A GRADING SYSTEM FOR *MEDICAGO SATIVA* HAY IN SOUTH AFRICA

By

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DEDICATION

I dedicate this thesis to my father, Gerrie Scholtz (Snr.) (20-09-1943 to 03-06-2006).

SCIENCES NOT FOUNDED ON EXACT EXPERIENCE AND MATHEMATICS ARE
EITHER DECEPTION OR MADNESS – A BANNER OF CHARLATANS, BLOWN
FULL BY THE WIND AFTER WHICH THE FOOLISH RABBLE FLOCKS.
LEONARDO DA VINCI (1452 – 1519)

PREFACE

This thesis is presented in the form of six separate articles, augmented by a general introduction and conclusion in an effort to eventually create a single unit. Although care has been taken to avoid unnecessary repetition some repetition has been inevitable.

The author herby wishes to express sincere thanks to the following establishments and persons who contributed to this study:

Mrs. Carol Keep for the grammatical editing of the thesis

My mother, parents-in-law, as well as other relatives and friends for their continuous interest and encouragement.

My wife, Celia, for the neat typing of the references, valuable commends and my son, Gerhard, for their love, loyal support and encouragement.

Our Heavenly Father, gratitude for His mercy in granting the opportunity, health and endurance to complete this work.

I, the undersigned, declare that this thesis submitted by me for the degree Ph.D. at the university of the Free State is my own independent work and has not previously been submitted by me at another university/faculty.

G.D.J. Scholtz
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May, 2008

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LIST OF ABBREVIATIONS

°C	Degrees centigrade of Celsius (temperature)
1/R	Reciprocal logarithm of reflectance
A	Amplitude
AA	Amino acids
ACP	Adjusted crude protein
ad libitum	Free access
ADF	Acid detergent fibre
ADF-CP	Acid detergent fibre-crude protein
ADF-N	Acid detergent fibre-nitrogen
ADICP	Acid detergent insoluble crude protein
ADIN	Acid detergent insoluble nitrogen
ADL	Acid detergent lignin
ARC	Agricultural research counsel
ATFI	Adjusted total forage index
BW	Body weight
C	Carbon
Cl	Chloride
Ca	Calcium
Ca/d	Calcium per day
CF	Crude fibre
CHO	Carbohydrate
cm	Centimetres
CNCPS	Cornell net carbohydrate and protein system
CP	Crude protein
Cu	Copper
CV	Coefficient of variation
DCAD	Dietary cation-anion difference
DCP	Digestible crude protein
DDM	Digestible dry matter

DE	Digestible energy
DEp	Digestible energy at production intake
DM	Dry matter
DMI	Dry matter intake
DOM	Digestible organic matter
ECP	Endogenous crude protein
EE	Ether extract
EE	Fat
EMS	Electromagnetic spectrum
EMT	Electronic moisture tester
eNDF	Effective neutral detergent fibre
eq.	Equation
etc.	*et cetera*
FA	Fatty acids
FCM	Fat corrected milk
Fe	Iron
FME	Fermentable metabolisable energy
FQI	Forage quality index
g	Gram
g/d	Gram per day
H	Hydrogen
H	Mahalanobis distances
ha	Hectare
HCL	Hydrochloric acid
HMSC	High moisture shelled corn
hr	Hour
Hz	Hertz
ICP	Insoluble crude protein
IVOMD	*In vitro* organic matter digestibility
IVOMD24	*In vitro* organic matter digestibility at 24 hours
IVOMD48	*In vitro* organic matter digestibility at 48 hours

I-VR	Cross validation coefficient of determination
K	Potassium
kd	Degradation rate
kg	Kilogram
kg/d	Kilogram per day
kp	Rate of passage
L	Lignin
L	Lag time
l	Litre
LMV	Lucerne milk value
LQI	Lucerne quality index
Lys	Lysine
MADF	Modified acid detergent fibre
Max	Maximum
Mcal	Mega calorie
MCF	Modified crude fibre
MCP	Microbial crude protein
ME	Metabolisable energy
MEfat	Metabolisable energy from fat
MEp	Metabolisable energy at production level of intake
Met	Methionine
Mg	Manganese
MJ	Megajoules
MJ/kg	Megajoules per kilogram
mm	millimeter
Mn	Manganese
MP	Metabolisable protein
MPLS	Modified partial least square regression
MSW	Mean stage by weight
MUW	Milk urea nitrogen
MW	Metabolic weight

N	Nitrogen
n	Number
Na	Sodium
NaSO4	Sodium sulphate
NDF	Neutral detergent fibre
NDF-CP	Neutral detergent fibre-crude protein
NDFD	Neutral detergent fibre digestibility
NDFD24	Neutral detergent fibre digestibility at 24 hours
NDFD48	Neutral detergent fibre digestibility at 48 hours
NDFn	Nitrogen free neutral detergent fibre
NDICP	Neutral detergent insoluble crude protein
NE	Nett energy
NEI	Net energy for lactation
NEIp	Nett energy for lactation at production intake
NEl	Nett energy for lactation
NFC	Non-fibre carbohydrates
NFTA	National forage testing association
NIR	Near infrared reflectance
NIRS	Near infrared reflectance spectroscopy
nm	Nano meter
NPN	Non-protein nitrogen
NRC	National research council
NSC	Non-structural carbohydrates
OC	Oil cake
OM	Organic matter
OMD	Organic matter digestibility
p	Normality
P	Phosphate
P<0.0001	Significant at 0,01% level of significance
P<0.01	Significant at 1% level of significance
P<0.05	Significant at 5% level of significance

pef	Physical effectiveness factor
peNDF	Physical effective neutral detergent fibre
ppm	parts per million
r	Correlation coefficient
R	Reflectance
r^2	Coefficient of determination
RDP	Rumen degradable protein
RFO	Relative forage quality
RFV	Relative feed value
RH	Relative humidity
RPD	Ratio of prediction to deviation
RR	Reticulo rumen
RUP	Rumen undegradable protein
S	Sulphur
SA	South Africa
SARA	Sub-acute ruminal acidosis
SD	Standard deviation
SEC	Standard error of calibration
SECV	Standard error of cross-validation
SEP	Standard error of prediction
SNV	Standard normal variant
SP	Soluble protein
td	Truly digestible
1x	Intake at maintenance
2x	Intake at 2x maintenance
3x	Intake at 3x maintenance
TDN	Total digestible nutrient
td	Truly digestible
TFI	Total forage index
TMR	Total mix ration
TTDOM	Total track digestible organic matter

UIP	Undegraded intake protein
US	United States
USA	United States of America
USDA	United States department of agriculture
v	Version
VFA	Volatile fatty acids
vs.	Versus
Zn	Sink
λ	Wavelength

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CHAPTER 1

GENERAL INTRODUCTION

This thesis is presented in the form of separate chapters, including a general introduction and conclusions in an effort to create a single unit. Although care has been taken to avoid unnecessary repetition some repetition has been inevitable.

Lucerne (*Medicago sativa* L.) is the most important hay crop in South Africa. According to Grönum *et al.* (2000) the current area planted with lucerne for hay production in South Africa is estimated as being between 208 000 ha and 240 000 ha. The average annual lucerne hay production in South Africa is approximately 3.8 million tons. Approximately 90% of the lucerne hay produced in South Africa is under irrigation. Grönum *et al.* (2000) mention that the estimated area planted with lucerne has remained more or less constant over the last few years.

One of the most important characteristics of lucerne hay is its high nutritional quality as animal feed. Jagusch *et al.* (1970) are of the opinion that lucerne hay is equal to, or even better than, most concentrates. Lucerne hay is an important roughage source for dairy cattle, and according to Grönum *et al.* (2000), the viability of the lucerne industry in certain regions depends to a large extent on the dairy- and ostrich industry. The animal feed manufacturing industry also recognises lucerne as one of the most important protein sources for animal feeds in South Africa. Hanson *et al.* (1988) report that lucerne contains between 15 and 22% crude protein on a dry matter basis, as well as all of the macro- and trace minerals and all the fat- and water soluble vitamins.

Van der Merwe & Smith (1991) mention that dry matter losses of sun-dried lucerne hay under good weather conditions could amount to 25%. When dry matter is lost, quality (nutritive value) is also generally reduced because of leaf losses. It is well known that lucerne leaves contain more nutrients than stems. Factors influencing the quality of lucerne hay have been studied intensively since as early as 1903 (Snyder *et al.*, 1903 as cited by Hanson, 1972). Several factors may influence the quality of lucerne hay, namely locality, climate, soil, fertilisation, water, harvest schedule, moisture content, loss of leaves, storage, disease, insects, weeds and cultivar (Wedin *et al.*, 1956; Gordon *et al.*, 1962; Anderson &

Thacker, 1970; Hanson, 1972; Temme *et al.*, 1979; Hanson *et al.*, 1988; Smith *et al.*, 1996; Cherney & Hall, 1997; Grönum *et al.*, 2000).

Most of the factors influencing lucerne hay quality can be controlled to some extent through proper management. For example, adjusting harvest dates can control maturity. Soil testing can identify optimum lime and fertiliser requirements. The highest quality species that suit the available soil resources may be chosen. Drying agents and preservatives may help to avoid rain-damaged forage. Although variety selection is very important for yield and persistence, it has relatively little effect on forage quality (Hanson *et al.*, 1988).

A very limited database currently exists on nutritive values for lucerne hay in South Africa (Scholtz, 2001). In addition, nutritional information on SA lucerne hay is also scarce with regards to nutrient fractions required for modern diet formulation and evaluation programmes, including the National Research Council (NRC, 2001) and the Cornell Net Carbohydrate and Protein System (CNCPS) (Tylutki *et al.*, 2007). This required information includes protein and carbohydrate fractions, detailed nutrient composition, predicted energy values and rumen degradability. There is a real need for more reliable data on the nutritive value of South African lucerne hay. Apart from the extension of the SA lucerne hay database, there is an urgent need to develop new accurate near infrared reflectance spectroscopy (NIRS) calibration equations for the different relative nutrients. Accordingly, it is important to establish whether NIRS may be relied upon as a viable replacement for chemical analysis in determining South African lucerne hay feeding parameters used in modern diet formulation models.

Forage quality has been defined in various ways but often poorly understood (Ball *et al.*, 2001). According to Erasmus (2000) the roughage quality of a feed refers to the voluntary intake and the efficiency of utilisation of the relevant nutrients in the specific feed. Linn (1992) contends that high quality feeds should have a consistent nutrient content, high nutrient availability, an absence of mould or other toxic substances, adequate physical characteristics as in the case of roughage to stimulate rumination, able to be readily consumed by animals, and result in animal production that meets or exceeds expectations. Ultimately, Ball *et al.* (2001) define forage quality as the extent to which forage has the potential to produce a desired animal response; thus, production potential. The quality of lucerne hay can vary considerably in accordance with the many factors influencing it. This

variation in quality hampers the efficient utilisation of lucerne hay in animal diets. One of the major problems in the lucerne industry is the implementation of an accurate standardised national grading system. Frequently, high-quality and low-quality are offered at the same price. In many sales, buyers evaluate lucerne hay only on a visual basis which does not always indicate feed quality accurately. This, and many other factors, has led to the stagnation of prices paid for lucerne hay and may mean that the value of lucerne has been underestimated. In a survey by Grönum *et al.* (2000) producers indicated that a grading system which could be implemented effectively, in terms of application and cost, needs to be in place. This could lead to the price of lucerne reflecting its true value. Various methods are available for the evaluation of lucerne hay quality.

A quality evaluation (grading) system for lucerne hay includes different aspects namely: sampling, sample handling and preparation, analytical analysis (chemical and/or near infrared spectroscopy) and a quality model to evaluate its production potential. According to Scholtz (2001) the successful implementation of a quality evaluation system (model) for lucerne hay in practice should, *inter alia* satisfy the following requirements:

a) It should be simple to carry out.

b) It should be able to be implemented in a relatively short time.

c) It must be accurate.

d) Visual (subjective) judging must be part of the system. Chemical analysis does not identify mould and foreign materials that may be present in lucerne hay.

e) Objective measurement should be mainly put into practice.

f) It should be acceptable for all participants.

The legitimacy of any analysis report rests on obtaining a representative sample that accurately reflects the quality of a particular batch (lot) of hay; in other words, what the animal will consume. Thus, it should be truly representative of the lot in every sense, including chemical composition, physical constitution, and presence of foreign material. Taylor (1997) has assumed that even a good representative sample provides only an estimate of the average quality of a hay lot. Forage analysis results are the most prone to sampling and sample preparation error due to their physical nature. Because of the physical nature of forage, especially lucerne hay, analytical results are the most subject to sampling and sample preparation error. Accordingly, Williams & Norris (2001) demonstrate that sampling-related error can account for 60 to 70% of the overall error of testing.

Sampling equipment and procedure for lucerne hay are well documented in the literature (Bath & Marble, 1989; Martin *et al.*, 1992; Putnam, 1998; Sheaffer *et al.*, 2000). Recently, Putnam (1998) introduced a standardised protocol to assure a representative sample of hay. This procedure is currently being implemented by the national forage testing association (NFTA) in the US. On the other hand, there is a shortcoming regarding sample preparation, and especially moisture and dry matter (DM) losses during the grinding of lucerne hay. Most of the research in this regard has been done with grains (Williams & Norris, 2001). It is of the utmost importance that the final milled sample accurately represents the nutritive value of the lucerne hay as fed. The effectiveness of using electronic probe-type testers as an alternative in assessing moisture content of lucerne hay in the baled form also needs urgent investigation.

The accurate prediction of nutrient composition is essential for optimal animal production and limited nutrient losses to the environment (Tylutki, 2002). As emphasised by Snyman & Joubert (1992) the estimation of forage quality from published tables, although of great value, is inaccurate and may lead to over- or underfeeding with respect to production needs. Thus, the use of recognised published tables such as the NRC (2001), is not an accurate enough alternative to forage testing. On the other hand, laboratory analysis is laborious, expensive, environmentally unfriendly and time-consuming so that results often become known only after consumption by the animal. NIRS facilitates timely nutrient analysis. However, this technique requires a sufficient number of samples and reliable wet chemistry results of a specific population to develop robust calibration equations. Therefore, precise and accurate NIRS calibration equations are of utmost importance to assess lucerne hay quality by means of an appropriate quality model. Many of these models require parameters that have not been calibrated locally for South African lucerne hay.

Various mathematical models for assessing the comparative feeding value of forages have been developed over the history of forage quality evaluation research. These models include: Relative Feed Value (RFV; Rohweder *et al.*, 1976), Total Forage Index (TFI; Hutjens, 1995), Adjusted Total Forage Index (ATFI; Erasmus, 2000), Forage Quality Index (FQI; Moore *et al.*, 1984), Relative Forage Quality (RFQ; Moore & Undersander, 2002) and Lucerne Quality Index (LQI; Scholtz & Van der Merwe, 2006 –unpublised data). All the approaches assess

differences among forages from the standpoint of maximal dry matter intake (DMI) and digestibility. Energy is often the nutrient most limiting for a dairy herd and has received the most attention in forage evaluation systems (Weiss, 1993; Robinson *et al.*, 2004). Thus, the majority of the models available in the literature are based on digestible energy intake potential. Lucerne hay has always been perceived as an important source of protein in the South African animal feed industry. Accordingly, LQI based on RFV, includes CP as a model component. This model is currently used to evaluate South African lucerne hay. The LQI model has a further advantage of simplicity and the empirical equation was developed specifically for the local SA lucerne hay population. The other models were derived by regression equations (empirical) of numerous forage species and foreign populations. However, the merit of lucerne hay CP incorporated into a LQI, as with other CP containing grading systems (RFQ, TFI and ATFI) are questionable due to its poor utilisation by ruminants (Martin & Mertens, 2005). Mechanistic models, such as the summative total digestible nutrients (TDN) equation of Weiss *et al.* (1992) are based on nutritional uniform feed fractions and non- specific to a specific population. This model accounts for substantially more sources of variation than the above-mentioned models. The evaluation of these models to assess lucerne hay quality needs urgent investigation.

Lucerne hay is mostly used in dairy cattle diets (Grönum *et al.*, 2000). Therefore, a model to predict lucerne quality specifically for dairy cattle should be developed. This model may be used to rank lucerne according to its quality and/or feeding value for milk production. The inclusion of lucerne hay in formulations for dairy cattle is dictated primarily on the basis of their function as roughage (Zinn *et al.*, 2004). Furthermore, all mentioned models address only the chemical composition of the forage without considering the physical characteristics of the feed, animal factors and the inevitable associative effects. This stresses the necessity of considering the animal when evaluating and/or developing a model for lucerne quality grading. Ration formulation software models, such as the Cornell Nett Carbohydrate and Protein System (CNCPS), are available and integrate in a non-linear approach, nutrient intake, ruminal fermentation, intestinal digestion, absorption, metabolism of chemical analysis and mathematical models with cattle requirements for each production situation (Knowlton *et al.*, 1992; Fox *et al.*, 2000; Fox *et al.*, 2004). The CNCPS was evaluated with data from individually fed dairy cows from several independent studies (Fox *et al.*, 2004; Tylutki *et al.*, 2007). During these evaluations, CNCPS accounted for 86% of the variation in first limiting (ME or MP) milk production with a 1% bias (Tylutki *et al.*, 2007). The

feasibility of CNCPS as a tool to evaluate different chemical parameters and/or models to determine the quality of lucerne hay for milk production needs to be investigated. Accordingly, the CNCPS could be a valuable support in determining lucerne hay quality relative to animal performance.

The purpose of this study was to develop a national grading system for lucerne hay in South Africa by identifying the most appropriate sampling, as well as sample handling and preparation procedures, the most accurate NIRS – nutrient calibrations and an accurate, cost effective quality model.

In Chapter 3 the variation in nutritive value of South African lucerne hay was investigated.

The effect of the grinding procedure on the moisture and protein content of the final ground product was investigated in Chapter 4. Furthermore, the accuracy of electronic moisture testers was verified in this chapter.

The identification of useful NIRS predictive relationships from a pre-selected spectrally structured lucerne hay sample population in South Africa was investigated in Chapter 5.

In Chapter 6 milk predicted by the empirical and mechanistic CNCPS model for dairy cows was investigated as a criterion to identify different parameters (chemical and *in vitro*) and/or develop a model for lucerne hay quality grading.

Chapter 7 focused on the identification of models for assessing lucerne hay quality, using NIRS analysis and CNCPS milk production prediction as a criterion of accuracy.

CHAPTER 2

LITERATURE REVIEW

The literature review was intended as an extensive study on all aspects of lucerne hay, namely: origin, history, nutritive value, quality, etc., to support the writing of the different individual chapters. Although this is initially a more laborious way of finally producing a dissertation, it has the advantage of incorporating all aspects of ruminant nutrition, applicable to lucerne hay. Even though care has been taken to avoid unnecessary repetition in following this approach, some repetition is inevitable.

1. LUCERNE

Throughout its long history as a forage crop, lucerne has had many common names. A complete account of the derivation of the scientific and common names of lucerne has been given by Scofield (1908) as cited by Michaud *et al.* (1988). The ancient Greeks called it *medicai* and the Romans *medica*. According to Piper (1935) and Klinkowski (1933), it is still known as *erba medica* in Italy and the names *mielga* or *melga*, that still persist in parts of Spain, are probably also derived from the ancient classical names. In the principal areas where it is now cultivated however, the plant is known either as "lucerne" or as "alfalfa". The name "alfalfa" is of Arabic origin and means "the best fodder" (Westgate, 1908).

The name *lucerne*, variously spelled as *luzern*, *luserne*, and *lucern* may have a much more modern derivation than the word *alfalfa*. Piper (1935) suggested that the word was first used in 1587 in Southern Europe. This name was also formerly applied to the plant in the eastern parts of the United States and in Utah, but this name has practically given way to the name "alfalfa" which it was introduced by the Spaniards. The name lucern(e) is now in common usage in all European countries east of Spain, and also in South Africa. Common and local names given to lucerne indicate its widespread use and complement the chronicles of ancient people and their activities.

1.1 Origin

Common lucerne (*Medicago Sativa* L.) appears to be the only forage crop which was cultivated before recorded history. Accordingly, the accuracy with which its centre of origin can be deduced is limited by this distinction (Bolton, 1962). It is generally agreed however,

that the most likely centre of origin is in southwestern Asia, Iran, Transcaucasia, and the highlands of Turkmenistan (Westgate, 1908; Bolton, 1962). De Candolle (1919) as cited by Bolton (1962) states that "It (lucerne) has been found wild, with every appearance of an indigenous plant, in several provinces of Anatolia, to the south of the Caucasus, in several parts of Persia, in Beluchistan, and Kashmir". This general area would include the modern political divisions of Turkey, Syria, Iraq, Iran, Afghanistan, West Pakistan, and Kashmir. The oldest recorded reference to date indicates that lucerne was used as forage more than 3300 years ago (Michaud *et al.*, 1988). The Persians were apparently the first people who grew this plant around 490 B.C for horse, and cattle feed. Lucerne had been introduced into Spain by the Moorish invasions of the 8th Century, and was closely tied with the horse culture of the Iberian peninsula, and thereby with military power. Due to this linkage, lucerne most likely accompanied the Spanish colonial expeditions to South America and Mexico in the 16th Century, and is thought to have been introduced into present-day south-western US by early Spanish expeditions (Bolton, 1962).

1.2   Lucerne hay in South Africa

According to Bolton (1962) lucerne was brought from France to the Cape Colony in South Africa around 1850, where it soon became important on the large ostrich farms. When the area of ostrich farming declined, lucerne remained and has become widely grown on irrigated land throughout South Africa.

Lucerne hay (*Medicago Sativa* L.), often called the "Queen of forages," is the most important hay crop species for dairy cows in South Africa. According to Grönum *et al.* (2000) the current area planted with lucerne for hay production in South Africa is estimated as being between 208 000 ha and 240 000 ha. The average annual lucerne hay production in South Africa is approximately 3.8 million tons. Approximately 90% of the lucerne hay produced in South Africa is under irrigation. Grönum *et al.* (2000) mention that the estimated area planted with lucerne has remained more or less constant over the last few years.

One of the most important characteristics of lucerne is its high nutritional quality as animal feed. Jagusch *et al.* (1970) are of the opinion that lucerne is equal to, or better than, most concentrates. Lucerne hay is an important roughage source for dairy cattle and according to Grönum *et al.* (2000), the viability of the lucerne industry in certain regions depends to a large extent on the dairy and ostrich industry. The animal feed manufacturing industry also

recognises lucerne as one of the important protein sources of animal feeds in South Africa. Hanson *et al.* (1988) reported that lucerne contains between 15 and 22% crude protein on a dry matter basis, as well as all of the macro- and trace minerals and all of the fat- and water soluble vitamins.

The dairy feed industry utilises a large proportion of the lucerne hay crop produced in South Africa. Consequently, its nutritive value especially for dairy cattle is important. The quality of lucerne hay can vary considerably in accordance with the many factors influencing it. This variation in quality hampers the efficient utilisation of lucerne hay in animal diets. One of the major problems in the lucerne industry is the lack of a uniform national grading system. This and many other factors, have led to the stagnation of prices paid for lucerne and could mean that the value of lucerne is underestimated. In a survey by Grönum *et al.* (2000), producers indicated that a grading system, which could be implemented effectively in terms of application and cost, needs to be in place. This could lead to the price of lucerne reflecting its true value. Various methods are available for the evaluation of lucerne hay quality.

2. NUTRITIVE VALUE

The lactating cow is far more efficient in converting feed nutrients into human feed nutrients (milk) than any other ruminant grown for meat (Miller, 1979). The dairy feed industry is the largest consumer of lucerne hay in South Africa (Grönum *et al.*, 2000); thus its nutritive value for dairy cattle is of great importance. Ruminant feeds are not equal in their capacity to support the dairy cow functions of maintenance, growth, reproduction, and lactation. According to Van Soest (1994) feeds supply energy and other essential nutrients in the form of protein, vitamins, and minerals to the animal. Energy is often the most limiting factor for a dairy herd and has received the most attention in forage evaluation systems (Weiss, 1993; Robinson *et al.*, 2004). Some feeds' characteristics are related to physical form and have minor relation to indigenous chemical composition. Thus Van Soest (1994) concluded that animal response to a feed depends on complex interactions among the diet's composition (associative effects), its preparation, and the consequent nutritive value.

According to Raymond (1969) nutritive value as such, is conventionally classified by ruminant nutritionists and agronomists into three basic components: digestibility, feed consumption and energetic efficiency. Blaxter (1964) defined nutritive value as the result of chemical composition, digestibility and intake per unit time by an animal. Although nutritive

value of ruminant feeds can be defined in many ways, ultimately it is the energy value that is the most important to ruminant nutritionists, as it is the energy level of any diet that determines the maximal productivity of the ruminant animal to which it is fed (Robinson *et al.*, 2004). Kirilov (2002) pointed out that the feeding value depends not only on nutritive value, but also on the quantity of ingested forage, respectively energy and protein when the forage is fed on an *ad libitum* basis to animals. Thus Van Soest (1994) notes that intake is more relevant to animal production than digestibility.

3. FORAGES

3.1 Utilisation of forages

Forages have been described as bulky feeds which have relatively low digestibility. Although fermentative digestion of fibre is slow and incomplete, ruminants have developed many attributes that result in efficient digestion. The intake and digestibility of forage by dairy cattle directly affects their meat and milk production, as well as rumen function and animal health. The associative effects of forages on the utilisation of other dietary ingredients are of the greatest importance. For this reason Zinn *et al.* (2004) suggested that forages are often referred to as functional diets. Thus, their inclusion in formulations for dairy is dictated primarily on the basis of their function as roughage. However, forages are the foundation upon which good dairy nutritional programmes are built. Refining dietary balances of forages has provided an important way of optimising animal production (Van Soest *et al.*, 1991). Lucerne has one of the highest feeding values of forages. It has always been perceived as an excellent source of protein, but is sometimes under-estimated as an energy source.

Domesticated ruminants are able to convert forage nutrients into human food nutrients (Beerman & Fox, 1998 as cited by Fox *et al.*, 2000). This remarkable capability is possible because of the unique anatomy and adapted function of the ruminant stomach. The ability of ruminants to digest and utilise forages to meet their nutritional needs is well documented (Miller, 1979, Van Soest, 1994). Forages provide fibre in the diet which enhances proper digestion in forage consuming animals. Arana (1997) described fibre as the portion of the feedstuff that can limit digestion, requires cud chewing or rumination for particle reduction and occupies space in the rumen because of bulkiness, thus limiting intake. Ruminants have a highly developed and specialised mode of digestion that allows them to better access energy in the form of forages than other herbivores. Forage energy is generally cheaper than

concentrates; thus,, there is economic incentive to maximise the proportion of forage in *ad libitum* DMI of ruminants. Since dairy cattle often develop problems when fed mainly concentrates with zero to little fibre, fibre can be considered as an essential nutrient (Miller, 1979). In addition, Fox & Tedeschi (2002) suggested that the inclusion of high levels of fibre in backgrounding diets, prevents excessive fat deposition during early post weaning growth, manipulates the marketing date, and controls acidosis in high energy finishing diets.

Digestion of non-cell wall organic matter fractions (nonstructural carbohydrates plus protein and lipid) is comparatively high (>80%) in forages (Zinn *et al.*, 2004). However, digestion of the cell wall fraction (neutral detergent fibre; NDF), ranges between 40 and 70% (Zinn *et al.*, 2004). Plant cell walls which are measured as fibre cannot be effectively digested by animals. The small intestine lacks the enzymes to digest these fibre fractions (MacRae & Armstrong, 1969), but it can be fermented by microorganisms in the rumen (Mertens, 2002). Plant cell walls comprise a complex array of carbohydrate fraction including hemicellulose, cellulose, and lignin, as well as pectin that impart rigidity and structural stability needed for growth (Mertens, 1992). Although cellulose is the predominant component of plant fibre, it is important to recognise that the cellulose microfibrills are tightly bound to covalent bonding in a matrix of other fibre components, particularly hemicellulose and lignin (Jeffries, 1990). Analogous to reinforced concrete, digestion of cellulose is limited by this hemicellulose-lignin encasement.

3.2   Forage quality

The basic requirement for forage in the diet is to maintain healthy rumen function; however, forages can also deliver other nutrients as well (Stokes, 2002). Ball *et al.* (2001) notes "Forage quality is defined in various ways but often poorly understood. It represents a simple concept, yet encompasses much complexity". Some researchers define it as relative, depending on one's perspective regarding market conditions and intended use (Orloff & Marble, 1997). It has been defined in many ways, including protein, fibre, minerals, fats, sugar, starch, anti-nutritional compounds, olfactory factors, total digestible nutrients, and other physical and/or chemical components (Robinson *et al*, 1998; Lacefield, 2004). Kirilov (2002) defined lucerne quality as a generalising concept covering chemical, morphological and physical composition, digestibility, intake energy and protein value. However, each of these has merit but all fall short of clearly defining forage quality. Robinson & McQueen (1992) suggested that quality is ambiguous as a descriptive term when applied to forages in

ruminants. It is generally considered to be positively correlated with both voluntary feed intake and digestibility. According to Erasmus (2000) roughage quality of a feed refers to the voluntary intake and the efficiency of utilisation of the relevant nutrients in the specific feed. Lacefield (2004), supported by Felton & Kerley (2002), defined forage quality as the extent to which forage has the potential to deliver a desired animal response, thus production potential. Linn (1992) is of the opinion that high quality feeds should have a consistent nutrient content, high nutrient availability, absence of mould or other toxic substances, adequate physical characteristics as in the case of roughage to stimulate rumination, readily consumed by animals, and result in animal production that meets or exceeds expectations. Zinn et al, (2004) described forage quality as a complex function of its nutrient composition (energy, protein, minerals and vitamins), chemical-physical characteristics of its fibre (fragility of the cell walls), acceptability (palatability), and associative interactions with other dietary ingredients. They also stress that acceptability (palatability) and associative interactions should be recognised as a major factor influencing forage quality. These definitions acknowledge the necessity of considering the animal. Thus, since forages are predominantly used by livestock as a source of nutrition, forage quality is an expression of the characteristics that affect consumption, nutritional value and the resulting animal performance. It is obvious that the quality and nutritive value of feeds can be regarded as synonymous.

The quality of the forage has a great deal to do with the dietary fibre content. Mertens (2003) notes "dietary fibre is unique among feed constituents because it is defined only on a nutritional basis (that is, in terms of the digestive and physiological effects that it elicits) but must be measured chemically". Thus, the nutritional definition for dietary fibre is key to method relevance". Zinn et al. (2004) defined forage as feedstuff containing 35% or more fibre (NDF). However, this minimum fibre has been poorly defined. According to Grant (1997) fibre should be of proper quality and particle size to ensure maximum dry matter intake (DMI), normal rumen fermentation and milk fat synthesis, proper muscle tone in the digestive tract, and ruminal pH greater than 6.0. The fibre (NDF) component of forages represents a major source of energy; however, less than 50% of this fraction is readily utilised by the animal (Hatfield et al., 1999). Ruiz et al. (1995) supported by Hartnell et al. (2005) concluded that a measure of digestibility of fibre would help explain differences in fibre quality among dietary sources. It is also well known that feeding higher levels of concentrate cannot substitute for lower forage quality (Staples, 1992). Ward (2005, Ward, R.T., Pers.

Comm., Cumberland Valley Analytical Services, Inc., 14515 Industry Drive, Hagerstown, MD 21742, USA) contends that if lignin is used to evaluate forage quality, it should be viewed in the context of neutral detergent fibre (NDF), since digestibility is a function of the lignin-fibre interaction. Lignin as a tool in evaluating forage quality was however criticised by him due to its unreliable relationship with digestibility. Forage fibre has often been considered to be a negative component of forages and feeds in general, being associated with the reduced energy content of the forage, reduced intake potential and reduced milk production (Robinson, 2005). However, it is now widely recognised that the nutritional quality of forage fibre varies both within and among forages and that it is possible to select fibres that both maintain rumen function, by stimulating chewing, while having faster rates of digestion in the rumen, thus giving them a higher energy value and intake potential to dairy cows. According to Van Soest (1994) forage quality is indicative of several contrasting factors; namely the supply of plant cell wall, its optimum digestibility and rate of digestion.

Factors influencing the quality of lucerne hay have been studied intensively since as early as 1903 (Snyder *et al.*, 1903 as cited by Hanson, 1972). Several factors can influence the quality of lucerne hay namely, locality, climate, soil, fertilisation, water, harvest schedule, maturity, curing, moisture content, loss of leaves, handling, storage, disease, insects, weeds variety, method of sample collection and differences between laboratories (Wedin *et al.*, 1956; Gordon *et al.*, 1962; Anderson & Thacker, 1970; Hanson, 1972; Temme *et al.*, 1979; Hanson *et al.*, 1988; Smith *et al.*, 1996; Cherney & Hall, 1997; Grönum *et al.*, 2000). Of these, the maturity stage at harvest influences lucerne quality the most (Llamas-Lamas and Combs, 1990; Ball *et al*, 2001; Martin & Mertens, 2005). Research conducted by Antoniewicz *et al.* (1995) has shown that the biggest changes in crude protein (CP), crude fibre (CF) and NDF content in lucerne hay took place between the budding and blooming stages. Decreased digestibility in forages is associated with an increase in cell wall content (Brink and Fairbrother, 1994) that continuously decreases in digestibility with maturation (Sanderson *et al.*, 1989). However, Llamas-Lamas and Combs (1990) reported that beyond some stage of lucerne maturity, rate of digestion is no longer affected. Hoffman *et al.* (1993) found that rumen protein degradability decreased as NDF and acid detergent fibre (ADF) increase and CP decreased. In contrast, Broderick *et al.* (1992) reported that maturity had no effect on lucerne hay protein degradability when an *in vitro* measurement was used. Broderick *et al.* (1992) suggested that drying or storage conditions may influence degradability of protein in baled lucerne hay.

Various mathematical models for the prediction of lucerne hay composition and nutritive value depending on plant age exist (INRA, 1981, 1988). The estimation of lucerne hay morphological development by index called Mean Stage by Weight (MSW), introduced by Kalu & Fick (1981), provides the possibility of obtaining an exact, numerical expression of the stage of lucerne. On this basis, mathematical models were developed to predict the content of CP, NDF, ADF and digestibility of lucerne hay (Kalu & Fick, 1983).

Most of the factors influencing lucerne hay quality can be controlled to some extent through proper management. For example, adjusting harvest dates can control maturity. Soil testing can identify optimum lime and fertiliser requirements. The highest quality species that suits the available soil resources should be chosen. Drying agents and preservatives may help to avoid rain-damaged forage. Although variety selection is very important for yield and persistence, it has relatively little effect on forage quality (Hanson et al., 1988).

Lucerne hay is low in fibre and high in protein compared to other forages, which makes it an excellent complement for grains and other forages in dairy diets (Martin & Mertens, 2005). According to Hoffman et al. (1998) dry matter intake (DMI) by dairy cows of diets containing grass was lower than those of diets containing lucerne hay. Lucerne varieties possess a unique proportion of structural to non-structural components, which may explain in part why it is consumed at such high levels (Yu et al., 2003).

According to Elizalde et al. (1999) the difference in NDF content between lucerne hay and grasses (i.e., Timothy) can be accounted for by the difference between NDF and ADF, which is primarily hemicellulose. Hoffman et al. (1993) reported that there is a trend of higher NDF accumulation in grasses (i.e., Timothy, orchard grass, perennial ryegrass and brome grass) compared with legumes (i.e., lucerne, red clover and birdsfoot trefoil).

The intrinsically brittle nature of legumes allows higher NDF intake for a given forage or diet NDF (Rayburn & Fox, 1993). Thus, although NDF digestibility of grasses was often greater than that of legumes, due to its higher proportion of lignin (Hoffman et al., 1998; Zinn et al., 2004), the physical characteristics of the fibre (legume hay) cause it to be very brittle and breakable (Waghorn et al., 1989). Because of this characteristic and due to lucerne's lower fibre content (Hartnell et al., 2005), a rapid rate of passage from the rumen (Stokes, 2002;

Zinn *et al.*, 2004; Martin & Mertens, 2005) occurs, and thus less distension. Distension is a great limitation on DMI for high producing dairy cows (Allen, 2000). Dulphy and Demarquilly (1983) suggested that the extent and mode of lignification of cell walls determines the resistance to mechanical degradation during mastication and microbial degradation in the rumen and thus the rate of degradation. The passage rate of lucerne hay in a beef cow is approximately 36 hours compared with up to 70 hours for lower quality forages (Balliette & Torell, 1998). Oba & Allan (1999) also reported a significant interaction between neutral detergent fibre digestibility (NDFD) of the forage family (grasses vs. legumes) on DMI and fat corrected milk (FCM). In addition, lucerne particles have a shorter buoyancy period than grass particles, further increasing their rate of clearance from the reticulo rumen (RR) (Allen, 1996). According to Siciliano-Jones & Murphy (1991) most particles in the RR are buoyant because of retained grasses. Slower digestion and passage rate limit intake, and thus lower total nutrient intake and digestibility. However, Plascencia *et al.* (2003) as cited by Zinn *et al.* (2004) reported that when either elephant- or sudan-grass were to replace 45% of the NDF provided by lucerne hay in a lactation diet for lactating Holstein cows, there were no forage source effects on DMI, milk yield and milk efficiency, although the percentage of milk fat was greater when grass hay was included in the diet.

Van Soest (1987) described five features of lucerne hay which makes it superior to grasses, namely, it incurs only a small depression in digestibility with higher intake; it has a moderate neutral detergent fibre content; higher cell wall density leads to higher intakes; it has a high buffering capacity and a moderately fast rate of fermentation. Wilson and Hatfield (1997) observed that lucerne stems have significant anatomical features that influence their wall structure and digestion characteristics. According to the hypothesis of Wilson & Mertens (1995) the anatomical structure of cells and tissues in grasses may be of greater importance than cell wall chemistry in determining the rate and extent of fibre digestion, because anatomical structure significantly influences cell wall accessibility to rumen microbes. However, anatomical restrictions to cell wall digestion in legumes are limited (Wilson & Hatfield, 1997).

Another reason lucerne hay may be superior to grasses is that it contains a higher concentration of pectin. Pectin is unimportant in grasses, but legume forages contain significant amounts of pectin (Van Soest, 1982). According to Martin & Mertens (2005) lucerne hay stems contain 10 - 12% pectin as a component of the cell wall. Jung & Engels

(2002) indicated that pectin content of lucerne cell walls declines as the stems mature and cell wall concentration increases. Although a component of cell walls, pectin has some very desirable nutritional characteristics. Hall (1994) noted that it is a highly digestible, fermentable carbohydrate energy source. According to Fox *et al.* (2000) pectin is more rapidly degraded than starches. During its fermentations it appears not to produce lactic acid, tends not to depress ruminal pH, and it barely ferments when ensiled. Thus, it does not result in acidosis like rapid fermentable starch (Hatfield & Weimer, 1995); this is partly due to depressed fermentation at low pH. Pectin is predominantly fermented by fibre digesting rumen bacteria (*Succinivibrio dextrinosolvens, Lachnospira multiparus*), whereas starch is primarily fermented, especially at low pH, by *Streptococcus bovis* (Van Soest, 1994). Thus, not only are the species, but also the end products of fermentation dissimilar. There is however, considerable overlap of function such that disappearance of one species or group is not likely to have much effect on overall rumen function (Van Soest, 1994).

Ward *et al.* (1957) confirmed earlier studies that showed that lucerne ash stimulated the digestibility of low quality roughage in sheep. Compared to grasses, lucerne has a rich mineral profile.

4. SAMPLING AND SAMPLE PREPARATION OF LUCERNE HAY

4.1 Sample preparation

According to Williams & Norris (2001), sample preparation is defined as the transformation of the sample into the form in which it will be analysed, without causing any changes in functionality or composition (other than in moisture content). This procedure includes sampling, grinding or some other form of size reduction, blending, sub-sampling and storage.

4.2 Sampling procedure

Williams & Norris (2001) supported by Bath & Marble (1989) noted that sampling is the most important single source of error in any chemical or physicochemical analysis of agricultural commodities and of most food products and ingredients. A major problem with forage analysis at all stages of growth lies in sampling and sample preparation. The validity of any type of analysis rests on obtaining a representative sample that accurately reflects the quality of a distinct lot of hay. According to Williams & Norris (2001) sampling and sample preparation can account for 60 - 70% of the overall error of testing results. Thus, an analysis is only as good as the sample submitted. Harlan *et al.* (1991) noted that the combinations of

normal variation in lucerne hay with inadequate sampling techniques are against the widespread adoption of scientific ration formulation. Ideally, the sample should be truly representative of the total population in every sense, including chemical composition, physical constitution and the presence of foreign matter. Representative sampling of forages is even more complicated in which care has to be taken to protect the natural ratio of leaves and stems. Groenewald & Koster (2005) suggested that the stem/leaf ratio in dried lucerne hay could easily be altered by careless sampling and sample preparation.

Lucerne hay is often sold by lot, which is defined as lucerne coming from a single cutting, a single field and variety, harvested within a 48-hour period and is generally less than 200 tons (Bath & Marble, 1989; Putnam, D.H., 2005, Pers. Comm., Dept. of Agronomy and Range Science, University of California, Davis, CA 95616, USA). Allen & Caddel (1990) proposed several factors to be considered when determining a lot size namely, forage species, stage of maturity, cutting schedules, soil type, soil fertility, presence of weeds, harvest conditions and storage effects. According to Sheaffer et al. (2000) variability within a lot may lead to inaccurate quality assessment if sampling is not done properly. Several studies have been conducted on sampling procedures for small (20 to 40 kg) rectangular bales (Martin et al., 1992), large (400 kg) rectangular bales and large round bales (Lauriault et al., 1998). There is however, little information available on sampling milled lucerne hay, which is commonly sold in South Africa. Currier et al. (1984) reported a distinct pattern of high leaf concentration on one side and high stem concentration on the other side of small rectangular bales. This within-bale variation was confirmed by Martin et al. (1992) who reported large differences among sampling sites on small bales. Accordingly, Martin et al. (1992), supported by Putnam (1998), recommended random sampling at least 20 bales per small bale lot to compose a representative sample for quality assessment of a particular lot. Each of the randomly selected 20 bales should be sampled once per bale, with the probe entering at right angles near the centre of one end. In the case of large rectangular bales, Sheaffer et al. (2000) found 12 randomly chosen bales, sampled once per bale and on any location on the bale, to be adequate for quality characterisation of a lucerne hay lot. Allen & Caddel (1990) proposed that a minimum of 10 representative large, round and rectangular, bales should be randomly selected. From each bale two cores should be collected.

Putnam (1998) proposed a standardised sampling protocol (now adopted in most parts of the USA) to assure a representative sample of hay. This standardised protocol includes factors

such as the identification of a single lot of hay, when to sample, the type of coring device used, random sampling, number of cores taken, technique used, sample amount, handling of samples, sub-sampling and choice of laboratory. Research has shown that if these guidelines are followed, sampling variation can be reduced to an acceptable level and reliable results can be obtained (Putnam, 1998; Scholtz & Van der Merwe, 2005 -unpublished data).

4.3 Grinding

Transformation of the sample into a laboratory-acceptable state for reference analysis often calls for some type of size reduction (Williams & Norris, 2001). Grinding is another major cause of variation in analytical (chemical and NIR) results (Groenewald & Koster, 2005). According to Williams & Norris (2001) this is one of the most important phases of analytical work and should be assigned to competent and conscientious workers.

4.3.1 Grinder type and screen size

According to Michalet-Doreau & Cerneau (1991) grinding increases the degradation of protein in feeds. This varies however with the type of feedstuff. In addition, Richards *et al.* (1995) found that the type of mill used to grind the sample also affects *in vitro* digestibility. *In vitro* digestibility is known to be a function of the surface area available to microbial activity. Several feeds were tested for starch digestion and reported different rankings when samples were ground through a 1-mm screen with Udy mill (Udy Corp., Fort Collins, CO) compared to a Wiley mill (Fisher Scientific, Inc., Chicago, IL). Accordingly, Ward (2005) found that NDF digestibility values of samples ground by a knife mill (Wiley) (1 mm screen), were about 92% of that from a cyclone mill (Udy). Ward (2005, Pers. Comm) stated that knife type mills tend to cut larger average particle sizes than the cyclone mill that shred particles which produce a finer end product; thus, a larger surface area available to microbial activity. For forages, Williams (2007) recommended the Christy/Norris 8-in. (Christy, Ltd., Scunthorpe, England) and the Wiley No.4 (Fisher Scientific, Inc., Chicago, IL) to be the most suitable. These grinders are preferred due to their convenient larger sample access for grinding large samples, or large numbers of samples of forages (Williams, P., 2008, Pers. Comm., PDK Grain, 5072 Vista View Crescent, Nanaimo B.C V9V 1L6, Canada). However, the specific grinder used in sample preparation is not important, provided that the same grinder is used for future NIRS analysis (Williams, 2001).

Several researchers confirmed that screen size, ranging from 2.0 to 0.1mm, might influence *in vitro* digestibility (Cone *et al.*, 1989; Richards *et al.*, 1995; Stern *et al.*, 1997; Ward, 2005). Samples are usually ground finely to ensure a homogeneous sample for chemical (reference) and/or near infrared reflectance (NIRS) analysis. However, Mould (2003) is of the opinion that this will influence the impact of particle size on degradation kinetics and loss of fine particles in the filtration process. Therefore, he recommended the evaluation of substrates in the form in which they are found in the rumen or offered to the animal, or a slightly reduced particle size in the case of forages. However, grinding could be a problem when examining lucerne hay due to its high leaf and stem segregatory nature, making the sample non-homogeneous.

The mean particle size, particle size distribution and therefore the diffuse reflectance signal of the NIR instrument can be markedly affected by the condition of the grinder used in sample preparation (Williams & Norris, 2001). Cyclone sample mills need periodic replacements of the Carborundum ring around the grinding chamber and of the 1.0-mm screen. According to Williams & Norris (2001) the surface of the Carborundum ring becomes progressively polished, where at the same time, the holes of the 1.0-mm screen tend to increase in size by the passing of samples. Thus, the net result is an increase in particle size. Williams & Norris (2001) also noted that small impeller mills need fairly frequent replacement of the impellers, especially if they are used to grind fairly hard commodities.

4.3.2 Moisture loss during the grinding process

According to Williams & Norris (2001) the moisture content of whole grain is usually significantly higher than that of the ground grain upon which the reference analysis is based. This phenomenon is found in almost all materials, especially lucerne hay. For some purposes, it is important to report the moisture content of lucerne hay as received rather than on a constant- moisture basis. This calls for accurate determination of lucerne hay moisture in the bale form and/or in the ground form. According to Williams & Norris (2001) the moisture status of the sample affects the accuracy of analysis before and after grinding. Variability in moisture among samples of the same commodity causes significant differences in mean particle size, particle shape, and particle size distribution. Furthermore, Williams & Norris (2001) are of opinion that the composition on the moisture content of the ground grain used in reference analysis must be recomputed on the basis of the original moisture content of the whole grains (or other materials). This will also apply for lucerne hay.

