest fibre, due to microbial fermentation that releases energy for animal use. In the ruminant, fermentation takes place primarily in the rumen. As fibre digestion takes place, microbial protein is produced in the rumen and together with undegradable protein digested in the small intestine where the resulting amino acids are then absorbed and utilised by the animal. Fibre is the major energy source for the ruminant as well as the microbes inhabiting the forestomach and large intestine (Tamminga, 1993).

Energy provision is not the only function of fibre. Fibre plays an important role in maintaining the necessary stratification in the rumen, by stimulating rumen wall contractions and providing adhering surface for the microbes. The adhering surface prevents the flushing out of microbes from the rumen too early (Tamminga, 1993). The amount and size of fibre particles is important for healthy rumen function and will determine the speed of digestion as well as feed intake by the ruminant. The fibre mat present in the rumen consists mostly of long fibre particles. The fibre mat acts like a sieve allowing the passage of smaller particles, but retaining longer particles for further digestion. This mechanism is called the selective retention mechanism. There are two major constraints on fibre digestion, which can play a role in animal performance. The first is digestion rate. According to Allen and Mertens (1988) digestibility is directly related to digestion rate. Therefore, digestion rate is a constraint on fibre digestion and factors altering this rate will affect digestibility. The optimal fibre digestion is dependent on intrinsic characteristics such as chemical composition and physical structure. Tissue morphology will also influence microbe accessibility and digestion rate (Akin, 1986). The second constraint is passage rate which is influenced by selective retention. Fibre digestibility decreases as passage rate increases. Passage rate is inversely related to rumen volume at a certain level of intake. Other factors that can affect the passage rate include particle size, rumen motility and specific gravity (Ehle, 1984; Sissons *et al*. 1984).

Forage quality can also be influenced by many other factors such as weather and season, therefore forage has extremely variable digestibility. Cell wall digestibility is the primary determinant of animal productivity, efficiency and profitability (Jung and Allen. 1995) and digestibility, to a large extent, drives feed intake. Intake is negatively influenced, when a diet of low digestibility is fed, because passage rate will be slower, therefore the rumen will stay filled for longer. Bearing in mind that fibre represents a significant fraction of ruminant diets, therefore their productivity will be limited by the ability to consume and digest the fibrous part of a diet (Allen and Mertens, 1988). If a greater percentage of the total potential energy stored in forages is available to the ruminant, it would have a significant economic impact and increase profitability through reduced off farm feed purchases. The productivity of animals and the profitability of any farm business are ultimately linked to animal nutrition, which in turn is determined by intake and nutritive value of the diet.

2.3 Cell wall composition and development

Energy from forages is mainly acquired from cell wall fermentation in the rumen. Therefore, high intake and high cell wall digestibility is required for optimal production. The structural organization of plants influences intake through control of forage particle breakdown, the nature of

particles produced and passage rate through the rumen. The growth and development of a cell wall is divided into two phases: primary cell growth and secondary cell wall growth (Jung and Allen. 1995). Primary cell growth is where the plant cell wall increases in size through cell wall elongation. During this phase the cell wall consists of polysaccharides, proteins and phenolic acids. The second phase starts when cell wall elongation ceases and secondary cell wall thickening starts (Jung and Allen. 1995). The additional material that is deposited during this phase contains more cellulose than xylans. Pectins are not deposited, but lignin deposition takes place during the secondary phase (Terashima *et al*. 1993). Lignin deposition begins in the middle lamella; therefore the lamella will have the highest concentration of lignin. This may be the reason why rumen microbes degrade cell walls from the lumen outwards. It may also explain why the middle lamella is never completely digested (Jung and Allen, 1995).

Cell wall and NDF concentration of grasses and legumes are similar, but the NDF content in legumes are much lower than the cell wall concentration (Jung and Allen, 1995). Lignification of legume leaves as maturity proceeds, depresses digestibility. The decrease in digestibility is less in legumes than in grasses due to less noncore lignin that can bind to the available polysaccharides and decrease availability for the microbes. Stems and leaves of immature legumes have similar digestibility, but as maturity increases the stem digestibility decreases markedly, where leaf digestibility changes little (Minson, 1990).

Legumes have a higher iNDF/ dNDF ratio as well as different degradation rates of dNDF compared to grasses. The extent of legume DM and OM digestion are greater than that of grass, due to different cell wall concentrations. More rumen digestion occurs when legumes are consumed with lower intakes and passage and digestion rates are greater for legumes than for grasses (Beever *et al*. 1986). Grass contains ferulic acid and a small amount of p-coumaric acid that are esterified to arabinoxylans that are present in the primary wall (Jung and Allen, 1995). Legume cell walls contain small amounts of phenolic acids that are also found in grasses (Jung *et al*.1992) and contain more pectins and cellulose compared to xylans than grasses. The lignin content of legume cell walls is greater than grasses, but the lignin is not scattered in legumes as in grasses. The shift in lignin type during maturation is the same for all forage species (Jung and Allen, 1995). Leaves of grasses have a higher concentration of cell walls than legume leaves. During the maturation of grasses, the lignin content increases markedly, but is does not increase that much in legumes (Wilman *et al*. 2000).

All forages contain p-coumaric acid esters. Grasses have higher concentrations than legumes, but p-coumaric acid esters are greater in tropical grasses than C_3 grasses. Perennial grasses (C_3) grow in temperate climates and have a higher ratio of mesophyll to vascular tissue than C_4 grasses growing in tropical and subtropical climates (Akin *et al*. 1989). Mesophyll is the most easily degraded by

rumen microbes. The mesophyll in C_3 grasses is readily penetrated because of the openness of the tissue due to infrequent points of attachment between the cells (Hanna *et al*. 1973). Therefore, C_3 grasses are more digestible than C_4 grasses, due to different proportions and arrangements of tissue. The proportions and arrangements differ due to differences in photosynthetic pathways (Akin, 1986). The parenchyma bundle sheath in C_3 species is a distinct structure and the cell wall is also rapidly degraded. The parenchyma in C_4 species are rigid, thick-walled structures that are slowly degraded by rumen microbes (Akin *et al*. 1989). Tissues that give a positive reaction with a histochemical stain for lignin are the least digestible and relatively large amounts will reduce forage quality (Jung and Allen, 1995). Digestion for grasses is slower than legumes because lignin is localized in legumes but not in grasses. According to Mertens and Loften (1948), the lag time for C_3 grasses are longer and the digestion rate of C_4 grasses are slower than C_3 grasses and legumes. The reason for this might be that hydration and microbial attachment take longer for C_4 grasses. According to Minson (1990), particle breakdown characteristics are responsible for the differences in voluntary intake between C_3 and C_4 grasses.

Fibre content as well as the degree of lignification will affect particle breakdown, mastication and rumination; therefore those two factors will influence the suitability of particles to exit the rumen (Minson, 1990). Particle breakdown during rumination is greater for C_3 grasses than C_4 grasses, due to a greater degree of susceptibility for fracture. As grasses mature, there is an increase in lignification, a decrease in digestibility and a reduction in voluntary intake. Continued efforts to fully understand the chemistry of forage cell walls, digestive physiology and the metabolism of forage fed ruminants will lead to increased and more strategic forage use contributing to increased efficiency of animal production.

2.4 Effects of NDF on the ruminant

Analysis of fibre in forages is critical in ruminant nutrition, because diets usually contain large amounts of forage and as mentioned previously, the fibre fraction affects feed intake and animal performance (Jung *et al*. 1997). Gravimetric methods such as neutral detergent methods are the most popular methods of fibre analysis. The aim of feed evaluation methods is to describe the intrinsic feed factors determining the degradation characteristics because ration evaluation systems take into account the extrinsic factors altering degradation kinetics. The concept behind the detergent fibre analysis is that plant cells can be divided into less digestible cell wall and mostly digestible cell walls (Huhtanen *et al*. 2006). Van Soest developed a system, where the two fractions can be successfully separated by the use of two detergents: a neutral detergent and an acid detergent system (Van Soest *et*

al.1991). According to Huhtanen *et al*. (2006) the neutral detergent divides the feed into a soluble fraction that is completely available and a fraction that is slowly degraded by microbes. Neutral detergent fibre is the most common measure of fibre used for animal feed analysis, but it does not represent a uniform group of chemical components (Raffrenato, 2011). Neutral detergent fibre forms part of the cell wall and consists of cellulose, hemicellulose and lignin. Different types of forages have variable digestibility in the rumen (Mertens, 2000).