4.3.3   Cleaning of grinders

Cleaning of grinders between samples avoids sample-to-sample contamination. Some grinders, such as the cyclone and hammer mill type are self-cleaning when grinding low fat content samples. Williams (2006) reported that up to 2% or more of a sample of grain may remain in a Falling Number KT-3100 or similar grinder after grinding. These fine particles are usually of different composition to the blended ground material, and should be completely removed by brushing and added to the milled sample before blending (Williams, 2006).

4.4   Blending and sub-sampling

One of the major breakthroughs in modern NIR technology is that instruments are now available that can accurately and effectively analyse samples without prior grinding (Groenewald & Koster, 2005). Blending and sub-sampling before grinding entail a careful study of the original sample to ensure that it is thoroughly blended and that the sub-sample abstracted for analysis is truly representative of the population from which the original sample was drawn (Williams & Norris, 2001). Blending is important in all substances which tend to stratify due to changes in temperature (Williams & Norris, 2001), or due to segregation between stem and leaf material in especially lucerne hay. Bath & Marble (1989) are however of opinion that most problems involving chemical analysis of duplicate samples sent to different laboratories, have been traced to hand mixing and sub-sampling of the cored sample prior to grinding. Accordingly, they stated that the sifting of fine leaves and stem parts cannot be avoided, and samples can not really be considered to be duplicates when divided in this way. This is most likely especially valid for day-to-day routine applications. This finding is however in contrast to the results of Groenewald (2005 -unpublished data) who reported successful splitting of unground samples. In his experiment five separate well-mixed unground samples were each carefully split into two separate portions. Each of the ten portions was then analysed on a NIR instrument (PERTEN DA7200, Perten Instruments AB, Sweden) to determine the repeatability of the two duplicate portions of each of the five samples. An average variation of 0.5% was achieved between the two portions of each of the five samples. It must be stated however that this was a special case where extreme care was taken by a very experienced person. The same repeatability may not easily be achieved for routine applications.

Blending of the ground sample is an equally important part of sample preparation to improve accuracy. The ground sample stratifies in the grinder receptacle and must be thoroughly blended before testing by reference or NIR (Williams & Norris, 2001). Williams & Norris (2001) recommend the stirring of the ground contents in a sample jar, 15-20 times before loading the sample cell (NIR) or weighing (reference method). This procedure becomes increasingly important as the sample size increases.

4.5   Sample presentation for reflectance

According to Williams & Norris (2001) everything that has happened to the sample up to the time that it is scanned in the NIR instrument will be embodied in the sample and recorded in the spectrum. During the preparation of samples subjected to NIR or analytical analysis it is important to select a method that minimises loss of moisture and maintains the integrity of the constituents of analytical interest.

An advantage of NIR testing is that the sample size that can be scanned by the instrument is usually much larger than the sample that can be subjected to reference analysis (Williams, 2006). Therefore, the larger the sample used for analysis, the more representative it should be of the population.

Drying and grinding procedures are especially important due to the fact that water is a strong absorber of NIR light, and particle size affects the shape of the spectrum (Stuth *et al.*, 2003). Variance in ambient (Stuth *et al.*, 2003) and sample temperature (Groenewald, C.A., 2007, Pers. Comm., Divisional Director, Scinetic, Centre of Scientific Technology, a Devition of Afgri Operations. 252 Jean ave., Centurion, South Africa) also influences the scanning of samples. As with particle size, temperature affects the shape of the spectra causing the spectrum to be misinterpreted (Williams *et al.*, 1982).

Internal fans achieve cooling in most NIR instruments (Williams & Norris, 2001). If these stop working for any reason or the ambient temperature rises above 35 °C (Groenewald, 2007, Pers. Comm.), results can become erratic due to temperature fluctuation and the overheating of some of the components. On the other hand, results may also be affected if the ambient temperature falls too low. Sensitivity to the temperature of the detectors is an important reason why temperature affects NIR instrument performance (Williams & Norris,

2001). Most, but not all, NIR instruments are sensitive to vibrations and variation in sample and ambient temperatures (Groenewald, 2007, Pers. Comm.).

Furthermore, when samples at various temperatures are analysed in sufficient quantity, they will change the internal temperature of the instrument. The influence of sample temperature on the accuracy of NIR analysis of ground wheat has been well documented (Williams *et al.*, 1982). Limited information was however, available in the literature on the influence of sample temperature on NIR prediction of ground and unground lucerne hay. In some cases, temperature sensitivity may be compensated for by incorporating samples at different temperatures into the calibration and validation sample set (Williams & Norris, 2001). In the case of instruments that do not require the samples to be ground, cold samples enter the instruments. These samples may be below freezing, and the analysis of a series of them cools the instrument, leading to further significant errors (Williams & Norris, 2001). The same applies in reverse to the analysis of samples at very high temperatures, such as freshly ground lucerne hay. The warming of samples due to the action of grinding is inevitable, especially in lucerne hay. According to Groenewald (2007, Pers. Comm.) not only the temperature, but also the charging of the sample molecules due to the grinding process, has an effect on the NIR scan. Since prediction errors have been reported from the testing of immediately freshly ground samples as distinct from samples analysed several hours after grinding, it is possible that the temperature and oxidation status may also be factors (Williams & Norris, 2001). Sampling errors occur at several places in the calibration-validation process as outlined by Williams & Norris (2001) in Table 1.

Ideally, samples should be scanned to record spectra and chemically analysed at the time of receipt (since that is the time at which they will be scanned when the calibration has been developed), and stored for subsequent checking of the NIR instrument. Spectra retain their properties forever, whereas samples may not (Williams & Norris, 2001).

Table 1   **Sources of sampling error in straw and forage (Williams & Norris, 2001)**

Commodity	Sources of sampling error
Straw and forage	Physical composition
	Dimensions (length, etc.)
	Leaf/stem ratio
	Stage of maturity
	Moisture content
	Sub-sampling for sample preparation
	Blending of withdrawn sample
	Sample identification
	Blending of prepared (usually ground) sample
	Sub-sampling for actual laboratory analysis

The relative humidity (RH) of the atmosphere may have a strong influence on NIR instrument performance (Williams, 2006). This phenomenon was first noted by Davies & Grant (1987), who noted that changes in RH of the atmosphere change the noise level of a near infrared spectroscopy (NIRS) instrument, particularly in the areas of maximum absorption by water bands. As with temperature sensitivity, RH sensitivity may be compensated for by developing calibrations under conditions in which the RH can be varied during the period of calibration development (Williams & Norris, 2001). Thus, to minimise the impact of ambient temperature and relative humidity, some NIRS instruments need to be housed and maintained in an environmentally controlled room on a solid surface with controlled lighting, whereas others can operate under factory conditions where there are vibrations and temperature variations (Groenewald & Köster, 2005).

4.6 Sample storage

Storage of samples is an important aspect of sampling. Samples should be protected from change, especially moisture, between the sampling/sample preparation stages and analysis by NIRS or reference methods (Williams, 2006). Samples stored at 4-5°C in heavy duty plastic bags, further protected by tightly lidded plastic pails, can be preserved without significant change for several years (Williams & Norris, 2001). According to Williams (2006) a sufficient sample should be brought out each time to last for a few days, and should be allowed to equilibrate to room conditions before scanning by NIRS.

5.   NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

Near-infrared spectroscopy (NIRS) is a long-established, and now mature, technology. Norris and his colleagues developed the first application of NIR to measure water, oil and protein in grains and seeds (Norris & Hart, 1965 as cited in Givens *et al.*, 1997). Once its potential was established, the application quickly spread, at first to forage and animal feeds, then to the lucrative fields of pharmaceuticals and petrochemicals.

The NIRS method of analysis has several advantages over conventional analytical methods namely, simplicity of sample preparation, speed, multiplicity of analysis with one operation, its non-destructive nature, its requiring no reagents, and its non-consumption of the sample (Hannaway *et al.*, 1992; Stuth *et al.*,2003). The disadvantages of the NIRS method are instrumentation requirements, dependence on calibration procedures, complexity in choice of data treatment, and lack of sensitivity for minor constituents.

5.1   Theory of near infrared spectroscopy

5.1.1   *The electromagnetic spectrum (EMS)*

NIR technology involves the interaction of electromagnetic radiation (in the form of waves) with matter in very different ways (Groenewald & Köster, 2005; Stuth *et al.*, 2003). The wavelength of a wave is the distance between the two peaks or high points and is indicated by the symbol λ (Groenewald & Köster, 2005) in Figure 1.
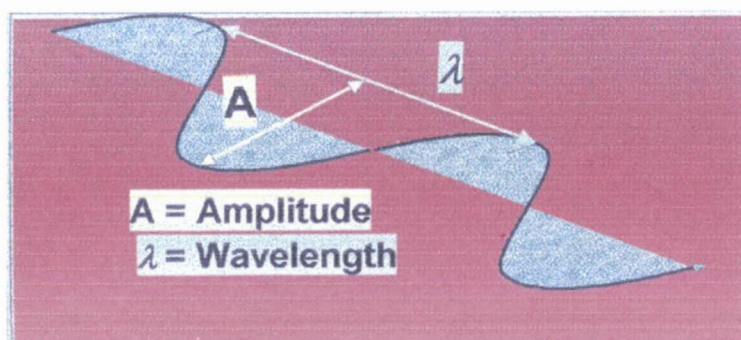
Figure 1   Typical wave propagating through space (Groenewald & Köster, 2005)

A wavelength (λ) in the NIR spectrum is normally measured in nanometer (nm) where 1000nm = 0.001mm (Shadow, 2000 as cited by Groenewald & Köster, 2005). The electromagnetic spectrum (EMS) consists of photons of different energies (Stuth *et al.*, 2003). The EMS ranges from the short (<1.0 mm), high frequency (10 10 to 10 24 Hz) gamma-rays

to long (>10 mm), low frequency (<3 x 10 11 Hz), radio waves. The part of the spectrum lying between the visible region and the infrared region is known as the NIR region ranging from 750nm to 2600 nm (Figure 2) (Groenewald & Köster, 2005).
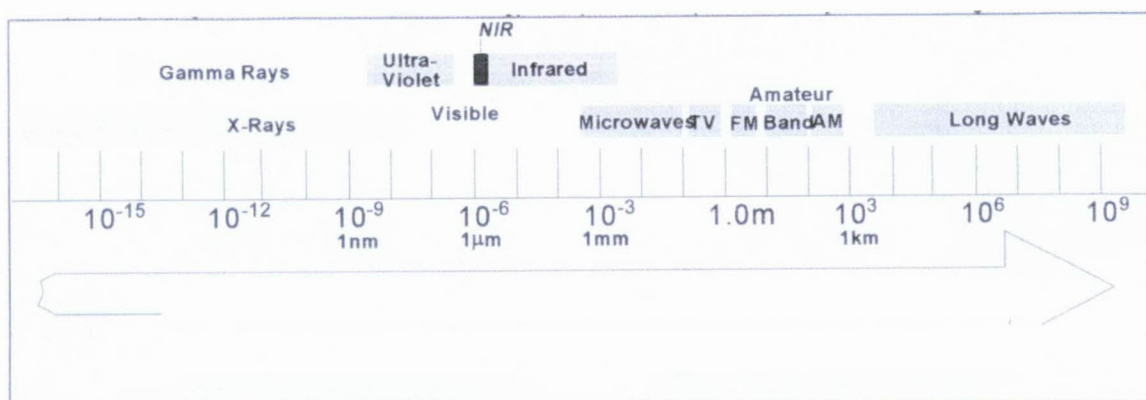
Figure 2　**The electromagnetic spectrum (Shadow, 2000 as cited by Groenewald & Köster, 2005)**

Higher energy photons cause electron shifts, and lower energy photons result in molecular vibrations (Murray & Williams, 1987). The sample to be analysed is bombarded with NIR rays of different wavelengths (Groenewald & Köster, 2005). Carbon (C) and hydrogen (H) chemical bonds in organic molecules when struck by NIR light (Figure 3) vibrate, stretch, and bend at frequencies similar to the vibrations of the EMS found in the NIR region (Figure 2).
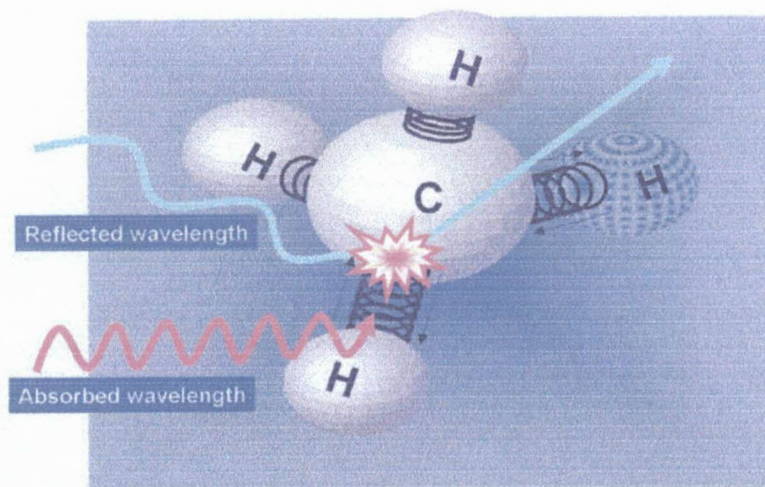
Figure 3　**Vibrating bond between carbon (C) and hydrogen (H) atoms (Groenewald & Köster, 2006)**

Wavelengths which correspond to the frequencies at which the particular bond is vibrating, are absorbed, whereas others are scattered and reflected (Figure 4).

incident radiation
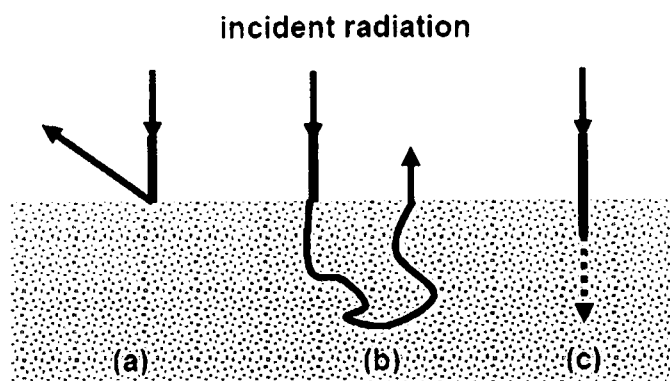
Figure 4   **Diagrammatic representation of specular (a) and defuse (b) reflectances, and absorption (c) of near infrared radiation from a sample (Givens *et al.*, 1997)**

5.1.2 *Measurement of the absorbance of radiation by a sample*

The NIR spectrometer projects a known quantity of NIR light onto matter and then records the reflectance from that matter, storing the information on a computer. The depth of penetration into the matter is about 2mm (Hruschka, 1987). According to Groenewald & Köster (2005) the depth of penetration of the light beam into the substance is not determined by the position of the detector, but rather by the strength of the light source. Apart from the chemical features of the sample to be analysed, physical attributes, especially particle size, also affect NIR spectra in the form of "scatter of radiation". Scatter is the dispersion of reflected light from the surface of a sample particle without penetrating the sample (Figure 4), and it can be a significant portion of the observed variation in NIR spectra (Givens *et al.*, 1997). While the Beer-Lambert law generally describes the relationship between radiation diffusely reflected (Figure 10) from a solid sample and characteristics of that sample, the path length of diffusely reflected radiation cannot be predicted because it is scattered by random reflections, refractions and diffractions within the sample (Dryden, 2003). Therefore, the variation within NIR diffuse reflectance spectra are mainly a result of (1) non-specific scatter of radiation, (2) variable path length and (3) the chemical composition of the sample (Barnes *et al.*, 1989). Thus, while the characteristics of NIR reflected from a sample can be used to predict certain sample characteristics, each application of this type must be obtained by calibration.

5.2 Creation of calibration equations

Calibration is the process of creating a spectro-chemical prediction model (Shenk & Westerhaus, 1996). Calibration consists of both physical and electronic steps. The process begins with obtaining a sample set of the desired material, i.e. hay, silage or faeces. The calibration set should be well distributed, representing the range of expected variation in the constituent of interest. This variation could be (1) temporal, e.g. date or time of collection; (2) spatial, e.g. range site or geographic location; or (3) biological, e.g. cultivars or stage of growth (Stuth *et al.*, 2003). According to Williams & Norris (2001) the spectrum contains all of the information that makes up the sample, including chemical composition and the interactions among constituents that cause the sample to possess its unique physical properties and functionality. Williams & Norris (2001) suggested that the spectrum would be the same, irrespective of the instrument used to record it.

Williams & Norris (2001) recommended the selection of a calibration population by using both spectral and reference data, rather than selection using spectral data alone. They described three steps to follow: first, list samples from lowest to highest reference data; second, remove access samples with similar composition; and third, prepare a calibration set and preferably two validation sets. Once the selected laboratory reference data and spectra are obtained and coordinated, mathematical and statistical procedures are performed. According to Groenewald & Köster (2005) the instrument has to be trained to recognise different products and constituents. In essence, the process relates chemical information contained in the spectral properties of a substance to chemical (or physical) information revealed by reference laboratory methods. Thus, calibration equations can be used to estimate the chemical composition of corresponding samples of unfamiliar composition (Stoltz, 1990).

Cozzolino & Moron (2004) described the technique as the correlation between chemical properties, as determined by defined reference methods, and absorption of light at different wavelengths in the near infrared region. The near infrared region contains information concerning relative proportions of C-H, N-H and O-H bonds, which are the primary constituents of the organic molecules in forages (Osborne *et al.*, 1993; Coleman & Murray, 1993). However, although the reflectance of NIR radiation is related to the sample's organic chemistry, there is no chemical or physical relationship between the analyte under

consideration and the wavelength(s) which may be selected to predict it (Villalobos *et al.*, 1991).

Prediction of some minerals in forages by NIRS may be possible through their association with organic complexes, chelates, and pigments such as chlorophyll (Givens & Deaville, 1999; Ruano-Ramos *et al.*, 1999). The NIRS relies on calibration, which utilises absorbencies at many wavelengths, to predict composition of a feed sample (Batten, 1998). According to the Beer-Lambert law, absorption of a constituent, and therefore diffuse reflectance (R), is linearly related to concentration. According to Stolz (1990) the calibration process establishes this relationship in the form of a multiple linear regression equation for each parameter. The goal is to derive a predictive equation that can quantify the constituent of interest by using a NIRS alone, and bypassing the laboratory methods. The standard error of calibration (SEC) is obtained by predicting the samples that have been used in the calibration.

5.3   Validation

Calibration equations are routinely validated against another set in which reference (laboratory) analytical values have been determined. Thus, equation validation is conducted to assess the predictive ability of the selected calibration equation (Stuth *et al.*, 2003). According to Williams (2006) there are three main methods for evaluation (validation) of calibration models.   These are cross-validation, test-set validation and prediction of completely unknown samples.   Cross validation is favoured by many NIR experts, due mainly to the computation time saved (Shenk & Westerhaus, 1996; Williams, 2006).   It involves elimination of samples from the calibration set, either in groups or individually.   The sample or group of samples is then predicted, using the equation that has been developed using the remaining samples, and replaced in the original sample set.   This exercise is then repeated with all samples or sample groups. The standard error of cross-validation (SECV) is used to judge the predictive ability of a calibration equation. According to Williams (1987) slope and bias are also important when evaluating equation performance. However, because cross validation is carried out on the same overall sample set, it suffers from the fact that it does not provide information on any biases, or slope changes.

The test-set validation involves setting up a validation set of the same general type or population, but selected and removed from the same population used for the calibration

sample set. The test-set evaluation provides all of the statistics necessary for a true evaluation, in that it gives the slope, intercept and bias data, as well as the r^2 and standard error of prediction (SEP) statistics. The SEP is used to obtain an independent measure of the calibration equation accuracy. This kind of validation is best suited for large populations, whereas cross-validation is best suitable for small populations.

The ultimate evaluation of a calibration is to validate the equation with unknown samples. A set of these samples should then be submitted for reference analysis to verify the true efficiency of the calibration as an analytical tool.

The ratio of prediction to deviation (RPD) statistics has been introduced fairly recently as a means of relating SEP to the SD of the reference data of the samples used in validation (Williams, 2006). According to Williams & Norris (2001) RPD simplifies the interpretation of SEP.

$$RPD = SD \text{ (validation samples)}/SEP \hspace{3cm} \text{(eq. 1)}$$

High values for the RDP indicate efficient NIR reflectance predictions, as shown in Table 2. It is evident that the SEP should be much lower than the SD, hence a RPD of preferably 5 or higher. According to Williams and Norris (2001) (Table 3) r^2, the bias and the RDP are the most meaningful statistics used to evaluate the performance of calibrations.

Table 2   The ratio of prediction to deviation (RPD) statistics (Williams & Norris, 2001)

Ratio of Prediction to Deviation (RPD) value	Classification	Application
0.0 - 2.3	Very poor	Not recommended
2.4 - 3.0	Poor	Very rough screening
3.1 - 4.9	Fair	Screening
5.0 - 6.4	Good	Quality control
6.5 - 8.0	Very good	Process control
8.1+	Excellent	Any application

Table 3   Guidelines for interpreting r^2 (Williams & Norris, 2001)

Value of r	r^2	Interpretation
Up to ± 0.5	Up to 0.25	Not usable in near-infrared reflectance calibration
±0.51-0.70	0.26-0.49	Poor correlation: reasons should be researched
±0.71-0.80	0.50-0.64	OK for rough screening; more than 50% of variance in y accounted for by x
±0.81-0.90	0.66-0.81	OK for screening and some other 'approximate' calibrations
±0.91-0.95	0.83-0.90	Usable with caution for most applications, including research
±0.96-0.98	0.92-0.96	Usable in most applications, including quality assurance
±0.99 +	0.98+	Usable in any application

The SD should be compared with the mean reference results to evaluate the significance of the values (Williams & Norris, 2001). This is achieved by the coefficient of variation (CV).

$$CV = (SD(population) \times 100)/Mean (population) \qquad \text{(eq. 2)}$$

Guidelines for interpretation of the coefficient of variability (CV) statistic are shown in Table 4. The size and interpretation of the CV depends partly on source of the data (Williams & Norris, 2001). They recommended that the CV of reference testing for constituents such as protein or moisture contents, to be close to 1.0%. For quality assurance applications, the CV should be about 1.0-1.5%. Values of 2-3% are acceptable for general use and those higher than 3% should be investigated to determine the reason. Values of up to 5% may accrue from the determination of the reproducibility of functionality parameters.

Table 4   Guidelines for interpretation of the coefficient of variability (CV) statistic (Williams & Norris, 2001)

CV Value (%)	Reference Tests, Protein, etc.	NIR[a] Reference Constituents	NIR[a] Reference Functionality, etc.
Up to 0.5	Exceptional	...[1]	...[1]
0.6-1.0	Excellent	Exceptional	...[1]
1.1-2.0	Very good	Excellent	Exceptional
2.1-3.0	Good	Very good	Excellent
3.1-4.0	Fair	Good	Very good
4.1-5.0	Poor	Fair	Good
5.1+	Needs investigation	Poor	Fair

[a] NIR = near-infrared reflectance

[1] unlikely to be attained

Over-fitting of data involves using a large number of wavelengths giving a highly accurate equation according to statistics such as the r^2, the standard error of cross validation (SECV), or the regression F value (terms or factors). These over-fitted equations however, may not accurately predict analyte concentrations when applied to data other than those used to derive the equation. Bertrand *et al.* (1987) pointed out that the problem of over-fitting is directly related to a high correlation between absorbencies at different wavelengths, as well as to the equation, recognising features of the calibration data set which are not representative of the data which will be used in predictions (Hruschka, 1987). According to Hruschka (1987) over-fitting is identified by standard errors of calibration (SEC) that are much less than the standard error of reference (SEL), significant differences between reference and NIR values, and a standard error of prediction (SEP) greater than twice the SEC (Williams, 2006). Because the spectral data have to be calibrated with the reference method, the calibration error cannot be smaller than the error on reference data. Biston *et al.* (1989) proposed a selection of equations by lowest SEP, rather than lowest SEC, to avoid over-fitting of data.

Failure to include all sources of variation may lead to outliers in subsequent analysis. An outlier is identified as a sample that does not conform to the majority of the population in terms of spectral data and differs from such population by more than three times the SEP (Williams & Norris, 2001). According to Williams & Norris (2001) there are two main types of outliers. These are spectral outliers and analytical outliers. When an outlier appears during the development or validation of a calibration, it is important to verify that it is really an outlier. This can be done by reanalysing and rescanning of the particular sample. If the sample remains an outlier after retesting by NIRS and reference methods, it is a spectral outlier.

Software systems for outlier detection are all based on spectral characteristics and, in most cases, rely on differences in Mahalanobis distances. According to Williams & Norris (2001) Mahalanobis distances is defined by a distance measured based on a set of multivariate (the training data) that are used to describe it and whose Euclidean length varies according to the direction in space in which it is being measured. The equivalent Euclidean length is large in direction (i.e. dimensions) where the data are spread out and small in directions where the data are compact. Since the Mahalanobis distance depends on the data, it changes as the defining data change.

Several software packages include methods for the detection and elimination of outliers. The "global H" statistics introduced by InfraSoft International (Port Matilda, PA) identify samples with H values of above 3.0 (three times the SEP) as potential outliers. However, it should be noted that the inclusion of some outliers in calibration sets can improve the integrity of the calibration with regard to future samples that resemble the outlier, but affect the magnitude of the constants (Williams & Norris, 2001). Thus, outliers are important in the development of calibration equations by showing areas of deficiency in the sample sets. Different factors causing outliers are presented in Table 5. Moisture is of great concern with regard to contributing to outliers in lucerne hay, due the nature of hay sampling (Par. 4.3). Moisture status includes moisture content and degree of hydration. The degree of hydration of complex molecules such as proteins may cause changes in the special orientation of the molecules (Williams & Norris, 2001). They emphasise that this could cause samples containing these molecules to interact differently with the irradiating light from other samples.

Table 5   Factors causing outliers (Williams & Norris, 2001)

Chemical composition
Interaction among constituents
Wavelength selection
Moisture status
Physical texture
Particle size and shape
Bulk density
Sample preparation technique
Sample orientation in cell
Sample temperature
Number of wavelengths used in calibration
Mathematical treatment of log(1/R) data

5.4   Factors that may affect accuracy of NIRS results.

Most instrumental, sample and operational sources of error in NIR testing are summarised in Table 6. Drying and grinding procedure are important because water absorbs NIR radiation strongly (Baker *et al.*, 1994) and sample particle size affects the shape of the spectrum. Scanning conditions should be as uniform as possible with respect to ambient temperature. As with particle size, temperature affects the shape of the spectra influencing the scattering of radiation as it passes through the sample (Givens *et al.*, 1997). Small particles scatter infrared radiation more that large particles (Hruschka, 1987).

Table 6   Sources of error in Near-infrared (NIR) technology

A	**Instrument Sources**	
	1	*Wavelength scale*
	2	*Photometric scale*
	3	*Instrument temperature control*
	4	*Cell covers*
	5	*Relative humidity of the atmosphere*
	6	*Instrument to instrument differences*
	7	*Sample presentation system*
B	**Sample sources**	
	1	*Chemical composition*
		A Interactions among constituents
		B Influence of chemical constituents on physical condition of material
		C Moisture status of material
	2	*Bulk density*
	3	*Physical texture of sample*
	4	*External factors (weather etc.)*
	5	*Sample temperature*
	6	*Ambient temperature*
	7	*Conversion factors*
	8	*Whole grain application*
		A Kernel (seed) size
		B Pathlength
		C Sample access
		D Colour
		E Moisture content
		F Foreign material
		G Temperature
C	**Operational sources**	
	1	*Calibration practice*
		A Number of samples
		B Sample selection
		C Accuracy of reference analysis
	2	*Sample preparation*
		A Sampling and sub-sampling
		B Grinder type
		C Grinder condition
		D Blending after grinding
	3	*Sample storage*
		A Before preparation
		B After preparation
	4	*Sample cell loading*
		A Mixing
		B Packing
		C Cleanup between samples
	5	*General carelessness*

However, Robert *et al.*(1986) suggested that correction for variation in particle size can be done by a regression approach. More radiation is absorbed by large particles, giving higher log (1/R) values and this effect is greater at those wavelengths that are absorbed more strongly.

5.5   Size of calibration set

The optimum size for calibration data sets has not been resolved. Williams (1987) suggests 35 to 40 for simple calibrations. Research conducted by Hsu *et al.* (1998) has shown that the number of calibration samples for NIR could be reduced from 302 to 65 without substantial reduction in prediction ability. According to results from Abrams *et al.* (1987), supported by Hoffman *et al.* (1999) and Williams & Norris (2001) a calibration set numbering greater than 100 was necessary for accurate calibration equations.

5.6   Common calibrations for different species

5.6.1   Forages

Garcaí & Cozzolino (2006) reported the potential of NIR to predict the chemical composition of different forage plant species. However, Groenewald & Koster (2005) emphasised that universal calibrations would rarely represent a true reflection of samples from local areas and usually need adjustment. Marten *et al.* (1984) proposed that the greater diversity of chemical structure in a multi-species sample population requires more complex statistics in development of a calibration equation. However, they concluded that several species can be evaluated simultaneously by universal calibrations, almost as well as single specie equation.

Mathison *et al.* (1999a) suggested that the prediction accuracy of NIR in prediction might be improved by using discrete populations rather than combined calibrations (different forage populations).

Universal equations have been tested under several circumstances. With the exception of NDF, Mathison *et al.* (1999b) found no improvement in the accuracy of prediction by including barley silage and barley hay in a calibration set along with barley straw samples. Similarly, Antoniewicz *et al.* (1995) found higher r^2 values for calibrations within grass or lucerne, than for a combined set of forages. This indicated a greater diversity of chemical structure in these multi-families sample population. However, they support the possibility of

a useable common calibration for different species. Marten *et al.* (1984) also reported that different species can be evaluated concurrently by an appropriate calibration nearly as well as single species. Several authors have indicated that broad-based calibration models for forage chemical components have the potential to offer accuracy comparable to single specie (specific) calibrations (Abrams *et al.*, 1987; García & Cozzolino, 2006). It seems that different opinions exist regarding the accuracy of prediction when a single calibration for different species is used.

5.6.2 Compound feeds

In contrast to a simple nutrient, digestibility of a feed is a complex parameter which is more prone to animal variation. Compound feeds are further spectrally complicated due to the wide choice of ingredients and number of possible combinations (De Boever *et al.*, 1995). However, universal equations on compound feeds can be a valuable tool if used with caution for screening purposes (Groenewald, 2007, Pers. Comm.).

5.7 Direct and indirect methods of measuring forage quality

5.7.1 Direct

NIRS has been successfully used to predict the nutritive value of forages and hays through direct scanning of the forage samples (Holechek *et al.*, 1982). The ash content represents both available and unavailable minerals to the animal. Crude protein is one of the most commonly measured components of forages. The primary cause of these good predictions is the strong -N-H absorptions in the NIR region. ADF and NDF can be estimated due to variations in -C-H and -O-H bonds (Stuth *et al.*, 2003). Although several lignin calibrations have been reported for lucerne hay (Reeves, 1988a), Reeves (1988b) suggested a generally lower precision in estimations of lignin than protein or ADF. Mixed results have been reported on forage fats, primarily because forages contain relatively small concentrations with low variance leading to poorer correlations (Berardo *et al.*, 1997; Park *et al.*, 1998). Minerals do not absorb in the NIR region due to their inorganic status. However, Clark *et al.* (1987) pointed out that mineral detection by NIR is possible due to complexes formed with organic compounds. Narrow ranges and low concentrations in forages also obstruct the prediction of minerals by NIR (Stuth *et al.*, 2003). Digestibility calibrations are based on a diverse set of reference procedures, including both *in vivo* and *in vitro* techniques (De Boever *et al.*, 1986; Reeves, 1988a). According to Baker *et al.* (1994) digestibility calibrations can be sensitive to residual moisture in samples. They suggested that calibrations developed with

samples containing moisture of less than 80g per kg DM have been more accurate than those with higher moisture. As with NDF, digestibility is a property of forage and not a chemical parameter, thus prediction with NIRS can be more complex. Accordingly Stuth *et al.* (2003) defined NIRS prediction of digestibility as a prediction of a predicted component of feed. However, research has demonstrated that NIRS has been applied successfully to the prediction of digestibility (Russel *et al.*, 1989; Givens *et al.*, 1992a). However, it should be noted that digestibility is influenced by the amount of feed constituents (such as ADF and NDF), the patterns of chemical bonding between cellulose and lignin (O'Keeffe *et al.*, 1987), and also by non-feed factors such as the level of feed intake and the rate of passage of feed through the digestive tract (Dryden, 2003). Thus, digestibility predictions are usually for a given time point and only represent potential digestibility. However, these may not be predicted well by NIRS techniques or any other conventional chemical method (Dryden, 2003).

5.7.2 Indirect

The application of NIRS technology to indirectly predict the quality parameters of a feed via faecal scans is well documented by Lyons & Stuth (1992). As diet chemistry changes, the by-products of digestion (plant residue, microbial bodies, secondary metabolites, slough tissue, etc.) also change (Stuth *et al.*, 2003). Thus, a strong relationship between the secondary products in the faeces and characteristics of the primary product (i.e. diet) may exist.

5.8 Monitoring quality control on the instrument

Monitoring and quality control are needed to confirm the accuracy of both the instrument and the calibration. According to Stuth *et al.* (2003) monitoring is carried out in two steps. First, diagnostics are run weekly and it is verified that the instrument meets manufacturer specification. Second, and most importantly, a check cell test is run daily. These tests are not only important to detect instrument drifting, but also ensure reliable and consistent evaluation of the predicting performances of different instruments within a network (Groenewald, 2007; Pers. Comm.).

5.9 Transferability of calibrations among instruments (Portability of calibration equations)

Transferability of calibrations means using a single calibration on a network of instruments. Thus, all of the instruments on a specific network should produce identical spectra from a given sample, as well as the same result from that given sample. According to Williams & Norris (2001), this can be achieved by networking the instruments, using the same calibration in all of them. All the instruments are then "standardised" to a "Master" instrument, from which the output of all of the instruments involved in the network can be monitored and adjusted. Calibration transfer in practice is an important aspect of the suitability of an NIR instrument for a particular organisation (Williams & Norris, 2001). A calibration performed on a particular instrument cannot be expected to apply to any other instrument. Two instruments are unlikely to be exactly alike, no matter what make or model of instrument. Differences in internal geometry (temperature, electrical voltage, vibration, sample preparation) lead to differences in performance of different instruments (Dryden, 2003). Inherent differences among discrete filters have been recognised from the outset of NIR technology, and small, but significant, differences occur among diode array instruments and even among monochromators (Williams & Norris, 2001). In addition, the software systems used for calibration development vary widely among instruments, but the principles are universal. Calibrations can be transferred manually, by floppy disk, modem transfer, or by e-mail over long distances. After transfer, the accuracy of the calibration in the new instrument can be verified by analysing check samples.

The portability of prediction equations between instruments has been investigated by several authors (Dryden, 2003). Shenk *et al.* (1985) demonstrated that the prediction equations for protein, ADF, NDF, lignin and *in vitro* dry matter digestibility (IVDMD) of legumes and grasses could be used by other instruments of the same model with acceptable accuracy and precision. On the other hand, Williams & Krischenco (1986) could not duplicate protein determinations while using two similar instruments, and Shenk & Westerhaus (1985) found that quite different instruments produced equations with different numbers of wavelengths and predictive performances. Similar data are presented by Valdes *et al.* (1987). They reported similar r^2 and SEP for IVDMD in whole-plant maize from different instruments, but noted that protein estimations differed. Givens *et al.* (1997) warned however, that even instruments which have been matched by the manufacturer, may still behave differently, especially with more complex equations.

5.10 Operating environment

Groenewald & Köster (2005) emphasised the influence of environmental conditions on various instruments. Some NIR instruments can operate under factory conditions where there are vibrations and temperature variations. Other instruments are more suited only to laboratory conditions and may require specific air-conditioned and temperature controlled environments and vibration free operational areas.

5.11 Calibration maintenance

Any calibration must continually be checked and updated. According to Groenewald & Köster (2005) the main reason for calibration verification and updating is not instrument related but rather related to sample composition. A robust calibration is usually built over several seasons to incorporate changes in environmental conditions, cultivars or a host of other external factors such as pesticides used, weed control, degree of maturity, moisture variation and many others (Dersjant-Li & Peisker, 2005 as cited by Groenewald & Köster, 2005). These factors may affect the reliability of NIRS results if they are not included in the calibration data set. Accordingly, it is imperative to establish proper calibration verification and updating procedures by sending samples on a regular basis for chemical analysis to confirm that the calibration is still applicable for that particular product (Groenewald & Köster, 2005). The NIRS predictive equations are an ongoing process; thus, at any time, validation of any equation, even a robust one, could in many cases be improved upon at a later date.

6. MODELS FOR ASSESSING FORAGE QUALITY

Forages are the primary resource of nutrition of ruminant livestock throughout the world. Therefore, the estimation of their nutritional value has been of primary importance for the prediction of animal performance and the subsequent development of the livestock industry in the past few decades. Two important nutritive standards used in feed formulation are energy (digestible energy; metabolisable energy) and protein (rumen degradability). However, several researchers reported that the fundamental characteristic of rations for dairy cattle, on which all other nutrients are structured, is energy content (Harlan *et al.*, 1991; Robinson, 2005). The importance of energy to animal production and its impact on limiting the level of lucerne that can be fed to dairy cattle would suggest that energy might be the most important criterion to use in evaluating the quality of lucerne hay. Nevertheless, it is

not possible to chemically analyse a sample of lucerne hay to determine its energy value for dairy cows, as energy is an integration of multiple components (Weiss *et al.*, 1992; Daccord *et al.*, 1996 Robinson, 1999). Several systems for evaluation of forage energy value have been developed during the last 20 years (INRA, 1978). Because variability in chemical composition and available energy content usually is much greater for forages than for concentrates, the majority of equations have been derived from data of forages (Minson, 1982). The classical *in vivo* technique is difficult, slow and expensive and for that reason, different laboratory methods simulating the processes of digestion in the rumen are used. The *in vitro* laboratory methods used on the basis of rumen fluid (Tilley & Terry, 1963), the method on the basis of forage enzymatic degradability (De Boever *et al.*, 1986) or *in vitro* gas production of forage incubated in rumen fluid (Menke & Steingass, 1988) are well documented. Kirilov (2002 -unpublished data) reported a good relationship between the *in sacco* technique and lucerne *in vivo* digestibility and intake. Estimated available energy utilising regression equations (Minson, 1982) is probably the most common and practical method used today by many nutritionists. These equations are based on the negative relationship between fibre concentration and available energy (Weiss *et al.*, 1992).

A method which allows rapid and accurate estimates of lucerne energy value and nitrogen degradability is needed. The bottom line criteria for quality modelling are to make an objective assessment of chemical analyses that gives nutritionally useful information related to animal performance. Accurate prediction of nutritive value requires a representative sample, accurate laboratory analysis and improved feed evaluation methods (Undersander *et al.*, 2005).

The question of defining forage quality with a single value or number has been raised by several researchers (Coppock, 1997; Putnam, 2004; Hall, 2005). Van Soest (1967) reviewed the ideal nutritive predictor concept and stated that such a predictor should be easily, inexpensively, and precisely measurable; uniform (among forages) in digestibility; and consistent in nutritive value of absorbed end products. According to Harlan *et al.* (1991) lignin and neutral detergent soluble ought to be ideal predictors because of their essentially 0 and 100% digestibilities, respectively. However, it is questionable whether one single criterion would be acceptable for judging all hays. The eventual purpose for which hay is destined is a critical factor in determining the appropriate evaluation method. As the production function for which the hay will be used changes, the relative importance of the

various criteria could also vary accordingly. The best single measure of forage quality is animal productivity.

Ration formulation software models are available that predict microbial yield, undegraded feed protein and metabolisable energy and protein derived from a particular diet to further improve the accuracy of ration formulation (Sniffen *et al.*, 1992; Fox *et al.*, 2000; Tylutki *et al.*, 2007). Models for lucerne hay grading do not need to be sophisticated mathematically, but must be realistic in terms of their dependence on, and correspondence to, biological principles and theories. Therefore, the main focus on developing a grading model for lucerne hay, using different analytical parameters, should be on relevance and reproducibility of the parameters.

6.1   Single component models

Historically, equations based on a single nutrient have been the most popular means to estimate the energy contents of forages (Weiss, 1998). According to Minson (1982) these equations are dependant on a statistical relationship between digestibility and the chemical characteristics of feed.

6.1.1   Total digestible nutrients (TDN)

Digestibility is a functional component of plant tissue that reflects the nutritive value to the animal and significantly influences the intake levels of ruminants (Stuth *et al.*, 2003). Total digestible nutrients (TDN) describe the ability of the animal to digest the nutrients in lucerne hay and to convert these nutrients into energy (Robinson, 1999; NRC, 2001; Putnam, 2004). Weiss *et al.* (1992) described TDN as the term for available energy. The TDN prediction currently used in California and several western states in the US (Robinson & De Peters, 2002; Putnam, 2004) are based on research done by Bath & Marble (1989). Bath & Marble (1989) noted, as others before them during the 1970s, that ADF contains a high proportion of the indigestible fibre components, namely lignin and cutin. Therefore, there was a strong inverse linear relationship observed between ADF level of lucerne hay and its TDN value. Based on University of California Davis (UC Davis) research with cattle and sheep (completed by Garett) during the 60s and 70s (Robinson, P.H., 2005, Pers. Comm., Department of animal Science, on Shield Avenue, University of California, Davis, CA 95616-8521, USA), Bath & Marble (1989) proposed that the best ADF based equation to predict the TDN value of California lucerne hay was:

$$TDN \ (\% \ of \ hay \ DM) = 82.38 - (0.7515 \ x \ ADF\%) \qquad (eq. \ 3)$$

This equation, referred to as the "Western States Equation", has been adopted in California and most of the Western States as the official equation for TDN calculation (Robinson, 1999; Robinson, 2005). Several researchers and nutritionists used TDN for the calculation of net energy for lactation (NEl) and other energy estimates.

6.1.2   Merit of total digestible nutrients (ADF-TDN equation)

TDN, as with other historically ADF-TDN equations, is a linear measurement (function) derived from ADF (USDA, 2004). Putnam (2004) suggested that the use of ADF directly would be a more rational approach to hay marketing than the use of the calculated TDN.

Energy estimates such as TDN, although important, are essentially interpretations of data, rather than data themselves. Using TDN to evaluate forages can over-estimate its energy value compared to grains and other concentrate feeds (Bath & Marble, 1989). Forages generate more heat in their digestion process, leaving less of the digestible energy available for productive purposes.

The tendency is to rely on the basic similarity of fibre, within a forage type, to develop energy prediction equations for each forage type. According to Robinson (2005, Pers. Comm.) it is not possible to validate the "Western States Equation" biologically as it was based on hays grown in the central valley of California in the 1970s. Therefore, the application of this equation to current grown hays, as well as hays grown in other areas, is questionable. Lucerne cultivars, fertilisation, and other management and processing practices have changed dramatically during this period and it seems likely that the ADF: NDF ratios in current cultivars differ from those of cultivars grown in the 1960s and 1970s. In addition, Putnam (2004) in a literature survey revealed that the average USA dairy cow now produces >60% more milk than a dairy cow in 1974. Thus, given the developments and improvements in dairy cattle genetics and feeding, results obtained by earlier researchers with regard to development of energy prediction equations for lucerne hay, are likely to be considered obsolete in the near future.

Robinson (1999) is of opinion that within a few years in most of the developing world, ADF as with crude fibre (CF), will be considered outdated for purposes other than as its originally defined role as a preparatory step for the determination of lignin. However, the analysis also allows the content of acid detergent insoluble nitrogen (ADIN) to be identified; thus offering an estimate of undegraded protein small intestine availability, an input in many current protein systems (Mould, 2003). Others have found the modified ADF analysis that uses a slightly stronger acid and longer treatment period, to show a much greater ability to predict forage digestibility (Moss & Givens, 1990; Givens *et al.*, 1992b).

Robinson (1999) recommend the substitution of ADF with NDF as NDF is considered a more clearly defined cell-wall fraction, and captures all the structural fibre. This is unlike ADF that only captures 70 to 85% of the structural fibre. Since NDF is the slowest digestible portion of the plant, its increase will reduce the energy level of the hay. The ADF-TDN approach is not mentioned in the NRC (2001).

Other recommended equations used to predict the TDN value of California lucerne hays are, according to Robinson (2005), the following:

$$TDN = 97.36 - (0.68627 \times NDF) - (0.27333 \times CP \quad r^2 = 0.75 \qquad \text{(eq. 4)}$$
$$TDN = 90.21 - (0.69137 \times ADF) - (0.16483) \times CP \quad r^2 = 0.73 \qquad \text{(eq. 5)}$$
$$TDN = 85.37 - (0.52179 \times NDF) \qquad\qquad r^2 = 0.71 \qquad \text{(eq. 6)}$$
$$TDN = 83.49 - (0.58531 \times ADF) \qquad\qquad r^2 = 0.70 \qquad \text{(eq. 7)}$$
$$TDN\ (90\%\ DM) = ((82.38 - (0.7515 \times ADF\%))*0.9\ (Putnam, 2004) \qquad \text{(eq. 8)}$$

Robinson (2005) concluded that there is very little difference in the predictive accuracy of NDF and ADF in lucerne hay due to the close relationship between them. The addition of CP to the equations to predict TDN provides only a slight increase in predictive accuracy. In California, dairy hay has generally been marketed on the basis of TDN expressed on a 90% dry matter basis (Mathews & Putnam, 1998). Putnum (2004) however, stressed the standardisation of the TDN calculation, since widely varying TDN calculations create conceptual problems in the marketplace. In a study compiled by Undersander *et al.* (1993), ADF equations were found to slightly overestimate TDN in lucerne hay with a low concentration of ADF (29%). TDN was however, accurately estimated in samples with a higher ADF content.

The estimation of TDN in mixed forages (lucerne and grass) is prone to error due to its population specificity when using ADF-TDN equations. The statistical relationship between ADF and digestibility is different for different forages (Weiss, 1998). Harlan *et al.* (1991) reported a higher intercept and greater negative slope when ADF equations are used for grasses than for legumes. This accordingly, complicates the estimation of TDN of mixed grass and legume forages. Van Soest *et al.* (1991) stressed that while statistical associations with digestibility have been obtained, there is no chemical basis for this finding.

Moe *et al.* (1965) mentioned that single-component models do not consider the variable effect of DMI on digestibility. Another major limitation of the use of a single measurement to estimate energy is its insensitivity to changes in the concentration of other nutrients (Weiss, 1998). A single component equation also assumes either that the nutrient in the model is the only factor affecting digestibility, or that the measured nutrient is perfectly correlated with all other factors affecting digestibility; however, such an assumption is questionable (Weiss, 1998; Weiss, 2002). The use of single-component equations is limited to a specific feedstuff (e.g., lucerne), geographic areas and climate conditions (Weiss, 1993), and therefore population dependent. Furthermore, few equations based on ADF are available for concentrate feeds due to low variability in ADF concentration. Thus, according to Weiss (1998) these conditions reduce the statistical precision of models based on ADF and increase the sensitivity to analytical error.

Putnam (2005, Pers. Comm.) noted that it is unlikely that ADF or NDF alone is adequate to predict the full dimensions of feeding quality.

6.2   Multiple component models

Multiple component models based on feed consumption can be empirical or mechanistic (Weiss, 1998). Empirical equations are population specific multiple regression equations utilising numerous feed parameters regressed against digestibility. Mechanistic models are based on population independent nutritional uniform fractions, such as constant digestibility. According to Weiss (1993) the statistical precision of most empirical multi-component models is significantly better than for single component models. They are also less sensitive to analytical error than are single component models; however, because of co-linearity among

independent variables, empirical multiple component models are more prone to population specificity.

6.2.1 Relative feed value (RFV)

Relative forage quality was developed by the Hay Marketing Task Force of the American Forage and Grassland Council (Rohweder *et al.*, 1978). Relative feed value (RFV) as indicated in Table 7 combines the important nutritional factors of intake and digestibility and expresses them as an index (Rohweder *et al.*, 1976). The RFV system was developed for comparing forages on the basis of energy. Unlike the Bath and Marble (1989) TDN equation, which is valid only for lucerne hays grown in the central valley of California, RFV is purported to be valid for any forage (legume, grass, and legume-grass) anywhere, since it incorporates the variable ratios of ADF/NDF that exist among forages (Robinson, 1999). It has no units, thus allows comparisons between forages (Hannaway & Ballerstedt, 1988). RFV, which is an estimate of overall forage quality (Table 7), is calculated from estimates of dry matter intake (DMI) from NDF and digestibility or energy level (digestible dry matter (DDM) from ADF) of forages on a dry-matter basis. RFV increases as percentage ADF and NDF decrease.

Dairy farmers can use RFV to decide which hay should be fed to various groups of cattle. It is thus an index used to allocate forages to the proper livestock class with a given level of production performance. Coppock (1997) proposed the following guidelines in this regard:

Relative feed value	*Use*
Over 170	Excellent forage but should be limited to half of the forage dry matter. Maize silage is excellent diluting forage.
140 to 170	Forage for high producing cows as sole forage.
120 to 140	Forage for lower-producing cows, young heifers, or diluted with high-quality roughage for high producers.
100 to 120	Dry cows (check calcium levels) and other heifer feed (add energy such as maize silage or grain).
Under 100	Older heifer forage if supplemented properly. One alternative is to sell to a beef cow operation.

Table 7 **Proposed quality standards for legume, grass, and legume-grass mixed hays (Coppock, 1997)**

Quality standard [a]	CP[b]	ADF%[b]	NDF[b]	DDM[c]	DMI% of BW[d]	RFV[e]
Prime	>19	<31	<40	>65	>3.0	>151
1	17-19	31-35	40-46	62-65	3.0-2.6	151-125
2	14-16	36-40	47-53	58-61	2.5-2.3	124-103
3	11-13	41-42	54-60	56-57	2.2-2.0	102-87
4	8-10	43-45	61-65	53-55	1.9-1.8	86-75
5	<8	>45	>65	<53	<1.8	<75

[a] Standard assigned by Hay Market Task Force of the American Forage and

Grassland Council (Rohweder et al.,1976)

[b] Analyses associated with each standard: CP = Crude protein; ADF = Acid detergent

fibre; NDF = Neutral detergent fibre

[c] Digestible dry matter (DDM%) = 88.9 - 0.779 ADF (% of DM)

[d] Dry matter intake (DMI, % of body weight) = 120 / forage NDF (% of DM)

[e] Relative feed value (RFV) = [(DDM X DMI)/1.29] , the divisor, 1.29, was chosen

so that the standard reference hay for RFV index, full bloom lucerne, has a value of 100

(Rohweder et al., 1976). The constant, 1.29, was the expected DDM intake, as % body

weight (BW), for full bloom lucerne based on animal data (Rohweder et al., 1978)

Relative feed value is most valuable for animals using high roughage diets such as dairy cows and growing animals, because the RFV provides an index to rank roughage according to its digestible energy intake potential (Coppock, 1997). The demand for low-fibre, high quality hay has intensified, given that dairy cows are much more productive, and rations significantly different from those in the 1970s (Putnam & Undersander, 2006). Thus, in 2002 the USDA Market news developed a set of guidelines for hay quality designation into 5 categories in an attempt to synchronise the hay grading across the US (Table 7) (Getz, J., 2005, Pers. Comm., USDA Market News, 1428 S Pioneer Way, Moses Lake, WA 98837, USA). According to Putnam and Undersander (2006) supplementary subjective hay quality attributes are often included in the hay quality guidelines, since laboratory measurements do not predict all of the attributes of quality (Table 8).

Table 8 **USDA Quality Guidelines of hay quality for reporting economic data of lucerne hay (not more than 10% grass) adapted in 2002 (Putnam & Undersander, 2006)** [a]

Category [b]	ADF [c]	NDF [c]	RFV [d]	TDN [e]	TDN (90% DM) [f]	CP [c]
				---%---		
Supreme [1]	<27	<34	>180	>62	>55.9	>22
Premium [1]	27-29	34-36	150-180	60.5-62	54.5-55.9	20-22
Good [1]	29-32	36-40	125-150	58-60	52.5-54.5	18-20
Fair [1]	32-35	40-44	100-125	56-58	50.5-52.5	16-18
Utility [1]	>35	>44	<100	<56	<50.5	<16

[a] Guidelines are used along with visual appearance to determine quality

[b] Categories assigned by USDA-Market News (Putnam & Undersander, 2006)

[c] Analyses associated with each standard:; ADF = Acid detergent fibre; NDF = Neutral detergent fibre; CP = Crude protein

[d] Relative feed value (RFV) = [(DDM X DMI)/1.29] , the divisor, 1.29, was chosen

so that the standard reference hay for RFV index, full bloom lucerne, has a value of 100 (Rohweder et al., 1976). The constant, 1.29, was the expected DDM intake, as % body weight (BW), for full bloom lucerne based on animal data (Rohweder et al., 1978)

[e] Total digestible nutrients (TDN) = ((82.38 – (0.7515 X ADF)) (Bath & Marble, 1989)

[f] Total digestible nutrients (TDN) = ((82.38 – (0.7515 X ADF)) X 0.9

[1] Physical descriptions of hay quality to be used in combination with laboratory test results for lucerne hay quality categories (USDA-Market News):

Supreme: Very early maturity, pre-bloom, soft fine stemmed, extra leafy. Factors are indicative of a very high nutritive content. Hay has excellent colour and is free of damage.

Premium: Early maturity, i.e., pre-bloom in legumes and pre-head in grass hays, extra leafy and fine stemmed- factors indicative of high nutritive content. Hay is green and free of damage.

Good: Early to average maturity, i.e., early to mid-bloom in legumes and early heads in grass hays, leafy fine to medium stemmed, free of damage other than slight discolouration.

Fair: Late maturity, i.e., mid- to late-bloom in legumes, head in grass hays, moderate or below leaf content, and generally coarse stemmed. Hay may show light damage.

Utility: Hay in very late maturity, such as mature seed pods in legumes or mature heads in grass hays, coarse stemmed. This category may include hay discounted due to excessive damage and heavy weed content or mould. Defects will be identified in market reports when using this category.

6.2.1.1 Dry matter intake (DMI)

Research shows that DMI is regulated by various mechanisms and that these mechanisms are not mutually exclusive but interact together, influencing satiety and hunger (Iilius & Jessop,

1996). Kirilov (2002) defined forage DMI as a resultant value from palatability, physical, and chemical characteristics. Allen (2000) proposed several diet physical and chemical characteristics that can affect DMI including fibre content, ease of starch and fibre hydrolysis, particle size, particle fragility, fat concentration and characteristics, protein amount and ruminal degradability. Ellis *et al.* (1988) reported these numerous dynamic interactions which are involved, such as attributes of the animal, the forage and the gastrointestinal microorganisms. Van Soest (1965) suggests that forage NDF concentration was the highest related to DMI of forage by sheep compared to other chemical measurements. NDF intake is a good predictor of DMI in Holstein cattle, as proposed by Mertens (1987). Mertens (1994) proposed that NDF be used to define the upper and lower limits of DMI. A high inclusion of NDF in diets would cause rumen fill to limit DMI, whereas energy intake feedback inhibitors limit DMI at a low NDF concentration. Mould (2003) suggested that NDF relates best to feed intake as it represents the total insoluble fibre matrix, with lignin and associated phenolics most often indicated as the components limiting digestibility. The NDF method was designed initially to isolate the insoluble dietary fibre components in plant cell walls: cellulose, hemicellulose and lignin (Van Soest & Wine, 1967). Rohweder *et al.* (1978) demonstrated that NDF content is the best single chemical predictor of DMI. According to Robinson & De Peters (2002) NDF limits intake by increasing the bulk of the diet, thereby requiring the cow to spend more time eating and ruminating per unit of diet consumed. Rayburn & Fox (1993) reported DMI prediction to be the most accurate and least biased when forage NDF was included with body weight (BW), fat corrected milk (FCM) and days in milk. The National Forage Testing Association (NFTA) proposed an equation for estimating voluntary intake of forages (Moore & Kungle, 1999). Most current prediction equations for determining forage quality base their DMI prediction on the NFTA equation. This NFTA prediction equation is based on the assumption that NDF intake is a constant 1.2% of body weight (Moore & Undersander, 2002). The concept of constant NDF intake is based on studies conducted by Mertens (1987) who found a positive relationship between NDF intake and the bulk density of feeds. Merten's results suggested that daily NDF intake was $1.2 \pm 0.1\%$ of body weight per day in diets with the ability to yield a daily maximum of 4% fat corrected milk (FCM). However, Ruiz *et al.* (1995) reported that intake of NDF as a percentage of BW was not constant but increased linearly from 1.15 to 1.32 as dietary NDF concentration increased. In addition, Moore and Undersander (2002) criticised the concept of constant NDF intake and stated "Extrapolation of data on high-concentrate mixed diets (where associative effects decrease

forage intake) to forages fed alone does not seem to be rational or justified". This was confirmed by Roseler *et al.* (1997) who found less than 1% of the variation in DMI explained by dietary NDF in models predicting DMI of lactating cows fed high energy diets ranging from 25 to 42% NDF (DM). Other researchers also found NDF intake to be variable when grasses and legumes were fed alone to lactating cows (Beauchemin, 1996) compared to mixed diets (Rayburn & Fox, 1993). Sanson & Kercher (1996) found a very small correlation between observed DMI of lucerne hay and DMI predicted from NDF using the NFTA equation. In addition Reid *et al.* (1988) and Mathison (1990) have demonstrated poor relationships between intake and NDF when a range of forages was evaluated over several years ($r^2 = 0.05$ to 0.33 and 0.06 to 0.24, respectively). Similarly, in a review by Minson (1990) r^2 values relating NDF and intake ranged from 0.14 to 0.81. Harlan *et al.* (1991) reported r^2 values of 0.09 to 0.46 between NDF and DMI per 100 kg body weight in non-lactating dairy cattle. Moore *et al.* (1996) evaluated the intake prediction equations of NFTA and NRC (NRC 1984; NRC 1996) and concluded that 15 to 71% of the published DMI prediction equations were not acceptable. Some of this discrepancy is due to the use of an equation derived from sheep intake data to predict cattle intake data (Moore & Undersander (2002). Moore *et al.* (1996) demonstrated that multiple regression equations using two or more laboratory analyses (ADF, CP and NDFD) provide a higher percentage of acceptable estimates of intake than did equations using a single analysis. Lippke & Herd (1990) developed a model (FORAGVAL) that predicted intake and gain from CP and ADF. Moore & Kunkle (1999) developed a multiple regression equation including TDN, ADF and CP. In contrast with other research, they concluded that equations including ADF provide more acceptable predictions of intake (higher r^2) than NDF. This is in agreement with Harlan *et al.* (1991) who reported a linear relationship between *ad libitum* DMI and ADF ($r^2 = 0.38$) of hay, somewhat higher than for DMI vs. NDF ($r^2 = 0.27$); however, both were higher than for DMI vs. lignin ($r^2 = 0.19$). Thus it was speculated that the cellulose content of hay crops may be more closely related to *ad libitum* DMI than other fibre components.

6.2.1.2 *Digestible dry matter (DDM)*

Acid detergent fibre (ADF) has long been used to estimate energy content of forages (Undersander & Moore, 2004). Many studies have shown that the concentration of ADF in forages correlates negatively with digestibility (Minson, 1982; Harlan *et al.*, 1991). Most energy prediction models used by commercial testing laboratories have been based on this relationship (Weiss, 1998). Using ADF to estimate energy presumes that all ADF has the

same digestibility, although they may differ in ash, lignin, or cellulose. Van Soest (1964) described a highly significant (P<0.0001) negative relationship between ADF content and the extent to which legumes are digested. This is in agreement with the findings of Cilliers and Van der Merwe (1993) (P<0.0001; r^2 = 0.74) using veld herbage to investigate the relationship between ADF and *in vitro* organic matter digestibility (IVOMD). Rohweder *et al.* (1976) also stated that ADF is the chemical assay of choice to estimate the *in vitro* dry matter digestibility (IVDMD) of a wide range of forages, including lucerne hay. In contrast with these results, Scholtz (2001) reported that ADF predicted *in vitro* organic matter digestibility (IVOMD) less accurately (r^2 = 0.47) than NDF (r^2 = 0.57).

Moore & Undersander (2002) reported significant differences between observed and predicted DDM values. This is in agreement with Reid *et al.* (1988) who reported that ADF, when used for comparisons across forages and years, is not an accurate predictor of dry matter digestibility (DMD), organic matter digestibility (OMD) (Aufrere & Michalet-Doreau, 1988) or digestible energy (DE) content of forages (Mathison, 1990). Van Soest *et al.* (1978) demonstrated that ADF accounted for only 56% of the variability in digestible dry matter (DDM). Furthermore, they noted that neither ADF nor NDF were related to DDM in aftermath cuttings (r = -0.20). In contrast to these results Mertens (1980) proposed the use of NDF to estimate the energy value of feeds. Mertens (1994) reported a generally negative relationship between NDF content and both the degradation and intake of forage. This was confirmed by Van der Merwe & Fair (1999 -unpublished data) where 50 lucerne hay samples, in a preliminary study, indicated that the NDF content can be used to predict IVOMD with a significantly (P<0.0001) high degree of accuracy (r^2 = 0.80). Therefore, it would seem that, compared to NDF, ADF is a less accurate estimator of energy value or IVOMD (Scholtz, 2001). Weiss (1993) however, considered the use of empirical equations based on ADF and NDF to estimate available energy, to be theoretically incorrect and lacking adequate precision. Abrams (1988) is of opinion that more than half the error in predicting forage digestibility from ADF was associated with the selection of an unacceptable equation.

6.2.1.3   Merit of the relative feed value model

According to Grant (1994) it is important to note that RFV is only an energy intake index and does not take into account either protein (which is more expensive), fibre digestion (Moore & Undersander, 2002), or minerals in roughage. Protein level has to be evaluated separately from RFV and is not part of the RFV equation, as indicated in Table 7.

Since energy is often the most limiting nutrient for high producing dairy cows, much emphasis is placed on the accurate prediction of forage digestibility. Various researchers have questioned the reliability of ADF or NDF as predictors of digestibility. Sanson & Kercher (1996) supported by Moore & Coleman (2001) noted that the predictors of DMI (eq. 9) and DDM (eq. 10) explained very little (1 and 20%, respectively) of the variation observed in DMI and digestibility in lucerne hay. Moore & Undersander (2002) stressed that DDM is not a conventional measure of available animal energy requirements and feed energy concentration.

$$DMI = 120/NDF \qquad\qquad\qquad (eq.\ 9)$$

$$DDM = (88.9 - 0.779 \times ADF) \qquad\qquad (eq.\ 10)$$

The Californian TDN equations, as described by Robinson (2005), and RFV are primarily fibre based systems, since they depend upon ADF and NDF measurements. These systems based upon ADF and NDF have the advantage of simplicity, and can successfully identify major differences between hay lots. However, they may fail to differentiate important differences in forage quality within a critical range of interest where prices are dramatic (Putnam, 2004). Animals do not have nutritional requirements for RFV units. This means that nutritionists must convert the RFV value to an energy value by re-calculating through the fibre fractions (Robinson, 1999).

RFV and the traditional TDN are both based upon either ADF (TDN as calculation from ADF), or a combination of ADF and NDF (RFV). This calculated value places an added dimension of complexity into the market system (Putnam, 2005, Pers. Comm.). Putnam (2004) demonstrated that the relationship between the NDF level of lucerne hay and the calculated RFV value (Figure 5) was virtually perfect ($r^2 = 0.99$). This is supported by study of the USDA (2004) where a data set of 15 states showed that 97% of the variation in RFV was explained by NDF.

The chart shows: y = -220.99Ln(x) + 967.48, R² = 0.9715, with axes RFV (y) and NDF (x).
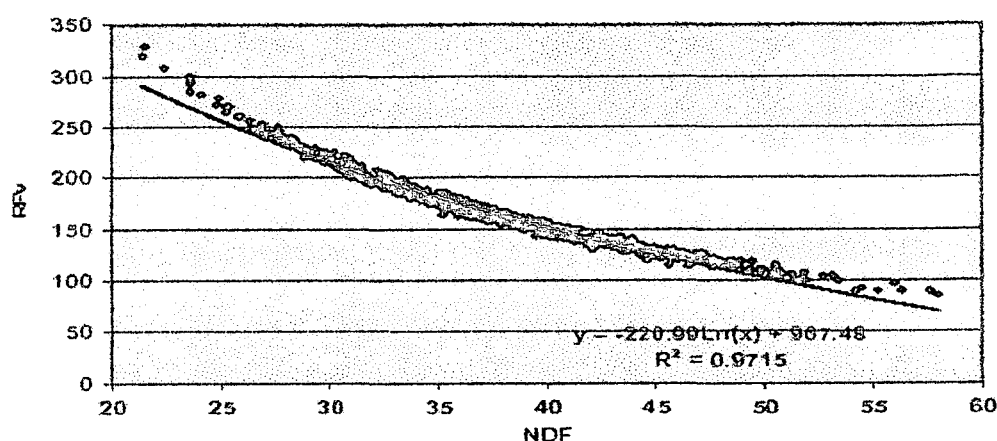
Figure 5   **Relationship between NDF and RFV (Putnam, 2004)**

Recently Putnam (2004) evaluated luceme data from multiple states in the US and found ADF and NDF to be closely correlated (Figure 6) in luceme hay ($r^2 = 0.92$). This corresponds to work done by Robinson and De Peters (2002) where a correlation of $r^2 = 0.83$ was noted. Thus, regardless of the value of the concept of TDN and RFV, these market methods are equivalent to the use of either ADF or NDF measurements alone.

Hoffman (2004) examined a large database of forage energy contents. The empirical equation (RFV) accurately predicts the average of the database but cannot precisely predict the energy content of any single forage in the database. This is in agreement with earlier findings of Comb *et al.* (1998) that regression equations used to predict forage digestibility (from ADF or NDF) could under- or over-estimate the digestibility of any single forage by as much as 25 to 30%.

The chart shows: y = 1.0206x + 5.2725, R² = 0.9156, with axes NDF (y) and ADF (x).
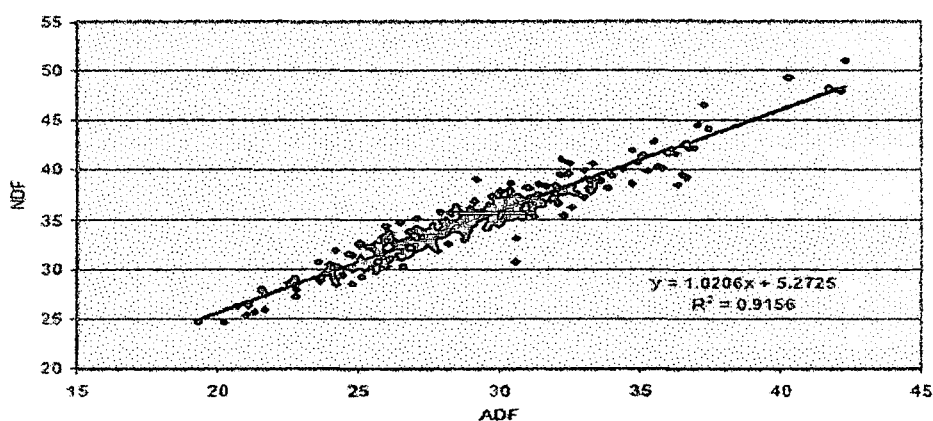
Figure 6.   **Relationship between ADF and NDF in samples from the western states of US, representing a wide range of samples (Putnam, 2004)**
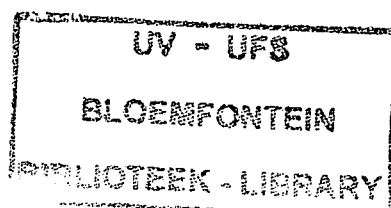
RFV was based on the concept of digestible dry matter intake relative to "standard forage" (Undersander & Moore, 2004). Data sets used to develop empirical equations should be carefully defined and continuously updated. "Lucerne" samples sent for analyses are frequently mixes of lucerne and grass that are not well defined. Energy value estimates from applying single variable regression equations like RFV in this case, become questionable (Combs *et al.* 1998; Weiss, 1994). According to Weiss (1993), supported by Combs *et al.* (1998), empirical equations are population specific. Beauchemin (1996) found NDF intake to be variable for grasses compared to legumes fed alone. In addition, Robinson (2005) suggested that correlations between ADF and digestibility in a feed (that impacts their energy levels) do not hold among feeds, because of chemical differences of feed fibres. Furthermore, lack of fit of equations to data sets different from the one on which they were developed, suggests that equations should be specific for different types of forages or forage mixtures (Moore & Undersander, 2002). Weiss (1993) reported a lower prediction error when one equation was used for grasses and another for legumes. However, this was in contrast to the results of Placencia *et al.* (2003) as cited by Zinn *et al.* (2004) who reported that when different forages are substituted into the diet on an equal NDF basis, differences among forages in feeding value become non-appreciable. These researchers concluded that net energy values of individual forages are largely additive.

Putnam (2004) concluded that predictions of energy or a single "feeding value" are highly dependent upon animal and feed type. These calculations are more risky as a marketing tool than the use of measured values directly for pricing forages. Zinn *et al.* (2004) suggested that RFV does not appraise or define forages as their primary role as roughages, while Robinson (1999) argued that RFV is neither relative nor a feed value within lucerne hay. Furthermore, the guidelines as described in Table 7 and Table 8 have disadvantages. According to Putnam and Undersander (2006) the primary one is that individual hay lots may be categorised in one category by one measurement, but not by another. Additionally, the categories themselves create a problem with hay lots that are in between two categories. It should also be remembered, that the forage quality descriptions in Table 7 are relative and may not reflect the economic or nutritional value of lucerne in a given production situation (Martin & Mertens, 2005).

6.2.2 Forage Quality Index (FQI)

The Florida Extension Forage Testing Program recommended forage quality index (FQI) from 1982 to 2002 (Moore *et al.*, 1984). FQI combine voluntary forage intake and TDN content into one value (Moore & Sollenberger, 2002) where voluntary TDN intake is used as a multiple of the TDN requirement for maintenance as illustrated in equation 11. According to Moore and Undersander (2002) it is assumed that TDN is equivalent to *in vitro* organic matter digestility (IVOMD) (Tilley & Terry, 1963), when digestible ether extract in forages is negligible.

$$FQI = TDN\ intake,\ g/MW/29 \qquad (Moore\ et\ al.,\ 1984) \qquad (eq.\ 11)$$

where: TDN intake, (g/MW) = DM intake, (g/MW) x TDN, % of DM/100

DM intake = 120.7 - 0.83 x NDF (% of DM)

MW = Metabolic weight = $W_{kg}^{0.75}$

TDN = Total digestible nutrients

OM = Organic matter

TDN (% of DM) = OM (% of DM) x OM digestibility (%)/100

IVOMD = *In vitro* OM digestion (Tilley & Terry, 1963)

NDF = Neutral detergent fibre

DM = Dry matter

29 = Maintenance TDN requirement for sheep (29g/MW)

Forages with FQI values above 1.0 are expected to support animal performance in proportion to the FQI value (Moore *et al.*, 1991). When FQI is less than 1.0, weight loss would be expected whereas, FQI of 1.0 would result in neither gain nor loss of weight (Moore & Undersander, 2002). The FQI required to meet an animal's requirement for TDN is determined by the size and production level of the animal (Moore *et al.*, 1991). Selection of TDN as the available energy expression made it possible to use FQI (as with RFV) both for relative comparisons among forages and for predicting animal performance.

6.2.2.1 Merit of the forage quality model

Moore & Undersander (2002) evaluated the FQI prediction equation using the animal and laboratory data of cool and warm season grasses (Moore *et al.* (1996). They observed that the mean predicted DMI (60.4 ± 4.8 g/MW) was much lower and less variable than the observed DMI (87.1 ± 13.3 g/MW). Observed DM intake (g/MW) could be predicted from

the DM intake prediction regression equation with a small accuracy (r^2 = 0.39). This low prediction accuracy was explained due to the use of an equation derived from sheep data to predict cattle feed intake. However, Moore & Undersander (2002) proposed a comparable divisor (value) for cattle of 36 g/MW, derived from data on growing cattle (NRC, 1984). Moore & Undersander (2002) concluded that the small r^2 values illustrated that NDF is not an acceptable predictor of the intake of forages when fed alone.

Moore & Undersander (2002) reported a 22% bias in predicted TDN (r^2 = 0.71). The predicted TDN (55.0 ± 3.7%) was similar to the mean observed TDN (55.0 ± 5.4%.). Moore & Undersander (2002) concluded that although the relationship between observed TDN and TDN predicted from IVOMD was stronger than that between observed digestible dry matter (DDM) and ADF, differences between observed and predicted TDN values were not repeatable in several other experiments.

6.2.3 Total Forage Index (TFI)

The RFV system does not use protein in calculating its value, and forages higher in protein may be undervalued by this system. One alternative is to incorporate a protein index into the RFV system to reflect more accurately the nutritive value of the forage. Hutjens (1995) uses the term Total Forage Index (TFI) to describe an index which builds on RFV by adding a protein value and a physical value as follows:

TFI   =   RFV + (%crude protein × x)   (eq. 12)

Where x = protein multiplier from 1 to 6 based on the importance of protein in the diet of the animal.

Hutjens (1998) stated that a protein index value can be calculated in two ways. The first method is based on the value of the protein in soy bean meal or another protein feed source. The second method described by Hutjens (1998) is more subjective because the user assigns a protein multiplier (from 1-6) based on the importance of protein in the diet of the animal. A lower multiplier (1-2) would apply to heifers' rations when protein needs are lower or when rations are based on high quality hay (some protein may not be used effectively). A high multiplier (5-6) would reflect rations where supplemental protein is needed or protein is relatively expensive.

Method two has the advantage of the user determining the importance of protein needed in the ration, independent of protein prices. Once a multiplier has been selected, it is multiplied with the percentage of crude protein in the forage to calculate a protein index. After developing a protein index, it is added to the RFV, which represents the TFI value of the forage. The formula can be modified depending on the protein value, the importance of energy value versus protein, and other ration factors. The comparable values of hay according to the RFV and TFI evaluation systems, compared by Scholtz (2001), are shown in Table 9. From the results in Table 9 it is evident that CP incorporated in the TFI equation had a minor effect on the price of hay.

Table 9   Price of hay based on different evaluation systems (models)

Quality standard of forage	Relative feed value	TFI value
Prime (24% CP, RFV 170)	R 600,00	R 613.30
Prime (24% CP, RFV 190)	R 670,60	R 657,10
Prime (20% CP, RFV 160)	R 564,70	R 551,18
Prime (22% CP, RFV 160)	R 564,70	R 571,30
One (18% CP, RFV 140)	R 494,10	R 487,30
One (16% CP, RFV 140)	R 494,10	R 467,20

6.2.3.1   Merit of the total forage index model

The addition of a protein index to the RFV brings a more complete expression of nutrient value to the forage (Hutjens, 1995). TFI does not include measures of protein quality for ruminant animals. In this regard, McDonald *et al.* (1995) emphasised that the crude protein fraction contains variable amounts of non-protein nitrogen. This led to the use of true protein instead of crude protein but this was unsatisfactory, since no allowance was made for the nutritive value of the non-protein nitrogen fraction.

Allen (2000) also noted by others, criticised the inclusion of CP in the RFV model due to its low correlation with digestibility and intake, and its considerable variation. Most data indicate that lucerne hay contains much less undegraded intake protein (UIP) than concentrate feed protein sources (NRC, 1985; Broderick *et al.*, 1992; Hoffman *et al.*, 1993; Griffen *et al.*, 1994). Evidence from several experiments indicates that lactating dairy cows use protein in lucerne inefficiently. Broderick & Satter (1998) supported by Martin & Mertens (2005) reported that immature lucerne is high in protein, but the protein is rapidly

fermented in the rumen to ammonia, leading to excessive non-protein nitrogen. Wastage of forage protein caused by ammonia overflow occurs when fermentable energy is insufficient to support the microbial growth required to utilise the excess nitrogen (Beever & Siddons, 1986). Excessive nitrogen is not only wasteful but also costs the cow energy to excrete, may reduce productive performance, and contaminates the environment. This is in agreement with earlier feeding studies by Broderick (1995). Because of this, Martin & Mertens (2005) proposed dairy diets containing predominantly lucerne formulated to contain 1 to 3 percent units more protein.

6.2.4 Relative Forage Quality (RFQ)

According to Moore & Undersander (2002) evaluations of intake and available energy prediction equations (TDN, RFV and FQI) may be unreliable for ranking forages and provide inaccurate intake and available energy values for diet formulation. Differences in animal performance when forages with a similar RFV index were fed are well documented (Undersander & Moore, 2004). Moore & Undersander (2002) have developed the RFQ index in an effort to address these shortcomings. The objective of RFQ was to successfully differentiate between the market value of hays that are different in feeding value but have the same fibre concentration. According to Zinn et al. (2004) this index also takes into consideration the differences in digestibility of the fibre fraction and can be used to predict animal performance and match animal needs more accurately. Moore & Undersander (2002) kept the same concept and format as for RFQ, except that TDN replaced DDM. Furthermore, NDF digestibility (NDFD) is part of the TDN and DMI calculation. RFQ also showed a similar mean and range as RFV and were closely correlated with each other (n = 29, r = 0.99). The correlation between RFQ and FQI was 0.97 (n = 71) and the regression equation, as follows: FQI = 0.0125 x RFQ + 0.097; n = 118, r^2 = 0.95 (Moore & Undersander, 2002). According to Peterson (2002), RFQ index relates to forage quality when forages are fed alone to non-lactating animals.

$$RFQ = (DMI_{legume}, \% \text{ of } BW) \times (TDN_{legume}, \% \text{ of } DM) / 1.23 \qquad (eq. 13)$$

where: DMI = Dry matter intake (eq.14)

TDN = Total digestible nutrients (eq. 16)

1.23 = devisor used to adjust the equation to have a mean and range

similar to RFV (Moore & Undersander, 2002)

Moore & Undersander (2002) developed the divisor, 1.23, from data on 29 forages (Moore *et al.*, 1996; Moore *et al.*, 1999), having animal observations on intake and both DDM and TDN by cattle. They reported a correlation of $r^2 = 0.99$ (n = 29) between DDM and TDN intake. The no-intercept regression TDN on DDM intake gave a slope of 0.95. Thus, multiplying the RFV divisor, 1.29, by 0.95 gave the RFQ divisor, 1.23.

6.2.4.1   *Dry matter intake (DMI) Neutral detergent fibre digestibility (NDFD)*

There are two equations for predicting dry matter intake (DMI) depending on forage type:

DMI calculations for lucerne, clover and legume/grass mixtures are calculated from the equation proposed by Mertens (1987).

$$\text{DMI}_{\text{Legume}} = 120/\text{NDF} + (\text{NDFD}_{48} - 45) \times .374 / 1350 \times 100 \qquad \text{(eq. 14)}$$

where: $\text{NDFD}_{48} = $ *In vitro* NDF digestibility at 48 hours (% of NDF)

45 = an average value for fibre digestibility of lucerne and lucerne/grass mixtures as proposed by Oba & Allen (1999).

DMI is expressed as % of body weight (BW), NDF as % of DM and NDFD as % of NDF

6.2.4.2   *Neutral detergent fibre digestibility (NDFD)*

The new $\text{DMI}_{\text{Legume}}$ equation, proposed by Moore & Undersander (2002), adjusts intake for digestible fibre (NDFD). Several researchers have demonstrated the effect of digestibility of the fibre on DMI (Mertens, 1987; Undersander & Moore, 2004; Zinn *et al.*, 2004). Oba & Allen (1999) reported that forages with high NDF digestibility might increase DMI when physical fill limitations to feed intake exist. Increased NDFD may result in reduced physical fill in the rumen over time and allow greater voluntary DMI (Dado & Allen, 1995). Rumen fill is affected by both NDF content of the diet and by forage NDF digestibility (Oba & Allen, 1999). Mertens (1987) reported that the energy content and fill effect are inversely related; thus they will always intersect at a point that predicts maximal DMI for a given level of production. However, Allen (2000) suggested that NDFD measured *in vitro* using a constant incubation time was a significant indicator of filling effects of NDF, but not

necessarily an index of energy content. Allen & Mertens (1988) suggested that NDF digestibility is a function of the potential digestible fraction and its rate of digestion and rate of passage. Oba and Allen (1999) reported that enhanced ease of NDF hydrolysis might stimulate rapid disappearance of NDF from the rumen, reduce physical fill, and allow greater voluntary feed intake.

According to Ruiz et al. (1995) digestibility of fibre has a significant influence on DMI and milk production. In a study conducted by Oba & Allen (1999) they reported that a one unit rise in NDFD (in vitro) was associated with a 0.17kg/day rise in DMI (digestibility and intake are related), 0.23 kg/day increase in milk yield, 0.25 kg rise in 4% FCM yield and a 0.03 kg/day increase in body weight. These concepts were confirmed in a trial conducted by Hoffman & Bauman (2003) (Table 10). In addition, Ivan et al. (2005) reported increased DMI and 4% FCM production when silage with higher NDF content and digestibility was substituted for silage with lower NDF content and digestibility. Hovell et al. (1986) however, contends that different degradation characteristics of different forages may complicate the choice of which parameter of degradability is best used as the predictor of voluntary intake.

Table 10 **Dry matter intake, NDF intake and milk yield of early-mid lactating dairy cows fed diets containing different levels of NDFD. (Hoffman & Bauman, 2003)**

Item	Dietary NDFD, % of NDF		
	45%	50%	55%
Dry matter intake, kg/day	20.5	22.1	23.3
NDF intake, kg/day	8.5	8.6	9.8
Milk yield, kg/day	33.5	34.7	35.1

Oba and Allen (1999) concluded that because of the use of NDF digestibility in diet formulation, it should be measured routinely to assess forage quality. The relationship between NDF and DMI (NDFD adjusted) in Figure 7 is expected because intake estimates, NFTA and RFQ, are based on NDF.
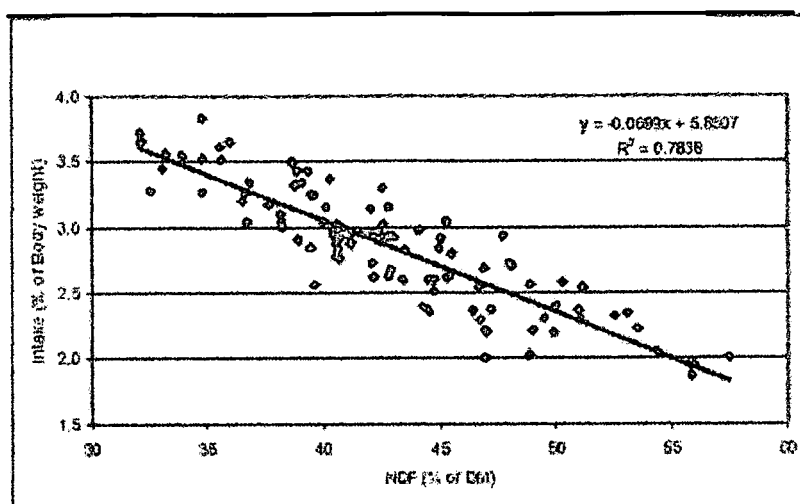
Figure 7   NDF vs Intake (1.2% BW plus NDFD adjustment) for lucerne and grass-legume mixtures (Undersander & Moore, 2004)

6.2.4.3   Total digestible nutrients (TDN)

According to Combs *et al.* (1998) digestibility of forages, thus energy value can be determined in four different ways. *In vivo* studies, however not practical as a routine analysis, are the reference standards by which all alternative methods are compared. Alternative methods are based on empirical relationships between forage fibre and digestibility and/or RFV (Rohweder *et al.*, 1978) and ADF-TDN (Bath & Marble, 1989), *in vitro* assays to directly measure forage digestibility (Weiss, 1998) or summative equations such as TDN (Weiss *et al.*, 1992). The summative approach measures the principal components in the forage that contribute to energy. Each component is multiplied by its respective digestion coefficient and added together. Thus, total digestible nutrients (TDN) are a cumulative value of digestible protein, crude fibre, non-fibre carbohydrate, and fat.

The traditional TDN as described by the NRC (1966) as a vague term that does not accurately describe the plants available energy. The National Research Council in the last revision of Nutrient Requirements for Dairy Cattle (2001) revised the historical TDN equation based on the summative equation of Weiss *et al.* (1992), assuming intake at one times maintenance or 1X:

$$TDN_{1X} = tdCP + (tdFA \times 2.25) + tdNDF + tdNFC - 7 \qquad \text{(eq. 15)}$$

Where: TDN_{1x} = summative equation of total digestible nutrients assuming intake at one times maintenance

tdCP = truly digestible CP for forages (tdCPf)

  = CP x exp[-1.2 x (ADICP/CP)]

tdFA = truly digestible fatty acids

  Note: If EE < 1, then FA = 0

tdNDF = truly digestible neutral detergent fibre

  = $0.75 \times (NDF - L) \times [1 - (L/NDFn)^{0.667}$

  or = *in vitro* neutral detergent fibre digestibility at 48hr ($NDFN_{48}$)

Where: NDFN = NDF - NDICP

tdNFC = truly digestible non-fibre carbohydrates

  = 0.98 (100 - [(NDF - NDICP) + CP + EE + ash])

  where: ADICP = acid detergent insoluble N x 6.25 = acid detergent insoluble crude protein

    L = acid detergent lignin

Total digestible nutrients (TDN) for lucerne, clovers and legume/grass mixtures are calculated based on the newest NRC recommendations (NRC, 2001) using *in vitro* estimates of digestible NDF (not those calculated from lignin) as follows:

TDN_{legume} = (NFC*.98) + (CP*.93) + (FA*.97*2.25) + ($NDFn * (NDFD_{48}/100$) – 7 (eq. 16)

  where: NFC = non-fibrous carbohydrate (% of DM) = 100 – (NDFn + CP + EE + ash)

    CP = crude protein (% of DM)

    FA = fatty acids (% of DM) = EE - 1

    EE = ether extract (% of DM)

    NDF = neutral detergent fibre (% of DM)

    NDFn = nitrogen free NDF = NDF – NDICP, also estimated as NDFn = NDF*.93

NDF-CP = neutral detergent insoluble N x 6.25 = neutral detergent insoluble crude protein

NDFD$_{48}$ = 48-hour in vitro NDF digestibility (% of NDF)

7 = metabolic faecal TDN determined by Weiss *et al.* (1992)

Figure 8 suggests a poor relationship (r^2 = 0.11) between ADF (used by RFV to estimate energy) and TDN (including NDFD measured *in vitro*) as recommended by NRC (2001). This correlation explains the reason why RFV is not a good predictor of the energy in forage. According to Undersander & Moore (2004) switching from ADF to TDN and using ash estimates have improved accuracy of diet balancing from 60 to 90%.
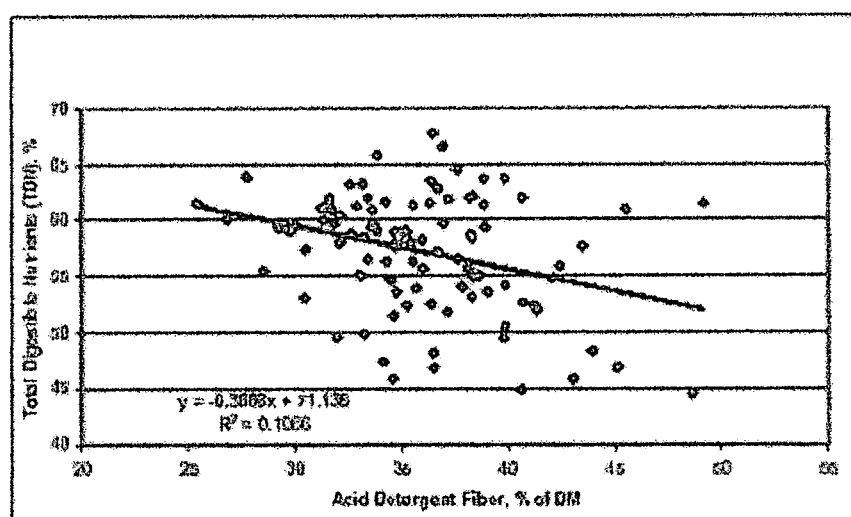
Figure 8   Comparison of ADF to TDN (NRC 2001) (Undersander & Moore, 2004)

6.2.4.4   Non-fibre carbohydrates (NFC)

The term non-structural carbohydrate (NSC) is often used interchangeably with non-fibre carbohydrate (NFC) but is analytically determined and might be slightly different from NFC. According to Hall (2005) the balance between fibre and NFC, especially the profile of NFC (sugars, starch, pectins, etc.), has drawn more interest due to their influence on animal performance and digestion (fermentability) of other feeds. Starch fermentation, rather than sugar, in the rumen seems to support more production of microbial protein, a major source of protein to the cow (Hall & Herejk, 2001). Broderick *et al.* (2000) found that increasing the sugar content of diets increased both DMI and butterfat production. In addition, sugar may

also be responsible for an increase or reduction in fibre digestibility, depending on the degradable protein concentration in the diet (Heldt *et al.*, 1999).

The effect of diurnal variation on the concentration of NFC in lucerne hay is well documented (Fisher *et al.*, 2002; Burns *et al.*, 2005; Mayland *et al.*, 2005). Harvesting lucerne hay at sundown rather than at dawn, takes advantage of the plants' potential to accumulate carbohydrates, as a result of photosynthesis during the daytime (Bowden *et al.*, 1968). This increase in NFC is associated with better *in vitro* digestibility (Burns *et al.*, 2005) and increased preference by sheep, goats and cattle (Fisher *et al.*, 2002; Mayland *et al.*, 2005). Thus, potentially improved daily DMI of more digestible forage might result in improved daily animal responses.

6.2.4.5 Crude protein (CP)

The benefit of lucerne crude protein (CP) is over-estimated by conventional grading systems (RFQ, FQI) due to its poor utilisation (Martin & Mertens, 2005) by ruminants. The mean undegraded intake protein (UIP) value reported by the NRC (1989) for lucerne hay is 28% (SD = 7). The rapid degradation in the rumen by microbes results in excessive excretion of nitrogenous waste by the animal (Martin & Mertens, 2005). Evidence from several experiments indicates that the protein in lucerne is utilised inefficiently by lactating dairy cows (Broderick *et al.*, 1992). However, in contrast with these findings, Robinson (1998) suggested that lucerne hay has a relatively high proportion of rumen undegradable protein (RUP), which makes it a high quality protein for dairy cows. Robinson (2008, Pers. Comm.) suggested a potential degradable fraction (PDF) of about 57% for lucerne hay. When accepting a rate of passage of 5%/h, the RUP would be between 33 and 35% of PDF, or a total RUP of 25 and 32% of CP.

6.2.4.6 Fatty acids (FA)

It has long been recognised that the two key factors that determine the energy value of forages for cattle are its content of fat, due to its high energy value, and the digestibility of its total structural fibre (i.e., NDF), due to its high level in forages (Robinson, 2005). According to Andrews *et al.* (1991) fat is used more efficiently for production under specific circumstances than carbohydrate. Van der Merwe and Smith (1991) concluded from the composition of ether extraction fraction, that it could be misleading and does not represent

the true fat or oil content of a feed. This was in agreement with the findings of Weiss *et al.* (1992) who reported that the ether extract (EE) fraction of forages has a high concentration of non-nutritive substances. Weiss *et al.* (1992) suggested that the ether extract (EE) may also contain a significant concentration of indigestible waxes, resins and essential oils. Weiss (1993) is of opinion that the inclusion of fat and ash, due to a high energy density and zero energy status respectively, in a model could make the estimation of available energy more appropriate for most feeds. The true digestibility of fatty acids (FA) is dependant on their concentration in the diet (Palmquist, 1991). However, forages, and especially lucerne hay, have a narrow range and low concentrations of EE which will not greatly compromise the accuracy and precision of forage energy estimation when using average values (Scholtz, 2001; Weiss *et al.* (1992).

6.2.4.7   Ash

According to Putnam (2004) ash has been shown to range up to 10 percentage points or more and may be of real value in predicting animal performance. With the ash value of hays, is important to identify hay lots with considerable soil contamination and/or hays with above-normal mineral concentration (Putnam, 2004). Hoffman (2003) stresses the importance of ash measurement in predicting the energy value of forages. Because ash has no energy value (Weiss, 1998), the measurement error of ash will result in exactly the same prediction error in the forage energy content.