Animal performance is affected by NDF digestibility, independent of the dietary NDF concentration. According to Hoffman *et al*. (2001), there are several important reasons why forages are evaluated for NDF digestibility; firstly research demonstrated that lactating dairy cows will consume more DM and produce more milk when forages are fed with higher NDF digestibility. Secondly, ADF and lignin have been used to determine potential NDF digestibility, but research indicated that ADF and lignin do not account for all the variation in NDF. Thirdly, a summative approach is used in the 2001 Nutrient Requirements of Dairy cattle (NRC, 2001), where protein, fat, non-fibre carbohydrates (NFC) and NDF are summed. Therefore a prediction of NDF digestibility is required to use a summative energy prediction for forages. Oba and Allen (2000) conducted a trial where they concluded that if forages of different *in vitro* digestibility are fed, at similar NDF concentration, significant increases in DMI and milk production were found. Van Soest (1994) found that a faster disappearance of the NDF fraction from the rumen because of increased digestion rate or passage rate can reduce rumen fill therefore allowing a greater voluntary feed intake. A prediction of NDF digestibility is essential when using a summative energy prediction for forages (Hoffman *et al*. 2001). In short, the rate of NDF degradability as well as potential NDF degradability are the most important feed characteristics that can be used to determine the value of a ration, due to the large variation in NDF concentrations and degradation rates among feeds (Huhtanen *et al*. 2006).

The accurate and precise predictions of intrinsic digestion parameters are critical for NDF prediction of rumen digestibility and intake. The CNCPS model (Fox *et al*. 1992; Russell *et al*. 1992; Fox *et al*. 2004) as well as the Nordic model (Danfær *et al*. 2006) demonstrate the importance of digestion rate and the extent of NDF digestion. Models of different complexity can describe digestion and passage in ruminants (Raffrenato, 2011). The kinetic parameters should be limited to intrinsic characteristics of cell walls in order to be useful in the rumen models available. Therefore, it is important that methods used for determining digestion characteristics, reflect the potential nutrient degradation in the ruminant.

2.5 Lignin characteristics and analysis

Lignin is the primary component limiting digestion of polysaccharides in the rumen, therefore it is critical for nutritionists to know the lignin value of a feed or forage. Dekker *et al*. (1972), concluded that degradation resistance of particles are due to the physical protection by lignin, rather than structural differences within the carbohydrate polymers. Lignin is a secondary cell wall component that decreases the potentially digestible fibre fraction in forages (Traxler, 1997). Lignin concentration is influenced by many factors such as temperature, light intensity, water availability, maturity, latitude, harvest and storage methods (Traxler *et al*. 1998). It has been demonstrated that both lignin composition and lignin concentration are responsible for limiting digestion in forages (Jung and Allen, 1995). Lignin composition changes from guaiacyl-type lignin to syringyl-type lignin as the forage matures. It was found that the BMR-mutation in annual C_4 plants, like maize, reduces lignin concentration and there is a shift in lignin composition to a more guaicyl-rich poly. The BMR mutation is phenotypically by the presence of a brownish pigment on the leaf and in the stem (Aguilar, Poliana Batista de *et al*. 2014) Jung and Allen, (1995) therefore concluded that improved cell wall digestibility in BMR-mutants is due to a lower lignin content and lower syringil-type lignin. The syringil-type lignin has a more linear structure, thereby protecting more polysaccharides from digestion (Jung and Allen. 1995). Other wall polysaccharides include cellulose, hemicellulose and pectin. Cellulose consists of covalently linked cellubiose unit chains, held together by hydrogen bonds (Akin *et al*. 1989). It is available for digestion by rumen microbes at different rates (Chesson, 1948); therefore, inhibition in the extent of digestion can be due to other factors in the cell wall (Akin *et al*. 1989).

Microbial hemicellulase can hydrolyze the glycosidic bonds which mean that hemicelluloses can be digested by microbes (Akin *et al*. 1989). Arabinose and lignin act as digestion inhibitors, but lignin is considered the more important inhibitor. Lignin content increases with plant maturity and different plant species have different amounts of lignin. Lignin can be defined chemically or from a functional point of view that lignin has in a plant (Raffrenato, 2011). From a functional perspective, lignin provides structural support to the cell wall facilitates water transport and protects the cell wall polysaccharides against degradation. Methods for lignin analysis differentiate phenolic acids by acid solubility. Acid detergent lignin (ADL) is determined by adding a subsequent step in the ADF procedure. Lignin is isolated from ADF through the use of 72% sulphuric acid, where the remaining carbohydrates are hydrolysed. According to Raffrenato *et* al. (2009) ADL represents more polymerized than phenolics that may behave more nutritionally uniform and impact the extent of NDF

digestion in anaerobic conditions. Several attempts have been made to predict iNDF from lignin concentration (Chandler, 1980; Traxler *et al*. 1998) that will be discussed in the following section.

2.6 Importance and the prediction of indigestible neutral detergent fibre

The energy content of forage is known as the single most important factor in predicting animal performance. A part of the forage cell wall is unavailable to microbial digestion in the ruminant, even if the total tract residence time is increased to infinity (Huhtanen *et al*. 2006). This portion is called iNDF. The digestibility of the remaining fibre, potentially digestible NDF, determines the digestibility of NDF. Therefore potentially digestible neutral detergent fibre (pdNDF) can be calculated as follows: pdNDF = NDF – iNDF. Forage digestibility is therefore constrained by the digestion rate of pdNDF (Van Soest, 1994).

Indigestible neutral detergent fibre is one of the more important chemical measurements to determine the net energy (NE) value of a feed (Krämer *et al*. 2012). Ellis *et al*. (1999), defines iNDF as an ideal nutritional entity, because it is digested at the rate of zero and it represents the non-digestible part of NDF (Jancík *et al*. 2008). Indigestible NDF has been described as the most important factor affecting the total diet organic matter digestibility (Nousiainen *et al*. 2004) and it plays an important role in contributing to rumen digesta load. According to Ellis *et al*. (1999), the determination of iNDF should be included in all the basic feedstuff analysis because it has a predictable digestibility. A close empirical relationship exists between silage iNDF and organic matter digestibility, therefore iNDF can be a useful entity in predicting the nutritive value of a forage (Nousiainen *et al*. 2003). The Nordic model dairy cow metabolism module demonstrates the importance of iNDF estimation on OM and NDF digestibility, rumen NDF pool and microbial N flow (Raffrenato, 2011). Simulations were done by Danfær *et al*. (2006), which indicated the profound effects of these parameters, named above, on DM digestibility and therefore on the supply of energy and microbial protein. The iNDF concentration of forage is influenced by plant species (Rinne *et al*. 2006) and maturity (Kuoppala *et al*. 2004). The iNDF concentration presents an important indicator of the quality of grass cell wall carbohydrates and it can be a good predictor of *in vivo* roughage digestibility (Jancík *et al*. 2008). Analytically, iNDF can be estimated by fitting kinetic models that describe the disappearance of NDF over digestion time (Waldo *et al*. 1972; Robinson *et al*. 1986; Weimer *et al*. 1990). According to Mertens (2000), the estimation of iNDF is not a mathematical or modelling concept, but it is rather a critical biological principle upon which the concept of digestion kinetics is based.

Digestion rates are often calculated without subtracting the indigestible fraction or by using a fraction that was determined from too short fermentation time. The fermentation used for iNDF

estimation plays a major role in the prediction of digestion rates. The subtraction of iNDF, from earlier time points, will result in the prediction of greater than true digestion rates. Where the subtraction of iNDF from later time points (little to no iNDF), will result in less than true digestion rates. Mertens (1977) has shown the effect of fermentation time chosen to estimate iNDF on digestion rate, using an ln-linear approach. Any error in estimating indigestibility will bias the estimate fractional rate and lag time, because they are estimated using the ln-linear regression (Mertens and Loften, 1948; Moore and Cherney, 1986, Raffrenato, 2011). Mertens (1977) conducted a trial where he concluded that iNDF can be accurately estimated by using a 96h residue for the estimation of digestion rate, but there are results from *in situ* trials (Robinson *et al*. 1986) and *in vitro* trials (Van Soest *et al*. 2005) that indicate that in most cases digestion is not completed by 96 hours. Fitting models to degradation curves with increased residuals (up to 40 days) and using incubation times that are progressively reduced indicated that there are two common misestimates. The misestimates are underestimation of the degradable fraction and overestimation of the rate constants; therefore extended incubation times are needed (Robinson *et al*. 1986).