6.2.4.8   Neutral detergent fibre crude protein (NDF-CP)

Crude protein that is insoluble in neutral detergent represents the slowly degraded protein in feeds (Fox *et al.*, 2003). Higher NDF-CP values are usually associated with grasses, due to the higher NDF concentration (Van Soest, 1994) compared to lucerne. In addition, the nitrogen content of NDF in feeds is greatly increased by heating, which promotes denaturation of albumins (Van Soest, 1994). According to Weiss *et al.* (1992) NDF-CP comprises less than 10% NDF for forages not exposed to heating. Van Soest (1965) and Mertens (1973) stressed that only a fraction of NDF-CP is associated with lignin; thus not to be considered as a contaminant of NDF. However, Hoffman (2003) pointed out that the overall influence of NDF-CP on summative energy equations is small.

6.2.4.9   Neutral detergent fibre digestibility (NDFD)

Acid detergent fibre (ADF) has been used to estimate digestible dry matter (DDM) (par. 6.2.1.2) for the last 25 years (Peterson, 2002). It was, however, intended as a preparatory step for lignin analysis (Van Soest et al., 1991). When using any fibre determination (ADF) to estimate digestibility, the assumption is made that there is a constant linear relationship between fibre concentration and digestibility (Undersander & Moore, 2002). This was criticised by Moore et al. (1998). There is considerable variation in the in vitro digestibility of the dry matter (IVDDM) relative to the ADF content ($r^2 = 0.037$) for lucerne and grass-lucerne mixes (Undersander & Moore, 2002). The in vitro and in situ estimates of digestibility have long been recognised as being more closely related to animal performance than chemical extractions (Weiss, 1994). The NRC (2001) for dairy animals recognises this and recommends the use of digestible fibre. Lundberg et al. (2004) cited by Hoffman (2004) demonstrated that using ADF as predictor of IVOMD accounted for 0.61 of the variance in IVOMD in maize silage, compared to a 0.98 coefficient of determination between summative predicted TDN and IVOMD when NDFD was used as the digestion coefficient for NDF. This demonstrates the improved capability of summative equations (Weiss et al., 1992) using NDFD as an energy predictor.

The dramatic impact of NDFD content of forage on the energy value of the diet is well documented (Ruiz et al., 1995; Oba & Allen, 1999; Hoffman & Bauman, 2003). Increased NDFD may result in increased energy density of diets and microbial N production (Oba & Allen, 2000). However, Oba & Allen (1999) mentioned the difficulty of quantifying the effect of NDFD of forages on animal performance due to confounding factors found in individual experiments. Some of the confounding factors included greater particle fragility (Waghorn et al., 1989) and shorter rumen retention time (Allen, 1996) of legumes compared to grass.

The NRC (2001) provides two options to estimate the NDF digestibility. The first approach utilises the feed lignin (acid detergent lignin (ADL)) content as an indication of lignification within a plant species and it is negatively associated with NDF digestibility. This is supported by a study in which Herrero et al. (1996) notes that lignin is the best single chemical compound most commonly associated with the impairment of digestion. However, Mould (2003) demonstrated that because of the complex nature, its association with NDF

and the lack of a standardised procedure, lignin content shows a large variability relative to degradation. Conrad *et al.* (1984) derived a mathematical model based on the surface law of a geometric object to predict the proportion of NDF available for digestion from the NDF and lignin values of the feed (eq.17). According to the surface law of Conrad *et al.* (1984) the influence of lignin on the digestibility of NDF is more significant at lower than at higher lignin concentrations. However, Jung *et al.* (1997) found a weak relationship between acid detergent lignin and *in vivo* digestibility in sheep for maize silage. Weiss *et al.* (1992) modified the equation for truly digestible NDF (tdNDF) given by Conrad *et al.* (1984) to account for neutral detergent insoluble crude protein (NDICP). They also derived a considerably lower coefficient of 0.75 than the 0.96 derived by Girard and Dupuis (1988), who also failed to include neutral detergent insoluble nitrogen (NDIN).

Available NDF = $1-[\text{lignin}^{0.67}/(\text{NDF})^{0.67}]$ (eq. 17)

The second method includes a 48 h *in vitro* estimate. This is a direct determination of NDF digestibility and can substitute the lignin-based estimate. Linn (2003) pointed out that that the NRC (2001) NDFD equation (using lignin) does not consider feed type. Lignin does affect the digestibility of NDF; however, the effect is variable with feed type and/or the environment in which it is grown. Moore & Undersander (2002) proposed the 48h *in vitro* system to directly determine NDF digestibility and to estimate TDN concentrations of forages fed alone, rather than the lignin approach. Wisconsin research has shown for forages that the 48 hour *in vitro* NDF digestibility does not substitute directly for the calculated NDFD value. Mould (2003) suggested that because of the complex nature of lignin, its relationship with NDF and lack of a standardised procedure, lignin content when expressed on a DM basis, shows a large variability relative to degradation. Similarly, Robinson *et al.* (2004) found a superior relationship between *in vitro* NDFD ($r^2 = 0.76$) compared to lignin ($r^2 = 0.40$) concentration and *in vivo* digestibility in sheep.

Zinn *et al.* (2004) stated that ruminal NDF digestion is not an intrinsic function of the forage *per se*, but rather a complex function of the complete diet. Ruminal fibrolitic capacity (growth of cellulitic bacteria) diminishes with decreasing ruminal pH (Russell & Wilson, 1996), and ruminal pH is dependent on the effective NDF (eNDF, %) content (Pitt *et al.*, 1996). Thus, according to Zinn *et al.* (2004) ruminal fibre digestion might be more limited by ruminal fibrolitic capacity than by the native degradability of the fibre. This is in

agreement with Alvarez *et al.* (2004) who observed that most ($r^2 = 0.96$) of the variation in ruminal ADF digestion was explained by changes in ruminal pH.

High yielding (55kg/d) cows are challenged to meet their energy requirements, and DMI of these cows is probably limited by distension of the reticulo-rumen (RR) to a greater extent than for lower yielding cows consuming the same diet (Oba & Allen 1999; Allen 2000) Distension in the RR can be reduced by decreasing the forage NDF content and including forages with highly digestible NDF (Allen, 2000), thereby increasing the energy concentration of the diet. Accordingly, Allen (2000) recommended that forages with the highest NDFD should be allocated to the highest producing dairy cows on the farm.

6.2.4.10 Merit of Relative Forage Quality (RFQ)

According to Moore & Undersander (2002) RFQ has the following advantages:

1. RFQ may be translated into energy requirements for maintenance and production.
    a. Multiplying RFQ by .0123 gives an estimate of TDN intake (% of BW).
    b. TDN concentration may be converted to NE concentration.
    c. DM intake can be calculated by dividing TDN intake by TDN concentration.
    d. Both DM intake and TDN can be used as inputs for several nutritional models.
    e. The TDN component has a value by itself when forages are fed in restricted amounts or in mixed diets.

2. Development of a new index provides the opportunity for flexibility in choice of equations for predicting DMI and TDN. These equations should however, be specific for different types of forage.

3. Associative effects between forages and concentrates that influence forage intake and digestibility can be predicted from estimates of forage TDN intake when fed alone (Moore *et al.*, 1999).

4. RFQ can be converted to FQI:

$$FQI = .0125 * RFQ + .097 \qquad \text{(eq. 18)}$$

A significant advantage of the summative equation (TDN) over the empirical equation (RFV)

is that the summative approach is population independent (Combs *et al.*, 1998). Results of a feeding trial conducted by Combs *et al.* (1998) suggested that digestibilities predicted by the *in vitro* digestion approach and the TDN summative approach (Weiss *et al.*, 1992) were similar to the digestibility observed *in vivo*. The RFV empirical approach (Rohweder *et al.*, 1978) was less accurate than the summative and *in vitro* approaches.

A great advantage of the RFQ equation is not just an improvement in the ability to rank forages based on more specific quality components, but the fact that it can be applied to various forage types. The higher NDF content of grasses is always biased against forage material that had contained grass. The old RFV system simply looked at levels of ADF and NDF. By including NDF digestibility, various hay crop mixtures, including grass, may be adequately compared by this single index (Ward, 2005).

When Moore & Undersander (2002) designed RFQ, they wanted approximately the same mean and range as RFV so that RFQ could substitute RFV without making economic and other management changes. The relationship between RFV and RFQ is set out in Figure 10.
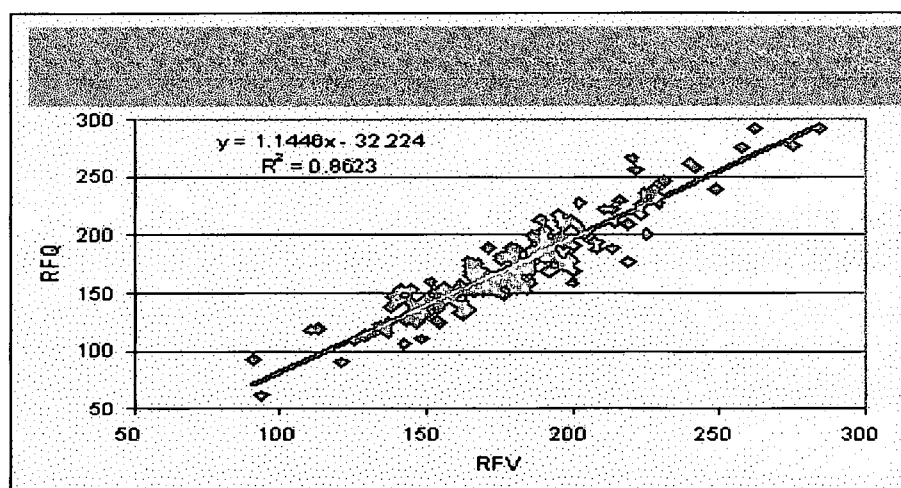
Figure 10. **Comparison of RFQ and RFV for about 200 lucerne hay, haylage, and baleage entries from 20 states and two Canadian provinces (Undersander, 2002).**

The overall coefficient of determination (Figure 10) was high ($r^2 = 0.86$) due to the large range of data values. However, the RFQ of individual samples varied by as much as 40

points higher or lower than RFV, and 22% of the samples varied by 20 points or more (Undersander, 2002).

Current lucerne quality description models (TDN, RFV, TFI, FQI and RFQ) presume quality increases to infinity. This is not true because exceptionally high quality can provide too much protein and not enough NDF in a particular dairy ration. Although the energy content will be relatively high, it still is much lower than the energy content of maize. Diets containing very high quality lucerne hay with low NDF content should contain very high forage levels to meet the cow's fibre requirements. This reduces the energy density of the diet because there is little room left for grain, which makes it difficult to formulate rations that meet the protein, energy, and fibre requirements of dairy cows (Martin & Mertens, 2005). Thus, lucerne hay loses its primary role as a functional component of the diet.

Zinn et al. (2004) noted that current adopted laboratory approaches (RFV, LQI and RFQ) for assessing forage quality, especially lucerne hay, are not favourably accepted by nutritionists. They suggested that the models are not sufficiently accurate in the assessment of practical differences between forages of similar classification, and they do not appraise forages with respect to their primary role as the functional component of the diet, namely roughages. In addition, Moore & Undersander (2002) recognise the limitation of RFQ and recommend further research to identify forage specific equations that will provide more acceptable predictions of DMI and TDN. However, Hoffman et al. (2001) stressed that NDF concentration of forages is still a dominant factor in determining overall forage quality.

The accuracy of predicting DMI, however, is one of the most concerning limitations in most models. Because of the complexity of factors controlling feed consumption, accurate prediction of daily DMI is difficult. According to Crampton et al. (1960) variations in livestock production are more highly correlated with DMI than to ration digestibility. It is also well accepted that forages (including lucerne) with a high energy concentration are consumed in larger amounts than poor quality forage, and high quality forage is essential for high milk yields (Holter et al., 1997). The close relationship between DMI and the milk yield of dairy cows is well defined by research (Roseler et al., 1997; Voelker et al., 2002; Hristov et al., 2005). According to the Hristov et al. (2004) DMI is driven by milk production when lactation diets are fed. This concept is supported by the lag between peak milk yield (MY) and peak DMI during the early stages of lactation (NRC, 2001). Recently Hristov et al.

(2005) evaluated the intake of individual nutrients as predictors of MY and milk protein yield (MPY) in Holstein dairy cows. They concluded that models based on the intake of nutrients significantly improved prediction of MY and MPY in Holstein dairy cows, compared with DMI alone. It should also be noted that intake and digestibility are non-independent variables. Although intake is directly related to digestibility, the digestive capacity of animals is inversely related to feeding level, with the magnitude of this effect greater with concentrate feeds than poor quality, long or coarsely chopped forages (Mould, 2003). Thus, the definition of the NRC (2001), which stated that intake of nutrients are a function of DMI and nutrient density of the diet.

For a number of individual feeds, the TDN value cannot be determined directly as it cannot be the exclusive ingredient in a diet (Linn, 2003). Thus, accuracy in calculating the TDN of lucerne hay in a diet of mixed feeds is questionable because of associative effects. In addition, forages are known to produce more heat of fermentation and methane production than grains (Eastridge, 2002). Thus, the TDN system underestimates the energy of grain relative to the energy of forages.

RFQ does not account for differences in starch availability. The NFC differs in how it affects animal performance and digestion of other feeds (Hall, 2005). The NFC fraction in lucerne is roughly half pectin (Hall, 1998); whereas sugars, starches, organic acids, and other reserved carbohydrates such as fructans make up the other half. The unique property of pectin was discussed in par. 3.2. Starch fermentation in the rumen may support more production of microbial protein, than does sugar (Hall & Herejk, 2001). Broderick *et al.* (2000) reported that increasing sugar content of diets increased DMI and butterfat production. Sugar can also influence fibre digestibility depending on how much degradable protein is in the diet (Heldt *et al.*, 1999).

Ward (2005, Pers. Comm.) stressed that *in vitro* evaluation may vary significantly between laboratories. Variation may be due to equipment used, sample grind size, fermentation lag, rumen fluid source and incubation time Accordingly, Allen (1996) proposed that differences in NDF digestibility of forages vary with *in vitro* incubation time. Moreover, Zinn *et al.* (2004) stated that length of time needed for the degradative process of NDF, rather than the digestibility thereof is the more limiting aspect, especially in dairy formulations where forage inclusion rates are necessarily high (40 - 60%). Mould (2003) showed that the use of animals

to provide microbial inoculums for the *in vitro* procedure introduces additional sources of error due to variations within animals (time effect), time of sampling relative to feeding, basal diet, etc; Cone *et al.* (1989) made similar observations. According to Stern *et al.* (1997) *in vitro* starch disappearance was greater when ruminal fluid was obtained from a concentrate fed cow than from one that has been hay fed. However, they also reported that ranking among feeds to be independent of the donor cow diet. Richard *et al.* (1995) demonstrated that the type of mill and screen size used to grind the sample had a significant influence on *in vitro* digestibility. Different *in vitro* laboratory methods on the basis of ruminal fluid (Tilley & Terry, 1963), the method on the basis of forage enzymatic degradability (De Boever *et al.*, 1986) or *in vitro* gas production of forage incubated in ruminal fluid (Menke & Steingass, 1988), are also potential sources of error within and between laboratories. Moreover, the static nature of the *in vitro* procedure does not ideally simulate the *in vivo* situation with regard to substrate /rumen fluid ratio and the retention or incubation time (De Boever *et al.*, 1996).

There is however, debate in the industry about the appropriateness of 48hr vs. the 30hr and 24hr *in vitro* measurements. Several researchers recommend that the incubation time of *in vitro* NDFD should be reduced from 48hrs (NRC, 2001) to 30hrs (Robinson, 2005, Pers. Comm.) or 24hrs (Tylutki, T.P., 2007, Pers. Comm., Agricultural Modeling and Training Systems, LLC, 416 Davis Road, Cortland, NY 13045, USA). The rationale is that a shorter incubation time is more appropriate to describe the digestion potential of NDF for lactating dairy cows, as feed is not retained in the rumen of a high producing dairy cow for a 48-hour period (Hoffman & Combs, 2004). The 48hr NDFD recommendation of the NRC (2001) is to calculate TDN content of forages at a maintenance intake level. Hoffman *et al.* (2003) proposed that the 30hr incubation period might be a better indication of the amount of NDF digested by a cow at maintenance level. According to this researcher a 48-hour incubation period will result in a slight over-prediction of NDFD. Hoffman and Combs (2004) are of opinion that the 48hr NDFD (NDFD48) assay has the advantage of repeatability between, and within laboratories, and the availability of a large database (NRC, 2001) for most feeds. However, Hoffman *et al.* (2003) reported a strong positive relationship between 30hr and 48hr NDFD values across all forages ($r^2 = 0.84$). Within forages they found NDFD48 to be higher than 30hr NDFD (9-12 and 2-5 percentage units as a % of NDF for grasses and legumes respectively).

Earlier studies by Miller and Oddoye (1989) reported that voluntary DMI of conserved forages was best described by the disappearance of NDF after 12 hours of incubation, reflecting lag phase, potential degradability and rate of degradation. This was supported by Alexandrov *et al.* (1995) who reported that most of DM and CP in lucerne hay disappear during the first 6-12 hours of incubation in the rumen. Balliette and Torell (1998) reported that the passage rate of lucerne hay is approximately 36 hours versus the 70 hours required for grass hay.

An alternative approach from the adaptations of the classical Tilley and Terry (1963) *in vitro* rumen digestibility system, is the *in vitro* gas method (Menke *et al.*, 1979; Menke & Steingass, 1988; Pell & Schofield, 1993) commonly used by California researchers (Robinson *et al.*, 1999; Robinson, 2005, Pers. Comm.). An important difference is that the rate of forage digestion can be measured. The production of gaseous products is monitored continuously by the use of computerised pressure sensors (Pell & Schofield, 1993). According to Herrero *et al.* (1996) the technique is gaining popularity due to its simplicity, low cost and high reproducibility of obtaining a dynamic description of the nutritive value feeds, while at the same time allowing more samples to be analysed.

In a wider sense, however, the issue of different incubation times is somewhat irrelevant because changing the incubation time of the assay reduces the amount of NDF digested; therefore, NDF digestibility values obtained from 30- and 48-hour digestions cannot easily be compared to NDFD values listed in the NRC 2001 (Hoffman 2003).

Hall (2005) suggested that the balance of rumen degradable protein (RDP) and rumen undegradable protein (UDP) is important to meet protein requirements of the dairy cow. RFQ does not distinguish between protein fractions (RDP, UDP), which are important when balancing a diet. Therefore, it assumes that all legumes have equal degradability in the rumen. In addition Harlan *et al.* (1991) are of opinion that in the proximate analysis system, digestible CP (N x 6.25) is assumed to have equal energy weight with carbohydrates; however, according to Van Soest (1982), 30 % of the N in some forages, and much of the faecal N, is in the form of non-protein nitrogen (NPN), which has variable amounts of associated energy. Linn (2003), stressed that the over-estimation of the energy concentration in diets can be increased by feeding higher protein diets to dairy cows.

6.3 The Cornell Net Carbohydrate and Protein System (CNCPS)

All the models previously discussed addressed only the chemical composition of the forage without consideration of the physical characteristics of the feed, animal factors and associative effects. These effects have been acknowledged by Sniffen *et al.* (1992) when they developed the Cornell Net Carbohydrate and Protein System (CNCPS) model using a combination of mechanistic and empirical sub-model approaches, assuming steady state conditions. It also uses statistical representation of data that describes the aggregated response of whole model compartments (Fox *et al.*, 1995).

The CNCPS model has been designed to evaluate diets and cattle performance based on animal, environmental and feed composition information in diverse production situations (Fox *et al.*, 2004; Tylutki *et al.*, 2007). According to Fox *et al.* (2004) predicted animal requirements account for different animal physiological states (lactation, pregnancy and growth), body reserves and environmental effects. The CNCPS also uses feed carbohydrate and protein degradation (k_d) and passage (k_p) rates to predict extent of ruminal fermentation, microbial protein production, post ruminal absorption and total supply of metabolisable energy (ME) and metabolisable protein (MP) to the animal (eq. 19) (Fox *et al.*, 2004). The inclusion of this equation (eq. 19) in the CNCPS model makes the model non-linear and thus dynamic.

$$D = k_d/(k_d + k_p) \qquad\qquad (eq.19)$$

According to Tylutki *et al.* (2007) the latest version (6) of the CNCPS (CNCPS v6), represents an evolution of the model that was first published in 1992 by Russell *et al.* (1992), Sniffen *et al.* (1992) and Fox *et al.* (1992).

The CNCPS further assumes that all feeds are composed of carbohydrate, protein, fat, ash and water (Sniffen *et al.*, 1992). Protein and carbohydrate DM fractions are further subdivided into chemical composition, physical characteristics, ruminal degradation and post ruminal digestibility characteristics. CNCPS uses these fractions and their digestion rates to compute the amount of structural and non-structural carbohydrates available for each of the carbohydrate microbial pools (Sniffen *et al.*, 1992).

6.3.1 Chemical composition

The carbohydrate (CHO) and crude protein (CP) sub-fractions are fractionated by physical or chemical methods according to the latest version (6) of CNCPS (CNCPSv6) principles (Tylutki et al., 2007). Table 11 summarises the analytical methods and calculations used to determine the carbohydrate and protein nutrient pools.

Table 11   CNCPSv6 carbohydrate and protein nutrient pools[a]

Item	Pool	Units	Calculation or analysis method
Acetic, propionic, butyric	CA1	%DM	HPLC
Lactic	CA2	%DM	HPLC
Other organic acids (OAA)	CA3	%DM	Book values
Sugar	CA4	%DM	Dubois et al. (1956 Ralph)
Starch	CB1	%DM	Holm et al. (1986 Ralph)
Soluble fibre	CB2	%DM	NFC-CA1-CA2-CA3-CA4-CB1
Neutral detergent fibre (NDF)	CB3	%DM	NDF-NDFCP-CC
Lignin	CC	%DM	(NDF x Lignin x 2.4)/100
Non-protein nitrogen (NPN)	PA	%DM	NPN x (SolCP/100) x (CP/100)
Soluble protein	PB1	%DM	SolCP x CP/100 – PA
	PB2	%DM	CP - (PA - PB1 - PB3 - PC)
Neutral detergent fibre crude protein (NDFCP)	PB3	%DM	NDFCP- ADFCP
Acid detergent fibre crude protein (ADFCP)	PC	%DM	ADFCP

[a] Description of pools adopted from Tylutki et al. (2007)

6.3.1.1   Protein fractions

Feed protein is partitioned into three fractions, namely non-protein nitrogen (NPN), true protein, and unavailable nitrogen (Van Soest et al., 1981). Pichard & Van Soest (1977) described these fractions as fraction A (NPN), B (true protein), and C (bound true protein), respectively. Fraction B is further partitioned into three sub fractions (B1, B2, B3) according to their intrinsic rates of degradation in the rumen (Van Soest et al., 1981; Khrishnamoorthy et al., 1983). The protein (P) fraction B1 (PB1) is free amino acids and small peptides which are rapidly degradable with a degradation rate (k_d) of 100-200%/h. Fraction PB2 is the difference between buffer insoluble protein and fraction PB3 which is fermented in the rumen at a lower rate than PB1, thus intermediately degradable with a k_d of 5-12%/h, and some could escape to the lower gut. Fraction PB3 is believed to be the slowest degradable of the three PB fractions with a k_d of 1-15%/h. Because of its association with plant cell walls, PB3

to a large extent escapes to the lower intestines. Fraction PC is the acid detergent fibre-crude protein (ADF-CP) which is considered to be resistant to microbial and animal enzymatic breakdown and thus undegradable and unavailable to the animal. All passage rate equations were adopted from Seo *et al.* (2006).

6.3.1.2 *Carbohydrate fractions*

Feed carbohydrates, based originally on Sniffen *et al.*(1992), were further subdivided in CNCPSvs.6 to: CA1 (acetic, propionic and butyric acids), CA2 (lactic acid), CA3 (other organic acids), and CA4 (sugars) with degradation rates (k_d) of 0%, 5%, 3% and 40-60%/h respectively. Both fractions CB1 (starch) and CB2 (soluble fibre) have an intermediate k_d of 20-60%/h. Fraction CB3 (available NDF) has a slow k_d of 3-6%/h and fraction CC (lignin x 2.4) is totally undegradable. The passage rate (k_p) equations were adopted from Seo *et al.* (2006). Carbohydrate and protein fractions in feeds are then summed to determine the intake of each fraction (Sniffen *et al.*, 1992).

6.3.2 *Physical characteristics*

According to Weiss (1998) physical characteristics of feeds have a significant affect on digestibility and passage rate. According to Van Soest (1994) the quality of a feed is considerably changed by its physical characteristics, although it may be relatively independent of its chemical composition. In the CNCPS, the growth rate of bacteria that digest available fibre carbohydrate (FC) and NFC depends on rumen pH, which is predicted from diet NDF and physical characteristics of feed, stimulating chewing and rumination (Fox & Tedeschi, 2002). The physical characteristics of feed are described as effective neutral detergent fibre (eNDF) as published by Sniffen *et al.* (1992). The effectiveness of NDF is described by Smith and Waldo (1969) and Mertens (1986) as the percentage NDF remaining on a 1.18 mm screen after dry sieving. Several adjustments were made for density, hydration and degree of lignification of NDF within classes of feed.

The purpose of the effective fibre concept was to meet the minimum fibre requirements that would maintain minimum milk fat percentage (Mertens, 1997). Fox *et al.* (2000) describes this term as the properties of forage that will stimulate chewing and salivation, rumination and rumen motility. According to Zinn *et al.* (2004) the eNDF of forage did not only represent its particular functionality in promoting digestive function, but also represents the character of the forage that can limit energy intake, thus negatively influencing performance.

It is important however, to note that dietary NDF concentration is poorly related to "fibre effectiveness" (Garcia & Kalscheur, 2005).

Mertens (1997) further proposed that there should be differentiation between eNDF and physical effective NDF (peNDF). He described peNDF as the physical characteristic of fibre (primarily particle size) that influences chewing and the biphasic stratification of ruminal contents (floating mat of large particles on mat of liquid and small particles); whereas, eNDF was defined as the sum of the total ability of the NDF in a feed to replace the NDF in forage in a diet so that the percentage of milk fat produced and rumen pH is effectively maintained. However, it has been proposed that low pH is related more closely to high concentrations of NFC than to low effective fibre (Fox & Tedeschi, 2002).

Conceptually, peNDF is related to roughage value (Sudweeks et al., 1981), fibrousness characteristic (Balch, 1971) fibrosity index (Sauvant et al., 1990), and physical structure (Norgaard, 1986), because all are related to chewing activity. The eNDF values in the CNCPS version 6. have been revised to correlate with the proposed peNDF values of Mertens (1997). The NRC (2001), however, is of opinion that the application of this concept is limited due to the lack of established requirements and standard, validated methods to measure effective fibre of feeds. This precluded NRC (2001) from establishing specific recommendations. Although the NRC (2001) does not use peNDF *per se*, it does acknowledge the importance of particle size through the use of forage NDF. A number of studies on the effects of peNDF on feed intake, digestibility, and milk production and composition have also been conducted (Yansari et al., 2004; Yang & Beauchemin, 2005) that produced inconclusive results due to differences in measuring peNDF. However, recently several authors (Yang & Beauchemin, 2006; Yang & Beauchemin 2007b) validated the use of the original Penn State Particle Separator (PSPS) with 3 sieves (19-, 8- and 1.18mm) to provide a better description of the variation in dietary physical effectiveness and the potential of the diet to promote chewing and prevent ruminal acidosis.

The CNCPS uses the NDF content of the diet and physical properties of the NDF to predict pH. The eNDF was found to be an adequate predictor of rumen pH (Pitt *et al.*, 1996), namely rumen pH = $5.425 + 0.04229 \times \%eNDF$ for eNDF < 35% DM; ($r^2 = 0.52$).

6.3.3 Dry matter intake (DMI)

The CNCPS calculates nutrient supply from actual DMI, or empirical equations are provided to predict DMI when intake is unknown. Equations specifically developed for each production class are used (Fox *et al.*, 2004). DMI is adjusted for the effect of the environment in which the cattle are housed (Tylutki *et al.*, 2004). Other factors affecting predicted DMI include body weight, breed, age, diet energy density, milk production, pregnancy, body fat, humidity and other lot conditions (Tylutki *et al.*, 2004).

Research has demonstrated that the CNCPS intake model predicted DMI to within 2% of actual values (Kolver *et al.*, 1998; ADAS, 1998). Much emphasis is placed on adequate information on feed composition and DMI, for the accurate prediction of nutrient requirements and performance under specific conditions (Fox *et al.*, 2000; Fox *et al.*, 2004).

6.3.4 Predicting supply of energy and protein

The advantage of mechanistic models such as the CNCPS is that they are more applicable to a variation of feeds and account for more sources of variation, than do single-component equations (Weiss, 1998). The CNCPS has been used successfully on beef and dairy cattle farms to evaluate and formulate rations (Fox *et al.*, 2004; Tylutki *et al.*, 2007). Several researchers assessed the ability of the CNCPS to predict lactation performance in dairy cows (Stone, 1996; Ruiz *et al.*, 2001; Ruize *et al.*, 2002; Fox *et al.*, 2004; Recktenwald & Van Amburgh, 2006; Tylutki *et al.*, 2007). In an evaluation with individually fed dairy cows, the CNCPS accounted for 90% of the variation in actual milk production with a 1.3% bias (Fox *et al.*, 2004).

CHAPTER 3

THE NUTRITIVE VALUE OF SOUTH AFRICAN *MEDICAGO SATIVA* L. HAY

1. INTRODUCTION

Lucerne (*Medicago sativa* L.) hay is a very important roughage source for livestock in South Africa (Van der Merwe & Smith, 1991). Grönum *et al.* (2000) estimated that approximately 3.8 million ton of lucerne hay is produced annually in South Africa. A large proportion of this lucerne hay crop is utilised by the dairy industry. Consequently, its nutritive value for especially dairy cattle is important.

According to Blaxter (1964) nutritive value is the result of chemical composition, digestibility and intake per unit time by an animal. Linn (1992) mentioned that high quality feeds should have in addition to a high nutrient content, a consistent nutrient content, high nutrient availability, absence of mould and other substances, adequate physical characteristics as in the case with roughages to stimulate rumination, readily consumed by animals that results in production meeting or exceeding expectations. Currently most end users of lucerne hay are still purchasing their lucerne hay by means of a subjective evaluation criteria (free from objectionable odours,-dust and mould; absence of foreign material; leafiness; green colour), whereas actual nutritive value should be the main criteria. Several objective measurements (single or combined chemical analysis) could however be a more accurate indication of nutritive value.

Lucerne hay quality (nutritive value) varies considerably and is influenced by factors such as, harvesting at specific physiological stages, climatic factors, edaphic factors such as soil conditions, leaf losses during haymaking, storage and feeding practice, diseases and insects, weeds, lucerne cultivar, moisture content during storage, water supply and fertilisation (Bezeau & Sonmor, 1964; Hanson *et al.*, 1988; Putnam *et al.*, 1997). Furthermore lucerne hay grown on many farms in South Africa are not from pure stands but are mixtures of lucerne and grasses of botanical composition (*Cynodon Dacylon* L.; *Ornithogalum spp.*; *Moraea spp.*) that is unknown or difficult to estimate. Given that South African dairy rations could contain up to 40% lucerne hay, milk production variability can be partially related to lucerne hay. Therefore,

variation in quality of South African lucerne hay hampers the accurate formulation of ruminant diets especially for dairy cattle.

Van Wyk *et al.* (1955) published the first nutritive values for lucerne hay in South Africa, based on only 38 samples. This was followed by Van der Merwe (1970) who classified the quality of lucerne according to stage of harvesting (bloom stage). Van der Merwe (1970) emphasised that the nutritive value of roughages can vary considerably. Therefore their values (crude protein, crude fibre, fat) can serve at best as a general guideline in diet formulations and should be verified by actual analysis. These values also do not include later developments in nutrition like analysis for acid detergent fibre (ADF), neutral detergent fibre (NDF) and the extent of protein and fibre degradation. Mertens (1992), McDonald *et al.* (1995) and NRC (2001) reported values in this regard. The only South African particulars in this regard in the available literature is that of Erasmus *et al.* (1990), one average based upon three samples from various locations. In addition, information is also lacking on nutritional value of South African lucerne hay with regards to nutrient fractions required for modern diet formulation programs including National Research Council (NRC) (2001) and the Cornell Net Carbohydrate and Protein System (CNCPS) (Tylutki *et al.*, 2007). Information is required on protein and carbohydrate fractions, detailed nutrient composition, predicted energy values and rumen degradability. This clearly illustrates the urgent need for more reliable data on the nutritive value of South African lucerne hay.

The objective of this study was to evaluate the variation- and expand the existing and limiting nutritive value database of lucerne hay in South Africa.

2. MATERIALS AND METHODS

2.1 Sampling

During 2004 and 2005, samples of lucerne hay (n = 600) (*Medicago sativa* L.) were collected from 600 cuttings at different times in the season (September 2004 to April 2005) from several commercial irrigation farms in the main lucerne producing areas (sites) in South Africa (Cradock, Douglas, Groblershoop, Hartswater and Vaalharts irrigation scheme, Hopetown and Upington). These sites represent a wide range soil characteristics (texture, organic matter, N and P content, pH) and farm management practices. The objective was to obtain as wide as possible range of samples that would vary more in chemical and digestible properties than

would lucerne sampled from a distinct area, thus representing the South African lucerne population. The origin of lucerne hay samples for chemical analysis and estimates of digestibility are shown in Figure 1.
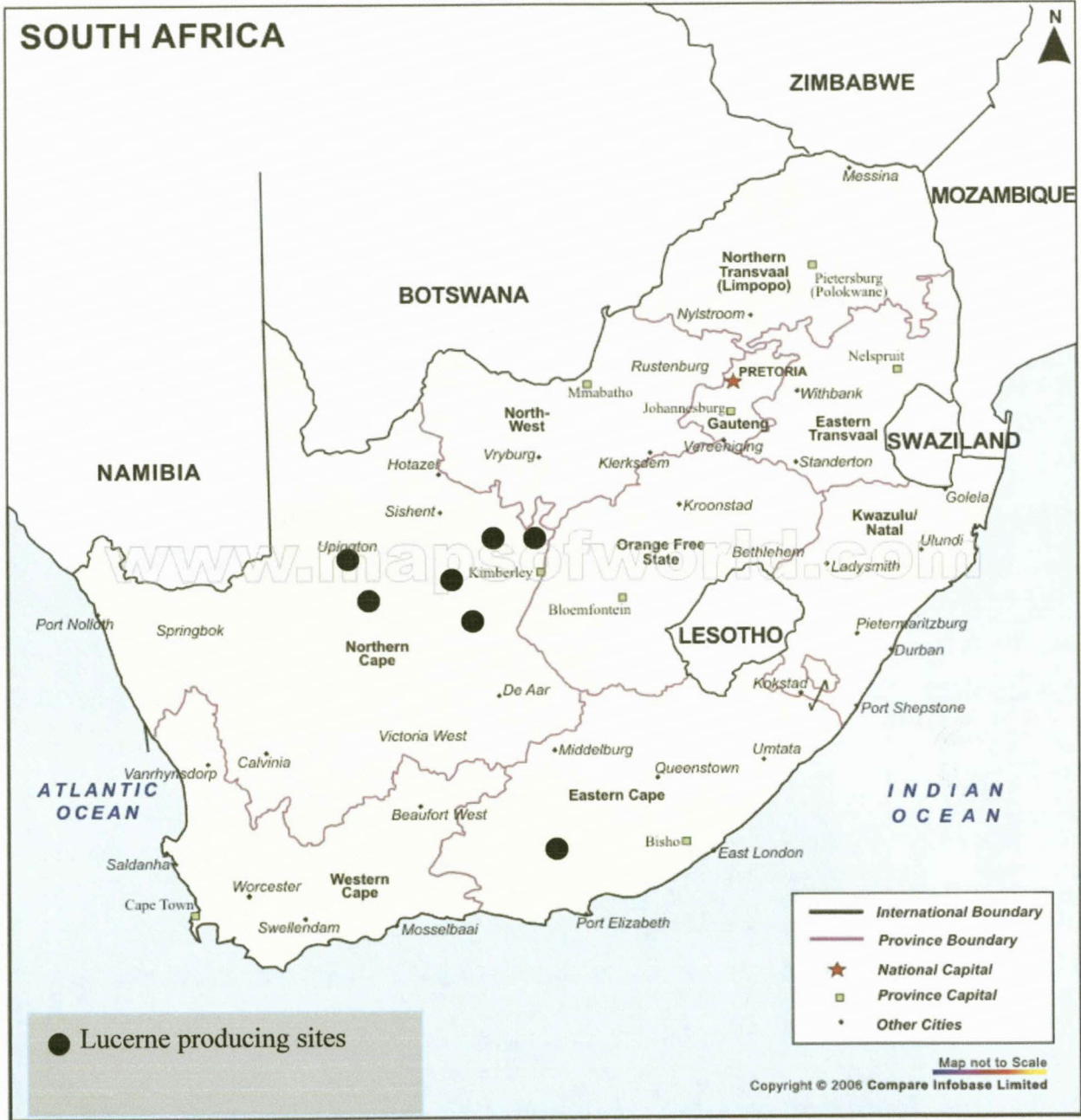
Figure1. **Origin of lucerne hay samples (Mapsofworld, 2006)** (www.mapsofworld.com)

Each lucerne hay sample was a composite of 20 core samples randomly collected, from a lot, with a LTC (Lucerne Tech Consult CC., Hopetown, South Africa) forage core sampler to ensure a statistically valid sample (Martin *et al.*, 1992). A lot included the same species,

variety, cutting, land and time (Putnam, D.H., 2005, Pers. Comm., Dept. of Agronomy and Range Science, University of California, Davis, CA 95616, USA).

The 20 cores of each lot were thoroughly mixed and milled under controlled conditions through a 1-mm screen using a LM 3100 mill (Perten Instruments AB, Huddinge, Sweden). Representative duplicate samples were obtained using a sample splitter (Perten Instruments AB, Huddinge, Sweden) with 12 flutes of 15 mm slot sizes (Perten Instruments AB, Huddinge, Sweden). The samples were sealed in airtight containers and stored in a refrigerator at 3°C for subsequent near infrared reflectance spectroscopy (NIRS) scanning and chemical analysis.

2.2 Chemical analysis

The required number of samples to represent the South African population was selected by scanning the 600 milled lucerne hay samples with a NIR-System 5000 monochromater (NIR Systems, Silver Spring, MD, USA) resulting in 168 samples representing a wide spectral range. These 168 samples were analysed by Cumberland Valley Analytical Services, Inc., (Maugansville, Maryland, USA) by means of wet chemistry. Crude protein (CP), ash and acid detergent fibre (ADF) contents were determined by means of the procedures described by AOAC (2000). Dry matter (DM) were determined by a two-step process: First step: Partial dry matter adapted from Goering & Van Soest, 1970, second step: Modified to 105°C for 3 hours as described by the National Forage Testing Association recommendations, 2002 (Ward, R.T., 2005, Pers. Comm., Cumberland Valley Analytical Services, Inc., 14515 Industry Drive, Hagerstown, MD 21742, USA). Neutral detergent fibre (NDF) and acid detergent lignin (ADL) were determined according to the procedures of Goering & Van Soest (1970) with a modification to the NDF procedure, which exclude sodium sulphite (neutral detergent solution) and includes ∞-amylase to hydrolyse the starch component (Dowman & Collins, 1982). Soluble protein (SP) was determined by the borate-phosphate procedure as detailed by Krishnamoorthy et al. (1982). Acid detergent insoluble crude protein (ADICP) and neutral detergent insoluble crude protein (NDICP) were determined by measuring the CP content of the ADF and NDF residue respectively using a Leco FP-528 Nitrogen Combustion Analyzer (Leco. 3000 Lakeview Avenue, St. Joseph, MI 49085). Fat (EE) was analysed according to the method described by the AOAC (1990). Starch was determined by a modification of the method of Holm et al. (1986) as cited by Hall (2000) and sugar by Dubois et al. (1957), as

cited by Hall (2000). Metals (Fe, Mn, Zn, Cu) and minerals (Ca, P, Mg, K, Na, S, Cl) were analysed by means of the procedures described by AOAC (2000).

Cellulose and hemi-cellulose concentrations were estimated as the difference between ADF and ADL concentrations and NDF and ADF concentrations respectively. Dietary cation-anion difference (DCAD) was calculated using the equation as described by Beede *et al.* (1992).

$$DCAD = (\%Na/0.023 + \%K/0.039) - (\%Cl/0.0355 - \%S/0.016) \qquad \text{(eq. 20)}$$

2.3 Nutrient fractions

The carbohydrate (CHO) and crude protein (CP) sub-fractions were fractionated by physical or chemical methods according to the Cornell Net Carbohydrate and Protein System version 6 (CNCPSv6) principles (Tylutki *et al.*, 2007). Table 1 summarised the analytical methods and calculations used to determine the carbohydrate and protein nutrient pools.

All chemical analysis was performed in duplicate and the results expressed on a dry matter basis.

2.4 *In vitro* digestibility

In vitro organic matter digestibility at 48 hours (IVOMD48) was estimated using the two-stage rumen fluid-pepsin technique described by Tilley & Terry (1963). For the estimation of *in vitro* organic matter digestibility at 24 hours (IVOMD24) the technique was however modified to 24 hours without sulphite (neutral detergent solution) or pepsin/hydrochloric acid (HCL) treatment, but with the inclusion of amylase (Ward, 2005, Pers. Comm.). Rumen fluid was collected from several early to mid lactation cows consuming a high production total mix ration (TMR) of lucerne haylage, maize silage and high moisture shelled corn (HMSC) (Ward, 2005, Pers. Comm.). The 24 hours and 48 hours *in vitro* digestibilities of NDF (NDFD24 and NDFD48, respectively) were determined using the modified Tilley and Terry procedures as described above.

Table 1   **CNCPSv6 carbohydrate and protein nutrient pools[a]**

Item	Pool[b]	Units	Calculation or analysis method
Acetic, propionic, butyric	CA1	%DM	HPLC
Lactic	CA2	%DM	HPLC
Other organic acids (OAA)	CA3	%DM	Book values
Sugar	CA4	%DM	Par. 2.2
Starch	CB1	%DM	Par. 2.3
Soluble fibre	CB2	%DM	NFC-CA1-CA2-CA3-CA4-CB1
Neutral detergent fibre (NDF)	CB3	%DM	NDF-NDFCP-CC
Lignin	CC	%DM	(NDF x Lignin x 2.4)/100
Non-protein nitrogen (NPN)	PA	%DM	NPN x (SolCP/100) x (CP/100)
Soluble protein	PB1	%DM	SolCP x CP/100 - PA
Complex protein	PB2	%DM	CP - (PA - PB1 - PB3 - PC)
Neutral detergent fibre-crude protein (NDF-CP)	PB3	%DM	NDFCP- ADFCP
Acid detergent fibre-crude protein (ADF-CP)	PC	%DM	ADFCP

[a] Description of Cornell Net Carbohydrate and Protein System (CNCPS) pools adopted from Tylutki *et al.* (2007)

[b] Characterisation of carbohydrate fractions are as follows: *CA1 (acetic, propionic and butyric acids), CA2 (lactic acid), CA3 (other organic acids), CA4 (sugars) (degradation rates of 0%, 5%, 3% and 40-60% per hour for CA1, CA2, CA3, and CA4, respectively. Fraction CB1 (starch (degredation rate of 20-60%/h)), CB2 (soluble fibre) (degradation rate of 20-60%/h). Fraction CB3 (available NDF) that has a slow degradation rate of 3-6%/h and fraction CC (indigestible NDF) (degradation rate of 0%/h). The passage rate equations were adopted from Seo et al. (2006).*

Protein was sub-divided into: fraction PA, PB1, PB2, PB3 and PC. Fraction PA is non-protein nitrogen (NPN), which is rapidly degradable with an assumed degradation rate to be affinity. Fraction PB is true protein (TP), which is further sub-divided into three fractions PB1, PB2 and PB3, which have different rates of degradation in the rumen (Sniffen et al., 1992.). Fraction PB1 is peptides which is rapidly degradable with a degradation rate of 120-400%/h. Fraction PB2 is the difference between buffer insoluble protein and fraction PB3 which is fermented in the rumen at a lower rate than PB1, thus intermediately degradable with a degradation rate of 3-16%/h, and some could escapes to the lower gut. Fraction PB3 is believed to be the slowest degradable of the three PB fractions with a degradation rate, for forages, equal to fraction CB3 (3-6%/h). Because of its association with plant cell walls, PB3 to a large extend escapes to the lower intestines. Fraction PC is the ADFCP which is considered to be resistant to microbial and the animal enzymatic breakdown, thus un-degradable and unavailable to the animal. All passage rate equations were adopted from Seo et al. (2006)

According to Mould (2003) the use of the neutral detergent solution in the *in vitro* procedure is meant to remove any contamination, however, the sulphite has the potential to remove

undegraded substrate that leads to overestimation of degradation, especially when using short incubation periods. For this reason Ward (2005, Pers. Comm.) excluded the use of the neutral detergent solution in the modified *in vitro* technique. The exclusion of the pepsin/HCL treatment was due to hypothesising that bacterial contamination was non-significant.

All *in vitro* procedures were performed in triplicate and the IVOMD results were expressed on a dry matter (DM) basis, whereas NDFD was expressed as a percentage of NDF.

2.5 Energy units

Metabolisable energy (ME), ME from fat (ME$_{fat}$) and fermentable metabolisable energy (FME) were estimated using equations from McDonald *et al.* (1995), while estimated energy content for total digestible nutrients at 1X maintenance (TDN$_{1x}$), digestible energy at 1X maintenance (DE$_{1x}$), digestible energy at production level of intake (DE$_p$), metabolisable energy at production level of intake (ME$_p$) and net energy for lactation at production intake (NEl$_p$) were estimated from the NRC (2001) model. In this approach the prediction of TDN$_{48}$, NDFD determined at 48 hours by the *in vitro* procedure was used to calculate truly digestible NDF (tdNDF), whereas TDN$_{lig}$ was predicted using lignin to calculate tdNDF (NRC, 2001). The total track digestible organic matter (TTDOM) was calculated according to the NRC (2001) equation (Appendix A).

2.6 Statistical analysis

Statistical analyses were performed using SAS 9.1.3 Service Pack 4 (2002-2003). Descriptive statistics namely the mean, standard deviation (SD), coefficient of variation (CV), minimum and maximum values were calculated for the quantitative variables.

3. RESULTS AND DISCUSSION

3.1 Dry matter and energy

The chemical composition and digestibility of the selected lucerne hay samples are presented in Table 2

Table 2   **Chemical composition and digestibility of 168 SA lucerne hay samples**[a]

Chemical Analysis	Minimum	Mean	Maximum	SD[b]	CV[c]
Dry matter (%)	86.46	92.69	94.42	1.1	1.19
Organic matter (%)	70.48	87.03	92.76	3.55	4.08
Acid detergent fibre (%)	21.26	33.22	47.28	4.53	13.62
Neutral detergent fibre (%)	28.89	44.06	65.93	6.79	15.41
Lignin (%)	4.32	7.35	16.25	1.69	23.03
Cellulose (%)	16.29	25.87	36.44	3.47	13.4
Hemicelluloses (%)	5.26	10.84	19.86	3.3	30.43
Pectin (%)	2.41	11.11	16.16	2.65	23.85
Sugar (%)	1.5	5.71	10.3	1.55	27.14
Starch (%)	1.09	2.03	3.94	0.57	28.13
Non-fibre carbohydrate[d]					
NFC_{NRC}[d] (%)	6.22	25.13	36.11	5.76	22.92
NFC1[d] (%)	0.0	20.37	34.23	7.33	35.98
NFC2[d] (%)	5.35	24.65	35.84	5.88	23.85
NFC3[d] (%)	6.28	25.55	36.7	5.86	22.94
CA4[e] (sugar) (%)	1.5	5.71	10.3	1.55	27.14
CB1[e] (starch) (%)	1.09	2.03	3.94	0.57	28.13
CB2[e] (soluble fibre) (%)	0.0	12.63	23.26	6.3	49.9
CB3[e] (available NDF) (%)	7.26	22.14	38.55	4.51	20.39
CC[e] (indigestible NDF) (%)	10.36	17.64	39	4.06	23.03
Fat (%)	1.18	1.83	3.14	0.32	24.44
$NDFD_{24}$[f] (% NDF)	26	35.87	54.06	4.71	13.13
$NDFD_{48}$[g] (% NDF)	30.93	41.57	62.21	5.13	12.34
$NDFD_{lig}$[h] (% NDF)	11.2	16.5	26.84	2.84	17.22
IVDMD[i] (%)	58.03	74.22	84.85	4.76	6.41
$IVOMD_{24}$[j] (%)	43.5	59.88	69.26	4.45	7.44
$IVOMD_{48}$[k] (%)	45.2	61.36	73.19	5.38	8.76
TTDOM1[l] (%)	49.8	52.8	102.6	9.4	17.80
TTDOM2[m] (%)	31.4	54.32	64.1	5.1	9.39

[a] All values are based on a DM basis; [b] Standard deviation; [c] Coefficient of variation; [d] Different non-fibre carbohydrate (NFC): NFC_{NRC} (NRC, 2001), NFC1 (Mertens, 1992), NFC2 (Van Soest et al., 1991) and NFC3 (Wang et al., 2001); [e] Carbohydrate fractions; [f] Neutral detergent fibre digestibility measuring in vitro rumen digestibility of NDF after 24 hours; [g] Neutral detergent fibre digestibility measuring in vitro rumen digestibility of NDF after 48 hours; [h] Neutral detergent fibre digestibility calculated by using lignin and NDF content of lucerne hay as primarily independent variables (NRC, 2001); [i] In vitro dry matter digestibility; [j] In vitro organic matter digestibility measuring in vitro rumen degradability of OM after 24 hours; [k] In vitro organic matter digestibility measuring in vitro rumen degradability of OM after 48 hours; [l] Total track digestible organic matter calculated by using Neutral detergent fibre digestibility after 48 hours; [m] Total track digestible organic matter calculated by using lignin

3.1.1 Dry matter

The moisture content of lucerne hay must be within a specific range for effective storage. Hay stored too wet (above 16%) will undergo pronounced fermentation with the production of heat (Bath & Mable, 1989). The feeding value may be greatly decreased because of mould or the loss of nutrients that occurs during extensive fermentation .

According to Table 2 the average dry matter (DM) content of lucerne hay in the present study was slightly higher than the approximately 90% reported by Morrison (1961), Van der Merwe & Smith (1991) and NRC (1996). Scholtz (2001) reported a corresponding average of 93% DM. According to Morrison (1961) the primary objective in haymaking is to dry the green plants to such an extent that the hay can be safely stored without heating unduly or becoming mouldy. Lucerne DM disappears in the heating process, reducing yield as well as digestibility. Heating above 52 °C will reduce the digestibility of protein, fibre and carbohydrate compounds (Bath & Marble, 1989). They also suggested that mouldiness and odours that decreases palatability are usually associated with lucerne that has been baled too wet and that subsequently heated to 47 °C to 52 °C, whereas hay that has been heated to 55°C to 60°C usually turn brown, and black when heating exceeds 66 °C. Hanson et al. (1988) mentioned that high-moisture lucerne hay (above 20% moisture) causes damage like heating, moulding and respiration losses of soluble carbohydrates and recommended a safe upper limit moisture content of 15%. However Smith et al. (1996) are of the opinion that the upper moisture level depends on the type of hay, density and size of bale, drying conditions after baling and other factors and recommended 18% for lucerne hay. In the present study the highest moisture content recorded (Table 2) was safely below the critical moisture level. Therefore heat and mould damage were unlikely to occur. However, in a preliminary study conducted by Scholtz & Van der Merwe (2007; -unpublished data) significant moisture losses of up to 46% (original moisture content) were detected during the milling process. Therefore the moisture values in Table 2 are probably not representative of the actual values before grinding.

3.1.2 Ash

Ash is an approximate measure of the mineral content and other inorganic matter that remains as post-combustion residue. The ash content may, however, contain material of organic origin such as sulphur and phosphorus from protein. McDonald et al. (2002) reported some loss of

volatile material in the form of sodium (Na), chloride (Cl), potassium (K), phosphorus (P), and sulphur (S) during the ignition process.

The mean ash content of lucerne hay in Table 3 was similar to the value reported by NRC (1989). Various researchers (Van der Merwe & Smith, 1991; Mertens, 1992; NRC, 2001) reported slightly lower values ranging from 7.5–11% ash. In the current study the ash content of lucerne hay averaged 12.97% and ranged from 7.25% to 29.52% (CV = 27%). The wide range observed in this study may be explained by soil contamination (exogenous ash) due to harvest practices (Abreu & Bruno-Soares, 1996). This is confirmed by Bath & Marble (1989), which noted that values above 14% for lucerne hay are usually an indication of soil contamination. Thus, it is evident from the data set that 24% of the samples in the present study were prone to soil contamination. Morrison (1961) suggested that lucerne hay has a higher mineral content than grains like maize and wheat. This is because of the accumulation of minerals in the leaves during growth, soil washed onto the growing plants by rain, and dust settling on the roughage before it is stored. Since ash has zero energy content, it is normally subtracted from an energy calculation, such as total digestible nutrients (TDN) (Weiss, 1993; Mooney, 2002). Thus using the average ash concentration instead of actual ash may increase error by up to 16 TDN units in the present study. Hoffman (2003) stressed the importance of ash measurement in predicting the energy value of forages. Accordingly the elevated maximum ash value of 29.52% in this study could have a detrimental effect on the energy value of such a hay lot. Ward et al. (1957) confirmed earlier studies, which showed that lucerne ash could stimulate the digestion of low quality roughage. This will however not apply for soil ash presenting lucerne hay

According to Putnam (2005, Personal Comm.), ash has been shown to range up to 10 % or more at any given fibre and may be of real value in predicting animal performance. The ash value is an indication of hay lots with a significant soil contamination and/or hays with above-normal mineral concentration (Putnam, 2004). Compared to grasses, lucerne hay has a rich mineral profile.

Table 3   **Mineral content and dietary cation-anion difference of 168 lucerne** *Medicago sativa* **L. samples**[a]

CHEMICAL ANALYSIS	Minimum	Mean	Maximum	SD[b]	CV[c]
Ash (%)	7.25	12.97	29.52	3.55	27.39
Dietary Catio-Anion Difference (meq/kg)	-1.30	26.70	59.91	13.41	50.23
Ca (%)	0.64	1.35	2.12	0.26	18.97
P (%)	0.15	0.30	0.42	0.05	17.18
Mg (%)	0.23	0.41	0.98	0.10	25.24
K (%)	1.06	2.53	4.27	0.64	25.11
Na (%)	0.05	0.25	0.71	0.12	49.16
S (%)	0.18	0.30	0.43	0.05	15.20
Cl (%)	0.32	1.07	1.95	0.36	33.90
Fe (ppm)	149	874	3138	584	67
Mn (ppm)	30	57	153	21	37
Zn (ppm)	23	36	75	8	23
Cu (ppm)	2	7	39	3	45

[a] All values are based on a DM basis

[b] Standard deviation

[c] Coefficient of variation

With respect to macro minerals in lucerne hay (Table 3), the sodium (Na) value varied the most, followed by chloride (Cl), manganese (Mg) potassium (K) calcium (Ca), phosphate (P) and sulphur (S). According to Hanson *et al.* (1988) Ca and Mg concentrations in lucerne hay are greater than for grasses at equivalent stages of maturity. The effect of low Ca diets on calcium homeostasis is well established and has been reviewed in depth (Goings *et al.*, 1974; Green *et al.*, 1981). According to Lean *et al.* (2006) the optimal dietary concentration in pre-partum diets to lower risk of milk fever is contentious. Oetzel (2000) and Thilsing-Hansen *et al.* (2002) noted that the practice of feeding very low levels of calcium pre-partum, <20 g/d, is effective in controlling hypocalcemia, whereas Lean *et al.* (2003) as cited by Lean *et al.* (2006) suggested a pre-partum intake of 60 g/d. This contrasts somewhat with the results of Goff & Horst (1997) who proposed that observed benefits of lowering dietary Ca pre-partum to approximately 50 g/d in preventing milk fever were, in part, the result of a reduction of dietary K rather than a stimulatory effect of low dietary Ca on calcium homeostasis. Thus, Goff (2000) concluded that calcium concentration in pre-partum diets had little influence on the incidence of milk fever when fed at levels above the daily requirements, approximately 30g of Ca/d.

Recently Lean *et al.* (2006) evaluated this phenomenon and concluded that an increase in Ca concentration from 0.5 to 1.0% in the pre-partum diet would increase the risk by 327%. The mean Ca value in Table 3 was slightly lower than the 1.47% reported by NRC (2001). Accordingly, it is evident that dry cows fed diets containing 100% lucerne hay, consuming at least 200 g of Ca/d, would be destined for incidences if milk fever.

The interrelationships among K, Na, Cl and S and its contribution to the dietary cation-anion difference (DCAD) (Eq. 20) of dairy diets are well documented (Beede *et al.*, 1992; NRC, 2001; McDonald *et al.*, 2002;). DCAD is an important barometer for the risk of milk fever and of the cow at parturition (Beede, 2003; Lean *et al.*, 2006). Oetzel (2000) recommended a dietary DCAD of approximately -15 meq/kg of DM. The DCAD (eq.20) for lucerne hay in the data set ranged from -1.3 to 59.91 meq/kg of dietary DM, averaging 26.7 meq/kg. The variation (CV = 50.23 %) of DCAD illuminates the importance of routine analysis of lucerne hay with regards to the acid-base balance of pre-partum dairy diets. In this regard the high K content of lucerne hay, which plays a mayor role in the dietary anion-cation balance of dairy diets is of great importance. Nearly all of the dietary K is absorbed by the cow, making it a very intoxicating alkalinising cation (NRC, 2001). Cows fed diets high in K and Na are in a relative state of metabolic alkalosis, which increases the probability of hypocalcemia. Recent advances in dry cow nutrition have clearly demonstrated the value of lowering the K intake in cows pre-partum (Goff, 1999). As a result, most nutritionists attempt to limit dietary K concentrations or acidify the cow via negative DCAD balances, however, these targets are not well defined (NRC, 2001). Lucerne and other legumes are known to accumulate K within their tissues to concentrations that are well above the requirements for optimal plant growth (between 1.7 and 2.0 % DM) (Stokes, 2002).

The average K content in the current study (Table 3) was lower than the mean values of 3.1% and 3.05% reported by Lanyon (1980) and Kelling *et al.* (2002) respectively, and resembled that of the NRC (2001) (2.37%). However, the maximum value corresponded with the highest value reported by Lanyon (1980). Factors that might have contributed to the K status in lucerne hay are: cutting height, as K tends to accumulate in the stems rather than leaves (Rominger *et al.*, 1975); maturity, K content decreases with advanced maturity; rain, K is easily leached from the plant when rained on between mowing and baling/chopping (Kelling *et al.*, 2002): Over-fertilising with potassium results in luxury consumption by the crop and can be undesirable to the health of the pre-parturient dairy cow (NRC, 2001).

The mean Na and Cl content in lucerne hay in the current study (Table 3) was higher than the 0.1% and 0.65% respectively reported by NRC (2001). Whereas the mean S values corresponded to the tabular values of NRC (2001) and Berger (1990).

According to Van Saun (1991) the macro-mineral profile for a close-up dry cow diet should be as follow (DM basis): Ca (0.36-0.41%), P (0.22-0.25%), Mg (0.22-0.25%), K (0.7-0.8%), Na (0.12-0.15%), S (0.19-0.21%) and Cl (0.24-0.26%). Whereas, the NRC (2001) recommends the following macro-mineral status for a standard close-up diet (DM basis) (cows entering second lactation or): Ca (0.43%), P (0.3%), Mg (0.39%), K (1.35%), Na (0.16%), S (0.18%) and Cl (0.42%). Thus, in a diet containing 40% lucerne hay (DM), the mean Ca (135 g/d), K (253 g/d), and Na (25 g/d) contribution from lucerne hay in the present study (Table 3) would contribute an abundance of these macro-minerals to these specific diets. The knowledge of daily intake (g/d) of these minerals is of more importance than the mineral status of the diet itself, due to variability in DMI of different diets. Lean *et al.* (2006) however concluded that critical assessment and control of all dietary macro minerals, and not only DCAD of the pre-partum diet, is essential in the prevention of milk fever.

Regarding trace minerals iron (Fe), manganese (Mn), sink (Zn) and copper (Cu), the values in the current study were generally higher than the tabular values in NRC (2001) and McDonald *et al.* (2002), except for Cu which had a lower value in this study. A large variation and standard error (SE) occurred for Fe (Table 3). This could probably be attributed to soil contamination as some areas in South Africa are known for high soil (exogenous) Fe content (Groenewald, C.A., 2007, Pers. Comm., Divisional Director, Scinetic, Centre of scientific technology, a devition of Afgri operations. 252 Jean ave., Centurion, South Africa). The trace-mineral profile for a close-up dry cow diet should be as follow (Van Saun, 1991): Fe (60 ppm), Mn (50 ppm), Zn (50 ppm) and Cu (12-18 ppm). The average Fe content in Table 3 would contribute 5 times more Fe than required for a close-up dry cow diet. Of great concern is the interfering of excessive dietary Fe with the absorption of other minerals, primarily Cu and Zn, which could lead to depletion of these minerals in cattle.

3.1.3 Organic matter (OM)

Organic matter is the material that is available for the digestion process (Van de Merwe & Smith, 1991). Compared to grasses, lucerne has a higher % ash (Ward *et al.*, 1957) and thus

lower % organic matter content. Many organic compounds contain mineral elements as structural components. Protein, for example, contains sulphur; and many lipids and carbohydrates contain phosphorus (Van Soest, 1994). The OM content of lucerne hay in this study (mean = 87.03%) had a low CV (Table 2) and could the average value be regarded as a reliable OM guideline.

3.1.4 Carbohydrates

Figure 2 illustrates the terminology of plant carbohydrates based on different solubility for each fraction. All these carbohydrate fractions are also present in lucerne hay as indicated in Table 2.
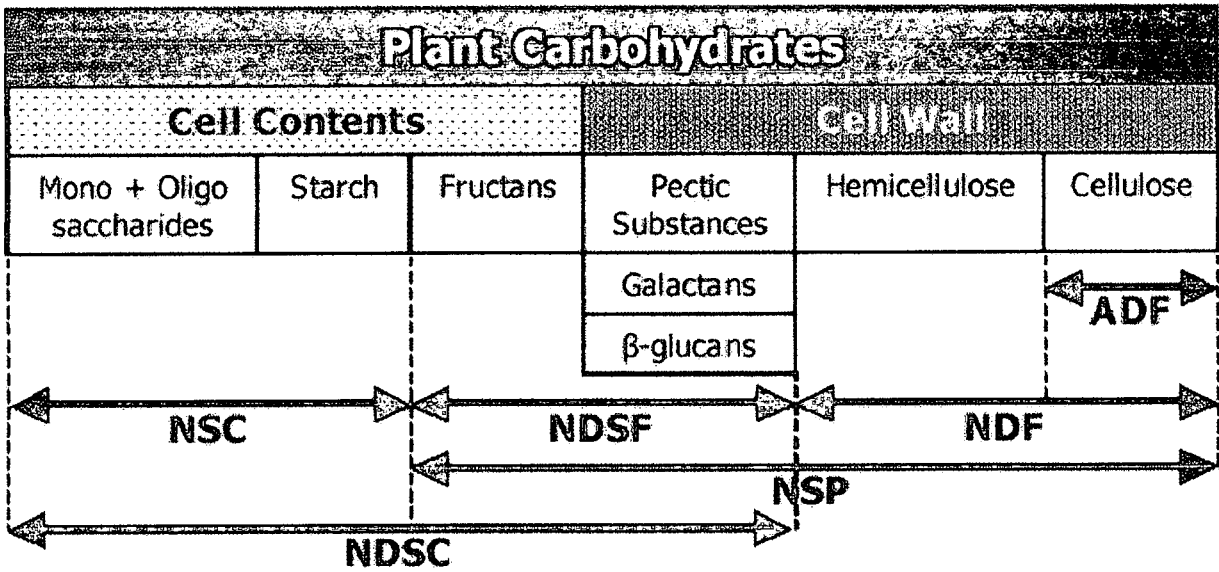
Figure 2   Plant carbohydrate fractions (Hall 2000)

Where:

NSC = Non Structural Carbohydrates

NDSC = Neutral Detergent Soluble Carbohydrates (also called Non-Fibre

Carbohydrates (NFC))

NSP = Non-Starch Polysaccharides

NDSF = Neutral Detergent Soluble Fibre

NDF = Neutral Detergent Fibre

ADF = Acid Detergent Fibre

Mono and Oligosaccharides are also called "Sugars"

NOTE: Lignin is present in ADF and NDF (not included here because it is not a

carbohydrate)

3.1.4.1   *Structural carbohydrates*

Structural carbohydrates are the physical stimulus that initiates rumination, thus playing an important role in maintaining the health and function of the rumen. According to Mertens (1992) structural carbohydrates are located in cell walls and provide the structural support needed for plants to grow upright. Plant cell walls (listed in the descending order of their degradability) consist of pectin, hemicellulose, cellulose and a non-carbohydrate polymer, lignin.

Pectin is unique in that it is rapidly fermentable (high k_d) resulting in 90-100% ruminal disappearance. Pectin, in contrast with hemicellulose, is not part of the cross-linked lignified matrix (Van Soest, 1982). Accordingly, it is the opinion of several researchers that pectin is best considered as a non-fibre carbohydrate similar to sugar or even qualify as its own entity (Van Soest *et al.*, 1991).

Several analytical techniques are available for the characterization of the structural carbohydrates (fibre fraction). The traditional measure for fibre according to the Weende analysis is crude fibre (CF) as described by Van der Merwe & Smith (1991). Crude fibre has been in use by nutritionist since the middle of the 19th century (Henneberg & Stohman, 1859 as cited by Hindrichsen *et al.*, 2006). Crude fibre is the residue remaining after successive boiling in dilute acid and alkali (NRC, 1989) and measures the cellulose and part of the lignin. A modified crude fibre (MCF), which includes the ash or mineral fraction, was used in some stages, notably in California during the 1950s, to evaluate lucerne (Baker & Ball, 2001). This test was developed in California as a more rapid test than the CF test. Hannaway & Ballerstedt (1988) proposed that MCF has a negative correlation with animal digestibility (more accurate than the standard CF analysis). However, according to several researchers (Van Soest, 1968; Lofgren & Warner, 1970, Mertens, 1982 and Robinson (1999) CF and its derivatives are an incomplete measure of the less digestible part of feeds as it fails to measure hemicellulose and most of the lignin. They suggest that acid detergent fibre and neutral detergent fibre are much more useful and accurate measures of the fibre component of feeds than are values for crude fibre. However, most countries still continues to require that CF levels be included in the guaranteed analysis of manufactured feeds (Tylutki, T.P., 2007, Pers. Comm., Agricultural

Modeling and Training Systems, LLC, 416 Davis Road, Cortland, NY 13045, USA). For these reasons, the lucerne hay was not analysed for CF.

a)   Acid detergent fibre

The ADF method (AOAC, 2000) has been used to evaluate the lucerne hay available energy. ADF is the plant fibre that remains after acid detergent removes part of the digestible cell wall material and the cell contents, and consists of cellulose, lignin, heat damaged proteins (ADF-N), and acid insoluble ash (Morse & Sedivec, 1990; Ball & Lauriault, 1999). In the United Kingdom the ADF method has been modified slightly (MADF) by increasing the duration of boiling and acid strength (McDonald *et al.*, 2002) to show a much greater ability to predict forage digestibility (Moss & Givens, 1990; Givens *et al.*, 1992b). According to McDonald *et al.* (1995) available data suggests that ADF is the measure of fibre that is the most highly correlated with the milk fat content. Thus for the purpose of the present study the lucerne hay was analysed for ADF.

Mean, minimum, maximum, standard deviation and coefficient of variation values for energy related analyses are shown in Table 2. These energy related analyses represents all chemical, digestibility or formula-based parameters associated with the energy value of lucerne. In the current study the mean ADF value for South African lucerne hay (Table 2) was almost identical to the values published by NRC (1996), NRC (2001) and Mertens (1992) and lower than the 39.98% and 37.5% reported by Erasmus *et al.* (1990) and McDonald *et al.* (2002) respectively. The variations in lucerne hay ADF values presented in Table 2 were larger than those found in the available literature. Maturity of the lucerne plant seems to have the biggest influence on the variation of chemical parameters and is presented as such in several textbooks (Van Soest, 1994; NRC, 1996). The larger variation found in the current study could be attributed to other management factors that influence the leave and/or inter-alia fibre content of the lucerne hay, and/or due to the robustness of the dataset. According to NRC (1996), ADF values ranged from 31.9% for lucerne hay at an early bloom stage to 38.7% at full bloom. Van Soest (1994) reported corresponding ADF values of 31% and 37% for lucerne hay cut at an early bloom- and full bloom stage respectively. Mertens (1992) published ADF values that ranged from 28.0% for early vegetative stage to 40.0% for full bloom.

b) *Neutral detergent fibre*

Robinson (1999) recommended the substitution of ADF with NDF as NDF is considered to be the more clearly defined cell-wall fraction that captures all the structural fibre. This is unlike ADF that only captures 70 to 85% of the structural fibre. McDonald *et al.* (1995) noted that NDF is the chemical component of foods that determines their rate of digestion the best. NDF is the plant fibre that remains after the removal of cell contents by neutral detergent solution. The NDF method was designed initially to isolate the insoluble dietary fibre components in plant cell walls (cellulose, hemicellulose and lignin) (Van Soest & Wine, 1967). While the NDF residue is often considered to represent these cell wall fractions, NDF is actually defined as a nutritional entity describing the variable and incompletely digestible portion of forages (Van Soest, 1994). Lucerne contains substantial amounts of pectin in their cell walls and is known to be solubilised and lost during neutral detergent extractions which result in NDF underestimating cell wall concentration of lucerne hay (Jung & Lamb, 2004). According to Mertens (1992) NDF is not a chemically pure entity, but represents substances (ADF fraction plus hemicellulose) in plants that are difficult to digest and break down into small particle sizes. Thus, incomplete pectin recovery in the NDF residue does not conflict with the nutritional definition of NDF.

Erasmus *et al.* (1990) found a mean NDF content of 42.8% for lucerne hay produced in South Africa. According to the results in Table 2 the mean NDF content of lucerne hay in SA is slightly higher. This also applies for a mean tabular value given by NRC (2001), namely 41.6%. The mean NDF value in the current study (Table 2) was close to the 45.3% and 43.9% value observed by Mertens (1992) and NRC (1996) respectively. In comparison with findings by Mertens (1992), this studies lucerne hay NDF exhibited higher variance. Results obtained by NRC (1996) indicated that NDF values ranged from 31.9% for hay cut at an early vegetative stage to 48.8% at full bloom. Mertens (1992) reported an even lower variation of 38.4% at early bloom and 52.1% at full bloom. The definition of a roughage source as proposed by Zinn *et al.* (2004) is a forage containing 35% or more NDF. Accordingly, several of the samples lower than the mean NDF in the SA population, technically won't fit this description (Table 2). As in the case of ADF, the larger variation of NDF values found in the current study could possibly be attributed to the influence of other factors than the cutting stage per se. The NDF content of stems could be as high as 70%, whereas leaf NDF content varies between 18 - 28% (Putnam, 2000). Therefore, excessive leaf loss during the harvesting process could have contributed to the variation. Another possible reason for the large variation

is the above normal temperatures recorded for the 2005 season, when the samples were collected. According to Putnam (2005, Pers. Comm.) forage of any species tends to be lower in quality if produced in warm region rather than cool region. This could also be partly explained by the change in leaf to stem ratio. In addition the high NDF values of lucerne hay found in the present study could restrict the inclusion level thereof in high yielding dairy cow diets. Allen (1991) recommended NDF levels between 25% and 30% in diet DM for high producing dairy cows, whereas Zinn *et al.* (2004) proposed a total of 29 to 32% NDF with the condition that 19% of dietary DM must be NDF from forage (NRC, 2001). Thus, inclusion of lucerne hay in these high producing dairy diets must contain a NDF content of 40% or less. Thus, only 33% of the South African population represented by Table 2 would fit this criterion, of which 8% of these samples contain less than 35% NDF, thus technically disqualified as a roughage, as previously noted by Zinn *et al.* (2004).

NDF content of the lucerne hay tended to vary more than ADF (Table 2), and probably can be accounted for by hemicellulose (CV = 30.4%) (the primary difference between ADF and NDF). From Table 2 it is evident that hemicellulose contributed approximately 25% to the average NDF content of lucerne.

c) ***Lignin***

Lignin may also be defined as non-carbohydrate substances that resist digestion (Van Soest, 1982). According to Van Soest (1994) lignin impacts the digestibility of the forage directly as indigestible material and indirectly as it inhibits digestion of chemically associated fibre. According to Hindrichsen *et al.* (2006) lignin has two main functions: it cements and anchors the cellulosic microfibrils and other matrix polysaccharides and stiffens the cell walls, thereby protecting them from rumen degradation and physical damage (Selvendran, 1984). Furthermore, the undigested lignin-polysaccharide residue acts as a ballast in the rumen and reduces forage intake (Waldo & Jorgensen, 1981). Thus, lignin tends to be the chemical component most commonly associated with the impairment of forage digestion (Van Soest, 1994). However, Van Soest (1994) pointed out that lignin has no effect on the digestibility of cell solubles (sugar and starch). Similar to ash, lignin also has theoretically no digestible energy (Weiss, 1998). Because lignin content of plant cell walls limits the availability of the polysaccharides (cellulose and hemicellulose) for enzymatic breakdown (Mansfield *et al.*, 1999), quantification of lignin is also of importance to lucerne quality models. Fox *et al.*

(2003) recommend the use of sulphuric acid determined lignin in the Cornell Net Carbohydrate and Protein System (CNCPS).

The mean lignin value (Table 2) of lucerne hay in the current study was almost identical to the tabular value of NRC (2001) and lower than the 9.9% DM reported by Yu *et al.* (2003). The minimum lignin value in the present study (Table 2) is slightly lower than the 5.3% of early vegetative cut hay, while the maximum value corresponds to the 17.0% of seeded cut hay given by the table values of Van Soest (1994). The minimum value in Table 2 could possibly be attributed to the presence of grass in the sample, because grass is known for its low lignin content. Variability in lignin was found to be the second highest (23%) of the fibre fractions.

Because of the complex nature of lignin, its relationship with NDF and the lack of a standardised procedure, lignin content when expressed on DM basis shows large variability relative to digestion (Mould, 2003). However, Van Soest (1993) reported that lignin when expressed on a fibre basis provided an improved association to digestibility across both legumes and grasses. Ward (2005) is of opinion that it is more descriptive to evaluate forage lignin in the context of NDF value, as lignin/NDF ratio (lignin as percentage of DM divided by NDF times 100), since digestibility is a function of the lignin-fibre interaction. Lignin content alone could be misleading, especially when comparing grass with legumes. For example 8.5% lignin is a much bigger concern in lucerne hay that test 35% NDF than grass hay that test 58% NDF due to the higher lignin content found in lucerne.

The average lignin/NDF (% of NDF) ratio in the current study (Table 4) was lower than the 18.26% ratio calculated from NRC (2001) values. Bethard (2002) is of opinion that forages with a lignin/NDF ratio larger than 18% often result in poor performance. Accordingly 20% of the samples evaluated in the current study were prone to poor rumen digestibility performance. However, the higher the association between lignin and fibre contents, the better its prediction of digestibility (Van Soest, 1994). This is in agreement with the findings of Traxler *et al.* (1998). In the present study lignin/NDF ratio (lignin as percentage of DM divided by NDF times 100) ranging more (Table 4) than the values calculated from the tabular values of Van Soest (1994), namely 14.7% for early vegetative stage to 24.8% for post bloom cut. The presence of grass in the samples of the current study could again have contributed to these differences.

Table 4   **Proportions of lignin, cellulose and hemicellulose of 168 South African** *Medicago sativa* **L. hay samples and their inter relationship to each other**

CHEMICAL COMPONENT	Minimum	Mean	Maximum	SD[a]	CV[b]
Lignin/NDF[1] (% of NDF[1])	8.8	16.7	27.5	2.4	14.4
Lignin/ADF[2] (% of ADF[2])	12.9	22.0	36.4	3.3	14.8
Cellulose/NDF[1] (% of NDF[1])	42.4	59.1	71.2	4.8	8.2
Cellulose/ADF[2] (% of ADF[2])	63.6	78.0	87.1	3.3	4.2
Hemicellulose/NDF[1] (% of NDF[1])	13.5	24.3	37.8	4.7	19.2
Lignin x 2.4/NDF[1] (% of NDF[1])	21.0	40.0	66.0	5.7	14.3
Lignin/Cellulose (Ratio)	0.2	0.3	0.6	0.1	21.4
Lignin/Hemicellulose (Ratio)	0.3	0.7	1.3	0.2	25.0
Hemicellulose/Cellulose (Ratio)	0.2	0.4	0.8	0.1	28.6

[a] Standard deviation

[b] Coefficient of variation

[1] Neutral detergent fibre

[2] Acid detergent fibre

A curvilinear relationship between lignin content of the NDF (Lig/NDF) and NDF digestibility has been reported previously (Van Soest, 1967; Jung & Vogel, 1986). This is in agreement with the results of Traxler *et al.* (1998). Lucerne fibre contains a high proportion of lignin relative to grasses resulting in low digestibility of NDF relative to grasses. However, due to lucerne's low fibre content (compared to grasses) and the rapid rates of digestion and passage, higher intake and digestibility could be expected (Van Soest, 1994; Martin & Mertens, 2005). Accordingly, the more grass present in the lucerne hay, the lower the lignin/NDF ratio would be. Lucerne hay grown on many farms in South Africa are not from pure stands but are mixtures of lucerne and grasses. The larger variation and lower mean of lignin/NDF (%NDF) found in the current study could probably be attributed to the influence of grass contamination other than the cutting stage per se.

The Cornell Net Carbohydrate and protein system (CNCPS) uses lignin to calculate unavailable NDF by using the equation, 2.4 times lignin concentration (Sniffen *et al.*, 1992). The value 2.4 was suggested by Chandler *et al.* (1980) as cited by Van Soest *et al.* (2005), based on long-term methane fermentations of 60-90 days on various waste materials (Van Soest *et al.*, 2005). The amount of NDF estimated by the 2.4 times lignin is assumed to be

obligately undegradable and thus have a rate of digestion of zero (Van Soest *et al.*, 2005). This means that a higher level of lignin decreases NDF availability, however it may also over predict the carbohydrate C fraction (CC) of feeds that are of low lignification (Fox *et al.*, 2003). The relative high variation (CV = 14.4) in NDF availability of lucerne hay in the present study can be observed in Table 4.

d)   Cellulose and hemicellulose

Cellulose and lignin are the primary components retained in the ADF residue, while hemicellulose is mostly estimated as the difference between the NDF and ADF concentrations of forages. Likewise, cellulose concentration is calculated as the difference between the ADF and acid ADL concentrations for a forage sample. Morrison (1980) demonstrated that ADF residue is contaminated with hemicellulosic sugar residues, while Theander & Aman (1980) reported a possible starch and protein contamination on the NDF residue which results in inaccurate estimates of both hemicellulose and cellulose concentration when calculated from detergent fibre residues. It is also well documented that ADL severely under estimates lignin content in forages (Hatfield *et al.*, 1994; Jung *et al.*, 1999; Hindrichsen *et al.*, 2006). Thus an expected over-estimated polysaccharide fractions (cellulose and hemicellulose) could be explained due to the fact that ADL values are used to calculate these fractions.

The mean cellulose and hemicellulose values for lucerne hay in Table 2 were generally in agreement with the values calculated from NRC (2001), and reported by Van Soest (1975). In the available literature the calculated cellulose and -hemicellulose fractions for lucerne hay ranged from 20.7% and 10% respectively for early vegetative to 29.6% and 14.0% cut past bloom (Van Soest, 1994), implying a much greater range for South African lucerne hay (Table 2).

The nutritional availability of cellulose and hemicellulose varies from total indigestible to complete digestible depending on the degree to which it is associated with lignin (Van Soest, 1994). Thus, a high lignin to cellulose (L/C) and lignin to hemicellulose (L/H) ratio is associated with low digestibility. The mean L/C ratio in the present study (Table 4) was slightly higher than the 0.24 reported by Van Soest (1976) as cited by Van Soest (1994) and similar to that of NRC (2001) (0.30). Van Soest (1976) as cited by Van Soest (1994) reported a L/C ratio that ranged from 0.18 to 0.3, which were much smaller than the observed range in the current study (Table 1). The mean L/H ratio in the present study was lower than the value

calculated from NRC (2001) values (0.86). The higher L/H ratio compared to the L/C ratio was expected because hemicellulose is more closely associated with lignin than any other polysaccharide (Sullivan, 1966) and is found mostly in lignified walls (Van Soest, 1994).

According to Van Soest (1994) availability of cellulose and hemicellulose could vary from total indigestibility to complete digestibility in some forage species. The maximum value of L/H in the present study confirms the possibility of total hemicellulose indigestibility in some lucerne hay samples. Overall these values suggested that cellulose and hemicellulose content in lucerne hay are to a large extent unavailable for digestion due to lignification. According to Van Soest (1994) digestibility of hemicellulose is directly related to that of cellulose and counter related to lignification. However, Van Soest (1994) is of opinion that cellulose possesses variability in nutritive quality (rate and extend of digestion) somewhat independent of lignification due to some intrinsic properties. Thus, it could be argued that the use of the lignin to ADF (%ADF) ratio (Table 4) for quality evaluation would be non significant due to the variability of cellulose quality and accordingly its large contribution to ADF in the present study (Table 4)

As expected, the hemicellulose to cellulose (H/C) ratio of the present study was lower than the ratio for grass species (0.67 to 1.24) and similar to the 0.39 ratio for lucerne hay reported by Van Soest (1973). NRC (2001) also reported a comparable H/C ratio for lucerne hay 0.37 thus, implying a relative constant H/C relationship between averages of lucerne populations.

From the results in Table 2 it is evident that the characteristic differences between and among the samples in the current study and values available in literature, with regard to lignin, cellulose and hemicellulose fractions, result in different relationships between lignin and digestibility.

3.1.4.2 Non-fibre carbohydrates

According to Mertens (1992) non-structural and structural carbohydrates refer to their function in plants. Sugars, starches, organic acids, and other reserve carbohydrates such as fructans, make up the non-structural carbohydrates (NSC) fraction and are major sources of energy for ruminants such as high producing dairy cattle (NRC, 2001). NSC and pectin are highly digestible and are generally increased in the diet at the expense of NDF to meet the energy demands of lactating dairy cows. Although the structural/non-structural classification of

carbohydrates is appropriate for describing plants, a slightly different classification of carbohydrates is needed to describe their nutritional characteristics (Mertens, 1992). They recommended that classification of carbohydrates into fibre or non-fibre fractions should be based on nutritional characteristics rather than on chemical composition or plant function. Non-fibre carbohydrates (NFC) represent the more rapidly digested fraction that includes pectins, starch and sugars. According to Hall (2005) the balance between fibre and NFC especially the profile of NFC (sugars, starch, pectins, etc.) has drawn more interest due to their influence on animal performance and digestion (fermentability) of other feeds.

Mertens (1992) suggested a convenient method of calculating NSC, which he termed non-fibre carbohydrates (NFC). NFC is calculated by difference as follows:

$$NFC_1\% = 100 - (CP + Fat + NDF + Ash) \qquad \text{(eq. 21)}$$

A variation in the classical calculation of NFC (eq.15) was proposed by Van Soest & Sniffen (1984) and Van Soest $et\ al.$ (1991) and recently adapted by the NRC (2001) namely:

$$NFC_2 = 100 - (CP + Fat + (NDF - NDF\text{-}CP) + Ash). \qquad \text{(eq. 22)}$$

Although the original NFC_1 equation is most commonly used, the alternative equation is preferred, because it corrects for CP in NDF and avoids subtracting NDF-CP twice (as part of CP and as NDF-CP). Van Soest & Sniffen (1984) stated that the insoluble protein in NDF is the slowest to be degraded and should therefore be excluded. However, this difference may be an analytical artefact given the absence of sodium sulphate and amylase in their NDF measurements. AOAC (2006) approved Mertens NDF method that includes sodium sulphate and amylase. This method removes most of the nitrogen from NDF. NSC is measured by enzymatic methods (Smith, 1969; Smith, 1981; Sarvar $et\ al.$, 1992) and is a distinct fraction. In some earlier studies by Van Soest $et\ al.$ (1991) and Sniffen $et\ al.$ (1992) no distinction between NSC and NFC was recognized. Smith (1992) proposed that NSC and NFC are interchangeable terms. This finding was in agreement with that of Sniffen $et\ al.$ (1992) which found that the calculated NFC is usually in close agreement with direct measured NSC. However several researchers (Mertens, 1988; Hoover, 1996 as cited by Bethard, 1997; Varga & Kononoff, 1999) reported that the concentrations of NFC and NSC are not equal for many feeds and the terms should not be used interchangeably. According to Bethard (1997) NFC

and NSC for lucerne hay were not significantly (P>0.05) correlated. Varga & Kononoff (1999) found a significant (P<0.001) difference between NFC (22% DM) and NSC (12.5% DM) for lucerne hay. Similar results for lucerne hay have been previously reported by Casper *et al.* (1990). Much of the difference is caused by the contribution of pectin and organic acids (Sniffen *et al.*, 1992; NRC, 2001). Hall (1998) found pectin in lucerne hay to represent roughly half of the NFC fraction. According to Martin & Mertens (2005) lucerne hay stems contain 10 - 12% pectin as a component of the cell wall. Jung & Engels (2002) indicated that pectin content of lucerne cell walls declined as the stems mature and cell wall concentration increases. Although a component of cell walls, pectin has some very desirable nutritional characteristics. According to Van Soest (1982) and Hall (1994) pectins are rapidly fermented in the rumen. According to Fox *et al.* (2000) pectin are more rapidly degraded than starches (Fox *et al.*, 2000) and support a microbial yield similar to starch (Schroeder, 2006), but their fermentation is depressed at low pH. During fermentation it appears to yield more acetate than other NFC (Strobel & Russell, 1986), tends not to depress ruminal pH, and barely ferments when ensiled. Although pectin is poorly recovered in the NDF residue (Van Soest, 1994), Jung & Lamb (2004) attempted to develop a prediction equation from NDF concentration in lucerne hay stems. Although the derived equation was significant, only 29% of the variation in pectin content of lucerne stems was accounted for by the NDF concentration. The quantification of pectin in the current study was estimated from NFC_1 as described by Mertens (2002).

Another variation of NFC was proposed by Wang *et al.* (2001) where NFC was calculated as follows:

$$NFC_3 = 100 - ((NDF\text{-}NDF \text{ protein}) + CP + Ash + (fatty \text{ } acids/0.9)) \qquad \text{(eq. 23)}$$

Total fatty acid concentration of feed samples was calculated as described by NRC (2001). For the purpose of the current study all versions of NFC were calculated and discussed (Table 2).

Currently, there is a very limited database of sugar and starch (NSC) analysis for lucerne hay. However, the mean sugar and starch presented in Table 2 was similar to the average sugar (6.8%) and starch (2.2%) analysis performed at the University of Florida (Hall, 2002). Alternatively, there is a comparative large database of NFC values because of the ease of calculation. The mean NFC_2 content of lucerne hay (Table 2) in the present study was lower than the 24.4% reported by Mertens (1992) whereas the NFC_1 value corresponded with the

latter. The NFC values from NFC1 were generally 4% higher than from NFC2, which is in line with the findings of Linn (2003). The NFC data of the current study varied widely while Mertens (1992) reported values that ranged only from 25.4% for lucerne hay cut at an early vegetative stage to 23.2% for hay in full bloom. According to Bethard (1997) widely divergent protein levels in lucerne hay may substantially increase the variation in NFC, as it is used in the calculation. The low minimum NFC value of lucerne hay found in the current study could possibly be explained by unavoidable plant respiration during the wilting process (Coblentz *et al.*, 2004). The latest NRC (2001) summarizes the relationships among minimum forage NDF, minimum dietary NDF, and maximum dietary NFC. The NDF fraction drives rumination whereas the NFC fraction determines fermentability (Grant, 2001). The interaction between these two fractions must be understood when formulating diets for high producing dairy cows. Bethard (1997) concluded that using NFC values in diet formulation should enable more precision in balancing carbohydrates in the diet. According to the results of the present study a high variation occurred in the NFC content of lucerne hay (Table 2). It is evident that an average value would be an unreliable measure to use in ration formulation. However Van Soest *et al.* (1991) suggested that this problem is greater with grass- or maize silage-based diets than with lucerne hay.

Broderick *et al.* (2000) reported that increasing sugar content of diets increased both DMI and butterfat production. Strobel & Russell (1986) suggested that fermentation of sugars usually generates more butyrate than any other NFC, and concentrations of propionate similar to starch. In addition, sugar can also be responsible for an increase or reduction in fibre digestibility, depending on the degradable protein concentration in the diet (Heldt *et al.*, 1999). Therefore given the different fermentation end products and large variation in lucerne hay sugar content (1.5 to 10.3% DM) (Table 1) sugar analysis should routinely be done. It has been proposed that low ruminal pH is related more to high concentrations of NFC or NSC rather than effective fibre. According to Cruywagen (2001) the inverse relationship between NDF and NFC is nearly perfect, complicating the decision in the ultimate cause of low ruminal pH. However, several researchers are of opinion that the lack of effective fibre and not the excessive concentration of NFC is primarily responsible for borderline acidosis and milk fat depression (Sudweeks *et al.*, 1981; Grant *et al.*, 1990). However, neither effective fibre (eNDF) nor physical effective fibre (peNDF) were determined and will be conducted in a further study.

The effect of diurnal variation on the concentration of NFC in lucerne hay is well documented (Fisher *et al.*, 2002; Burns *et al.*, 2005; Mayland *et al.*, 2005). Holt & Hilts (1969) reported that NSC concentration followed a non-linear daily trend, with the most rapid increase followed in the afternoon. Harvesting lucerne hay at sundown rather than dawn, takes advantage of the plants potential to accumulate carbohydrates, as a result of photosynthesis, during the daytime (Bowden *et al.*, 1968). This increase in NFC is associated with better *in vitro* digestibility (Burns *et al.*, 2005), increase preference by sheep, goats and cattle (Fisher *et al.*, 2002; Mayland *et al.*, 2005), thus potentially improved daily DMI of more digestible forage that might result in improved daily animal responses (Burns *et al.*, 2005). However, Coblentz *et al.* (2004) is of opinion that even though lucerne is cut under these conditions and wilted properly, the concentrations of NFC can fall to <8% of DM by the time the forage is baled due to unavoidable respiration during the wilting process. Accordingly it can be hypothesised that diurnal variation contributed to the observed variation in NFC.

3.1.4.3   Carbohydrate pools

The carbohydrate (CHO) fraction of feeds is composed of structural and non-structural carbohydrates, which could be part of the soluble or insoluble fractions. The proportions of this diversity of chemical fractions are largely a description of their degradation and factors influencing their availability to animal and microbial digestion, therefore important in determining the animal response to the forage (Van Soest, 1994). In the latest version (6) of CNCPS (CNCPSv6) the carbohydrate (CHO) pools have been expanded to eight fractions as indicated in Table 1. The carbohydrate fractions in the previous versions of the CNCPS were subdivided into four fractions: CA (sugars, organic acids, and oligosaccharides), CB1 (starch, soluble fibre), CB2 (available NDF) and CC (unavailable NDF) (Fox *et al.*, 2004). However, for the purpose of the current study fractions CA1, CA2 and CA3 were not included in Table 1 due to its absence in forage hay.