Indigestible NDF can be quantified by using *in vitro* and *in situ* methods. The measurement of *in vitro* fibre digestibility is based on the method developed by Tilley and Terry (1963) for determining *in vitro* DM digestibility (IVDMD). The *in vitro* procedure is performed in the laboratory. The procedure simulates digestion as it occurs in the rumen. The measurement of IVDMD has often been used to analyse feed, because there is a strong correlation with the *in vivo* digestibility of an animal (Holden, 1999). The Tilley and Terry method have been modified, by using different reagents, to improve the accuracy of the method. Several attempts have been made to determine iNDF using *in vitro* fermentations (Traxler *et al*. 1998; Van Soest *et al*. 2005). Traxler *et al*. (1998) used a fermentation time of 176hours, using flasks and filtration, where Van Soest *et al*. (2005) implemented a fermentation time of 240 hours, using plastic bottles and centrifugation. In both cases, samples were in direct contact with rumen fluid and buffer. The Daisy II apparatus (ANKOM Technology Corp. Fairport, NY) is a recent development that allows multiple feed samples to be analysed for IVDMD, thereby reducing labour demands and improving assay precision.

The *in situ* method was developed so that the samples can be exposed to a more realistic microbial environment. Indigestible NDF estimations can be obtained by conducting prolonged (> 10 days) ruminal *in situ* incubations (Huhtanen *et* al. 1994). A disadvantage of this method is that it can`t be routinely applied in commercial laboratories, because surgically altered animals are a requirement. Digestible NDF (dNDF) determination requires estimations of iNDF and NDF (Nousiainen *et al*. 2004). Several *in situ* trials have been performed, using 72 – 96 h incubation periods and a bag pore size of 43µm. The data has been fitted to the equations of Ørskov and McDonald (1979) used for

estimation of NDF degradation (Wilman *et al*. 2000). According to Nousiainen *et al*. (2004), this method is biased, due to lower microbial activity inside the bags than in surrounding digesta and the inflow and outflow of particles from the bags. These problems can be overcome by using a combination of bags with small pore sizes to decrease particle loss and by increasing incubation time. Nousiainen *et al*. (2004) determined iNDF using 12 day incubations, using nylon bags with pore sizes of 6 to 17 µm. It was concluded that the range of 6 to 17 µm is the best compromise to decrease particle inflow and outflow, but still allowing adequate microbial digestion inside the bags. The iNDF to NDF ratio also didn`t differ significantly, which further supports their conclusion. Mertens (1977), estimated iNDF by using a 96h residue. He concluded that 96h incubation is effective in estimating digestion rate, but results of other experimental trials indicated that digestion, in most cases, was not completed by 96h (Robinson *et al*. 1986; Van Soest *et al*. 2005). Rinne *et al*. (1997, 2002) increased the *in situ* incubation time from 96h to 288h and found that the iNDF concentration for grass silage at different maturities was reduced. Several factors are present which will cause variable results: the micro environment in the bag and the micro environment in the rumen differ as well as the enzyme activity which will cause different digestion results. This method is expensive because cannulated animals are required.

Lignin is known as the primary component limiting forage digestion and can be used to determine iNDF. There are several methods to determine the lignin content of forages including the Klason (KL) method and the acid-detergent lignin (ADL) method (Raffrenato *et al*. 2009). The lignin methods differentiate phenolic acids by acid solubility. Results indicated that the KL method resulted in higher values and represents total phenolic acids and little acid dispersible phenolics. Acid detergent lignin represents more polymerized phenolics which behaves in a nutritionally uniform manner and can impact the extent of NDF digestion. Raffrenato *et* al. (2009), reported that increased recovery of ADL and iNDF altered the relationship between iNDF and ADL, across forage species and within species. Several attempts have been made to predict iNDF from lignin content (Chandler, 1980; Traxler *et al*. 1998).

Chandler (1980) estimated iNDF by multiplying 2.4 with the lignin concentration after 90-120 hour fermentation. The CNCPS uses the 2.4 value as a ratio between ADL and NDF to estimate iNDF (Raffrenato *et al*. 2009). Van Soest (2005) concluded that the Chandler equation performed well when used to predict iNDF, since a satisfactory regression was found between the predicted and observed values ($R^2 = 0.94$). Indigestible neutral detergent fibre predictions will depend on the method used to determine lignin. Robinson *et al*. (1986) used Klason lignin to determine iNDF and concluded that the undegradable fraction was underestimated when compared to Chandler`s (1980) results. According to Van Soest *et al*. (2005), there is a relationship between lignin and digestibility; however, several

attempts to predict iNDF were unsuccessful due to high proportional errors in lignin and iNDF analysis, differences between forages as well as climatic factors. The variation in lignin assays might be a function of the filtering step (Raffrenato *et al*. 2009) therefore there is a need to estimate iNDF using its relationship with ADL and NDF. Prediction and analytical methods for iNDF are time consuming and expensive. Prediction equations compiled from chemical analysis are less expensive and faster, therefore this method is worth looking into (Jančík *et al*. 2008). Jančík *et al*. (2008) conducted a trial to determine the iNDF content of grasses and its prediction from chemical analysis.

Near Infrared Reflectance Spectroscopy (NIRS) measures the wavelength and intensity of the absorption of near infrared light by a sample. This method of analysis has been widely used in assessing various biological traits of forages (Deaville & Givens, 1998; Nousiainen *et al*. 2004). It has the potential to be used to accurately predict digestion parameters such as iNDF (Nousianen *et al*. 2004). Frequent calibration is essential to ensure accurate predictions. The downside of NIRS is that a large dataset is needed to ensure accurate calibration in order to accurately predict iNDF of feeds (Nousianen *et al*. 2004).

2.7 Hypothesis

The hypothesis for this trial is:

H_1: Accurate and precise prediction equations for iNDF prediction can be developed between the types, legume vs non legume and C_3vs C_4 forages.

H_0: Accurate and precise prediction equations for iNDF prediction can not be developed between the types, legume vs non legume and C_3vs C_4forages.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Introduction

This study was conducted at the University of Pretoria, Hatfield, Pretoria. Samples of forages used in this trial were collected and milled by Afgri Pty Ltd, Highveld, Centurion. Chemical analysis was done at Nutrilab, Department of Animal and Wildlife Sciences, University of Pretoria. This study was approved by the University of Pretoria Animal Ethics Committee, project number: ECO34-14.

3.2 Collection of samples

A total number of 124 milled samples were included in this trial. Table 3.1 contains a list of the 124 samples. The species and respective numbers of samples used were Oats (9), Sorghum (5), Maize (12), Lucerne (76), Ryegrass (5) and Eragrostis grass (4). The samples were classifies into type (silage, hay, green forage) and temperate (C_3) or tropical (C_4) forages. All samples were milled using a Wiley mill (Thomas Wiley Mills, Philadelphia, USA) equipped with a 1mm screen.

Table 3.1 Type and number of samples used in this trial

Common Name	Scientific Name	Sample number	Type	C_3/C_4
Oats	*Avena sativa*	9	Silage	C_3
Sorghum	*Sorghum bicolour*	5	Silage	C_4
Maize	*Zea mays*	12	Silage	C_4
Lucerne	*Medicago sativa*	67	Legume(Hay)	C_3
Ryegrass	*Lolium perenne*	5	Green/Hay	C_3
Eragrostis grass	*Eragrostis curvula*	4	Hay	C_4

3.3 Chemical analysis

Samples were analysed for DM, ash, NDF, ADF, ADL and 240h *in vitro* neutral detergent fibre digestibility. For all of the chemical analysis, the hot weighing procedure was used to record the sample weights. The procedure is described by Goering and Van Soest (1970). Four 50ml Berzelius beakers are heated in an oven at 105ºC and used to heat the balance. 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 removed and replaced by another warm beaker from the oven and the process repeated. After the balance was warmed up with four different heated beakers, Gooch crucibles were taken

directly from the oven and placed on the heated balance, and weights recorded when the balance stabilized (the reading goes up and then down, record the lowest weight). Cellulose and hemicellulose were estimated as the differences between NDF and ADF, and ADF and ADL, respectively.

3.3.1 Dry matter, organic matter and ash

All forage samples were analyzed for DM using the AOAC (2012) procedure 934.01. Porcelain crucibles were placed in an oven for an hour in order to completely dry the crucibles. The empty crucibles were hot weighed and weight recorded. One gram of each sample was weighed into the crucibles and placed in an oven for 24 hours at 105ºC. The crucibles were hot weighed and the weight recorded. The weight loss of each sample was determined by subtracting the empty crucible weights from the crucibles containing the dried samples. The DM was calculated as follows:

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

Organic matter content (OM) was calculated as the difference between DM and ash. Ash was determined using the AOAC (2012) procedure 942.05. The ash content 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 overnight. Crucibles were removed and placed in an oven for an hour at 105ºC. The crucible with the test sample ash were hot-weighed, and the ash content 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.

3.3.2 Neutral detergent fibre

The method used in this trial is described by Mertens (2002), which is the AOAC official method 2002.04. This method is based on using refluxing beakers/crucibles. 0.5g of each sample was weighed into a 500ml Berzelius glass beaker, with no pouring spout. Anhydrous sodium sulphite (0.5g) and 100ml NDF solution were added to the beaker containing the sample. Beakers were placed on the refluxing apparatus. The beakers were covered with round cold-water condensers to minimise evaporation. The heat setting was set on 2 until solution starts to boil and is then turned down to 1.5. As soon as the solution started to boil, 250µl α-amylase (Thermamyl, Ankom, NY – USA) was added. Beakers were removed one by one, one hour after boil. The solution was then poured into a 50ml fritted-disc Gooch crucible that was placed in a vacuum filter unit. The solution was filtered away and

the sample was repeatedly rinsed with boiling distilled water and rinsed twice with acetone. Two hundred and fifty µl α-amylase was added to the crucible with the first water rinse. Crucibles were placed in an oven at 105°C to dry overnight. Samples were hot-weighed the following day. Crucibles were then placed in a muffled furnace for 4 hours at 550°C. Samples were allowed to cool down overnight and was removed the following day and placed in a 105°C oven for an hour. Crucibles were then hot-weighed. The aNDFom was calculated as follows:

% aNDFom (DM basis) = [(Wf-Wa)/DM] x 100

Where: aNDFom is aNDF organic matter

DM: (g oven-dried matter weight)

Wa: crucible weights after ashing (g)

Wf: dried crucible weights after refluxing (g)

3.3.3 Acid detergent fibre

The method used in this trial is described by Raffrenato and Van Amburgh (2010). Each sample (0.5g) was weighed into a 500ml Berzelius beaker, without a pouring spout. Hundred ml ADF solution was added to each sample in the beakers which were placed on the refluxing apparatus. The beakers were covered with round cold-water condensers to minimize evaporation. The heat setting was set at 2 and after boiling lowered to 1.5. Beakers were removed one by one, one hour after boil. The solution containing the sample was poured into the 50ml fritted disc Gooch crucibles which were placed in the vacuum filter apparatus. The solution was filtered away and repeatedly rinsed with boiling distilled water and rinsed twice with acetone. The ADF was determined as follows:

% aADFom (DM basis) = 100 (Wf – Wa)/(S*DM)

Where: aADFom: aADF organic matter

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

S: as-is test portion weight (g)

Wa: crucible weights after ashing (g)

Wf: dried crucible weights after refluxing (g)

3.3.4 Acid detergent lignin

The ADF-filled Gooch crucibles were used to perform the ADL procedure. After recording the ADF weights, the Gooch crucibles were placed in a glass Pyrex tray. Each crucible was filled halfway with 72% sulphuric acid solution and a glass rod was placed in each crucible. The procedure lasts for 3 hours. The samples were stirred every hour and refilled with 72% sulphuric acid. The sulphuric acid that filtered out of the crucibles into the tray was removed and discarded. After three hours the crucibles were placed in a vacuum filter and the sulphuric acid filtered away. The remaining sample was thoroughly rinsed with boiled distilled water and twice with acetone. Crucibles were placed in an oven overnight at 105ºC and hot-weighed the following day. Samples were ashed in a muffle furnace for 4 hours at 550°C and thereafter left to cool down overnight. The following day the samples were removed and placed in an oven for an hour at 105ºC and thereafter hotweighed.

% aADLom (DM basis) = 100 (Wf – Wa)/(S*DM)

Where: aADLom: aADL organic matter

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

S: as-is test portion weight (g)

Wa: crucible weights after ashing (g)

Wf: dried crucible weights after refluxing (g)

3.3.5 240h *In vitro* neutral detergent fibre digestibility

The method used was described by Raffrenato and Van Amburgh. (2010). Rumen fluid was collected from two Holstein dairy cows at the University of Pretoria Experimental Farm. Each sample was weighed (0.75g) into 100ml Schott *in vitro* bottles. Two empty Schott bottles were included to serve as blanks. The remaining 70 bottles were filled with 40ml solution and placed in a waterbath at 39°C. Caps were placed on each bottle and CO_2 flushed through the system to make the bottles anaerobic. The medium turned from pink to clear, once the bottles were anaerobic. Ten milliliter of rumen fluid was added to the bottles using a syringe to inject the fluid through a small hole in the cap that can be closed. Samples were swirled every day. After 5 days the samples were re-inoculated with 10ml rumen fluid and 40ml medium solution. The medium had to be flushed with CO_2 before it was

added to the Schott bottles. After 10 days the samples were removed. Each sample was poured into a 500ml Berzelius beaker and the same procedure was followed as with the NDF extraction procedure, as was previously discussed, but no amylase was added during boiling and filtration.

3.4 Statistical analysis

The variability of iNDF was tested between type (silage, hay and green forage), species, and C_3/C_4 groups for significance between categories. A simple ANOVA was used for comparison between the different groups. Simple linear regression was conducted between iNDF concentration in DM as well as NDF and each of the explanatory cell wall fractions such as NDF, ADF and ADL. Stepwise multiple linear regression analysis with NDF, ADF, ADL in DM as well as NDF as explanatory variables in the model were completed for iNDF concentration in DM and NDF for the full dataset and within type, species and C_3/C_4 groups. A backwards selection procedure was used with the stepwise multiple linear regression analysis. The explanatory factors were tested for co-linearity using the variance inflation factor. For the simple regression analysis, differences among means with $P < 0.05$ were accepted as significant and differences with $0.05 < P < 0.10$ representing a tendency to significance. Akaike`s information criterion and R^2 were used for model evaluation and to find the best fit model. Another attempt to predict iNDF was done generating power function equations of the type $y = ax^b$, with y representing the ratio iNDF/ADL (DM basis) and x representing ADL/NDF. Prediction accuracy was tested and compared using correlations and mean square prediction analysis of Theil (1966) and Bibby and Toutenberg (1977).

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Chemical composition analysis

Many research groups have focused on forage type when investigating the relationship between chemical components and iNDF as well as NDF digestibility (Jung and Allen, 1995). The cell wall fractions or independent variables include NDF, ADF, ADF/NDF, ADL/NDF, ADL, iNDF, iNDF/NDF, hemicellulose, cellulose, hemicellulose/NDF, cellulose/NDF. Forage type had three sub categories, namely silage, hay and green forage. Species were separated into two categories: legumes and grasses and grasses were classified into C_3 and C_4 types. The last group that was analysed was categorized into three categories namely legumes, C_3 and C_4 forages.

The chemical composition in terms of NDF, ADF, ADL, hemicellulose, cellulose and respective ratios are presented in Tables 4.1a and 4.1b.

Table 4.1a Chemical composition of forages calculated separately for each category (g/kg DM).

	*n	NDF		ADF		ADL		ADF/NDF		ADL/NDF		iNDF	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Type													
Silage	39	51.1[a]	100.39	30.8	57.79	3.9	15.78	61.1	100.48	7.8	34.41	16.0	60.33
Hays	48	40.1[b]	115.87	31.2[a]	68.65	6.0	15.87	79.5	89.62	15.6	35.76	19.6[a]	50.13
Green forage	5	43.9[c]	48.45	27.6[b]	64.55	3.6	8.14	62.9	94.55	8.3	18.75	13.7[b]	65.89
Species													
Legume	76	54.9[a]	107.62	32.0[a]	66.78	3.9[a]	14.27	58.5[a]	52.03	7.2[a]	19.67	17.1	71.13
Grasses	48	39.3[b]	72.53	30.4[b]	64.22	6.0[b]	16.43	81.5[b]	65.53	16.2[b]	28.94	19.0	45.84
C3/C4													
C3	90	40.3[a]	101.59	30.8	65.54	5.6	17.32	77.8[a]	101.76	14.7	43.03	18.4[a]	50.762
C4	34	55.3[b]	111.77	31.6	65.47	39.9	16.67	57.3[b]	58.65	7.1	22.30	18.0[b]	75.864

Different superscripts within the same column indicate that groups differ ($P<0.05$), *n Number of samples

Table 4.1b Chemical composition of forages calculated separately for each category (g/kg DM).