Currently, there is a very limited database on the CHO fractions (CA4 and CB2) for lucerne hay to base recommendations upon. In Table 2 the CHO fraction showed relatively similar variation, except for CB2 (CV = 49.9%), which could be explained by the contribution of pectin to this fraction (Sniffen *et al.*, 1992) thus, its high concentration and variability in lucerne hay (par. 3.1.4). The highest average and widest range were reported for CB3 and CC, which had a similar CV. The CHO pool, expressed as a % of CHO (%CHO) of lucerne hay in the current study differed from the values reported by Yu *et al.* (2003) for different cutting

stages and varieties (Figure 2). Although a younger version of CNCPS (CNCPSv6) was used in the present study, all the fractions were calculated similar to that reported by Yu *et al.* (2003) except for the new fractions CA (CA1 - CA4), CB2, and CB3 (Table 1). Fraction CB2 of the older CNCPSv5 is calculated similar to the new CNCPSv6 fraction CB3. Mean CHO fraction values, expressed as %CHO, in the present study compared to Yu *et al.* (2003) were higher in CB1 (3.2 vs. 1.5% CHO) and CB3 (34.5 vs. 27.4% CHO), but lower in CC (27.4 vs. 35.1% CHO). The lower CC value obtained in the present study could be explained by the suspected grass contamination, which has lower lignin content compared to lucerne hay (Van Soest, 1994). No CA4 and CB2 values, for lucerne hay or grass, could be found in the available literature. The CA4 fraction (sugar) in Table 2 was already discussed in paragraph 3.1.4.
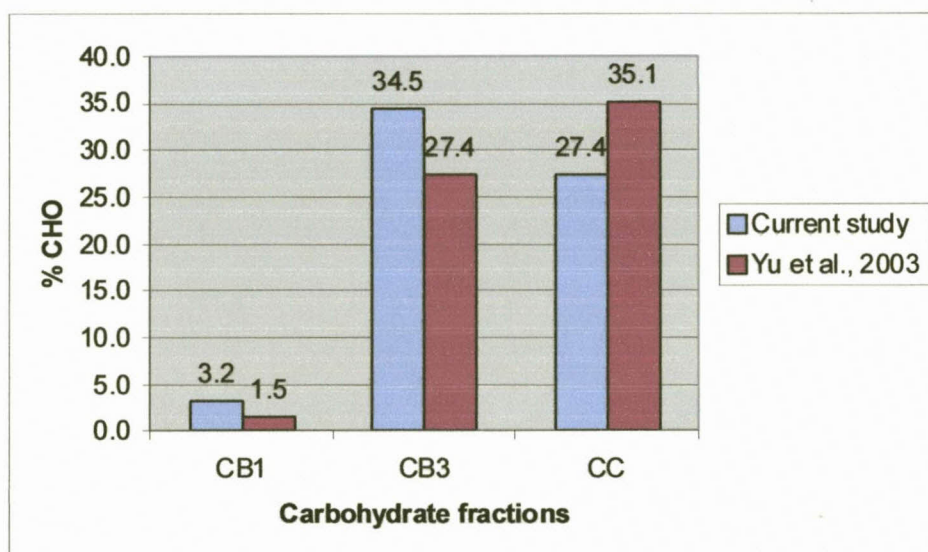
Figure 3   **Comparison of selected comparable carbohydrate (CHO) fractions (CB1 = starch, CB3 = available neutral detergent fibre and CC = indigestible neutral detergent fibre) between Yu *et al.* (2003) and the current study**

When comparing lucerne hay in the present study with timothy (*Phleum pratense L*), as reported by Yu *et al.* (2003), lucerne hay was higher in CB1 (3.2 vs. 1.34%) and CC (27.4 vs. 16.4%) but notably lower in CB3 (34.5 vs. 67.6%). These results indicate that lucerne contained higher starch (CB1) and less slowly degradable fibre (CB3). These results were however not supported by the literature (par. 3.1.4). Similar findings have also been reported by Yu *et al.* (2003). The higher undegradable cell wall fraction (CC) found in lucerne hay was

however expected due to the higher concentration lignin found in lucerne compared to grass (par. 3.1.4.1). The CB1 fraction of lucerne hay in the current study was three times greater than that in grasses (timothy) (Yu *et al.*, 2003). Elizalde *et al.* (1999) found similar results between lucerne and grasses (bromegrass and tall fescues). According to Yu *et al.* (2003) plant maturity, but not forage variety, had an influence on the CHO fractions. Accordingly, some of the variation of the CHO fractions reported in the present study could possibly be explained by stage of cutting.

It is evident from the result of the present study (Figure 3) that a minimum of 61.9% of CHO (CC + CB3) is totally unavailable for microbial usage. Although some of the CB2 is degraded in the rumen, the rest will make a further contribution to the CHO used in the small intestines. Hence, when including a significant amount of lucerne hay to a dairy diet, adding of adequate soluble CHO to supply in microbial demand would be inevitable.
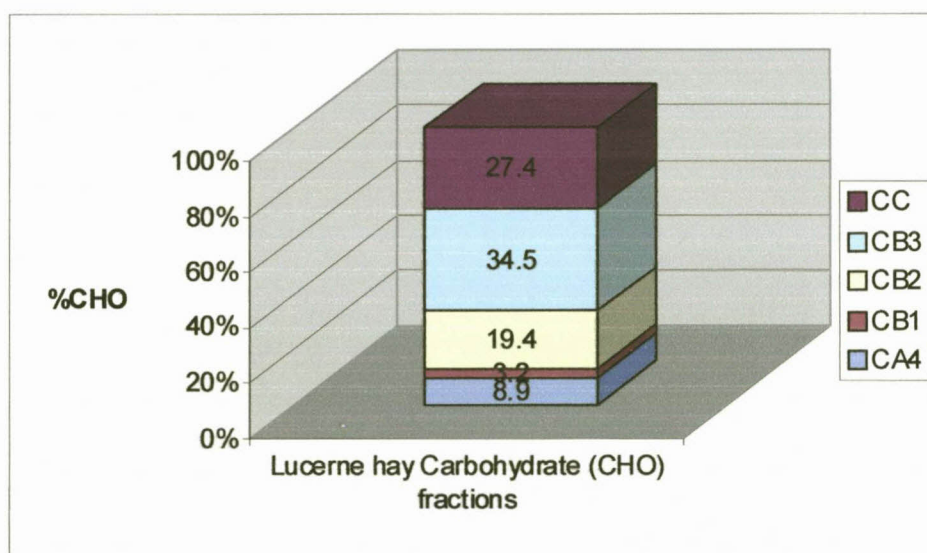
Figure 4   **Mean carbohydrate (CHO) fractions (CA4 = sugar; CB1 = starch; CB2; = soluble fibre; CB3 = available neutral detergent fibre; CC = indigestible neutral detergent fibre) of South African lucerne hay on a dry matter basis**

3.1.5 Crude fat

Crude fat is the amount of fat or oil extracted by ether extract or hexane in animal feed (AOAC, 1990). The extract or hexane soluble compound may include true fats and oils, fatty

acid esters, compound lipids and fat soluble vitamins or pro-vitamins such as the carotenoids, all of which may have nutritional value (Mason, 2000-unpublished data; Van der Merwe & Smith, 1991). However, (Mason 2000-unpublished data) suggested that hexane extract may also contain a significant concentration of indigestible waxes, resins and essential oils. Van der Merwe & Smith (1991) concluded from the composition of ether extraction fraction, that it could be misleading and does not represent the true fat or oil content of a feed. According to McDonald *et al.* (1995) crude fat contains 2.25 times the energy (caloric) value of carbohydrates and proteins. The estimated crude fat content of a feed is also used to formulate total dietary fat level (Smith, 1992) and calculate non-fibre carbohydrates (NFC) by difference (par. 2.2). It has long been recognised that fat is due to its high energy density, one of the key components determining the energy value of a forage for cattle (Robinson, 2005). According to Andrews *et al.* (1991) fat is used more efficiently for production than carbohydrate. Weiss (1993) is of opinion that the inclusion of fat and ash, due to a high energy density and zero energy status respectively, in a model could make the estimation of available energy more appropriate for most feeds.

The mean crude fat content (Table 2) of lucerne hay in the current study corresponds with the 1,8% reported by Van der Merwe & Smith (1991) for lucerne hay in South Africa. These values were however lower than the 2.3% and 2.5%, values published by Mertens (1992) and the NRC (2001) respectively. The NRC (1996) reported values that ranged from 2.9% at early bloom, to 2.6% at mid bloom and 3.4% at full bloom. However, Mertens (1992) observed that early vegetative hay contains 3.1%, early bloom 2.4% mid bloom 2.1% and full bloom 1.5 % crude fat. These values suggested that crude fat content decreased with maturity. From some of the literature cited it seems as if maturity has no definite influence on the crude fat content of lucerne hay. Van der Merwe & Smith (1991) also reported no particular pattern: pre-bloom 2.9%, early bloom 1.8%, full bloom 2.1% and past bloom 2.3%. According to Van der Merwe & Smith (1991) the fat content of feeds is the unstable part. The crude fat content of lucerne hay is however probably too low to cause any problems regarding oxidation and rancidity of fat during storage. Accordingly it is too low to significantly influence the content of available energy. Furthermore, the low fat content could result in dustiness and a lower feed intake when fed to animals. Weiss *et al.* (1992) also suggested that the crude fat in forages (including lucerne hay) may contain a significant concentration of indigestible waxes and resins, further lowering its available energy.

3.1.6 *In vitro* digestibility

The digestibility and intake are criteria, which give information about the forage nutritive value and its degree of utilisation by the animals (Kirilov, 2002). Since digestibility trials (*in vivo*) are laborious to perform, there have been numerous attempts made to determine the digestibility of foods by simulating in the laboratory the reactions, which take place in the alimentary track of the animal (McDonald *et al.*, 1995). The *in vitro* technique of Tilley & Terry (1963) is currently probably the most widely used method. According to McDonald *et al.* (1995) *in vitro* fermentation techniques make it possible to determine the digestibility of large amounts of small samples, as is the case in the present study. The *in vitro* and *in situ* (*in sacco*) estimates of digestibility have long been recognised as being more closely related to animal performance than chemical extractions (Weiss, 1994). Van Soest & Robertson (1985), however, stressed that the application of *in vitro* procedures to poor quality forage could lead to unsatisfactory results.

Several end point procedures have been reported to determine the extend of digestion or substrate utilization (Van Soest, 1994). These end point measurements include residual organic matter, neutral detergent residue, dry matter residue, cellulose disappearance, residue after pepsin digestion, gas production or volatile fatty acids. Several alterations and modifications, especially in the areas of fermentation end-product analysis and degradation dynamics, have been made to the original Tilley & Terry (1963) methodology (Mould, 2003). The time of batch fermentation is commonly 48 hours for digestibility evaluation, although other time periods that ranged from 3 hours to several hundred hours have been practices to calculate rate of fermentation (Van Soest, 1994). In the present study $NDFD_{30}$ has not been determined and will be investigated in a further study, whereas $NDFD_{24}$ and $NDFD_{48}$ were analysed due to its application in the recognised modern nutritional models CNCPS and NRC (2001) respectively.

Currently, there is a very limited database of $NDFD_{24}$ analysis for lucerne hay to base recommendations upon. Alternatively, there is a comparative large database of $NDFD_{48}$ because of its widespread usage by several researchers (NRC, 2001). The mean $NDFD_{24}$ and $NDFD_{48}$ showed a comparable variability (Table 2). The $NDFD_{48}$ seems to be higher than the $NDFD_{24}$ by 5 - 8 percentage units (% NDF) for lucerne hay in the current study whereas, Hoffman *et al.* (2003) reported less difference (2 -5%) between $NDFD_{30}$ and $NDFD_{48}$. These results, however, is in contrast with the findings of Yu *et al.* (2003) who reported similar

values for NDFD$_{24}$ and NDFD$_{48}$, thus claiming NDF in lucerne hay to be almost fully digestible within a 24 hour incubation period. This was however not the case in the present study (Table 2).

The mean NDFD$_{48}$ value (Table 2) of lucerne hay in the current study corresponds with the 40.3% for lucerne cubes reported by Robinson *et al.* (2004). These values were however lower than the 49.8% published by the Marshfield Soil and Forage Analysis Laboratory (Hoffman, 2003). The NDFD$_{48}$ data of the current study varies widely while the Marshfield Soil and Forage Analysis Laboratory (Hoffman, 2003) reported values that ranged from 44.2% for lucerne hay with a low NDF digestibility at 48 hours to 55.4% for hay with high digestibility. The NDFD$_{48}$ values as measured by the Marshfield Soil and Forage Analysis Laboratory are in close agreement with NDFD values listed in 2001 NRC (Hoffman, 2003). Thus, they noted that "if wet chemistry *in vitro* NDFD$_{48}$ values are to be used in summative energy equations, it is imperative that the normal range of NDFD values produced by the laboratory are appropriately related to NDFD values listed in the NRC (2001). These conclusions were however not supported by the results of the present study. This could probably be attributed to differences in geography, environmental conditions and harvesting practices found in SA compared to the USA. Consequently, the accuracy of the proposed summative energy equations (NRC, 2001) could be hampered by using the NDFD values of the present study. Accordingly the NRC (2001) stresses the use of correct procedures and conforming NDFD values for the credibility of the summative energy equation system. Hoffman & Combs (2004) reported a NDFD$_{48}$ variation from 36% to 75% for legume-grass mixtures from upper Midwest laboratories in the USA. Thus, the extreme high NDFD$_{48}$ variation in the present study could be explained by possible grass contamination, as illustrated by Figure 5. According to Hoffman (2003) legumes have generally less total NDF and lower NDFD values due to greater lignification as compared to grasses. The overall wider range in the present study, compared to the literature, could be due to the wider range of harvesting maturity levels (Martin & Mertens, 2005) and types of grasses included in the samples.
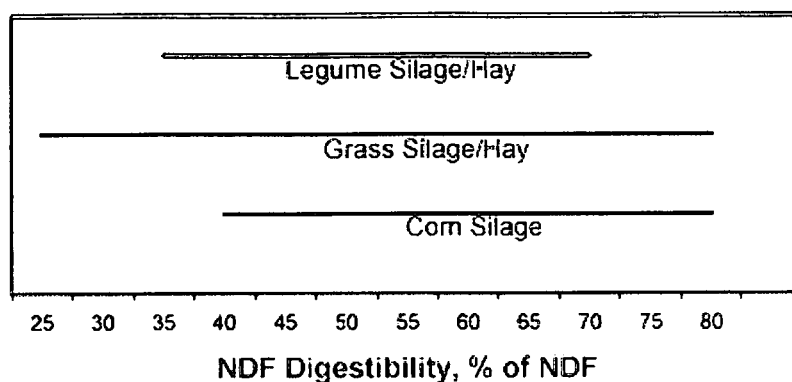
Figure 5   **Range in NDF digestibility of different forages (Reuss, 2001)**

Although many research on NDFD data have been reported, Oba & Allen (1999) mentioned the difficulty to quantify the effect of NDFD of forages on animal performance due to a variety of confounding factors found in individual experiments. Forages are usually supplemented differently in diets, which may lead to further confounding.

The NRC (2001) provides two options to estimate the digestibility of NDF for use in the TDN summative equation. The first method is NDFD (%NDF) as measured in a 48 hour *in vitro* incubation, as discussed earlier. The second approach utilises the lignin (sulphuric acid procedure) content of feed because lignification within a plant species can be negatively associated with NDF digestibility (par. 3.1.4.1). The majority researchers have concentrated on a single forage class when investigating the relationship between lignification and NDF digestibility (Jung & Vogel, 1986; Buxton & Russell, 1988). Several authors, however, have indicated that forage class is unimportant when estimating indigestible NDF from lignin content of the NDF (Conrad *et al.*, 1984; Weiss *et al.*, 1992; Traxler *et al.*, 1998). The mean NDFD value, estimated from lignin ($NDFD_{lig}$), in Table 2 was, however, remarkably lower than the laboratory analysed $NDFD_{48}$ in the present study and several other $NDFD_{48}$ values reported in the literature (NRC, 2001; Hoffman, 2003; Yu *et al.*, 2003). The range of $NDFD_{48}$ was double that of $NDFD_{lig}$, although the variation (CV) was relative similar. These results were however not supported by NRC (2001). The significant ($P<0.0001$) difference between $NDFD48$ and $NDFD_{lig}$ in the present study could greatly compromise the accuracy and precision of estimating TDN and metabolisable energy (ME) as proposed by NRC (2001). Thus, in contrast to the recommendation of NRC (2001), the present study has shown that $NDFD_{48}$ in lucerne hay did not corresponded with $NDFD_{lig}$ value calculated from the summative equation of Weiss *et al.* (1992). This was in agreement with the findings of several

researchers (Jung *et al.*, 1997; Linn, 2003; Robinson *et al.*, 2004) who also found a superior relationship using *in vitro* NDFD measurements as compared to using lignin to estimate NDFD. Mould (2003) concluded that because of the complex nature of lignin, its relationship with NDF and lack of a standardised procedure, it shows large variability when expressed on a DM basis. This calls for further investigation.

Hoffman *et al.* (2001) suggested that $NDFD_{48}$ could be converted to 48 hour *in vitro* dry matter digestibility ($IVDMD_{48}$) by the following equations:

$$IVDMD_{48} = 100 - ((100 - NDFD_{48}) \times (NDF/100)) \quad\quad\quad (eq. 24)$$

Therefore a strong relationship between NDF, $NDFD_{48}$ and $IVDMD_{48}$ is assumed. The calculated mean $IVDMD_{48}$ value in the present study was, as expected, higher than the $NDFD_{48}$ and $IVOMD_{24}$ values (Table 2). $IVDMD_{48}$, however, showed relative low variation (CV = 6.41) similar to $IVOMD_{24}$ (CV = 7.44). The $IVDMD_{48}$ (48.93%) reported by Yu *et al.* (2003) was significantly lower than the mean value calculated in Table 2. Hence, the use of the Hoffman *et al.* (2001) equation for estimating $IVDMD_{48}$ from $NDFD_{48}$ and NDF, in the present population (Table 2), is questionable.

Hvelplund *et al.* (1997) are of the opinion that organic matter digestibility (OMD) is the determinant factor of the energy value of a feed. According to McDonald *et al.* (1995) ME could be calculated from IVOMD as already stated. In this regard McDonald *et al.* (2002) proposed a commonly used formula to calculate ME for roughages fed to ruminants (Appendix A). The NRC (2001) dairy committee reported that total track digestible organic matter (TTDOM) could also be calculated by adjusting the contribution of fat to total digestible nutrients (TDN) by a factor of 1.25 (Appendix A).

Figure 6 illustrates the contribution of the different parameters to organic matter digestibility (OMD) compared to the structural carbohydrate contribution to NDFD. Due to the variability and influence of ash content in lucerne, as discussed in par. 3.1.2, estimation of organic matter digestibility seems theoretically sound in representing available energy. The reduced (24 hour) incubation time of the IVOMD assay was investigated for the reasons discussed earlier. It is also the opinion of Ward (2007, Pers. Comm.) that 24-hour IVOMD ($IVOMD_{24}$) opens greater opportunity to differentiate materials at this point as the differences/slopes comes together as it

moves towards the 48 hours incubation period. Thus, IVOMD24 would be more sensitive towards differentiating between samples than the classical 48hour IVOMD.
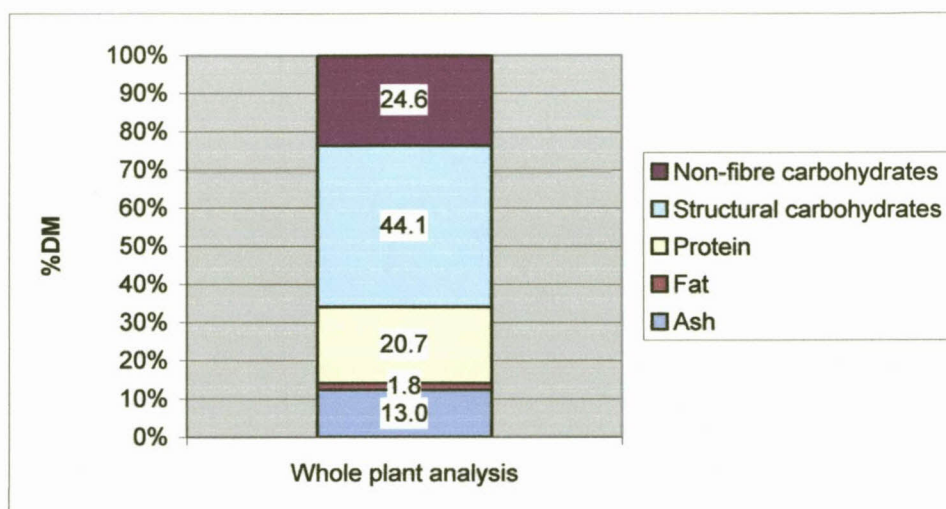
Figure 6 **Average structural and non-structural components of lucerne hay in the current study**

For the reasons mentioned above and the intrinsic characteristics of lucerne hay, the 24-hour IVOMD procedure was also investigated for the purpose of the present study. It is also however widely accepted that IVOMD is a reasonable assessment of *in vivo* digestibility (Lundberg *et al.*, 2004 as cited by Hoffman, 2004). This is however in contrast to the findings of Roberts *et al.* (2004) who reported IVOMD not to be a particularly good predictor of *in vivo* organic matter digestibility. Currently there is no database of IVOMD24 analysis for lucerne hay to base recommendations upon. The IVOMD48 database is, however, well documented in literature (Scholtz, 2001). Although the maximum values of lucerne IVOMD24 and IVOMD48 were fairly similarly higher (±26 and ±28, respectively) than the minimum values, their CV's (7.44 and 8.76, respectively) were surprisingly low compared to the other parameters. This was in contrast with the opinion of Ward (2007, Pers. Comm.), discussed earlier, regarding incubation time (24 vs. 48hr). Hence, from the results of the present study it is evident that *in vitro* incubation time (>24hr) had a minor effect on lucerne hay OMD.

3.1.7 *Energy units*

The mean total digestible nutrients, calculated from NDFD48, (TDN48) value indicated in Table 5 for lucerne hay in the present study was unrealistic higher than the values reported by NRC (2001) and Yu *et al.* (2003). On the other hand the total digestible nutrients, calculated

from $NDFD_{lig}$, (TDN_{lig}^2) value was similar (56.4%) and slightly lower (60.18%) compared to the tabular values of NRC (2001) (56.4%) and Yu *et al.* (2003), respectively. The large difference between TDN_{48}^1 and TDN_{lig}^2 obtained for lucerne hay in the current study is in disagreement with the recommendations of NRC (2001) that either $NDFD_{48}$ or $NDFD_{lig}$ could be used interchangeably in calculating TDN, irrespectively of the forage species. Several researchers (Jung *et al.*, 1997; Linn, 2003; Robinson *et al.*, 2004), however, found a superior relationship using *in vitro* NDFD measurements as compared to using lignin to estimate $NDFD_{lig}$ and ultimately TDN. This was, however, not supported by the results of the present study. The more realistic results obtained by using lignin, compared to $NDFD_{48}$, to calculate TDN (TDN_{lig}^2 and TDN_{48}^1), query the credibility of a universal $NDFD_{48}$ value across forage species (Table 5). The results of the present study however corresponds to the findings of Conrad *et al.* (1984), Weiss *et al.* (1992) and Traxler *et al.* (1998) who indicated that forage class is unimportant when estimating indigestible NDF from lignin content of the NDF.

NRC (2001) discussed the practical problems in predicting available energy for microbial growth, using rumen fermented OM of individual feedstuffs as a direct index. Accordingly they proposed an equation for calculating total track digestible organic matter (TTDOM) from TDN (Appendix A).

The mean $TTDOM_2$ value (Table 2) calculated from TDN_{lig}^2 was similar to the $IVOMD_{24}$ value of the present study, whereas $TTDOM_1$ calculated from TDN_{48}^1 related to neither $TTDOM_2$ nor $IVOMD_{24}$.

The energy content of lucerne hay as estimated from the NRC (2001) model are presented in Table 5. It was apparent that the use of TDN_{lig}^2 was more realistic relative to the tabular values of NRC (2001). Accordingly for the purpose of the present study the use of TDN_{lig}^2 was preferred for future associated energy equations.

The mean estimated energy values for lucerne hay in the current study was similar to all the tabular energy values presented by NRC (2001) (TDN_{lig}^2: 55.39 vs. 56.40%, DE_{1x}: 2.58 vs. 2.60), DEp: 2.37 vs. 2.38, MEp; 1.94 vs 1.96, NElp: 1.18 vs. 1.19Mcal/kg DM). Yu *et al.* (2003) however, reported higher lucerne energy values (%DM) for TDN_{lig}^2 (60.17 %), DE_{1x} (2.76 Mcal/kg), DEp (2.54 Mcal/kg), MEp (2.11 Mcal/kg) and NElp (1.29 Mcal/kg) than reported in Table 5. They also illustrated that the stage of cutting in lucerne had a significant

influence on energy values. These results again, illuminate fibre digestibility in determining the correct energy values for dairy cattle.

Table 5   Energy content of lucerne hay as estimated from the NRC (2001) model[a].

Energy unit	n	Min	Mean	Max	SD[b]	CV[c]
Total digestible nutrients$_{lig}$[1] (%)	168	53.35	80.46	104.06	9.71	12.07
Total digestible nutrients$_{48}$[2] (%)	168	31.63	55.39	65.72	5.40	9.75
Digestible energy $_{1x}$[3] (Mcal/kg)	168	1.51	2.58	3.07	0.24	9.30
Digestible energy p[4] (Mcal/kg)	168	1.39	2.37	2.81	0.22	9.28
Metabolizable energy p[4] (Mcal/kg)	168	0.95	1.94	2.39	0.23	11.86
Net energy for lactation p[4] (Mcal/kg)	168	0.48	1.18	1.49	0.16	13.56

[a] All values are based on a DM basis

[b] Standard deviation

[c] Coefficient of variation

[1] Total digestible nutrients calculated by using 48 hour *in vitro* NDF digestibility procedure (NDFD48) to calculate TDN

[2] Total digestible nutrients calculated by using NDFDlig, calculated from lignin, to calculate TDN

[3] Estimated energy content at 1 x maintenance level of intake

[4] Estimated energy at production level of intake

The average predicted ME values for lucerne hay in the present study (Table 6), as estimated from IVOMD (McDonald *et al.*, 2002), presented a lower energy value for lucerne hay than the 10.64 MJ/kg (DM) reported by Scholtz (2001), but still higher than the 8.2 and 9.1 MJ/kg (DM) given by McDonald *et al.* (1995) and MAFA (1990) as cited by the AFRC (1992) respectively. Van der Merwe & Smith (1991) also reported a lower value of 8.25 MJ/kg DM for South African lucerne hay.

From Table 6 it is evident that ME from fat (MEfat) had a small (± 7%) contribution to the total average ME content of lucerne. This is to be expected as the fat content of lucerne hay is low. This contribution tends to increase as the ME content of lucerne increases (± 6% for minimum to 18% for maximum). This is in agreement with the increasing difference between ME and FME as the ME and/or fat content of the lucerne hay increases.

Table 6   **Energy content of lucerne hay as estimated from the ARC model[a]**

Enery unit	n	Min	Mean	Max	SD[b]	CV[c]
Metabolizable energy (MJ/kg)	168	6.96	9.58	11.08	0.71	7.46
Metabolizable energy fat[1] (MJ/kg)	168	0.41	0.65	2.03	0.16	23.92
Fermentable Metabolizable energy (MJ/kg)	168	6.55	8.93	10.32	0.65	7.31

[a] All values are based on a DM basis

[b] Standard deviation

[c] Coefficient of variation

[1] Metabolizable energy from fat

According to McDonald *et al.* (1995) the yield of microbial crude protein is related to the energy available to the rumen micro-organisms in terms of fermentable metabolisable energy (FME). FME is expressed as MJ/kg DM, and calculated from the ME of feed or diet, minus the ME contributed from dairy fat. Allowances were made for fat content in both the ARC and INRA system (Parker, 2001). The mean FME value (Table 6) of lucerne hay in the current study was higher than the 7.4 MJ/kg DM and 8.2 MJ/kg DM values published by McDonald *et al.* (1995) and McDonald *et al.* (2002) respectively, and slightly lower than the value reported by Scholtz (2001) for South African lucerne hay. The minimum FME value corresponds to that of ME. Differences between ME and FME tend to increase as ME value rises. However, the difference between FME and ME values were small. This could again be attributed to the low fat content of lucerne hay.

The UK energy values presented in Table 6 were however non comparable to the values in Table 5, due to the different approach followed by NRC (2001) in that feed energy values is directly calculated from their nutrient composition.

3.2 Protein

3.2.1 Crude Protein

Proteins are complex organic compounds of high molecular weight (McDonald *et al.*, 1995). Traditionally, proteins in food for ruminant animals have been evaluated in terms of crude protein (CP) or digestible crude protein (McDonald *et al.*, 1995). According to Ball & Lauriault (1999) crude protein represents the nitrogen (nitrogen content x 6.25) fraction of the forage and measures true protein (amino acids), non-protein nitrogen (NPN) and also insoluble CP or acid detergent insoluble protein (ADIP). Foodstuffs contain numerous different proteins

and several types of NPN compounds. According to the NRC (2001) proteins are large molecules that differ in size, shape, function, solubility and amino acid (AA) composition. Proteins have been classified on the basis of their 3-dimensional structure and solubility characteristics (NRC, 2001). Amino acids (AA) are the building blocks of proteins (NRC, 1989). According to Polan (1992) it should be noted that AA values of feeds are expressed relative to crude protein. The absorption of essential amino acids from digested protein is vital to the maintenance, reproduction, growth and lactation of dairy cattle (NRC, 1989). The NRC (1996) indicates four different protein constituents, namely degradable and undegradable protein as well as soluble protein and non-protein nitrogen (NPN) constituents. Polan (1992) stressed that CP is of utmost importance in dairy rations and is often the first limiting and usually the highest priced nutrient.

Hanson *et al.* (1988) proposed that the quality of lucerne hay is closely related to its CP content, since it is related to stage of maturity and leafiness. Roughage is bulky and, because of presumed limitations of ruminal fermentation capacity and microbial growth, Polan (1992) suggested that the quality of especially the CP should be high. Although the term CP does not differentiate between NPN and true protein, this of little direct nutritional importance for ruminants which readily utilize both forms to generate microbial protein, especially in the case of lucerne CP (Mould, 2003; Martin & Mertens, 2005).

In the present study the average CP content was higher (Table 7) than the 16.7% and 18.83% on a moisture free basis, indicated by Van der Merwe & Smith (1991) and Scholtz (2001) respectively for South-African lucerne hay. Most of the mean overseas CP values found in the literature (Morrison, 1961; McCullough, 1994) were significantly lower. NRC (2001) reported a slightly lower (19.2%), and Elizalde *et al.* (1999) a similar average value as displayed in Table 7.

The higher CP value in the present study is probably from immature lucerne hay which is rapidly fermented in the rumen to ammonia and not used efficiently (Broderick & Satter, 1998). This usually leads to excessive excretion of nitrogenous waste by the animal. Because of this inefficient use of CP, Marten & Mertens (2005) proposed the inclusion of 1 to 3% units more CP in dairy rations, formulated mainly with lucerne hay as a forage source. In contrast Robinson (1998) suggested that lucerne hay has a relatively high proportion undegradable intake protein (UIP) (25 to 35% of CP), which makes it a high quality protein for dairy cows.

Table 7   Protein composition and utilisation of 168 lucerne hay samples[a]

Chemical analysis	Minimum	Mean	Maximum	SD[b]	CV[c]
Crude protein (% DM)	13.9	20.7	27.8	2.6	12.3
Soluble protein (% DM)	3.5	6.2	10.8	1.4	22.8
Soluble protein[1] (%CP)	16.5	30.0	47.3	5.6	18.6
Acid Detergent Fibre-Crude Protein (%DM)	0.7	1.7	11.8	1.1	66.2
Acid Detergent Fibre-Crude Protein[1] (%CP)	3.8	8.1	48.4	4.6	56.2
Neutral Detergent Fibre-Crude Protein (%DM)	0.9	4.3	15.3	2.7	63.9
Neutral Detergent Fibre-Crude Protein[1] (%CP)	3.8	20.4	59.2	11.9	58.0

[a] All values are based on a dry matter basis

[b] Standard deviation

[c] Coefficient of variation

[1] Variable expressed as a percentage of total crude protein

The variation of CP in Table 7 was higher than the values given by Van der Merwe & Smith (1991) and similar to the results reported by Scholtz (2001) for South African lucerne hay. According to Van der Merwe & Smith (1991) crude protein values for SA lucerne hay ranged from 14.4% for lucerne hay cut before bloom to 20.0% cut past bloom. This is almost identical to the before bloom (20.6%) and past bloom (14.6%) values of Morrison (1961). This change in CP concentration with advanced maturity could be explained by reduced leaf-to-stem ratio. Leaves are higher in CP than stems (Table 8), and the proportion of leaves in lucerne declines as the plant matures. Van Soest (1994) however, suggested that lucerne leaves maintain their quality as they aged, while grass leaves decline in quality. In addition, under adverse hay harvesting conditions leaf loss could have a detrimental influence on the leaf-to-stem ratio (Ball *et al.*, 2001), resulting inter alia the observed minimum values in Table 7. As mentioned before, not only the physiological stage, but also various other factors could influence the quality and consequently the protein content of lucerne hay.

Table 8   Leaf and stem crude protein content of lucerne (Collins, 1988)

Plant component	% of the whole plant	Crude protein (%)
upper leaf[1]	30.7	23.9
lower leave	12.8	21.8
upper stem[1]	6.5	13.4
lower stem	50.0	9.6

[1] Upper leaf and stem were taken from the last five internodes on each stem.

3.2.2 Protein fractions

According to Sniffen et al. (1992) the majority of models assumes that the soluble protein (PA and PB1) to be completely degraded in the rumen whereas some of the insoluble fractions (PB2, PB3 and PC) escape ruminal degradation depending on the influencing effect of digestion and passage. However, the different protein fractions differ in rate and extend of ruminal degradation, influencing the quantity of ruminally degraded and escape protein ingested by ruminants (Elizalde et al., 1999). As with the CHO fractions, protein fractions are also important in determining the animal response to a forage (Van Soest, 1994).

The protein fractions of lucerne hay, expressed on a CP basis (%CP), presented in Table 9 differed from the tabular values in NRC (2001). Fraction PA was lower (15.0 vs. 27.8% CP) and fraction PB (PB1, +PB2 +PB3) was higher (76.9 vs. 66% CP) and fraction PC was higher than the tabular value in NRC (2001). These results indicate that South African lucerne hay (Table 9) consisted of a higher concentration true protein as a percentage of CP than that in the U.S. reported by NRC (2001) (76.9 vs. 66% CP). Nitrate accumulation in forage is maximum in under cool, cloudy conditions, which reduce photosynthesis and the reduction of nitrate to amino acids (Van Soest, 1994). Accordingly, the sunny and longer day length found in South Africa could explain the higher percentage true protein reported in the current study.

Similarly, the higher temperatures reported in the lucerne producing areas of South Africa could probably attributed to the higher PC value reported in the present study compared to NRC (2001) tabular values. The nitrogen content of acid detergent fibre of forages has been correlated positively with lignin content (Van Soest, 1994). Higher environmental temperatures result in the increased lignification of plant cell walls and in specifically the stems (Van Soest, 1994).

The mean value for SP (% CP) in the current study were significantly lower than the 46.0%, 40.6% and 53.6% reported by Sniffen et al. (1992), Elizalde et al. (1999) and Yu et al. (2003) respectively. The maximum SP value in Table 9 related to the values for grass as noted by Sniffen et al. (1992). Elizalde et al. (1999) demonstrated that SP was a constant fraction (% CP) for lucerne as well as grasses as maturity advanced. This was in agreement with the findings of Hoffman et al. (1993) and Cherney et al. (1997) who reported that small changes in SP concentration occurred as maturity advanced. According to the findings of these researchers maturity did not contributed to the SP variation displayed in Table 9.

Table 9   **Protein fractions expressed as percentages of DM[1] and total CP[2] in 168 lucerne hay samples**

Fraction[a]	Minimum	Mean	Maximum	SD[b]	CV[c]
PA [1] (% DM)	1.8	3.1	5.4	0.7	22.8
PB1 [1] (% DM)	1.8	3.1	5.4	0.7	22.8
PB2 [1] (% DM)	1.7	10.2	16.3	2.3	22.6
PB3 [1] (% DM)	0.1	2.6	11.8	2.1	84.4
PC [1] (% DM)	0.7	1.7	11.8	1.1	66.2
PA [2] (% CP)	8.3	15	23.7	2.8	18.7
PB1 [2] (% CP)	8.3	15	23.7	2.8	18.7
PB2 [2] (% CP)	10.5	49.6	68.6	9.9	20.1
PB3 [2] (% CP)	0.3	12.3	52.7	9.9	80.4
PC [2] (% CP)	3.8	8.12	48.4	4.6	56.2

[a] Fraction PA is non-protein nitrogen (NPN), which is rapidly degradable with an assumed degradation rate to be affinity. Fraction PB is true protein (TP), which is further sub-divided into three fractions PB1, PB2 and PB3, which have different rates of degradation in the rumen (Sniffen *et al.*, 1992.). Fraction PB1 is peptides which is rapidly degradable with a degradation rate of 120-400%/h. Fraction PB2 is the difference between buffer insoluble protein and fraction PB3 which is fermented in the rumen at a lower rate than PB1, thus intermediately degradable with a degradation rate of 3-16%/h, and some could escapes to the lower gut. Fraction PB3 is believed to be the slowest degradable of the three PB fractions with a degradation rate, for forages, equal to fraction CB3 (3-6%/h). Because of its association with plant cell walls, PB3 to a large extend escapes to the lower intestines. Fraction PC is the ADFCP which is considered to be resistant to microbial and the animal enzymatic breakdown, thus un-degradable and unavailable to the animal. All passage rate equations were adopted from Seo *et al.* (2006)

[b] Standard deviation

[c] Coefisient of variation

[1] Protein fractions (A, B1, B2, B3, C) expressed as percentages of DM

[2] Protein fractions (A, B1, B2, B3, C) expressed as percentages of total CP

For the purpose of the present study SP (%CP) was assumed to contain 50% NPN (PA). The other 50% was represented by fraction PB1 (soluble true protein) (Tylutki, 2007, Pers. Comm.). However, fractions PA enter the rumen ammonia pool directly while PB1 has a rapid rate of digestion (k_d) and is nearly completely degraded in the rumen. Thus, with their aggregated low concentration (30% CP), individual identification of these two protein fractions would be irrelevant in this case. In accordance with the PA variation found in Table 9, NRC (2001) suggested that grasses and legume forages (eg. lucerne) contain the highest and most variable concentration of non-protein nitrogen (NPN). NPN is rapidly soluble in the rumen and is exclusively used by the micro-organisms. NRC (2001) further postulated that hays

contain higher amounts of NPN than the same feeds when fresh, because of the proteolysis that occurs during wilting. The proteolysis that occurs in forages during wilting is a result of plant and microbial proteases and peptidases. Plant proteases and peptidases are active in cut forages and are considered to be the principal enzyme responsible for the conversion of true protein to NPN in hays (Van Soest, 1994). Rapid wilting of cut forages slows down proteolysis and reduces the conversion of true protein to NPN (Garcia et al., 1989; Van Soest, 1994). This phenomenon possibly contributed to the variation of the fraction PA in lucerne hay as found in the current study.

The assumption that PA directly enters the rumen ammonia pool was however criticised by several researchers when evaluating high quality lucerne silage (Makoni et al., 1997; Ross & Van Amburgh, 2003). Fox et al. (2004) concluded that two thirds of the NPN in high quality silage should be included in the PB1 fraction. Elizalde et al. (1999) conducted a study, which demonstrated that fraction PA was not influenced by forage species or maturity. They reported an average of 22.6% across forages and maturity dates for lucerne, bromegrass and tall fescue. Accordingly it could be assumed that lucerne has a higher proportion of PB1 than did grasses due to its higher total SP compared to grasses. Licitra et al. (1996) is of opinion that most variation in PA content could be explained by experimental procedure. Several researchers reported a small contribution of fraction PB1 (5%) to total SP (Pichard, 1977; Krishnamoorthy et al., 1982) in forages; whereas most soluble protein in fresh pastures is rendered by fraction PB1 (Van Soest, 1982).

Fraction PC (Table 9) contains proteins closely bound to lignin, Mailiard products and tannin-protein complexes (Sniffen et al., 1992; Van Soest, 1994). This fraction is measured as acid detergent fibre-crude protein (ADF-CP) and appears to be highly resistant to rumen and lower intestinal track digestion (Krishnamoorthy et al., 1983). PC[1], ADF-CP, acid detergent fibre nitrogen (ADF-N), insoluble crude protein (ICP), unavailable protein and bound protein, all refer to the same fraction (Erasmus et al., 1990; Undersander et al., 1993; Linn & Martin, 1999; Tylutki et al., 2007). According to Van Soest (1994) ADF-N is the nitrogen remaining in the acid detergent fibre residue. While some occur naturally in all plant material (Mertens, 1979), ADF-N is generally considered to be an estimate of heat damage occurring during storage (high moisture or processing milling or pelleting). Nitrogen in excessive heated samples is highly resistant to microbial and mammalian enzymes (Krishnamoorthy et al., 1982; Van Soest, 1994). Several researchers (Goering et al., 1972; Mertens, 1979) suggested that the

non-heat-damaged ADF-N is related to the lignin (protein irreversibly bound to lignin) and to a fraction of protein in forages.

According to the results in Table 7 the average CP concentration of ADF (ADF-CP) (%DM) in lucerne hay was higher than the 1.38% and 1.31% (DM) mean reported by Scholtz (2001) and McDonald *et al.* (2002) respectively; and lower than the 2.5% and 2.3% (DM) found by Erasmus *et al.* (1990) and NRC (2001), respectively.

Mertens (1979) has shown that ADF-CP also exists in forages that have not been heated and observed that this non-heat-damage ADF-CP is related to the lignin and to a fraction of protein in forages. His work suggested that 5-12% of the N in non-heat-damaged forages is isolated as ADF-N (lower values for grasses than lucerne). This researcher also mentioned that the heterogeneity of ADF-N might explain the difficulty in measuring it (especially with near infrared reflectance spectroscopy). The lack of a constant biological availability value for ADF-CP is also a problem. Undersander *et al.* (1993) stressed the fact that heating can have both positive and negative effects on protein utilisation by the animal. Heating generally results in lowered digestibility of protein. Digestibility of ADF-CP varies from 0 to 60% (in distillers grain) depending on the feed ingredient and the time and intensity of heating (Van Soest, 1994). Mason (2000-unpublished data) suggested that a fixed proportion (e.g. 70%) of ADF-CP is unavailable whereas Yu (1974) reported an average digestibility of 34% ADF-CP for forages. Weiss *et al.* (1992) reported a corresponding ADF-CP digestibility for forages (30%). Undersander *et al.* (1993) further proposed that reducing the solubility of proteins at low heat inputs could compensate for the negative effect of heating by making them less degradable in the rumen. Therefore, if feeds are slightly heated it reduces the potential loss of protein in the rumen (as ammonia) and actually increases protein utilisation efficiency in the small intestines. Van Soest (1994) is however of opinion that although some products of NDF-CP might be absorbed from the small intestine, it might not be metabolised in tissues hence lost in urine.

With the concentration of ADIN expressed as a proportion of CP, it is possible to predict the true digestibility of the forage CP (Weiss *et al.*, 1983). Unavailable CP (ADF-CP) and N (ADF-N), when expressed as a percentage of total CP (ADIP and ADIN respectively) shows a high correlation with digestibility of forage CP (Thomas *et al.*, 1982). The mean ADIP value (Table 7) was higher than the 7.6% and 7.0% value measured by Erasmus *et al.* (1990) and

Coblentz *et al.* (1998) respectively. The mean PC^2 (%CP) values, which represents ADIP, reported for lucerne hay in the current study were generally higher than the 5.2% value obtained by Yu *et al.* (2003) for grasses, a result expected due to the higher lignin content of lucerne compared to grasses (Van Soest, 1994). A high CV was calculated for ADIP. ADIP values ranging from 3.5 to 13.6 and 8.3 to 14% in some roughages have been reported by Krishnamoorthy *et al.* (1982) and Janicki & Stallings (1988) respectively. This is much lower than the ADIP range found in the current study (Table 7) and those reported in the literature for lucerne hay (Yu *et al.* 2003). Therefore the influence of ADF-CP on protein availability of lucerne hay for ruminants seems to be significant. According to Mason (2001-unpublished data) most feed labs report an adjusted crude protein (ACP) value if heat damaged protein (ADIP) is higher than about 9% of total crude protein. However, Undersander *et al.* (1993) suggested no adjustment of CP when ADIP is less than 14%, partial adjustment when ADIP is between 14 and 20%, and complete adjustment for ADIP values above 20% of total CP. Linn & Martin (1999) recommended an adjustment for CP availability when the CP% in the ADF fraction increases above 12% of total CP.

In the present study 94.0% of the lucerne samples showed an ADIP content of less than 14%; 4.8% were between 14% and 20%, and 1.2% higher than 20%. It is obvious from these results that heat damage possibly occurred in only a small percentage (6%) of the samples. Scholtz (2001) reported similar results for South African lucerne hay. A high moisture condition during the baling process activates the process of spontaneous heating (par. 3.1.1) followed by the Maillard or non-enzymatic browning reaction (Coblentz *et al.*, 2004). This reaction involves the condensation of sugar residues with amino acids to form a brown indigestible polymer substance. This substance consists of approximately 11% nitrogen (N) and possesses many of the physical and chemical properties of lignin (Van Soest, 1994). The limited samples effected by the Maillard reaction in the present study is in agreement with the low moisture content found in the lucerne hay (Table 2).

The UK metabolisable (MP) system calculates the true digestibility of digestible undegradable protein (DUP) on the assumption that the ADF-N content is indigestible and that the remainder had a true digestibility of 0.9 (AFRC, 1992; McDonald *et al.*, 2002). Erasmus *et al.* (1990) reported that the available DUP is over-estimated if there is no correction made for ADF-N. However although ADF-N is assumed to be an indigestible fraction (AFRC, 1992; AOAC, 1979) it could be degraded slightly in the ruminal compartment (Weiss *et al.*, 1986; Van Soest,

1994), however poorly used by the ruminant animal (Sniffen *et al.*, 1992; Rebole *et al.*, 2001). It was also evident from the ADIP results in this study that there is no need to correct for ADF-N. The researchers of CNCPS (Fox *et al.*, 2003) acknowledge a possible PC digestibility as reported by some studies previously mentioned, however they assumed PC to have zero ruminal and intestinal digestibility for model purposes.

Fraction PB3 represents the cell wall associated proteins. This fraction is insoluble in neutral detergent but soluble in acid detergent (Neutral detergent fibre-crude protein (NDF-CP) minus ADF-CP). The terms NDF-CP and neutral detergent fibre-nitrogen (NDF-N) are expressed on a DM basis and refers to the same fraction. The mean NDF-CP value in Table 7 was surprisingly higher than the 3.1% and similar to the 4.2% (DM) of lucerne hay and grass-legume mixtures respectively, reported by NRC (2001). Yu *et al.* (2003) however, reported a lower NDF-CP (6.8%) value compared to the current study (Table 7). Higher NDF-CP values are usually associated with grasses, due to the higher NDF concentration (Van Soest, 1994) compare to lucerne. Thus, the higher values obtained for NDF-CP in the present study confirms the possibility of grass contamination, previously suspected with other parameters.

NDF-CP value, when expressed on a CP basis (NDIP) is presented in Table 7. It showed slightly less variation then NDF-CP (DM) (64% vs. 58%). The mean NDIP (%CP) values for lucerne hay in the current study and others (Sniffen *et al.*, 1992; Yu *et al.*, 2003) were generally lower than the mean NDIP values for grasses (26 to 34% CP) found in the literature (Sniffen *et al.*, 1992; Yu *et al.*, 2003). This was expected because lucerne and other legumes are characterised by low cell wall (NDF) relative to grass (Van Soest, 1994).