	*n	iNDF/NDF		Hemicellulose		Cellulose		Hemicellulose/NDF		Cellulose/NDF	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Type											
Silage	39	31.0	79.52	20.3[a]	70.88	26.9	48.24	38.9	100.48	53.2	76.98
Hays	48	49.8	77.36	8.8[b]	69.13	25.2[a]	59.07	20.5	89.61	63.9	69.25
Green forage	5	29.9	117.40	16.2[c]	50.20	24.0[b]	57.78	39.1	94.34	54.5	79.42
Species											
Legume	76	30.1[a]	81.05	22.8[a]	56.95	28.0[a]	57.07	41.5	52.02	51.2	46.91
Grasses	48	50.7[b]	57.89	68.1[b]	27.39	24.3[b]	51.60	18.4	65.53	65.4	58.89
C3/C4											
C3	90	46.7	106.72	9.4[a]	63.36	25.1	56.05	22.2[a]	101.76	63.2[a]	72.85
C4	34	31.5	48.23	23.7[b]	64.26	27.6	53.50	42.7[b]	58.65	50.2[b]	52.81

Different superscripts within the same column indicate that groups differ (P<0.05), *n Number of samples

The current dataset was analysed group wise by forage type, C_3/C_4 grasses, legume/grasses as it had an effect ($P < 0.05$) on cell wall fraction levels. Species within plant type affected NDF, ADF, iNDF and cellulose ($P < 0.05$), with silages having the highest NDF content. There was no difference between plant type with regards to ADF/NDF, ADL/NDF, iNDF/NDF, hemicellulose/NDF and cellulose/NDF levels. Hays and green forage differed with regards to ADF, iNDF and cellulose content ($P < 0.05$), where hays had the highest concentration of ADF, iNDF and cellulose.

Legumes contained the highest concentration of the following variables: NDF, ADF, hemicellulose, hemi/iNDF and cellulose, where grasses contained the highest concentrations of ADL, ADF/NDF, ADL/NDF, iNDF/NDF and cellulose/NDF. Significant differences in composition between legume and grasses were found for the variables NDF, ADF, ADL, ADF/NDF, ADL/NDF, iNDF/NDF, hemicellulose and cellulose. A difference ($P < 0.05$) was found in legume species within legume type for NDF, ADF/NDF, ADL/NDF, iNDF/NDF, and hemicellulose ($P < 0.0001$). A significant difference was found for ADF and cellulose levels ($P < 0.05$). No difference was found for ADL and iNDF.

The difference in variable composition between C_3 and C_4 were significant for NDF, ADF/NDF, iNDF, hemicellulose, hemicellulose/NDF and cellulose/NDF. A ($P < 0.05$) difference was found between C_3 and C_4 grasses with regards to hemicellulose levels ($P < 0.0001$). A significant difference was found for NDF, ADF/NDF and iNDF ($P < 0.05$). No differences were found for ADF, ADL, ADL/NDF, iNDF/NDF and cellulose levels between C3 and C4 grasses ($P > 0.05$).

4.2 Simple linear regression analysis

Tables 4.2 to 4.9 show the simple linear regression equations for the prediction of iNDF in DM for the forages analysed in this trial. Simple linear regression analysis was completed where iNDF served as the dependant variable. Groups were analysed according to type, legume/grass and C_3/C_4. All of the different forages were analysed to see if there was an effect across different groups of forages (Type, legume/grass, C_3/C_4). Best fit models for iNDF concentration in DM and related measurements for each group and category within each group are indicated in Tables 4.2 to 4.9 depending on the dependant variable used. The regression equations are all based on a limited number of samples.

Table 4.2 represents the simple linear regression results for the prediction of iNDF with NDF as the variable.

Table 4.2 Simple linear regression for the prediction of iNDF using NDF as dependent variable

	Parameter Estimates								
	n[a]	**Intercept**	**SE**	**P**	**Slope**	**SE**	**P**	**RSE**	**R^2**
All	124	81.49	16.64	<0.0001	0.23	0.04	<0.0001	49.32	0.24
Type									
Silage	39	[b]			0.32	0.01	<0.0001	41.84	0.94
Hays	48	[b]			0.48	0.01	<0.0001	40.63	0.96
Green	5	[b]			0.32	0.05	0.002	46.22	0.92
Species									
Legume	76	[b]			0.32	0.01	<0.0001	45.54	0.94
Grass	48	[b]			0.51	0.01	<0.0001	21.68	0.99
C3/C4									
C3	90	[b]			0.76	0.01	<0.0001	49.23	0.93
C4	34	[b]			0.34	0.02	<0.0001	46.48	0.94

[a] Number of samples, [b]Intercept not different from zero; model without intercept used,SE: Standard error, P: Significance level, RSE: Relative standard error, R^2: Correlation coefficient

Neutral detergent fibre includes the components, lignin, cellulose and hemicellulose. It is also characterized by the presence of iNDF, which is unavailable to microbial digestion (Raffrenato and Erasmus, 2013). It is regarded as a measure of cell wall concentration in plants (MacDonald *et* al. 2002). The availability of NDF is determined by the digestibility of the remaining fibre, after the indigestible portion is subtracted (Raffrenato and Erasmus, 2013). Individual groups, for example silage, hay, green, legumes and grasses had a better model fit, when looking at R^2, than all of the groups together, which can be seen in table 4.2. For the prediction of iNDF with NDF as the variable, grasses had the best model fit than all of the other groups, with a R^2 value of 0.99. Green forage had the worst model fit, with the lowest iNDF concentration, with a R^2 value of 0.92. Intercepts did not differ from zero and were not included in the models, except when samples were pooled (All). For all of the groups there was no significant difference in iNDF concentration when predicted using NDF as the variable, except for green forage ($P < 0.05$). Only five samples of green forage were included, therefore further research is needed to confirm these results. The same statement mentioned above is applicable for the rest of the regressions.

Table 4.3 represents the simple linear regression results for the prediction of iNDF with ADF as the variable.

Table 4.3 Simple linear regression for the prediction of iNDF using ADF as dependent variable

	Parameter Estimates								
	n[a]	Intercept	SE	P	Slope	SE	P	RSE	R^2
All	124	-24.26	15.48	0.13	0.67	0.05	<0.0001	36.16	0.60
Type									
Silage	39	[b]			0.53	0.02	<0.0001	40.51	0.95
Hays	48	[b]			0.62	0.01	<0.0001	30.35	0.98
Green forage	5	[b]			0.52	0.05	0.0007	34	0.96
Species									
Legume	76	[b]			0.55	0.02	<0.0001	47.48	0.93
Grass	48	[b]			0.62	0.01	<0.0001	24.25	0.99
C_3/C_4									
C_3	90	[b]			0.6	0.01	<0.0001	32.74	0.97
C_4	34	[b]			0.59	0.03	<0.0001	47.85	0.94

[a] Number of samples,[b]Intercept not different from zero; model without intercept used, SE: Standard error, P: Significance level, RSE: Relative standard error, R^2: Correlation coefficient

Acid detergent fibre consists of cellulose, lignin as well as lignified N and silica. According to MacDonald *et al*. (2002) there is a strong correlation between ADF content and digestion rate. Several studies have shown that there is a strong correlation between ADF and iNDF content. Jancik *et al*. (2008) and Traxler (1998) reported a strong correlation between iNDF and ADF, $R^2 = 0.71$. Jancík *et al*. (2008) predicted iNDF from ADF content with a R^2 of 0.69 for grasses. In this study, a R^2 value of 0.99 was found for iNDF prediction when using ADF as the variable for grasses. Acid detergent fibre had the best model fit for all the groups together, with a R^2 of 0.60. Individual groups, for example silage, hay, green forage, legumes and grasses had a stronger model fit, than the combination of the groups (All). For the prediction of iNDF with ADF as dependent variable, grasses showed the best fit than all of the other groups and legumes had the weakest model fit. Intercepts and slopes of the regression equations varied among all the plant types. For all of the groups there were no significant difference in iNDF concentration when predicted with ADF as the variable, except for green forage ($P < 0.05$).

Table 4.4 represents the simple linear regression outcome for the prediction of iNDF with ADL as the variable.

Table 4.4 Simple linear regression for the prediction of iNDF using ADL as dependent variable

	Parameter Estimates								
	n[a]	**Intercept**	**SE**	**P**	**Slope**	**SE**	**P**	**RSE**	**R^2**
All	124	63.56	10.61	<0.0001	2.24	0.19	<0.0001	38.65	0.54
Type									
Silage	39	[b]			3.89	0.17	<0.0001	76.79	0.93
Hays	48	[b]			3.17	0.07	<0.0001	39.19	0.96
Green forage	5	[b]			3.85	0.55	0.0022	45.5	0.93
Species									
Legume	76	[b]			4.23	0.16	<0.0001	76.46	0.94
Grass	48	[b]			3.08	0.05	<0.0001	29.23	0.98
C_3/C_4									
C_3	90	[b]			7.48	0.19	<0.0001	34.65	0.97
C_4	34	[b]			4.40	0.2	<0.0001	76.71	0.95

[a] Number of samples, [b]Intercept not different from zero; model without intercept used, SE: Standard error, P: Significance level, RSE: Relative standard error, R^2: Correlation coefficient

In Table 4.4, each group, for example silage, hay, green forage, legumes and grasses had a stronger model fit, compared to all of the groups together with an R^2 of 0.54. For the prediction of iNDF from ADL, grasses had the best fit model when compared to the other groups analysed and green forage had the worst model fit. Intercepts and slopes of the regression equations varied among all the forage types. For all of the groups there was no significant difference in iNDF concentration when determined with ADL as the variable, except for green forage ($P < 0.05$). Jancík *et al*. (2008) predicted iNDF content of grasses from ADL with a R^2 of 0.78, which was found to be the best predictor of iNDF for that trial. A R^2 value of 0.98 was found for grasses in this trial, where ADL was the second best predictor of iNDF content for grasses.

Table 4.5 represents the simple linear regression results for the prediction of iNDF with ADF/NDF as dependent variable.

Table 4.5 Simple linear regression for the prediction of iNDF with ADF/NDF as dependent variable

	Parameter Estimates								
	n[a]	**Intercept**	**SE**	**P**	**Slope**	**SE**	**P**	**RSE**	**R^2**
All	124	126.69	29.69	<0.0001	0.08	0.04	0.06	56	0.03
Type									
Silage	39	[b]			0.26	0.02	<0.0001	65.20	0.86
Hays	48	[b]			0.24	0.01	<0.0001	57.75	0.92
Green forage	5	[b]			0.22	0.04	0.0052	56.55	0.88
Legume/grass									
Legume	76	[b]			0.25	0.05	<0.0001	57.75	0.91
Grass	48	[b]			0.30	0.03	<0.0001	54.25	0.89
C_3/C_4									
C_3	90	[b]			0.23	0.01	<0.0001	49.74	0.93
C_4	34	[b]			0.31	0.26	<0.0001	77.46	0.85

[a] Number of samples, [b]Intercept not different from zero; model without intercept used, SE: Standard error, P: Significance level, RSE: Relative standard error, R^2: Correlation coefficient

Individual groups, for example silage, hay and green forage, expressed a better model fit ranging between a R^2 of 0.85 and 0.93 when compared to the combined groups (all) with a R^2 of 0.03. For the prediction of iNDF when using ADF/NDF, hays had the best model fit than all of the other groups and C_4 grasses had the worst model fit with R^2 of 0.93 and 0.85 respectively. Intercepts and slopes of the regression equations varied among all the forage types. For all of the groups there were no difference in iNDF concentration when predicted using the ADF/NDF ratio, except for green forage ($P < 0.05$).

Table 4.6 indicates the simple linear regression results for the prediction of iNDF using ADL/NDF as dependent variable.

Table 4.6 Simple linear regression for the prediction of iNDF with the ratio ADL/NDF as independent variable

	Parameter Estimates								
	n[a]	**Intercept**	**SE**	**P**	**Slope**	**SE**	**P**	**RSE**	**R^2**
All	124	139.29	13.55	<0.0001	0.34	0.1	0.001	54.25	0.09
Type									
Silage	39	[b]			1.81	0.14	<0.0001	74.97	0.81
Hays	48	[b]			1.19	0.05	<0.0001	66.14	0.89
Green forage	5	[b]			1.58	0.36	0.012	68.65	0.83
Legume/grass									
Legume	76	[b]			1.55	0.14	<0.0001	57.48	0.83
Grass	48	[b]			1.62	0.42	<0.0001	64.25	0.89
C_3/C_4									
C_3	90	[b]			1.19	0.04	<0.0001	58.48	0.91
C_4	34	[b]			2.48	0.17	<0.0001	66.03	0.89

[a] Number of samples, [b]Intercept not different from zero; model without intercept used, SE: Standard error, P: Significance level, RSE: Relative standard error, R^2: Correlation coefficient

In Table 4.6, individual groups, for example silage, hay, green forage and legumes had a better model fit when compared to the combined groups (all). For the prediction of iNDF when using ADL/NDF, C_3 had the best model fit compared to all of the other groups and silage had the worst model fit with R^2 of 0.91 and 0.81 respectively. Intercepts and slopes of the regression equations varied among all the forage types. For all of the groups there was no difference in iNDF concentration when determined using the ADL/NDF ratio, except for green forage ($P < 0.05$). In other trials, results of iNDF/ADL ratio were found to be between 1.5 and 6.2 with an average of 3.6. Suggesting that attempts to describe forage iNDF and from ADL content usually had low accuracy and precision (Harper and McNeil. 2015).

Table 4.7 represents the simple linear regression results for the prediction of iNDF with cellulose as dependent variable.

Table 4.7 Simple linear regression for the prediction of iNDF with cellulose as dependent variable

	Parameter Estimates								
	n[a]	**Intercept**	**SE**	**P**	**Slope**	**SE**	**P**	**RSE**	**R^2**
All	124	13.58	18.1	0.45	0.66	0.07	<0.0001	42.94	0.43
Type									
Silage	39	[b]			0.61	0.03	<0.0001	76.51	0.94
Hays	48	[b]			0.77	0.012	<0.0001	34.48	0.97
Green forage	5	[b]			0.59	0.06	0.0006	32.75	0.96
Legume/grass									
Legume	76	[b]			0.62	0.03	<0.0001	51.26	0.93
Grass	48	[b]			0.08	0.01	<0.0001	28.08	0.98
C_3/C_4									
C_3	90	[b]			0.72	0.02	<0.0001	39.39	0.96
C_4	34	[b]			0.67	0.04	<0.0001	52.76	0.93

[a] Number of samples, [b]Intercept not different from zero; model without intercept used, SE: Standard error, P: Significance level, RSE: Relative standard error, R^2: Correlation coefficient

Cellulose is an important structural component in cell walls of green forage. Individual groups, for example silage, hay, green, legumes had a better model fit, when compared to the combined groups of forages (all). For the prediction of iNDF when using cellulose as variable, grasses had the best model fit than all of the other groups and legumes and C4 grasses had the worst model fit with R^2 of 0.98 and 0.93 respectively. Intercepts and slopes of the regression equations varied among all the plant types. For all of the groups there was no significant difference in iNDF concentration when determined using cellulose as variable, except for green forage ($P < 0.05$).

Table 4.8 represents the simple linear regression results for the prediction of iNDF with hemicellulose as dependent variable.

Table 4.8 Simple linear regression for the prediction of iNDF with hemicellulose as dependant variable

	Parameter Estimates								
	n[a]	**Intercept**	**SE**	**P**	**Slope**	**SE**	**P**	**RSE**	**R^2**
All	124	173.95	8.94	<0.0001	0.07	0.06	0.22	54.49	0.012
Type									
Silage	39	[b]			0.75	0.05	<0.0001	62.02	0.87
Hays	48	[b]			1.49	0.11	<0.0001	58.48	0.68
Green forage	5	[b]			0.79	0.2	0.02	74.26	0.48
Legume/grass									
Legume	76	[b]			0.75	0.03	<0.0001	52.05	0.92
Grass	48	[b]			2.47	0.12	<0.0001	70.15	0.87
C_3/C_4									
C_3	90	[b]			1.35	0.10	<0.0001	115.19	0.64
C_4	34	[b]			0.76	0.05	<0.0001	54.00	0.92

[a] Number of samples, [b]Intercept not different from zero; model without intercept used, SE: Standard error, P: Significance level, RSE: Relative standard error, R^2: Correlation coefficient

In the rumen, both hemicellulose and cellulose can be slowly digested if these components are not protected by lignin. In Table 4.8, each group, for example silage, hay, green forage and legumes expressed a stronger model fit when compared to the combined forage groups. For the prediction of iNDF when using hemicellulose as variable, legumes had the best fit model fit, with a R^2 of 0.92, than all of the other groups and C_3 grasses had the weakest model fit with a R^2 of 0.64. Intercepts and slopes of the regression equations varied among all the plant types. For all of the groups there were no differences in iNDF concentration when predicted using hemicellulose as a variable, except for green forage ($P < 0.05$).

Table 4.9 represents the simple linear regression results for the prediction of iNDF with hemicellulose/NDF as dependent variable.

Table 4.9 Simple linear regression for the prediction of iNDF using hemicellulose/NDF as dependent variable

	Parameter Estimates								
	n[a]	**Intercept**	**SE**	**P**	**Slope**	**SE**	**P**	**RSE**	**R^2**
All	124	203.76	11.75	<0.0001	-0.08	0.04	0.06	56	0.03
Type									
Silage	39	[b]			0.39	0.03	<0.0001	73.31	0.48
Hays	48	[b]			0.48	0.04	<0.0001	86.91	0.48
Green forage	5	[b]			0.33	0.11	0.03	89.01	0.71
Legume/grass									
Legume	76	[b]			0.40	0.03	<0.0001	74.62	0.84
Grass	48	[b]			0.91	0.05	<0.0001	81.50	0.83
C_3/C_4									
C_3	90	[b]			0.66	0.04	<0.0001	101.42	0.72
C_4	34	[b]			0.41	0.04	<0.0001	48.49	0.84

[a] Number of samples, [b]Intercept not different from zero; model without intercept used, SE: Standard error, P: Significance level, RSE: Relative standard error, R^2: Correlation coefficient

Groups individually analysed, for example silage, hay, green forage, legumes had a stronger model fit, when compared to the combined groups of forages. For the prediction of iNDF when using hemiNDF as variable, legume and C_4 grasses had the best model fit, with a R^2 of 0.84,when compared to the other forages analysed and green forage had the weakest model fit with a R^2 of 0.71. Intercepts and slopes of the regression equations varied among all the plant types. For all of the groups there were no differences in iNDF concentration when determined using the hemicellulose/NDF ratio, except for green forage ($P < 0.05$).