The nitrogen content of NDF in feeds is greatly increased by heating, which promotes denaturation of albumins (Van Soest, 1994). According to Weiss *et al.* (1992) NDF-CP comprise less than 10% NDF for forages not exposed to heating. Accordingly it is evident from the results of the present study that 30% of the samples have undergone heating to some extent under these criteria. This is somewhat contrasting to the theory of the results obtained for ADIP, previously discussed, where accordingly only 6% of the samples of the current study were pregnable to heat damage. Van Soest (1994), however, is of opinion that the nitrogen content of ADF is not necessarily increased by heating only, but also requires the Maillard reaction to render protein recoverable in ADF. Furthermore, McBeth *et al.* (2001) found with

lambs a linear decrease and increase in digestibility coefficients for NDIP and ADIP respectively fed bermudagrass hay exposed to spontaneous heating.

The mean PB3[2] fraction for lucerne hay in the present study (Table 9) was slightly higher than the 10% reported by Sniffen *et al.* (1992) for lucerne pastures. Elizalde *et al.* (1999) reported an even lower PB3[2] value (3% CP) for fresh cut lucerne. These findings are however in contrast to the results obtained by Yu *et al.* (2003), who reported a higher proportion of rumen undegradable protein (RUP) in lucerne hay (PB3[2] = 27.16% CP) than expected. Sniffen *et al.* (1992) suggested that PB3 values are usually higher for hays than for grazed forages due to the typical higher NDF concentrations observed for forages harvested compared to when grazed in earlier stages of maturity. This was supported by the results in the present study and most literature. Both ADF-CP and NDF-CP (%DM) showed extreme variation (Table 7). This might be explained by the high variation found for PB3 (CV = 84.38%) in the current study. Elizalde *et al.* (1999) also reported lower PB3 (%CP) values for lucerne than in grasses (Bromegrass, Tall fescue), which was not the case for the current study.

A high percentage of fraction PB3 escapes rumen degradation because of its association with the cell wall (Van Soest, 1981; Krishnamoorthy, 1982; Sniffen *et al.*, 1992). This fraction contains prolamin and extensin type proteins that are assumed to be 80% digestible in the intestines (O'Connor *et al.*, 1993). The wide range obtained in Table 9 suggested that lucerne hay could make a major contribution to the RUP content of a diet. Therefore lucerne hay could be regarded as a high quality protein source for dairy cows.

According to Table 9 Fraction PB2[2] was the largest CP fraction (CV = 20.11%) in lucerne hay. Similar findings have also been reported by Sniffen *et al.* (1992) and Elizalde *et al.* (1999) for lucerne pastures and fresh lucerne, respectively. The mean PB2[2] (% CP) in the present study was higher than the 41.0% and 10.7% (%CP) reported by Sniffen *et al.* (1992) and Yu *et al.* (2003) respectively. Elizalde *et al.* (1999) reported a slightly higher PB2[2] value (51.6% CP) for fresh lucerne than the value in Table 9 for lucerne hay. It is evident from Figure 7 that the large difference in PB2[2] values between the present study and Yu *et al.* (2003) is due to the higher SolP and PB3[2] values reported by the latter. Elizalde *et al.* (1999) illustrated that fraction PB3[2] was greater in lucerne than in grasses (46% CP) (bromegrass and tall fescue). This finding was however not supported by Yu *et al.* (2003), who reported lower PB2[2] values for lucerne compare to grasses (10.7 vs. 33.5% CP). According to Sniffen *et al.* (1992) PB2[2] is

partly fermented in the rumen, while some escapes to the small intestines hence, contributing to passage of metabolisable protein (MP).
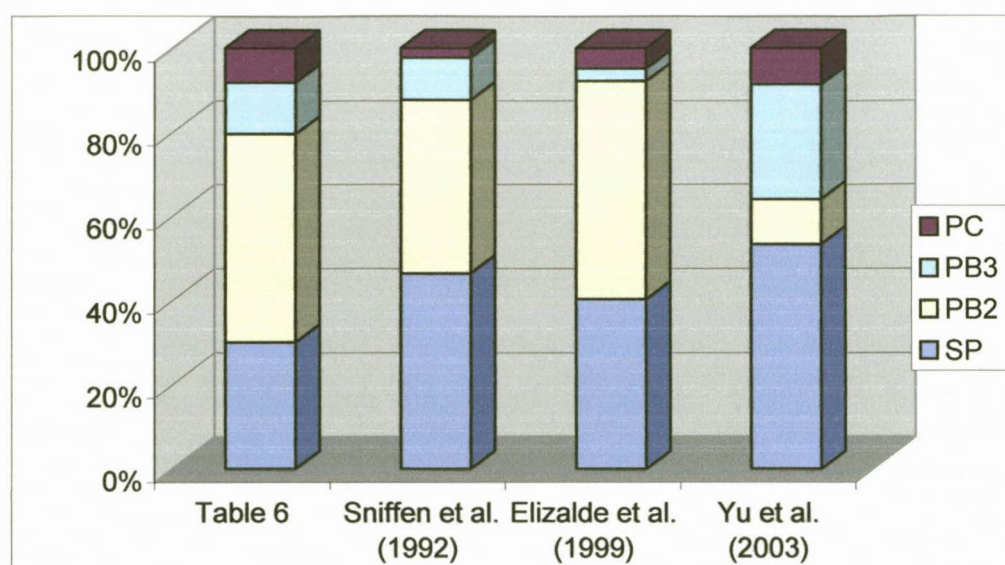
Figure 7. **Proportions of the soluble crude protein (SP) and insoluble CP (PB2, PB3 and PC) fractions expressed as percentages of total CP in different reports**

3.2.3 *Metabolisable protein (MP)*

Formulation of rations to meet the protein and amino acid (AA) requirements of ruminant livestock has developed from systems, which were based on digestible crude protein (ARC, 1980; NRC, 1984) to those which set out to express protein requirements in terms of metabolisable protein (AFRC, 1992; NRC, 1996; NRC, 2001). The NRC (2001) defined metabolisable protein (MP) as the true protein that is digested post-ruminally and the component AA absorbed by the intestine. MP is arguably the most important nutrient used in dairy diet formulation (St. Pierre & Weiss, 2007).

The proposed MP system of the AFRC (1992) adopts the principles of ARC (1980) and (1984), but with some amendments to the magnitude of factors in the system, in the light of subsequent published research and the results of specially designed experiments to test the original ARC recommendations. The main factors modified were rumen microbial needs for N and the digestibility of the efficiency of utilisation of absorbed AA to meet tissue amino acid needs.

The NRC (2001) proposed a somewhat different approach as the UK Metabolisable Protein System (AFRC, 1992) to calculate MP. Apart from the contribution of microbial crude protein

(MCP) and RUP as mentioned earlier, the NRC (2001) also emphasised the contribution (although to a much lesser extent) of endogenous crude protein (ECP) to MP in the small intestine. The CNCPS model as described by Sniffen *et al.* (1992) generates a changeable MP estimate for individual diet components based on protein composition, ruminal protein digestion rates, passage rates, bacterial yield, bacterial composition, and post-ruminal digestibilities of feed and bacterial fractions.

4   CONCLUSIONS

From the results of the present study it seems that the moisture content of lucerne hay was within the desired range for effective storage. Therefore heat and mould damage were unlikely to occur. This was confirmed by the low ADF-N content of the lucerne hay. However, the relative high dry matter content of lucerne hay in the present study could possibly be attributed to moisture losses during sample preparation and storage. Moisture losses during sampling preparation is of great importance when buying and selling hay and needs further investigation

Energy is important in the diets of high yielding lactating dairy cattle and is lucerne hay most apt to need supplementation in diets containing high levels of lucerne hay. Accordingly the importance of energy and/or energy related parameters to animal production stresses the importance of acknowledging its variation in the SA lucerne hay population.

The high ash content of lucerne hay emphasis its potential mineral contribution (especially Ca and K) to the diet. On the other hand the high ash content could be an indication of soil and dust settling on the lucerne before storage. Several lucerne hay samples measured an ash content above 14% which is usually an indication of soil contamination. Thus, it is evident from the data set that 24% of the samples in the present study were prone to soil contamination. Accordingly, the prominent high ash value of lucerne hay in this study could have a detrimental effect on the energy value of the composite diet due to the zero energy contribution of ash. The mean Ca value of the SA population proposed that cows fed diets containing 100% lucerne hay would be subjected to incidences of milk fever. In addition, the contribution of the high K content of lucerne hay in the present study to the DCAD of dairy diets further increased the probability of milk fever in close up dry cows. The mean Fe content of the South African lucerne hay population would contribute 5 times more FE than required

for close up dry cows. This high level of Fe in a diet might interfere with the absorption of primary Cu and Zn, which could lead to depletion of these minerals in cattle.

The high NDF values of some lucerne hay samples in the present study could restrict the inclusion level thereof in high yielding dairy diets. On the other hand, several of the samples lower than the mean NDF in the SA population had a NDF value lower than 35%, therefore these samples could nutritionally be disqualified as a roughage. In this regard, guessing NDF values too high or too low can have tremendous implication on intake, animal performance and health.

Although pectin was estimated from NFC, its intrinsic fermentation characteristic and high concentration in lucerne hay should be acknowledged when formulating dairy diets.

The probable overall effect of grass contamination on lucerne hay manifested in the majority of the energy related parameters. These parameters produced results with generally more variation than found in the available literature. This was expected due to the lower and higher lignin and NDF content, respectively, found in grasses compared to lucerne. The variations that exist in NDF, lignin, NFC and NDFD content of lucerne hay emphasise the importance of analysis to ensure accurate dairy diet formulation. This also applies for the rest of the nutritional energy parameters analytically analysed. Although the maximum $IVOMD_{24}$ and $IVOMD_{48}$ values of lucerne hay was respectively 25.76% and 27.99% units higher than the minimum, it showed surprisingly low CV's. Therefore the mean $IVOMD_{24}$ and $IVOMD_{48}$ values in the current study seem to be representative of the lucerne hay population. From these results it can be hypothesised that the application of the IVOMD procedure to lucerne hay forage could produce unsatisfactory results with regards to its relation to available energy and/or production potential.

From the results of the present study it is evident that the significant difference between $NDFD_{48}$ and $NDFD_{lig}$ could greatly compromise the viability of estimating TDN and metabolisable energy (ME) as proposed by NRC (2001). Thus, in contrast to the NRC (2001) recommendations, $NDFD_{48}$ seems to be unsuited to be used in the summative equation of Weiss *et al.* (1992).

The extremely high levels of CP found in the SA lucerne hay population are not always nutritionally desirable due to its high solubility. This could result in excessive levels of nitrogen in the rumen. Results from the present study revealed that the mean CP (% DM) and SP (% CP) content of lucerne hay was higher than those found in the literature. The variation in CP and other CP-fractions content of lucerne hay stress the importance of analysis to ensure accurate ration formulation.

The mean value for soluble protein (% CP) in the current study was significantly lower than the values reported in the literature. According to the findings of the present study the South African lucerne hay population contains a lower concentration of rapid degradable protein as reported by the NRC (2001).

It is obvious from the results of the present study that the influence of ADF-CP on the RUP of lucerne hay was negligible. Accordingly, due to the low mean ADF-CP content the influence of ADF-CP on CP availability is negligible. It seems from the results of the present study that heat damage possibly occurred only to a small percentage (6%) of the samples. This is in agreement with the low moisture content found in the tested samples. The higher NDF-CP values reported in the present study, again, confirms the possibility of grass contamination, previously observed with other chemical related parameters. In addition, 30% of the samples NDF-CP comprised more than 10% NDF. This implies that these samples might have undergone heating to some extend. The higher NDF content found in grass, together with its higher susceptibility to heat damage, explained the discrepancy found with ADF-CP with regards to heat damage. Both ADF-CP and NDF-CP (%DM) showed extreme variation in the current study. This emphasised the importance of identifying these fractions to quantify CP availability to ruminants.

From the results of the present study the variation in energy and protein composition as well as utilisation of nutrients in lucerne hay is evident. Accordingly, using average values obtained from standard books, in diet formulation are inaccurate. This emphasises the need for a rapid and accurate quality evaluation- and grading system for South African lucerne hay in practice.

CHAPTER 4

SAMPLE PREPARATION OF *MEDICAGO SATIVA* L. HAY FOR CHEMICAL ANALYSIS

1. INTRODUCTION

It is important that lucerne (*Medicago sativa* L.) hay trade is based on its forage quality. The validity of any analysis report rests on obtaining a sample that accurately reflects the quality of a particular batch (lot) of hay. Sampling and sampling preparation are key factors in successful analysis by any method. According to Williams (2006), a representative sample should provide the material for the accurate determination of the composition and functionality of the entire population. Thus, it must be truly representative of the total population in every sense, including chemical composition, physical constitution, and the presence of foreign material. Of all the available commodities, forage analysis results are the most prone to sampling- and sample- preparation-error due to their physical nature. Williams & Norris (2001) illustrated that sampling and sample preparation can account for 60 – 70% of the overall error of testing.

Sampling procedure for lucerne hay is well defined in the literature (Bath & Marble, 1989; Martin *et al.*, 1992; Putnam, 1998; Sheaffer *et al.*, 2000); however, very little research has been done on sample preparation for lucerne hay. According to Williams & Norris (2001), sample preparation is defined as the transformation of the sample into the form in which it will be analysed, without causing any changes in functionality or composition other than in moisture content. This process often calls for some type of size reduction.

Knowledge of lucerne hay moisture is, however, critical for proper harvesting and storage. Furthermore, water is weight and must be paid for when lucerne hay is bought and sold (Hunt & Pixton, 1974). Accurate moisture determinations are also important to convert nutrient content to a dry matter basis. Accordingly, errors in moisture determination are incorporated into other nutrient calculations, thereby affecting diet formulations (Thiex & Richardson, 2003). If the moisture content of lucerne hay is too high it could also hamper effective feed processing. Lastly, proper moisture concentrations in diets are necessary for optimum intake and performance of animals (Thiex & Richardson, 2003). Thus, an accurate

determination of moisture in lucerne hay is of the utmost importance. Several subjective, physical, analytical (chemical and near infrared reflectance spectroscopy) and industrial methods for determining the moisture content of lucerne hay exist. These methods also depend on proper procedures. Thiex & Richardson (2003) reported several sources of error that apply to all moisture methods namely: representativeness of laboratory and analytical samples; storage conditions of both laboratory and analytical samples; weighing errors; test portion size; room humidity; non-aqueous losses or interferences; grinding techniques (exposure to air, generation of heat, contamination, fineness of grind). According to Williams (2006), grinding of the sample during sample preparation results in moisture content that varies depending on the original moisture content, the type of grinder, and the number of samples ground at the same time.

Electronic probe type moisture testers operate on the principle of electrical resistance, utilising the relationship between the moisture content of the material and its conductivity (Shewmaker & Thaemert, 2004). These probe-type meters are, however, subject to error due to various external factors. These factors include variation in bale density, the type of forage, whether it is plant moisture or dew moisture and ambient temperature (Shewmaker & Thaemert, 2004).

Alternatively, chemical or near infrared reflectance spectroscopy (NIRS) analysis of moisture includes the transformation and reduction of the sample size by means of a laboratory grinder. Grinding is, however, a major cause of variation in moisture and other analytical results (Groenewald & Köster, 2005). According to Williams & Norris (2001) the moisture content of whole grain is usually significantly higher than that of ground grain upon which the chemical analysis (reference analysis) is based. This phenomenon is found in almost all materials, and more especially in hay such as lucerne hay. For some purposes it is important to report the actual moisture content of lucerne hay at trading. Furthermore, moisture loss during the grinding process is especially important for NIR scanning due to the fact that water is a strong absorber of NIR light. The degree of hydration may influence the optimum area of the NIR spectrum where the absorbers of specific constituents occur, thus affecting the whole NIR wavelength region (De Boever et al., 1996; Williams & Norris, 2001). Therefore, it could affect the predicted results of all the other parameters (CP, ADF, NDF, etc.).

The grinding procedure will not only alter the moisture status of the sample, but also effect changes in composition such as, inter alia, crude protein (CP) due to losses in the form of dust. According to Williams & Norris (2006) dust generated in the grinding process and the incomplete-ground residue in the grinder chamber is probably the largest cause of contamination between samples, especially in routine operations when a large number of samples is processed. Groenewald & Köster (2005) are of opinion that the product lost is not of the same composition as the product before grinding. Therefore, the final sample to be analysed might not be really representative of the product sampled for analysis (Groenewald & Köster, 2005). The question could thus be raised whether the final milled sample accurately represents baled lucerne hay of such sample, with regard to nutritive value as fed.

In the available literature no guidelines could be found for moisture and dry matter (DM) losses of lucerne hay during sample preparation. Most grinding statistics are based on grains (Williams & Norris, 2001) which lead to several assumptions regarding forages in general.

The objective of this study was to quantify the effect of the grinding procedure on the moisture and crude protein content of the final product. An additional aim was to investigate the accuracy of electronic moisture testers.

2.   MATERIALS AND METHODS

2.1   Lucerne hay samples

Forty-six samples of lucerne hay (*Medicago Sativa* L.) (n = 46) were obtained from several commercial irrigation farms at different locations in the Douglas and Hartswater area of South Africa (September 2006 to May 2007). The samples represented lots that were selected at different stages of maturity. As broad as possible a moisture range was obtained by means of a FARMEX electronic bale moisture probe (FARMEX, 1205 Danner Drive, Aurora, Ohio 44202). These samples were also obtained from different bale types namely small and large rectangular bales, as well as round bales. Each lucerne hay sample was a composite of 20 core samples (±12g dry weight) from the same cutting (lot), with a LTC (Lucernetech Consult CC., Hopetown, South Africa) forage sampler. Accordingly, the moisture reading of each sample was the average value of these 20 bales used to collect the core samples. In an effort to minimise moisture loss the unground samples were immediately

sealed in airtight containers and stored in a refrigerator below 5°C for subsequent grinding and chemical analysis.

2.2   Sample preparation and grinding losses

The 46 samples stored in the refrigerator below 5°C were exposed to room temperature (25°C) and a relative humidity (RH) of 56% for one hour to reach temperature equilibrium prior to grinding. Due to the nature of lucerne hay, care has to be taken to protect the natural ratio of leaves and stems. Every attempt was made in this study to obtain a representative sub-sample from each hay sample collected to obtain a representative moisture value for lucerne hay in the unground form. Each sample was thoroughly mixed and a representative sub-sample obtained (n = 46) for subsequent moisture analysis. The remainder of each sample was ground through a 1-mm screen using a LM 3100 laboratory grinder (Perten Instruments AB, Huddinge, Sweden) most widely used in South Africa. .

Separate samples (n = 5) were used to pre-warm the grinder to a constant temperature of 46°C before grinding the experimental samples (n = 46). The samples were milled for a total of ±100 seconds with a waiting period in between samples, lasting ±120 seconds each, to minimise temperature fluctuations within the sample and grinder. Temperature readings in the sample and grinding chamber were taken directly after grinding each sample, by means of a mercury thermometer. The material left in the grinding chamber after grinding each sample was quantitatively collected and stored in a refrigerator below 5°C for subsequent CP analysis. Additionally, the dust left in the dust bag originating from each sample was collected by thorough cleaning of the dust bag. The weight of the dust was determined by weighing the bag before and after grinding.

2.3   Chemical analysis

Moisture in the ground and unground samples (n = 46) was determined by forced-air oven drying at 98°C for 24 hours (AOAC, 2000). Crude protein content (CP: N x 6.25) of the residue in the dust bag and material remaining in the grinder were determined by means of the procedures described by AOAC (2000) using a Leco FP-528 Nitrogen Combustion Analyser (Leco. 3000 Lakeview Avenue, St. Joseph, MI 49085).

All chemical analysis was performed in duplicate and the results expressed on a dry matter basis.

2.4   Data analysis

Statistical analyses were performed using SAS 9.1.3 Service Pack 4 (2002-2003). Descriptive statistics namely the mean, standard deviation, coefficient of variation, minimum and maximum values were calculated for the quantitative variables. A complete randomised design was used. An analysis of the variance (ANOVA) was used to test for significant differences between groups. Dependent variables that were found to be significantly different ($P < 0.05$) were further subjected to Tukey's studentised range test (HSD). The Shapiro Wilk test was used to test for normality of the variables. Given that not all of the variables were normally distributed, the Pearson correlation coefficient was used to perform correlation analyses on the entire dataset. Furthermore, regression analyses were done separately for individual sources of variation (moisture and crude protein content of the unground samples).

3.   RESULTS AND DISCUSSION

3.1   Electronic moisture testers

The results when using an electronic moisture tester (EMT; probe) for lucerne hay are set out in Table 1. It was clear that the EMT was subject to error especially at high moisture levels in lucerne hay. An over-estimation of the moisture content of lucerne hay occurred at especially the maximum moisture level (Figure 1). These results were also reflected by the coefficient of variation values. Electronic moisture testers measure the resistance or conductivity in the hay.

Table 1   The effect of an electronic moisture tester and grinding on the moisture content of *Medicago sativa L.* hay

	Unground sample*	Ground sample*	Farmex[a]
Minimum (%)	6.8	5.8	8
Maximum (%)	18	10.6	37
Mean (%)	11.5	8.5	17
Standard Deviation	3.1	1.3	7.3
Coefficient of variation	27.4	16.1	41.8

* Normal distributed (p>0.05)

[a] Electronic moisture tester (FARMEX, 1205 Danner Drive, Aurora, Ohio 44202)

A small increase in bale density and/or moisture on the surface of the hay may increase conductivity dramatically (Shewmaker & Thaemert, 2004). This increase, due to surface moisture and bale density, results in an over-estimation of the moisture in lucerne hay. The average over-prediction of the moisture content in baled lucerne hay by the electronic moisture probe compared to unground samples, in Table 1 was in agreement with the 5% (% moisture units) reported by Shewmaker & Thaemert (2004). However, the average moisture content of the same samples after grinding was under-predicted by 8.5% (Table 1), when estimated by the electronic moisture probe.
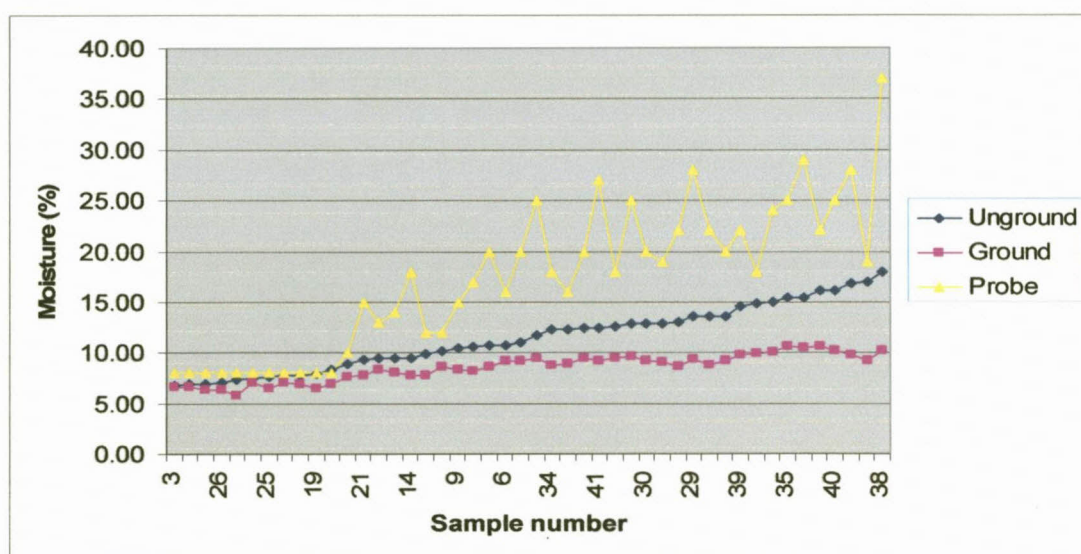
Figure 1   The effect of an electronic moisture tester (probe) and grinding on the moisture content of lucerne hay

The correlation between the moisture results determined on the unground samples and those with the EMT is shown in Table 2. Even though coefficient of determination between analytical measured moisture results on unground samples and values predicted by electronic moisture tester was significantly high ($r^2 = 0.79$, $P<0.0001$) (Table 2), the predicted values for higher moisture samples (>10 %) were found to be over-estimated in the current study (Figure 1). A slight improvement in the prediction of sample moisture prior to grinding from the electronic moisture tester was found by using a logarithmic equation ($r^2 = 0.8067$) compared to using a linear equation ($r^2 = 0.7877$) (Figure 2). It is clear that moisture values predicted by electronic moisture testers were more satisfactory at low moisture levels in lucerne hay bales. However, the EMT failed to accurately predict moisture content around

the critical moisture levels of 16% and higher. A significant positive correlation ($r = 0.84$; $P<0.001$) was also found between predicted moisture tester values and moisture loss due to the grinding process (Table 2).

Table 2   Correlation between different grinding products and chemical results

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
X1	1											
X2	0.91**	1										
X3	0.89**	0.88**	1									
X4	-0.08	-0.15	-0.03	1								
X5	-0.08	-0.14	-0.01	1.00**	1							
X6	-0.00	-0.09	-0.02	0.59**	0.61**	1						
X7	-0.12	-0.01	-0.04	0.50*	0.53*	0.96**	1					
X8	0.57**	0.44	0.49*	0.18	0.21	0.37	0.43*	1				
X9	0.72**	0.63**	0.64**	-0.01	0.04	0.13	0.24	0.91**	1			
X10	-0.39*	-0.26	-0.29	0.50*	0.48*	0.25	0.20	-0.37	-0.47*	1		
X11	0.70**	0.65**	0.62**	0.01	0.01	0.11	0.23	0.85**	0.97**	-0.23	1	
X12	0.97**	0.84**	0.84**	0.00	0.01	0.08	0.19	0.60**	0.72**	-0.34	0.70**	1
X13	0.92**	0.75**	0.78	0.11	0.14	0.20	0.32	0.64**	0.72**	-0.31	0.70**	0.98**

*$P<0.001$ and **$P<0.0001$

X1 Sample moisture unground

X2 Sample moisture ground

X3 Electronic moisture tester (Probe)

X4 Crude protein content of unground sample

X5 Crude protein content of ground sample

X6 Dust generated during the grinding process (g) and

X7 Dust generated during the grinding process (%); expressed as a percentage of the dry matter of the original unground sample

X8 Incomplete-ground residue collected from grinder chamber (g)

X9 Incomplete-ground residue collected from grinder chamber (%); expressed as a percentage of the dry matter of the original unground sample

X10 Crude protein content of dust generated during the grinding process (%)

X11 Crude protein content of dust generated during the grinding process (%); expressed as a percentage of the crude protein content of original unground sample

X12 Moisture loss due to the grinding process (g)

X13 Moisture loss due to the grinding process (%); expressed as a percentage of the moisture content of the original unground sample

An extensive study was performed by Scholtz & Van der Merwe (2007; -unpublished data) to evaluate two different types of electronic moisture testers used to predict the moisture content of lucerne hay. The preliminary data (data not shown) supported the variable results of Groenewald (2005; -unpublished data). As this is a further complicating factor, the use of an EMT needs further investigation.
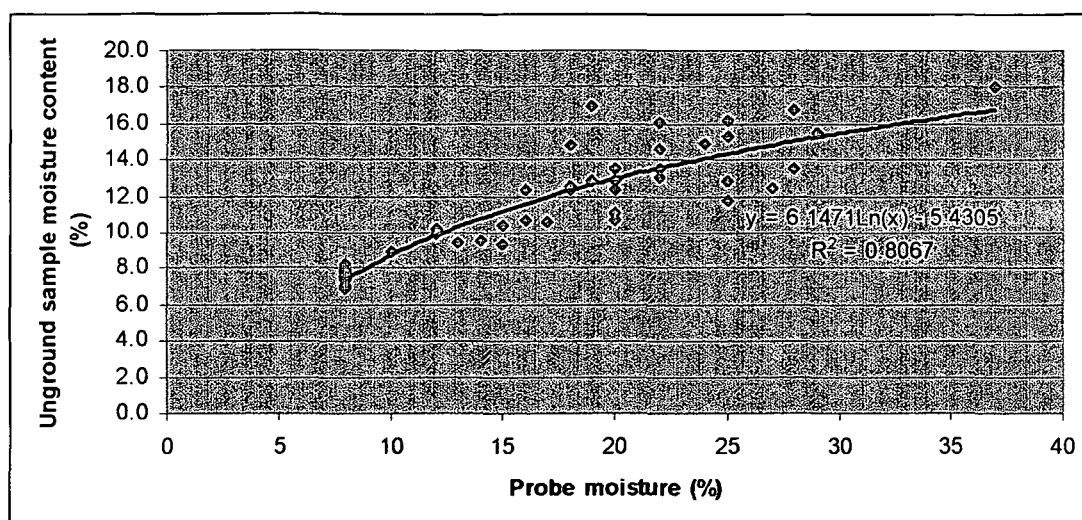
Figure 2 Relationship between moisture content measured analytically and by means of an electronic moisture tester (probe)

These results indicate that although electronic moisture testers provide an instantaneous moisture reading, they are subject to unacceptable error. Thus, they are not suitable when marketing hay, to identify high moisture in loads of baled lucerne hay.

3.2 Moisture loss

The moisture content of lucerne hay must be within a specific range for effective storage. Hay stored too wet (above 16%) will undergo pronounced fermentation with the spontaneous production of heat (Bath & Mable (1989). Therefore, accurate moisture determination in lucerne hay especially for trading purposes is of utmost importance.

From the results in Table 1 and Figure 3 is it evident that the grinding of lucerne hay resulted in moisture losses. Analysis of variance revealed significant (P < 0.0001) differences in the moisture concentration among the two treatments, which was also confirmed by Tukey's multiple tests. The mean sample moisture loss for the current study (27.5% of original moisture content) was high and somewhat unexpected. These losses were more pronounced for lucerne hay with high moisture content. In accordance with these results, Williams (2007) stated that moisture loss during the grinding process often causes under prediction of moisture content of the product sampled for analysis. According to Williams & Norris (2001) additional factors could influence the losses in moisture content namely the type of grinder, screen size, changes of revolutions per minute (RPM) of grinders, grinder maintenance and number of samples ground at the same time. According to the Shapiro-

Wilk test for normality, moisture content of lucerne hay samples was normally distributed (p > 0.05) across the range for both treatments (ground and unground). The results in Table 1 also show that grinding lucerne hay samples results in a lower CV of moisture content. Such lower variation was expected because heating generated by the grinding process of samples generally stabilises the moisture content of fibrous material.
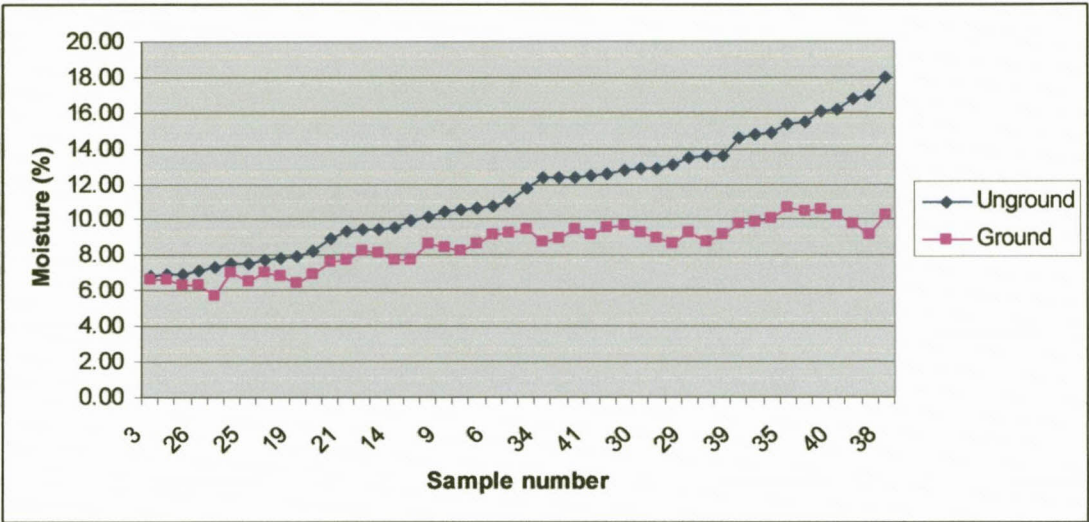
Figure 3   The effect of moisture content of lucerne hay on losses during grinding

The linear coefficient of determination (Table 2) between the moisture content of the ground and unground samples was significantly high ($r^2 = 0.83$, $P < 0.0001$). Figure 4 shows the exponential relationship between moisture content of lucerne hay samples in the ground and unground status. The graph suggest that 88% of the variation in moisture content of the original unground sample can be explained and calculated by moisture content of the ground sample using an exponential equation. This equation explained more of the variation in moisture content than the linear equation ($r^2 = 0.8372$). The highly significant ($P<0.0001$) positive exponential correlation again supported the finding that the higher the moisture present in the sample before grinding, the higher the moisture loss. However, the r^2 statistic is population dependent (Weiss, 1993) and would not necessarily be applicable to populations beyond this present study.
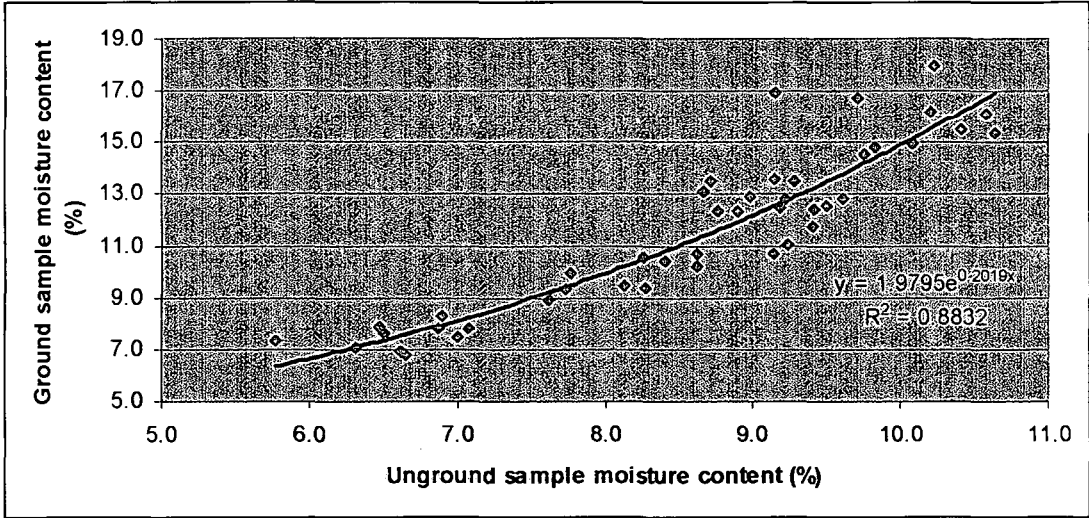
Figure 4   **Relationship between moisture content of lucerne hay samples in the ground and unground status.**

No research data could be found in the available literature on the extent of moisture loss during the grinding of lucerne hay. Moisture loss for some grains and lucerne hay during grinding, with the U-D Cyclone and LM 3100 grinders respectively, is summarised in Table 3.

Table 3   **Moisture loss during grinding process of grain (Williams, 2006) and lucerne hay**

Original moisture %	Moisture loss % Commodity			
	Wheat[a]	Dorum[a]	Barley[a]	Lucerne hay[b]
17	3.2	3.0	4.0	7.5
15	3.2	2.6	3.5	4.9
13	1.5	1.4	2.5	3.4
11	0.9	1.1	1.9	1.9
9	0.3	0.5	0.8	1.4

[a] Values adapted from Williams (2006)

[b] Values from the present study

The extent of moisture loss of all commodities was greater with the higher than the lower moisture levels. This could possibly be attributed to the difficulty with which higher moisture samples are forced through the 1mm sieve, resulting in higher heat generation, thus increasing moisture evaporation from the sample. There was however, an obviously higher

moisture-loss recorded for lucerne hay compared to grain. These losses become more prominent with increasing levels of original sample moisture (Figure 5).
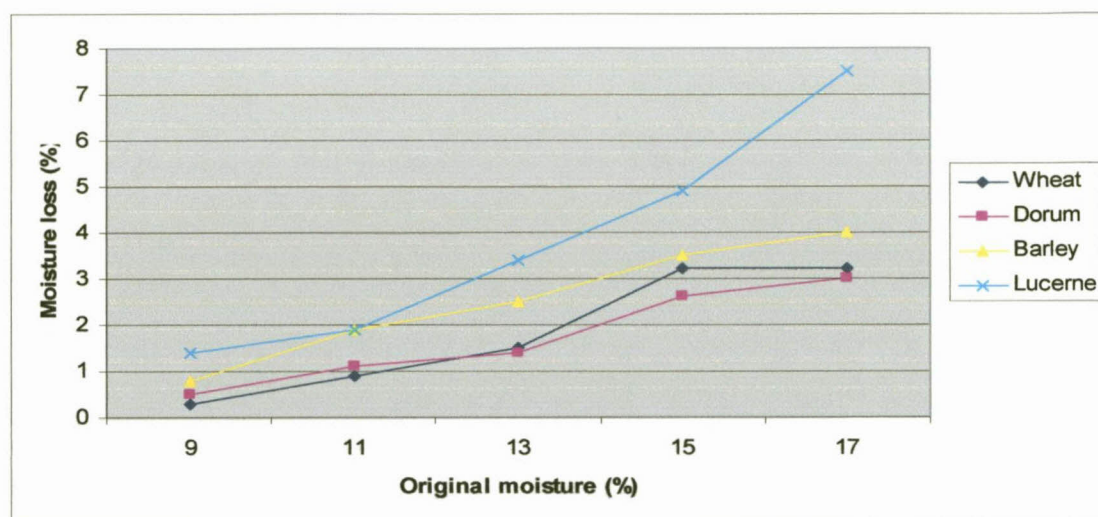
Figure 5   **Observed trends of losses in moisture content of grain and lucerne hay**

The higher moisture losses during grinding in lucerne hay compared to grains may probably be attributed to the differences in structural and non-structural carbohydrate contents. It could be expected that structural carbohydrates generate more heat and thus more moisture loss. Table 4 sets out the mean NDF and starch concentrations for lucerne hay and grain. From the results in Table 4 it may be hypothesised that the higher the NDF and the lower the starch concentration in a sample, the higher the moisture loss during the grinding process.

Table 4   **Mean NDF and starch values of different commodities [a]**

Commodity	Neutral detergent fibre	Starch and sugar
Lucerne	49	7.7
Barley [1]	20.1	59.9
Wheat [1]	12.4	70.1

[a] All values are based on a DM basis

[1] Values adapted from McDonald *et al*. (2002)

3.3   Sample loss

According to Williams (2007) the incomplete ground samples of grain residues from the grinder chamber are usually of different composition from the blended ground material, and should be completely removed by brushing and added to the ground sample before blending. However, for this study, and as part of an attempt to calculate the crude protein (CP) content

of the unground sample, this residue was quantitatively collected from the grinding chamber after the grinding of each sample, weighing and analysing for CP content.

The percentage of incomplete ground-residue collected from the grinder chamber and sample loss in the form of dust (dust bag) is presented in Table 5. The incomplete ground lucerne hay residues remaining in the grinder varied from 0.36 to 3.16% DM. The average loss was slightly lower than the 2% DM loss reported by Williams (2006) for grain, using a similar grinder.

Table 5   The proportion of incomplete ground residue and dust after sample grinding[a]

Component	Minimum	Mean	Maximum	SD[b]	CV[c]
Ground residue[1] (%)	0.36	1.39	3.16	0.69	49.64
Dust[1] (%)	0.25	1.07	3.40	0.75	70.09

[a] All values are based on a dry matter basis

[b] Standard deviation

[c] Coefficient of variation

[1] Variable expressed as a percentage of the original unground sample dry matter

In accordance with the ground residue the dust losses were low and highly variable (CV = 70.09%). The low value for ground residue and dust losses probably contributed to the high observed CVs. In the available literature no values for loss of sample due to dust that is generated in the grinding process could be found for any commodity.

Sample lost due to dust generated during the grinding process was non-significant (P>0.05) negatively correlated (r = -0.1) with moisture content of the unground sample (Figure 6). This non-significant (P>0.05) decrease in dust generated during the grinding of higher moisture samples was, however, expected due to a customary decrease in dust generation with a higher moisture content. The high observed CV observed for dust could have contributed to these results.

A significant (P<0.0001) positive correlation (r = 0.72) was observed between moisture content of the unground samples and the proportion incomplete ground residue collected from the grinder chamber (Table 2 and Figure 6). Such a correlation, although not high, was

expected because samples higher in moisture content are generally forced through the 1mm sieve with greater difficulty, leaving behind more of the sample in the grinding chamber.
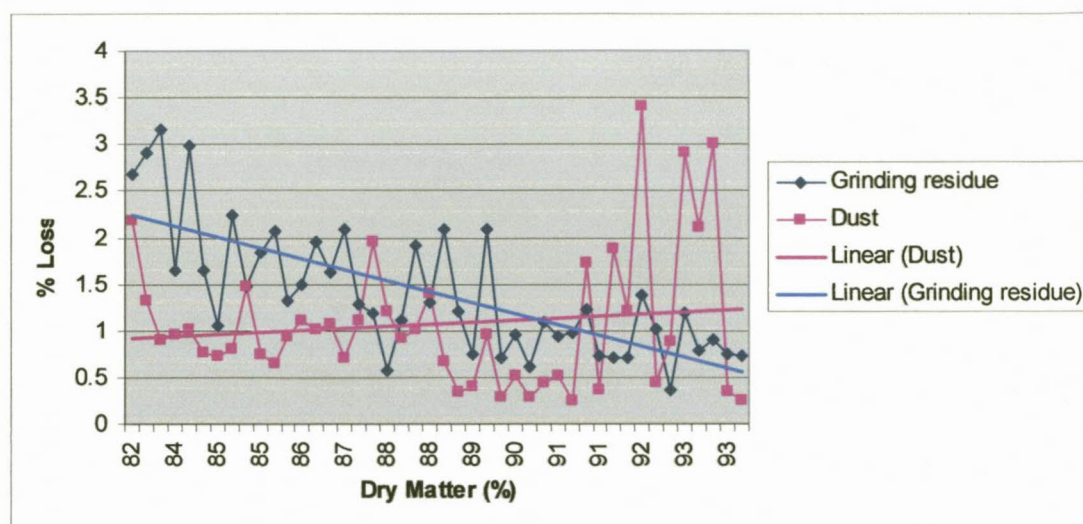
Figure 6   **Observed trends of losses in DM content of lucerne hay during the grinding procedure**

The CP content of different lucerne hay sample fractions is presented in Table 6. The variance of analysis revealed non-significant (P=0.6452, CV = 8.0094) differences in the CP content among the ground and unground samples, and is supported by Tukey's studendised range test. Surprisingly, CP content (% DM) of dust generated in the grinding process was significantly (P<0.0001) lower than that of the original unground sample (Table 6). This could probably be explained by the gusting of the lower specific gravity and CP-containing fibre particles into the dust bag during the grinding process. The CP content (%DM) of the incomplete grinding residue was also significantly (P<0.0001) lower than those of the ground and unground sample (Table 6). This is in agreement with the findings of Williams (2007) who also reported a different composition for incomplete grain residues compared to the grounded residue. On the other hand, as previously discussed, the incomplete ground residue should, in practice, be completely removed from the grinding chamber and added to the ground residue before blending. Thus, this fraction is of academic interest only.

Table 6   **Crude protein (CP) content of different lucerne hay fractions** [a]

Components	Minimum	Mean	Maximum	SD [b]	CV [c]
CP (UG) [1] (%)	15.72	19.78	24.83	1.57	7.94
CP (G) [2] (%)	15.74	19.95	25.11	1.61	8.07
CP content of dust [3] (%)	11.33	14.78	20.42	2.23	15.08
CP content of incomplete ground residue [4] (%)	12.14	15.13	19.14	2.13	14.08

[a] All values are based on a dry matter basis (n = 46 lucerne hay samples)

[b] Standard deviation

[c] Coefficient of variation

[1] Crude protein content of unground sample prior to grinding (Calculated as; CP content of ground sample (g/100g) + CP content in dust (g/100g) + CP content in incomplete ground residue (g/100g))

[2] Crude protein content of ground sample (residue)

[3] Variable expressed as a percentage of the dust dry matter content

[4] Variable expressed as a percentage of the incomplete ground residue dry matter content

A significant (P<0.001) but low negative correlation (r = -0.39) was observed between the moisture content of the unground sample and the CP content of the dust (Figure 7). It seems that the moisture content of the original unground sample could only explain 16% of the variation in the CP content of the dust. Accordingly, as previously discussed, no difference (P>0.05) was detected in dust losses during the grinding process due to the original moisture content.
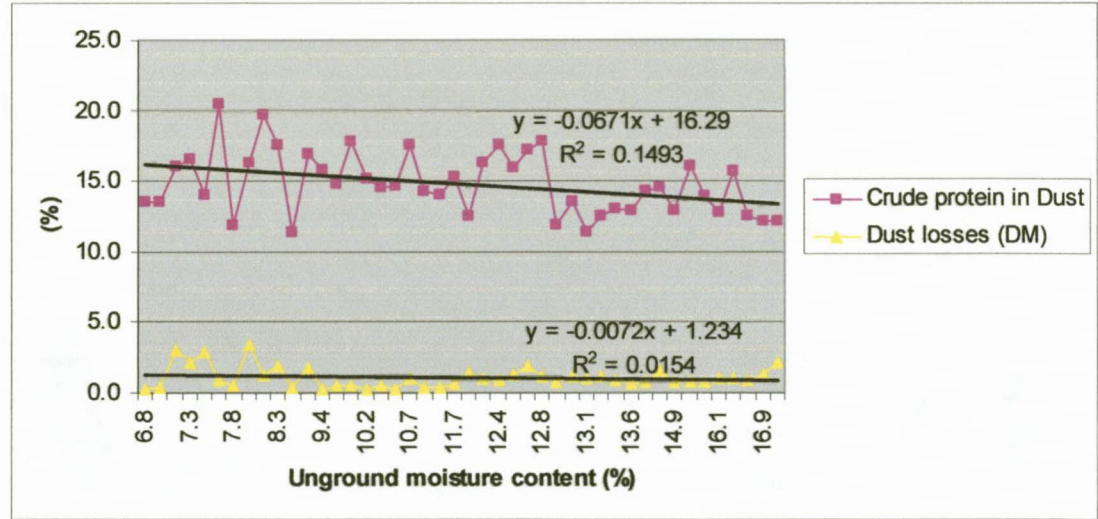
Figure 7   **Effect of original moisture content on loss of dust (expressed as a DM percentage of the unground sample) and CP content of dust.**

It is clear that because of the relatively small loss in dust (Table 5) and its lower CP concentration (Table 6), very little difference was observed in the CP content of lucerne hay samples with or without undergoing the grinding process. A virtually perfect positive relationship (r^2 = 0.99; P<0.0001) was observed between the CP content of unground and ground samples (Figure 8). Thus, based on these results, it is evident that dust generated in the grinding process had a non-significant (P>0.05) influence on the CP content of the end product.
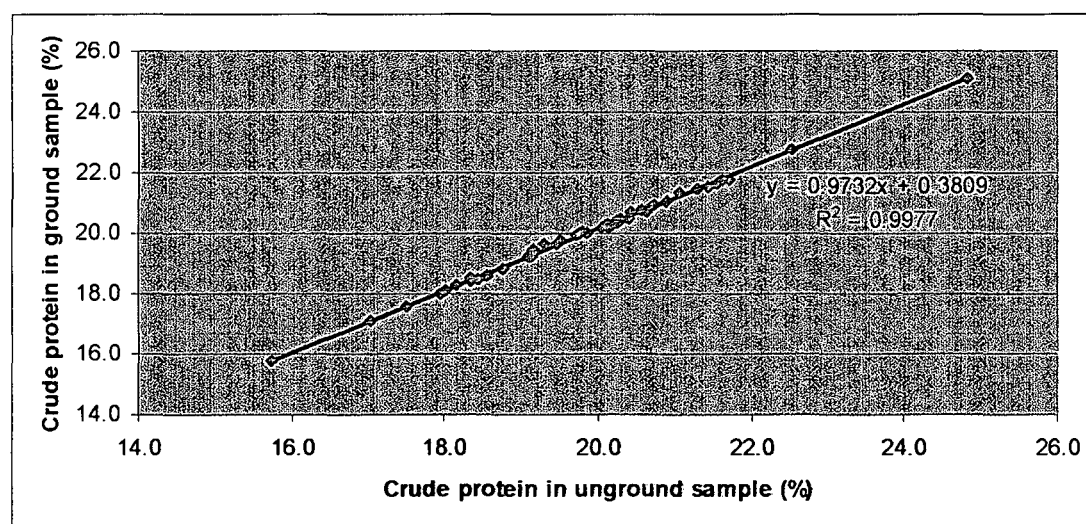
Figure 8   **Relationship between the CP content of lucerne hay samples in the unground and ground status**

The CP content of dust (%DM) and CP of unground sample (%DM) showed a weak positive relationship (r = 0.50; P<0.001). The actual CP content of dust (%DM) and ground residue showed a weak negative relationship (r = -0.47; P<0.001) (Table 2). The data of the incomplete ground residue collected from the grinder chamber are of academic interest only, as this residue is usually added to the ground sample as previously described.