In Table 4.10 is shown a summary off all the simple linear regression equations presented in Tables 4.2 to 4.9.

Table 4.10 Summary of all simple linear regression equations

Equation	RSE	R^2	P
All			
y = 81.79 + 0.23NDF	49.32	0.24	<0.0001
y = -24.26 + 0.67ADF	36.16	0.60	<0.0001
y = 63.56 + 2.24ADL	38.65	0.54	<0.0001
y = 126.69 + 0.08ADF/NDF	56.00	0.03	<0.0001
y = 139.29 + 0.34ADL/NDF	54.25	0.09	<0.0001
y = 13.58 + 0.66cellulose	42.94	0.43	<0.0001
y = 173.95 + 0.07hemicellulose	54.49	0.01	<0.0001
y = 203.76 – 0.08hemicellulose/NDF	56.00	0.03	<0.0001
Type			
Silage			
y = 0.32NDF	41.84	0.94	<0.0001
y = 0.53ADF	40.51	0.95	<0.0001
y = 3.9ADL	76.79	0.93	<0.0001
y = 0.26ADF/NDF	65.20	0.86	<0.0001
y = 1.81ADL/NDF	74.97	0.81	<0.0001
y = 0.61cellulose	76.51	0.94	<0.0001
y = 0.75hemicellulose	62.02	0.87	<0.0001
y = 0.39hemicellulose/NDF	73.31	0.48	<0.0001
Hays			
y = 0.48NDF	40.63	0.96	<0.0001
y = 0.62ADF	30.35	0.98	<0.0001
y = 3.17ADL	39.19	0.96	<0.0001
y = 0.24ADF/NDF	54.75	0.92	<0.0001
y = 1.81ADL/NDF	66.14	0.89	<0.0001
y = 0.77cellulose	34.48	0.94	<0.0001
y = 1.49hemicellulose	58.48	0.68	<0.0001
y = 0.48hemicellulose/NDF	86.91	0.48	<0.0001
Green forage			
y = 0.32NDF	46.22	0.92	<0.0001
y = 0.52ADF	34.00	0.96	<0.0001
y = 3.85ADL	45.50	0.93	<0.0001

y = 0.22ADF/NDF	56.50	0.88	<0.0001
y = 0.158ADL/NDF	68.65	0.83	0.012
y = 0.59cellulose	32.75	0.96	0.0006
y = 0.79hemicellulose	74.26	0.48	0.02
y = 0.33hemicellulose/NDF	89.01	0.71	<0.0001
Species			
Legume			
y = 0.32NDF	45.54	0.94	<0.0001
y = 0.55ADF	47.80	0.93	<0.0001
y = 4.23ADL	44.46	0.94	<0.0001
y = 0.25ADF/NDF	57.75	0.91	<0.0001
y = 1.55ADL/NDF	57.80	0.83	<0.0001
y = 0.62cellulose	51.26	0.93	<0.0001
y = 0.75hemicellulose	52.05	0.92	<0.0001
y = 0.4hemicellulose/NDF	74.62	0.84	<0.0001
Grass			
y = 0.51NDF	21.68	0.99	<0.0001
y = 0.62ADF	24.25	0.99	<0.0001
y = 3.08ADL	29.23	0.98	<0.0001
y = 0.30ADF/NDF	54.25	0.89	<0.0001
y = 1.62ADL/NDF	64.25	0.89	<0.0001
y = 0.08cellulose	28.08	0.98	<0.0001
y = 2.47hemicellulose	70.15	0.87	<0.0001
y = 0.91hemicellulose/NDF	81.50	0.83	<0.0001
C_3vsC_4			
C_3			
y = 0.44NDF	49.23	0.93	<0.0001
y = 0.60ADF	32.74	0.97	<0.0001
y = 7.82ADL	34.65	0.97	<0.0001
y = 0.23ADF/NDF	49.74	0.93	<0.0001
y = 1.19ADL/NDF	58.48	0.91	<0.0001
y = 0.72cellulose	39.37	0.96	<0.0001
y = 1.35hemicellulose	115.19	0.64	<0.0001
y = 0.66hemicellulose/NDF	101.42	0.72	<0.0001

C_4			<0.0001
y = 0.34NDF	46.80	0.93	<0.0001
y = 0.59ADF	47.85	0.94	<0.0001
y = 4.40ADL	44.71	0.95	<0.0001
y = 0.23ADF/NDF	77.46	0.93	<0.0001
y = 2.48ADL/NDF	66.03	0.89	<0.0001
y = 0.72cellulose	52.76	0.93	<0.0001
y = 0.76hemicellulose	54.00	0.92	<0.0001
y = 0.41hemicellulose/NDF	80.49	0.87	<0.0001

RSE: Residual standard error, R^2: Coefficient of variation, P: Significance level, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin

Table 4.10 summarizes all the single linear regression equations describing the relationship between iNDF and the chemical composition of the different forages used in this trial. According to the R^2 values and the residual mean square errors for the combined forage groups (seen as "all" in the tables), ADF represented the single best predictor of iNDF content with a R^2 value of 0.60, with ADL representing the second best predictor of iNDF content with a R^2 value of 0.54. Sixty samples were used in a trial by Jancík *et al*. (2008) that were separated into five different grass species for the predictiom of iNDF. They found that ADL is the single best predictor of iNDF content with a R^2 value of 0.78, with ADF having a R^2 value of 0.69. A possible explanation for the differences in results between our study and the Jancík study is that different methods were used to determine the ADF and ADL content. In the current trial a larger number of samples were used and there was no grouping of species, which may also contribute to the difference in results. The weakest single predictor of iNDF was hemicellulose with a R^2 value of 0.01 for the combined group of forages (seen as "all" in the tables). These results coincide with the results of Jancík *et al*. (2008) where hemicellulose was also the worst single predictor of iNDF with a R^2 value of 0.26. Krämer *et al*. (2012) reported that simple linear regression analysis for grasses revealed that lignin concentration in DM was the best single predictor of iNDF concentration. In the current trial iNDF prediction from grasses in NDF, ADF and ADL had the highest R^2 of 0.99, 0.99 and 0.98 respectively, when compared to other forages analyzed. Traxler *et al*. (1998) predicted iNDF from chemical components for C_3, C_4 grasses, legumes and combined forages. Correlation coefficient values of 0.63, 0.69, 0.66 and 0.77 were found, respectively. The current trial resulted in R^2 values of 0.97, 0.95, 0.94 and 0. 54 respectively. An explanation for the difference in results might be the method used for ADL analysis.

According to Table 4.10, iNDF prediction was the least accurate for legumes, when ADF was used as dependant variable, resulting in a R^2 value of 0.55. When considering the prediction equations in each category namely silages, hays and green forage, it was found that ADF was the best predictor of iNDF content for each of the types of forages with R^2 values of 0.95, 0.98 and 0.96 respectively. This is similar to the R^2 values of the combined group of forage ("All"), where ADF was also found to be the strongest predictor of iNDF content. The ADL/NDF ration was found to be the weakest predictor of iNDF for silage and green forageswith R^2 values of 0.81 and 0.83 respectively. The variable hemicellulose was the worst predictor of iNDF content in hays.

4.3 Multiple linear regression analysis

Tables 4.11 and 4.12 represents the multiple linear regression equations for the prediction of iNDF expressed on a DM and NDF basis, respectively, from chemical measurements resulting from the stepwise procedure. Multiple regression analysis was completed to evaluate the combined effects of the different cell wall fractions.

Table 4.11 Multiple linear regressions for iNDF (forage DM) based on cell wall chemical composition

		DM Basis		
Forage	**n[a]**	**Regression Equation**	**RSE**	**R^2**
All	124	-27.16 + 0.22NDF + 2.17ADL	28.48	0.75
Type				
Silage	39	-88.86 + 0.33NDF + 2ADL	27.52	0.48
Hays	48	3.5 + 0.23NDF + 1.6ADL	25.64	0.74
Green forage	5	-142.29 + 1.01ADF	11.34	0.98
Legume				
Legume	76	-121.26 + 0.4NDF + 1.48ADL	32.25	0.48
Grass	48	-15.53 + 0.34ADF + 1.15ADL + 0.46hemicellulose	18.51	0.84
C_3/C_4				
C_3	90	-3.95 + 0.33ADF + 1.49ADL	28.35	0.73
C_4	34	-114.34 + 0.4NDF + 1.78ADL	31.03	0.85

[a]Number of samples, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin, RSE: Residual square error, R^2: Coefficient of correlation

Table 4.12 Multiple linear regressions for iNDF prediction (forage NDF) based on cell wall chemical composition

		NDF Basis		
Forage	**n[a]**	**Regression Equation**	**RSE**	**R^2**
All	124	16.64 + 0.12NDF + 0.19ADFNDF + 1.7ADLNDF	64.3	0.72
Type				
Silage	39	16.86 + 0.32NDF + 1.69ADLNDF	52.85	0.58
Hays	48	271.26 + 145ADLNDF	57.72	0.45
Green forage	5	-671.47 + 0.9NDF + 0.91ADFNDF	20.98	0.98
Legume				
Grass	76	-49.2 + 0.43NDF + 1.6ADLNDF	55.62	0.55
Legume	48	357.72 + 0.92ADLNDF	51.69	0.21
C_3/C_4				
C_3	90	81.39 + 0.196ADFNDF + 1.6ADFNDF	65.11	0.64
C_4	34	-16.4 + 0.38NDF + 1.72ADLNDF	51.42	0.64

[a]Number of samples, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin, RSE: Residual square error, R^2: Coefficient of correlation

The stepwise multiple linear regressions resulted in the best model fit for iNDF prediction expressed on DM basis (R^2 = 0.75) as well as NDF basis (R^2 = 0.72), when compared to the simple linear regressions for the combined forages (All). Correlation coefficient values for the combined groups using simple linear regression ranged from 0.01 to 0.6, with hemicelluloses being the weakest predictor and ADF the strongest predictor of iNDF content. The stepwise multiple linear equation on DM basis for the combined forage group resulted in the lowest RSE value of 28.48g/kg. The variables used for iNDF prediction on DM basis include NDF, ADF, ADL and hemicellulose. The variables used for iNDF prediction on NDF basis include NDF, ADF/NDF and ADL/NDF. Green forage had the strongest model fit for iNDF prediction on DM basis with a R^2 value of 0.98 and a low RSE of 11.34 g/kg DM. This could perhaps be contributed to the low number of samples used, namely 5. More research will be needed, to verify the results from this trial.

When comparing results of iNDF prediction on a DM and NDF basis respectively, the regression equations developed on DM basis, resulted in the lowest RSE values, ranging from 11.34 to 32.25 g/kg DM, as well as the strongest R^2 value, ranging from 0.73 to 0.98. Regression equations developed on NDF basis resulted in RSE values ranging from 20.98 to 65.11 g/kg NDF and R^2 values ranging from 0.21 to 0.72. The simple linear regression equation for silage had a stronger model fit with R^2 values ranging from 0.81 to 0.98, when compared to the stepwise linear equations with R^2

values of 0.8 and 0.58 based on both DM and NDF respectively. Acid detergent fibre was the strongest single predictor for silage with a R^2 value of 0.95. Results from this study suggest that iNDF content in hays can be best predicted by simple linear equations, with R^2 values ranging from 0.68 to 0.98. Acid detergent fibre was the strongest predictor and hemicellulose the weakest predictor of iNDF. The R^2 values for stepwise linear equations on both DM and NDF basis were 0.74 and 0.45 respectively. Green forage iNDF content was best predicted by the multiple linear equations with a R^2 value of 0.98 on DM and NDF basis, when compared to the iNDF simple linear prediction equations. The linear regression equations resulted in R^2 values ranging from 0.71 and 0.96, with hemicellulose/NDF being the weakest predictor and ADL the strongest predictor. Readers are cautioned that only five replicates were used for analysis, therefore more research should be done to verify these results. Acid detergent lignin used as the variable for iNDF prediction for legumes had the best model fit (R^2 = 0.94), when compared to the R^2 values of the multiple linear equations on DM and NDF basis (R^2 = 0.8, 0.55 respectively). The results for iNDF prediction for grasses indicate that simple linear regression equation will more accurately predict iNDF content. Correlation coefficient values ranged from 0.83 to 0.99, with NDF and ADF as the best predictors. The multiple linear regression equations for grasses expressed on DM and NDF basis resulted on R^2 values of respectively 0.84 and 0.21. The C_3 and C_4 forages were best predicted by simple linear prediction equations, with R^2 values of 0.97 (ADF and ADL as variables) and 0.95 (ADL as variable). The multiple linear equations resulted in R^2 values of 0.85 and 0.64 on DM and NDF basis respectively.

CHAPTER 5

CONCLUSION

The feeding of quality forages is one of the most important aspects of ruminant nutrition. Previously, there was a lot of focus on concentrate feeding, but the production cost of high concentrate of feeding systems has increased dramatically over the past few years, therefore there has been a shift towards feeding more high quality forages.

The physical and chemical properties of forages impact on the nutritional value of a forage as well as the production performance of the animal. It is well known that the extent and duration of rumination depends on the proportion of cell wall content or NDF content. Neutral detergent fibre consists of a digestible fraction and an indigestible fraction. The indigestible fraction is defined as indigestible NDF, which remain indigestible even if rumen retention time is increased to infinity. Indigestible NDF plays a critical role in ruminant nutrition, contributing no energy to the diet and therefore should be taken into account when formulating rations. The iNDF should be subtracted from the NDF content, otherwise there will be an underestimation of the amount of energy available to the animal which can negatively impact on the production performance of the animal. The more iNDF in the forage, the lower the digestibility and possibly animal performance, therefore, it is critical to routinely take the amount of iNDF into account when formulating ruminant diets.

The results from this study suggest that prediction equations for the estimation of iNDF can be compiled from the cell wall content of a forage, avoiding laborious and costly analytical procedures. Results from this study has shown that prediction equations for the individual forage groups (silage, hay, green forage) are more accurate than prediction equations when using the combined groups (All). The dependant variable that resulted in the most accurate result was ADF, except for green forage where ADL was the variable that explained most of the variation in iNDF. As shown in Table 4.10, it is not appropriate to use the 2.4ADL calculation to obtain an iNDF estimate, since the ratios found between iNDF and ADL vary among species and also within species. Tables 4.11 and 4.12 contain the data from multiple linear regression analyses and is apparent that the prediction equations from the specific forage groups were more accurate than prediction equations for the combined forage groups. Tables 4.11 and 4.12 shows that the prediction equations that were NDF based, were more accurate than the prediction equations that were based on DM. Green forage had the highest prediction accuracy for both NDF and DM based analysis.

It can be concluded from the data in this trial that iNDF can be predicted from cell wall measurements, even when only using a single variable. As is the case with most research, a higher number of family or type-grouped samples will likely give more accurate and precise iNDF predictions. Accuracy and prediction may even increase when single species are considered instead of forage families and there is a need for the development of specie-specific equations. These equations will have to be evaluated based on a large quantity of iNDF estimations using long term *in vitro* fermentations, which will remain the reference procedure. Near Infrared Reflectance Spectroscopy (NIR) is another viable option for the estimation of iNDF, but not all laboratories have access to NIR technology. A large number of samples also need to be analysed in order to obtain accurate calibration when using NIR technology. Calibrations from other regions in the world are used in South Africa and accuracy and precision of these equations, when used with South African forages, are often unknown. Simple cell wall analyses have then the potential of being more economical and accurate and precise and most laboratories should be able to perform these analyses. Indigestible NDF estimation has become important especially when feeding high producing dairy cows to fine tune their rations and the use of the 2.4 factor or a default value used in many nutritional models or formulation programs is not justifiable anymore.

CHAPTER 6

CRITICAL EVALUATION

In order to develop accurate prediction equations for iNDF estimation, a wide range of forages that are used in ruminant nutrition need to be evaluated. A larger number of samples for every forage would ensure more accurate and repeatable results.

In this study, an additional filter was not placed in the Gooch crucibles during filtration, due to the vacuum pump being unable to filter out the solution in crucible. In future it is recommended to use additional filters to ensure that as much as possible of the remaining forage is captured.

The statistical models in this study are based on chemical composition as well as the number of samples used. With more samples and possibly more variability in chemical analysis values between samples, the models might change. Thus this study serves only as a basis for future studies and the development of accurate prediction equations, hopefully for the most common forage species that are used in South Africa.

The format of presenting the results is unfortunately in a repetitive fashion, but due to the design and objectives of the study, this was the most appropriate way to present the results. The author is aware of the excessive use of the same references but the lack of research data in this field contributed to this.

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