However, the effect of grinding on other chemical parameters that occur at higher concentrations in lucerne hay, such as NDF, might result in a different influence on the NDF content of the end product. The NDF content of dust collected from the grinding bag in the current study could not be determined due to the low quantity of dust produced. In an effort to get an idea of the influence of dust loss during the grinding on the NDF content of the end product, it was assumed that maximum 66% NDF could occur in the dust. This value equals the maximum %NDF found in the population of South African lucerne hay (Chapter 3, Table

2). From this value and the weight of dust observed in the grinder bag, the potential % unit loss of NDF in the form of dust was calculated.

The calculated % units of NDF lost in the 46 lucerne hay samples are presented in Table 7. It seems that a substantial loss of NDF could occur in the form of dust. Therefore, the NDF content of the end product could be changed. According to the mean NDF (0.71%) contribution of dust to the NDF (%) content of the end product, it could be hypothesised that dust generated in the grinding process could have a significant (P<0.05) influence on the NDF content of the end product. These findings are however, based on assumptions and need to be verified by further research.

Table 7   **Neutral detergent fibre content (NDF) of lucerne hay dust in grinder expressed as a percentage of the original unground sample [a]**

	Minimum	Mean	Maximum	SD[b]	CV[c]
NDF (%)	0.17	0.71	2.08	0.49	69.01

[a] All values are based on a dry matter basis (n = 46 lucerne hay samples)

[b] Standard deviation

[c] Coefficient of variation

4. CONCLUSIONS

From the results of the present study and those in the literature with lucerne hay and grain respectively, it seems that moisture losses during grinding are more prominent for lucerne hay (27.5% of original moisture content). Furthermore, these losses are more pronounced for lucerne hay with a high moisture content. Based on these results it is evident that analytical moisture standards for lucerne hay should be based on the original moisture content of samples in the unground state so as to be relevant in practice. The results of the present study suggest that the moisture content of the original unground sample may be calculated with high accuracy from the moisture content of the ground sample using an exponential equation. However, it should be noted that this relationship could be highly population specific due to the limited number of samples used in the current study.

Average sample losses due to incomplete ground residue in the grinder chamber and dust gathering in the dust bag were low. It was also found that the CP content (%DM) of dust generated in the grinding process was significantly lower than that of the original unground sample. Accordingly, the grinding process had no influence on the CP content of the end product. However, the effect of grinding on other chemical parameters warrants further investigation. The same applies to the contamination phenomenon between different commodities, especially animal by-products and forages, ground with the same grinder.

Results from the present study also point out that moisture values predicted by electronic moisture testers fail to accurately predict moisture content around the critical moisture level of 16% and higher.

CHAPTER 5

PREDICTION OF CHEMICAL COMPOSITION OF SOUTH AFRICAN *MEDICAGO SATIVA* L. HAY FROM A SPECTRALLY-STRUCTURED SAMPLE POPULATION

1.   **INTRODUCTION**

The dairy feed industry is the largest consumer of lucerne (*Medicago sativa* L.) hay in South Africa (Grönum *et al.*, 2000). As shown in Chapter 3 its energy and protein value could vary considerably and should be determined beforehand in order to compose a balanced diet. Fine-tuning of the nutrient supply to the individual requirements of the animals is essential to achieve their production potential and to avoid unnecessary mineral losses to the environment. Thus, an accurate prediction of the nutrient composition is essential to meet the animals' requirements and to avoid nutrient losses to the environment.

Although of great value in the past, the use of tables in the literature (NRC, 2001, McDonald *et al.*, 2002) as a guideline of the nutritive value of a feed is inaccurate and lead to over- or underfeeding of high producing animals like dairy cattle. Van Soest (1994) stated that values in published tables are often unreliable because of differences in geography and environment. Thus the use of recognised published tables like NRC (2001) is not a desirable alternative to forage testing. On the other hand, laboratory analysis is laborious, expensive and time consuming so that results often transpire late after consumption. With the recent introduction of near infrared reflectance spectroscopy (NIRS), fast evaluation of feeding quality is now a possibility.

The technique is based on the correlation between chemical properties, as determined by defined reference methods (chemical analysis), and absorption of light at different wavelengths in the near infrared region, measured by reflectance. The near infrared region contains information concerning relative proportions of C---H, N---H, and O---H bonds, which are the primary constituents of the organic molecules in forages (Osborne *et al.*, 1993 and Coleman & Murray, 1993). NIRS relies on calibrations, which utilise absorbencies at many wavelengths, to predict composition of a feed sample (Murray, 1986; Batten, 1998).

The precision and accuracy of the predictions are critical to the acceptance of NIRS as an analytical tool. In practice, the reproducibility of the NIRS method is usually equal, and often superior, to that of the reference method. Furthermore, NIRS has less variance in analyses of the same sample than laboratory analyses (Marum & Aastveit, 1990; Shenk *et al.*, 1979). However, it requires a large number of samples for calibration, thus significant initial start-up costs. Timely decision making on strategic use of nutritional supplements or adjustments in ration formulation to efficient sustain milk, meat, or fibre production is also facilitated by this method. A major advantage of NIRS is its ability to analyse samples rapidly, non-destructive, and its non-polluting technology. Thus as noted by Mark *et al.* (2002) it avoids the problem of organic and other chemical waste disposal, and there are few if any hazards associated with the technique because it uses no toxic or corrosive reagents.

Near infrared reflectance spectroscopy (NIRS) is a long-established, and now highly developed technology. Norris & Hart (1965) as cited by Givens *et al.* (1997) developed the first application of NIRS to measure water in grains and seeds. Norris *et al.* (1976) are also credited with the first application of NIRS to the analysis of forages. Their precisions were sufficiently high for them to claim "infrared reflectance has the potential for use in rapid evaluation of forage quality". Since then numerous workers have explored the use of NIRS for the prediction of both chemical composition and digestibility on lucerne hay (Atanassova *et al.*, 1994; Krachunov, 1998; Van Waes *et al.*, 1998). However, limited calibrations have been developed and published for South African grown lucerne hay (Stoltz, 1990; Snyman & Joubert, 1992; Scholtz, 2001). There is an urgent need to ascertain whether NIRS could be relied upon to analyse all the lucerne hay feeding parameters used in modern nutritional models. This is especially important in developing a model for lucerne hay grading in South Africa

The objective of his study was to identify useful predictive relationships from a pre-selected spectrally structured lucerne hay sample population in South Africa.

2. MATERIALS AND METHODS

2.1 Lucerne hay samples
Six hundred lucerne (*Medicago sativa* L.) hay samples were collected from several commercial irrigation farms in the main lucerne producing areas in South Africa, which

varied in location, soil characteristics (texture, organic matter, N content, pH) and farm management. The samples were collected and prepared as described in Chapter 3.

2.2   Scanning of lucerne hay samples

The software for scanning, mathematical processing, calibration and statistical analysis with the NIR spectrophotometer was supplied by Infrasoft International® software 3.01 version (ISI, Port Matilda, PA, USA). The 600 milled lucerne hay samples (approx. 10g) were scanned using 50 mm quarts cuvettes in an NIR-Systems Model 5000 scanning monochromator (Foss NIR Systems, Silver Spring, MD, USA) without replicates over a wavelength range of 1100–2498 nm at every 2 nm providing a total of 700 data points for each sample spectrum. The NIRS spectra for the selected South African lucerne hay population (n = 168) is shown in Figure 1. Two pairs of lead sulphide detectors collected the reflectance spectra and the reflectance energy readings were referenced to corresponding readings from a ceramic disk at 25°C. The spectrum of each sample was the average of 32 successive scans (16–32–16). Spectral data were stored as the reciprocal logarithm of reflectance (1/R). Spectra were also corrected for light scattering and sample particle size by using ISI Detrend and standard normal variate (SNV) (Barnes *et al.*, 1989).

2.3   Selection of representative samples

Samples for inclusion in the calibration sets were selected on the basis of spectral characteristics. These samples were checked for erroneous measurements and outliers, as extreme samples influence wavelength selection and coefficient size in the development of calibration equations. The Centre algorithm (Infrasoft International, Port Matilda, PA, USA) described by, Shenk & Westerhaus (1991) was used for this procedure. The required number of samples for calibration was chosen using the SELECT algorithm available in the WinISI software (Infrasoft International, Port Matilda, PA, USA) and developed by Shenk & Westerhaus (1991). The SELECT algorithm works by choosing a subset of samples with representative spectral characteristics of the whole population on the basis of their standardised *H* (Mahalanobis distances). A minimum standardised *H* distance of 1.0 between neighboring samples (spectra) was chosen, resulting in the algorithm selecting 168 representative samples (Calibration set: spectrally structured) from the population (*n*=600).
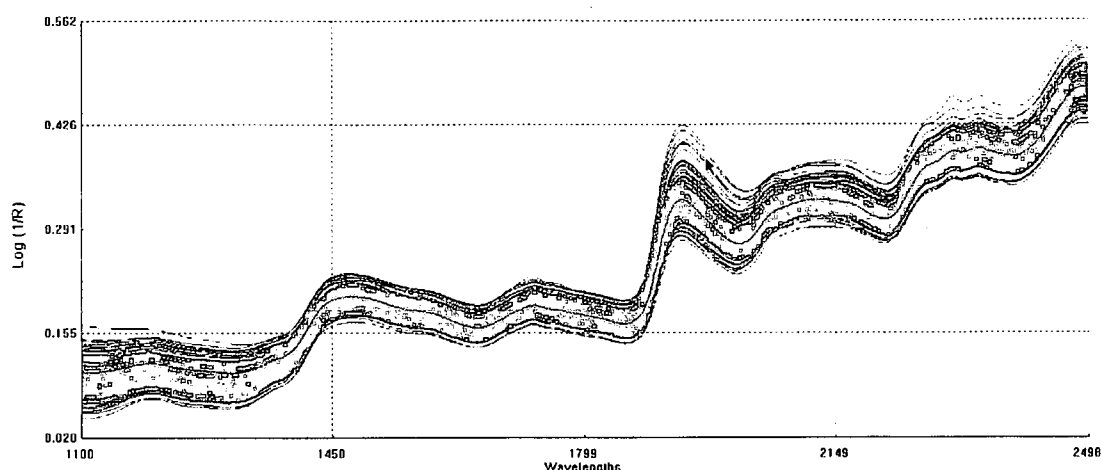
Figure 1   **NIRS absorption spectra of South African lucerne hay samples.**

2.4   Reference (chemical) analysis

The selected 168 samples were analysed in duplicate by Cumberland Valley Analytical Services, Inc., Maugansville, Maryland, USA, as described in Chapter 3 and Chapter 4, for the following parameters: dry matter (DM), crude protein (CP), soluble protein(SP), acid detergent fibre-crude protein (ADFCP), neutral detergent fibre-crude protein (NDFCP), lignin, fat, starch, sugar, *in vitro* neutral detergent fibre digestibility at 24 hours (NDFD24), and 48 hours (NDFD48), *in vitro* organic matter digestibility at 24 hours (IVOMD24), *in vitro* organic matter digestibility at 48 hours (IVOMD48), ash, calcium (Ca), phosphorus (P), magnesium (Mg), potassium (P), sodium (Na), sulphur (S) and chloride (Cl).

2.5   Calibration and validation

Calibration equations were developed from the 168 samples selected from the population set (n=600), using modified partial least square regression (MPLS) (Shenk & Westerhaus, 1993) with internal cross-validation (NIRS 2, 1995) to encompasses all of the chemical and spectral variation (Wiliams, 1987) and the physico-chemical characteristics that are likely to be found in the population (Williams & Cordiero, 1985).

Parameters in the mathematical processing were sought through trial and error in order to minimize the standard error of cross-validation, giving best results with the mathematical treatment 1, 4, 4, 1, which means 1: number of derivative of spectra, 4: extent of gap over which the derivative was to be calculated, 4: the smoothing of points, 1: second smoothing (almost never used and normally set as 1). In comparison with other regression methods, the

Westerhaus, 1994). Only terms were included in the equation that had an F statistic of more than 8 to minimize over-fitting of data.

The standard error of cross validation (SECV) was calculated by an internal validation of 33% of all samples randomly taken by the software routine, which was predicted by an equation based on a calibration with the remaining 67% of all samples. Since the cross-validation results are reliable indicators of equation performance (Shenk & Westerhaus, 1994), the lower SECV and standard error of calibration (SEC), together with their coefficient of determination (r^2) values, was the criteria used for the final selection of the equations to be tested further.

Another measure for equation selection was the number of H outliers when the constituents were predicted in the South African lucerne hay population. According to Shenk & Westerhause (1994) a low number of H outliers would indicate the span of the equation for predictive purposes. Murray (1993) as cited by Herrero *et al.* (1996) suggested that the number of H outliers when predictions are carried out, in a routine forage evaluation by NIRS, should not exceed 4%. Accordingly this was the limit used in the present study.

Williams (2007) stated that the r^2 and the ratio of prediction to deviation (RDP) are the most meaningful statistics for appraisal of analytical efficiency by NIRS. Other calibration evaluation statistics used in the present study included the SEC and SECV.

3. RESULTS AND DISCUSSION

3.1 Reference values

The chemical range, mean values, standard deviation (SD), coefficient of variation (CV) and normality (p) of the South African lucerne hay population are shown in Table 1. Selecting calibration samples by the automatic selection procedure (SELECT algorithm; Infrasoft International, Port Matilda, PA, USA), resulted in a more or less normal distribution across the range for some of the quality components. However, this procedure based on the H distance of samples may not guarantee the maximum range in every case (Volkers *et al.*, 2003). A wide variation in the chemical composition of South African lucerne hay (Table 1) was expected due to a wide range of varieties, seasons and growth stages, etc. (as discussed

Table 1   **Statistics of the reference tested parameters of the lucerne hay samples, used for NIRS calibration.**

Parameter[a]	n[b]	Mean	Minimum	Maximum	Range	SD[c]	CV[d]
Dry Matter (%)	160	92.72	89.82	95.62	5.80	0.97	1.04
Crude Protein (%)*	160	20.78	13.20	28.37	15.17	2.53	12.17
Soluble Protein (%)*	163	6.25	2.00	10.50	8.50	1.42	22.66
ADF-CP[e] (%)	159	1.52	0.07	2.98	2.91	0.49	31.88
NDF-CP[f] (%)	160	4.06	0.09	11.55	11.46	2.50	61.45
Acid Detergent Fibre (%)*	160	33.03	19.72	46.34	26.62	4.44	13.43
Neutral Detergent Fibre (%)*	161	43.77	24.37	63.17	38.80	6.47	14.78
Lignin (%)	160	7.13	3.25	11.01	7.77	1.29	18.16
Fat (%)	161	1.84	0.92	2.75	1.83	0.31	16.64
Starch (%)	164	2.00	0.36	3.65	3.30	0.55	27.43
Sugar (%)*	163	5.69	1.16	10.21	9.06	1.51	26.54
NDFD24[g] (%NDF)	163	35.80	22.27	49.33	27.05	4.51	12.59
NDFD48[h] (%NDF)	162	41.33	26.53	56.12	29.59	4.93	11.93
IVOMD24[i] (%)	161	60.07	47.54	72.60	25.06	4.18	6.95
IVOMD48[j] (%)	161	61.51	46.04	76.98	30.94	5.16	8.38
Ash (%)	157	12.75	3.21	22.30	19.08	3.18	24.94
Calcium (%)*	162	1.31	0.37	2.26	1.89	0.32	24.00
Phosphorus (%)*	163	0.30	0.14	0.46	0.33	0.05	18.20
Magnesium (%)	157	0.42	0.00	0.96	0.96	0.18	42.55
Potassium (%)*	159	2.50	0.46	4.54	4.08	0.68	27.22
Sodium (%)	162	0.25	0.00	0.61	0.61	0.12	47.24
Sulphur (%)*	157	0.31	0.12	0.49	0.38	0.06	20.33
Chloride (%)*	162	1.06	0.02	2.10	2.07	0.35	32.64

[a] All values are based on a DM basis

[b] Number of samples from calibration population used to create equation

[c] Standard deviation

[d] Coefficient of variation

[e] Acid detergent fibre-crude protein (ADF-CP) and neutral detergent fibre-crude (NDF-CP)

[f] Neutral detergent fibre-crude (NDF-CP)

[g] *In vitro* Neutral detergent fibre digestibility at 24 hours

[h] *In vitro* Neutral detergent fibre digestibility at 48 hours

[i] *In vitro* organic matter digestibility at 24 hours

[j] *In vitro* organic matter digestibility measuring at 48 hours

* $p > 0.05$ (normal distributed)

in Chapter 3) used to develop the NIRS calibration model. The CV values obtained in the current study are of the same order and magnitude as those of Scholtz (2001) for a population of 210 samples of South African lucerne hay. Thus, the spectrally selected population in the current study covered a good portion of the variability reported in the available literature (Chapter 3) for this species.

In the present study an attempt was made to classify the suitability of the reference methods for NIRS calibration, according to the relationship between the error in analysis and the spread in composition (Cozzolino & Moron, 2004). If a product shows a narrow range in composition, or if the error in estimation is large compared with the spread (as SD) in composition, then the regression finds increasing difficulty in finding stable NIRS calibrations (García & Cozzolino, 2006). Where the error exceeds one third of the SD of the population, regression can be misleading (Williams & Norris, 2001). Where SECV is equal or higher to the SD, the instrument is not predicting the reference values at all (Williams & Norris, 2001). Williams (2007) suggested that very low SD values for a population of reasonable size (60 or more) may indicate that the variance is so low that analysis is not necessary, except for quality control. Hence, fat content and several minerals in the present study might fit this criterion (SD ≤ 0.32).

From Table 1 it seems that the NDF-CP value varied the most followed by ADF-CP, starch and sugar concentration, respectively. According to Williams (2007) the CV of parameters should be at least between 10 - 12 % to represent a population. All of the parameters calibrated in the present study, except DM, IVOMD$_{24}$ and IVOMD$_{48}$ fulfil in this criterion. Although the maximum value of lucerne IVOMD$_{24}$ and IVOMD$_{48}$ were 25%DM and 30%DM higher, respectively, than the minimum these fairly low variations were somewhat surprising. Scholtz (2001) also reported an even lower CV value of 5.39% for IVOMD$_{48}$ for South African lucerne hay. The overall low CV for IVOMD could probably be explained by the relative constant susceptibility to degradation of organic matter (OM) in the rumen. The low CV and range of DM in the current study was due to the excessive moisture loss during the grinding process as discussed in Chapter 4 (par. 3.2). This led to the under prediction of the moisture content of especially high moisture lucerne hay.

3.2   Criteria for the prediction ability of calibration models

The most commonly used measures of the strength of the relationship between a dependent variable and independent variable are the coefficient of determination (r^2). However, the r^2

statistic is population dependent (Weiss, 1993). This means that, when used for comparison of different models that were derived from different populations, r^2 should not be the sole statistic used to evaluate the models (Weiss, 1993). Thus, Williams (2001) proposed the use of r^2 (Table 2) together with RPD (Table 3) as the most meaningful statistics for appraisal of analytical efficiency by NIRS. However, interpretation of RPD in the literature revealed mixed results. Williams (2001) suggested minimum requirements for RPD values of 3.1 – 4.9 for screening and 5.0 – 6.4 for quality assurance (Table 3). On the other hand Edney *et al*. (1994) considers RPD values of 2.5 – 3.0 adequate for screening, but values of 3.0 – 5.0 are require for quality assurance. Several authors are however of opinion that RPD values higher than 2.5 in any equation are required for an acceptable predictability (Williams & Sobering, 1993; Edney *et al*., 1994; Mathison *et al*., 1999).

Table 2   Guidelines for interpreting r^2 (Williams, 2001)

Value of r	r^2	Interpretation
Up to ± 0.5	Up to 0.25	Not usable in near-infrared reflectance calibration
±0.51 - 0.70	0.26 - 0.49	Poor correlation: reasons should be research
±0.71 - 0.80	0.50 - 0.64	OK for rough screening; more than 50% of variance in *y* accounted for by *x*
±0.81 - 0.90	0.66 - 0.81	OK for screening and some other "approximate" calibrations
±0.91 - 0.95	0.83 - 0.90	Usable with caution for most applications, including research
±0.96 - 0.98	0.92 - 0.96	Usable in most applications, including quality assurance
±0.99 +	0.98 +	Usable in any application

Table 3   The ratio of prediction to deviation (RPD) statistics (Wiliams, 2001)

RPD value	Classification	Application
0.0 - 2.3	Very poor	Not recommended
2.4 - 3.0	Poor	Very rough screening
3.1 - 4.9	Fair	Screening
5.0 - 6.4	Good	Quality controle
6.5 - 8.0	Very good	Process controle
8.1+	Exelent	Any application

The statistical results obtained from the calibration procedure are presented in Table 4. Performance on cross-validation, expressed as coefficient of determination (1-VR) and standard error of cross validation (SECV) are also shown in Table 4.

Table 4   **Calibration and prediction (cross-validation) statistics of quality parameters of South African lucerne hay.**

Parameter	SEC[a]	r²[b]	SECV[c]	1-VR[d]	SD[e]	RPD[f]
Dry Matter (%)	0.17	0.97	0.20	0.96	0.97	4.84
Crude Protein (%)	0.47	0.97	0.55	0.95	2.53	4.57
Soluble Protein (%)	0.52	0.87	0.68	0.77	1.42	2.08
ADF-CP [g] (%)	0.20	0.83	0.24	0.77	0.49	2.06
NDF-CP [h] (%)	0.73	0.91	0.84	0.89	2.50	2.96
Acid Detergent Fibre (%)	0.98	0.95	1.12	0.94	4.44	3.97
Neutral Detergent Fibre (%)	1.38	0.95	1.62	0.94	6.47	3.99
Lignin (%)	0.32	0.94	0.36	0.92	1.29	3.61
Fat (%)	0.13	0.81	0.17	0.71	0.31	1.82
Starch (%)	0.39	0.51	0.44	0.36	0.55	1.25
Sugar (%)	0.45	0.91	0.54	0.87	1.51	2.82
NDFD24 [i] (%NDF)	1.75	0.85	1.96	0.82	4.51	2.31
NDFD48 [j] (%NDF)	2.29	0.78	2.64	0.72	4.93	1.87
IVOMD24 [k] (%)	1.31	0.90	1.47	0.88	4.18	2.84
IVOMD48 [l] (%)	1.68	0.89	1.91	0.86	5.16	2.70
Ash (%)	0.83	0.93	1.02	0.90	3.18	3.12
Calcium (%)	0.16	0.74	0.21	0.58	0.32	1.54
Phosphorus (%)	0.03	0.78	0.03	0.66	0.05	1.71
Magnesium (%)	0.14	0.41	0.16	0.23	0.18	1.14
Potassium (%)	0.25	0.87	0.30	0.80	0.68	2.23
Sodium (%)	0.06	0.76	0.07	0.67	0.12	1.73
Sulphur (%)	0.05	0.24	0.06	0.16	0.06	1.08
Chloride (%)	0.07	0.95	0.09	0.93	0.35	3.74

[a] Standard error of calibration

[b] Coefficient of determination

[c] Standard error of cross-validation

[d] Cross validation coefficient of determination

[e] Standard deviation

[f] Standard deviation to standard error of cross validation

[g] Acid detergent fibre-crude protein

[h] Neutral detergent fibre-crude protein

[i] *In vitro* Neutral detergent fibre digestibility at 24 hours

[j] *In vitro* Neutral detergent fibre digestibility at 48 hours

[k] *In vitro* organic matter digestibility at 24 hours

[l] *In vitro* organic matter digestibility at 48 hours

Prediction of chemical composition depends on the choice and number of the chemical assays carried out on the feeds (Van Soest, 1994). The residual SEC's are partly related to the reproducibility of the analytical methods. According to Coates (2002) accuracy in the determination of reference values for use in NIRS prediction equations is critical, as the accuracy of the NIRS prediction is only as good as the reference values used for the calibration.

The coefficient of determination was influenced by the range in chemical data, as demonstrated by the differences in r^2 values for each component of the lucerne hay calibration. The r^2 usually is higher when the range of independent variables is wide instead of narrow (Neter & Wasserman, 1974). This is in accordance with studies carried out by Clark et al. (1987), Stoltz (1990) and Volkers et al. (2003).

For most parameters the cross-validation statistics were marginally inferior to those for the calibration, as demonstrated by the lower coefficient of determination (r^2 and 1-VR) value and higher standard errors. The SECV should theoretically always be higher than the SEC (Williams, 2007). In practice, however, the SECV may not always be higher than the SEC. For some applications, the precision of the NIRS instrument may be superior to that of the reference method. In the current study the SECV value for phosphorus was equal to the SEC value, whereas higher SECV value was reported for all the other parameters. According to Williams (1987) the SECV should not be more than 3 % of the mean reference value for that analyte. The highest value reported was 2.64% for neutral detergent fibre digestibility at 48 hours (NDFD48), whereas phosphorus (P) had the lowest (0.03) SECV.

The parameters in the present study with a RPD value lower than 2.5 included SP, ADF-CP, fat, starch, NDFD24, NDFD48 and all the minerals except Cl. These low unacceptable RPD values should be considered when identifying and/or developing a model for lucerne hay quality determination and grading.

3.2.1 Prediction of chemical composition (dry matter, fat, protein- and carbohydrate fractions) of lucerne hay

The accuracy (r^2) with which the NIRS were able to predict DM, CP, ADF and NDF of lucerne hay in this study compared favourably with results of other similar studies on South African lucerne hay (Stoltz, 1990; Snyman & Joubert, 1993; Scholtz, 2001). The r^2 values of

≥ 0.95 calculated in equation development and $1 - VR$ of ≥ 0.94 for cross-validation indicate that an excellent portion of the variance in DM, CP, ADF and NDF of lucerne hay were explained by the calibration models. This also applied to some extend for lignin and sugar (Table 4). From Table 2 it seems that these calibrations are useable for most applications including quality assurance. According to Table 3 the RPD values of all the mentioned parameters, with the exception of sugar could be classified as fair and applicable for screening. Relationships between chemically determined and NIR predicted values for DM, CP, ADF and NDF are graphically illustrated in Figure 2. The relationships for the other chemical parameters are included Appendix B1.

The highest calibration r^2 were obtained for CP and DM ($r^2 = 0.97$; Table 4). Moisture has a strong absorbance at about 1940 nm, affecting the whole NIR region (Williams, P. 2007, Personal communication, PDK Grain, 5072 Vista View Crescent, Nanaimo B.C V9V 1L6, Canada). Moisture status includes moisture content and degree of hydration (Williams, 2001). The degree of hydration of complex molecules such as proteins may cause changes in the particular orientation of the molecules. This could cause samples containing these molecules to interact with the irradiating light differently from other samples (Williams, 2001). Therefore, to ensure accuracy, the moisture content of the samples must be kept within the moisture range of the calibration (Winch & Major, 1980; Williams, 2007).

Total nitrogen (N) or CP (N x 6.25) is one of the most commonly measured components of forages and feedstuffs (Stuth *et al.*, 2003). Strong –N-H absorptions in the NIR region are the primary cause for the good calibration statistics (Table 4). The relatively high concentrations of CP, which in forage and feed can range from 3 to 50% DM, is also another contributing factor (Roberts *et al.*, 2003).

The measurements of detergent fibre fractions, namely ADF and NDF of lucerne hay, are also successful and stable NIRS calibrations occurred with SEC values of <1.4. This finding was confirmed by several authors (Clark *et al.*, 1987; Stoltz, 1990; Williams & Norris, 2001). Although these fibre fractions (ADF and NDF) are a "property" of forage and not constituents, it can still be estimated due to variations in -C-H and -O-H bonds in the range from 30 to 80%.
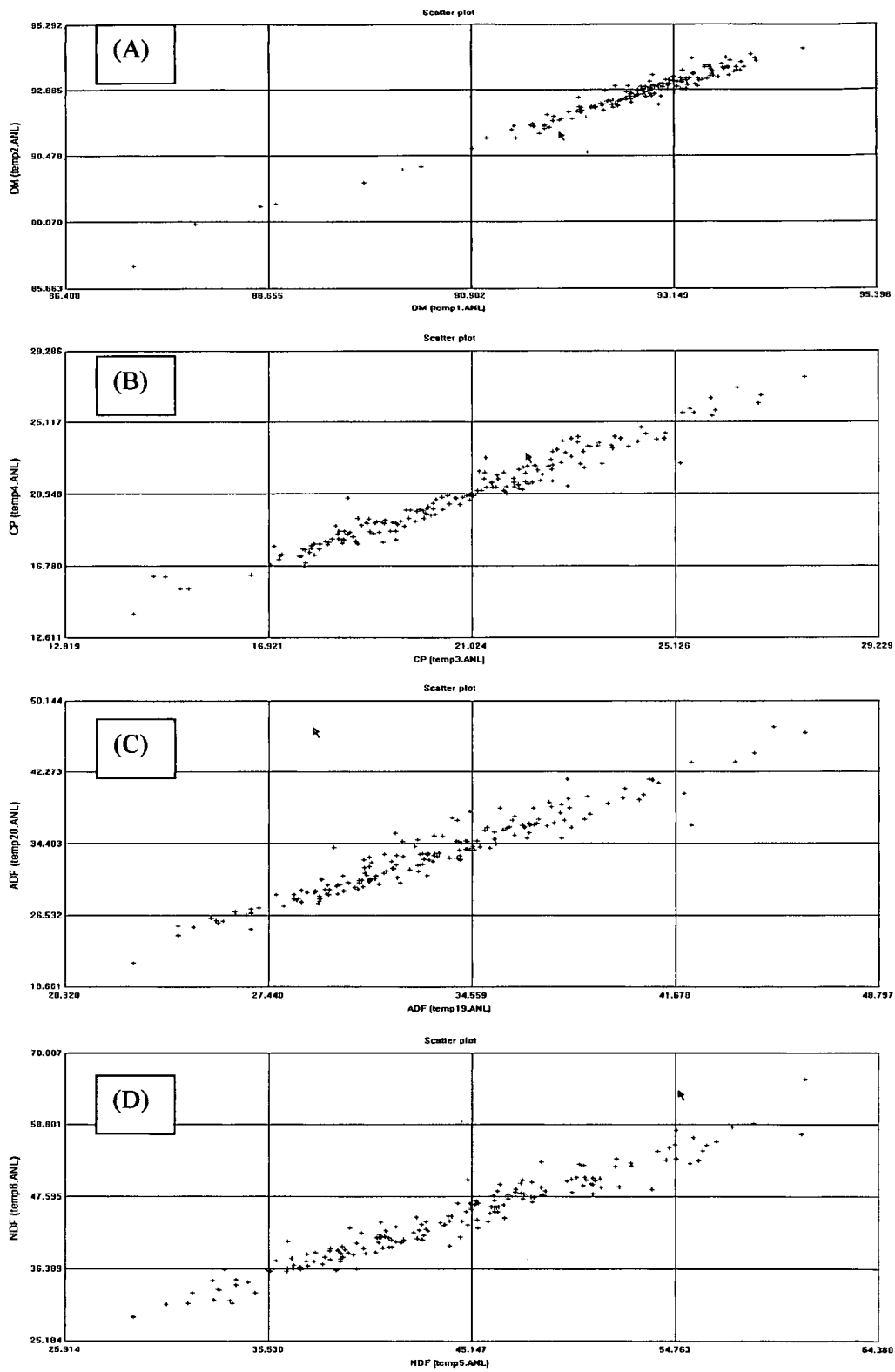
Figure 2 Near infrared reflectance spectroscopy (NIRS) predicted values versus reference values for lucerne hay (A = dry matter, B = crude protein, C = acid detergent fibre, D = neutral detergent fibre).

The high coefficient of correlation obtained for lignin (Table 4), which generally gives poorer results compared to the other chemical parameters analyzed (Berardo *et al.* (1997), was somewhat surprising. According to Hoffman (2004) lignin is an arduous laboratory assay and is usually not well predicted by NIRS. Murray *et al.* (1987) mentioned the NIRS signals at 1660 nm and 2266 nm directly arising from lignin. According to Givens (1993) it is mainly by this means that NIRS has the potential to predict digestibility.

According to the r^2 values in Table 2 and Table 4 the soluble protein (SP), ADF-CP, NDF-CP, fat and sugar calibrations were useable with caution for most applications, including research. The 1-VR values of these parameters were generally moderate ($\geq$ 0.71). The exception, however, were the poor cross-validation statistics for the starch content (1-VR = 0.36). The RPD values, however, range according to Williams & Norris (2001) from very poor to poor for these parameters. The RPD values calculated for NDF-CP and sugar calibrations met the requirements (>2.5) for an acceptable predictability as set by several authors already mentioned.

According to Hoffman *et al.* (2003) measuring NDF-CP is usually more problematic for NIRS. The overall influence of NDF-CP on the NRC (2001) summative energy equation is however small, therefore a qualitative assessment of NDF-CP using NIRS is acceptable and will not greatly compromise the accuracy and precision for the summative energy prediction for forages. ADF-CP showed a somewhat inferior prediction potential compared to NDF-CP. Mertens (1979) mentioned that the heterogeneity of ADF-CP might explain the difficulty in measuring it (especially with NIRS).

It was not possible to accurately calibrate equations for fat (r^2 = 0.81; RPD = 1.82). Forage measures of fat in the literature have had mixed results (Bernardo *et al.*, 1997; Park *et al.*, 1998). The overall poor predictive power of fat can be ascribe to the low concentration, narrow range and heterogeneous nature of crude fat found in lucerne hay (Stuth *et al.*, 2003). The relevancy of an accurate calibration equation for lucerne hay fat content is however questionable due to its low concentration in lucerne hay.

According to several authors (Williams & Sobering, 1993; Edney *et al.*, 1994; Mathison *et. al.*, 1999) the RPD obtained in the present study for DM content (4.84), CP (4.57), ADF

(3.97), NDF (3.99) and lignin (3.61) could be considered as good and suggested that NIRS calibration models might be used for routine analysis of these parameters. Moderate NIRS calibration models, adequate for screening, were found for NDF-CP (2.96) and sugar (2.82) whereas poor models, as already stated, were reported for SP (2.08), ADF-CP (2.06), fat (1.82) and starch (1.51). The poor calibration performance of ADF-CP (SD = 0.49), fat (SD = 0.31) and starch (SD = 0.55) could possibly be explained by lack of variation in these parameters for lucerne hay. However, according to Williams & Norris (2001) (Table 3) RPD values vary from very poor for starch, fat, ADFCP, SP; to poor for sugar and NDFCP; to fair for lignin, ADF, NDF, CP and DM.

3.2.2 Prediction of in vitro digestibility

It could be expected that statistics assessing the performance of the calibration equations were not as good when spectra were used to predict some nutritional attributes, such as digestibility kinetics. Digestibility is a characteristic, while protein is a chemical entity. Therefore, according to Stuth *et al.* (1999) NIRS prediction of digestibility represents a prediction of a predicted component of feed. According to Andrés *et al.* (2005) parameters measured by biological methods are subjected to higher uncontrolled variability due to multiplicity of sources of experimental and sampling error sources (e.g. basal diet, days, incubation runs, replicates). This can affect the predictive capability of NIR equations that are highly dependent upon the error of the reference method. Thus, the prediction ability of NIRS is expected to decrease with the complexity of parameter such as NDFD.

In addition, digestibility calibrations can also be sensitive to residual moisture in samples (Griggs *et al.*, 1999). Stuth *et al.* (2003) suggested more accurate equation development with samples containing moisture of less than 8% than those with higher moisture.

IVOMD24 and IVOMD48 results (Table 4) indicated an acceptable coefficient of determination (r^2 = 0.90) and showed a relative low SEC (1.31 and 1.68, respectively). This is not typical of such biological measures (Bruno-Soares *et al.*, 1998). Accordingly the 1-VR values (average = 0.87) were high. The RPD values of 2.70 to 2.84 are regarded by Williams & Norris (2001) (Table 3) as poor and only good for rough screening. It was however higher than the 2.5 set by several other researchers for an acceptable predictability (Williams & Sobering, 1993; Edney *et al.*, 1994; Mathison *et al.*, 1999).

The SEC for IVOMD48 in the present study was slightly higher than the 1.56 reported by Marten *et al.* (1984) and lower than the 2.4 reported by Stoltz (1990) for South African lucerne hay. This could be explained by a wider range of calibration data used in the current study compared to the data of Stoltz (1990) (30.94 and 23.65, respectively).

The IVOMD24 statistics, assessing the performance of the calibration equations compared well with that of IVOMD48. This was somewhat surprising as the influence of the lag time (L) on the 24 hour period was expected to have a more confounding effect on digestibility than the 48 hour period. Herrero *et al.* (1996) observed that the calibration and cross-validation of equations using NIR spectra were poorer for shorter incubation times. However the lag time (which by definition is mainly mathematical) could not be successfully predicted by either chemical composition or the NIRS (Andrés *et al.*, 2005). According to Herrero *et al.* (1996) it could be argued that changes in the proportions of the substrates may result in differences between the predictive ability of the different fermentation times. In such case, the lignin component, which is indigestible and well predicted by NIRS in the current study, would normally increase relative to the other cell wall fractions as fermentation time increases. However, the observed calibration performance between IVOMD24 and IVOMD48 in the present study did not support these assumptions and/or expectations.

The overall acceptable predictability of IVOMD could be due to NIR spectra containing information about not only the chemical components (i.e., chemical bonds and functional groups of the compounds), but also about other chemical and physical properties of the samples (Andrés *et al.*, 2005). Furthermore, 85% of the samples in the current study used for calibration contained less than 8% moisture and could therefore; according to Griggs *et al.* (1999), not hamper IVOMD calibration accuracy. As pointed out before, the degree of hydration of samples may cause changes in particular orientation of molecules thus, interacting differently with irradiating light.

It is however widely accepted that IVOMD is not a particularly good predictor of *in vivo* organic matter digestibility (Roberts *et al.*, 2004). According to Beever & Mould (2000) the use if NIRS to predict *in vivo* effects from limited chemical and *in vitro* data is likely to bring disrepute to a highly useful technique. *In vivo* digestibility differs from *in vitro* digestibility because it accounts for individual variability (Landau *et al.*, 2006). Several authors however suggested that *in vivo* organic matter digestibility (OMD) is the best reference method for

developing NIRS equations for OMD (Murray, 1993; Deville & Flinn, 2000; García & Cozzolino, 2006).

The r^2 values in Table 2 and Table 4 indicated that NDFD24, IVOMD24 and IVOMD48 were useable with caution for most applications, including research whereas NDFD48 was only applicable to screening and other approximate calibrations. The 1-VR values of these *in vitro* parameters were generally moderate to high (0.72 to 0.88). According to several authors previously mentioned, RPD values calculated for IVOMD24 and IVOMD48 calibrations met the requirements (>2.5) for screening purposes whereas calibration models for NDFD24 and NDFD48 failed. According to Williams (2001) the RPD values for NDFD24, IVOMD24, and IVOMD48 could be considered as poor and that these NIRS calibration models might only be used for very rough screening. According to Table 3 the NDFD48 NIRS calibration model could not be recommended for any application.

From Table 4 it seems that the calibration of the 24 hour NDFD marginally out-performed the 48 hour period. Development of an accurate and precise NIRS equations for the NDFD in lucerne hay has proven more problematic because of high levels of soil contamination and the heterogeneous nature (due to grass contamination) of the lucerne hay samples analysed in the present study (Ward, R.T., 2005, Personal Communication, Cumberland Valley Analytical Services, Inc., 14515 Industry Drive, Hagerstown, MD 21742, USA). A variable degree of soil contamination, which dilutes energy content, might influence the accuracy of NDFD calibrations (De Boever *et al.*, 1996). This greater chemical structure diversity in some of these multi-species samples undoubtedly accounted for a lower NIRS prediction ability of NDFD compared to pure lucerne hay samples (Marten *et al.*, 1984). Another probable reason is that *in vitro* fermentation characteristics of feeds are usually described by non-linear kinetics (France *et al.*, 1993 as cited by Herrero *et al.*, 1996). Accordingly, as mentioned by Herrero *et al.* (1996), the exponential nature of the *in vitro* kinetics equation altered the distribution and range of NDFD, therefore hampers a good fit by the multivariate and linear calibration methods used.

The relationships between *in vitro* determined and NIRS predicted values for IVOMD24 are graphically illustrated in Figure 3. Similar trends were observed for the other *in vitro* parameters (figures included in Appendix B2).
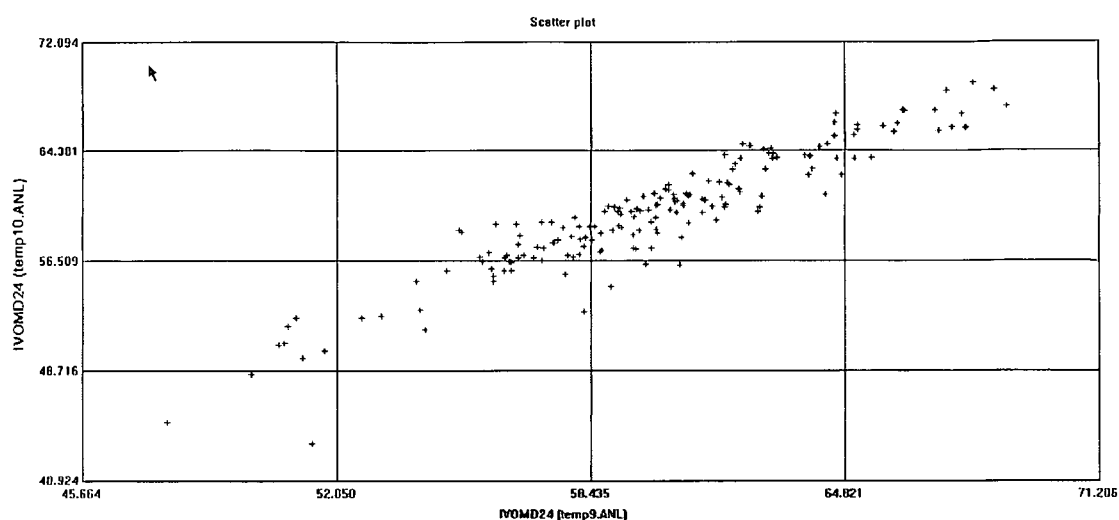
Figure 3 Near infrared reflectance spectroscopy (NIRS) predicted values versus reference values for in vitro organic matter digestibility at 24 hours (IVOMD24) (%) for lucerne hay.

With the exception of NDFD48 and NDFD24, *in vitro* digestibility parameters could be satisfactorily predicted from NIR spectra, with robust calibration equations (r^2 >0.80) and acceptable cross-validation (RPD>2.7 for IVOMD24 and IVOMD48) as set by several authors mentioned. However, according to Table 3 IVOMD24 and IVOMD48 calibrations could only be used for very rough screening. The RPD values for NDFD24 and NDFD48 calibrations are regarded as by Williams (2001) as very poor and not recommendable.

3.2.3 Prediction of minerals

The SEC, SECV and r^2 values indicate how well the equations will perform within the same population (Cozzolino & Moron, 2004). However, with minerals, the SEC and especially r^2 are not good indicators of calibration performance, because the NIRS not directly measuring the element (Malley *et al.*, 1999; Stuth *et al.*, 2003; Cozolino & Moron, 2004). According to Clark *et al.* (1989) the r^2 values for mineral determinations are governed more by the amount or variability (range in concentration) present than by direct relationship between concentrations change and absorption in the NIR region (Cozzolino & Moron, 2004). Stuth *et al.* (2003) stated that the low levels and narrow ranges in forage plants also hampers the estimation of most minerals by NIRS. Because of this narrow range, also observed in the current study (Table 1), some authors argue that mineral equations should be evaluated by

coefficient of variation (CV) rather than r^2 as the narrow range in concentration could render r^2 values misleading (Roberts *et al.*, 2003; Stuth *et al.*, 2003)

NIRS measures bonds within organic compounds, which are negatively related to organic materials. If the mineral matter is bound within organic compounds the distortion of the spectrum may be detectable at certain wavelengths, suggesting that NIRS could predict inorganic materials using their relationship between organic matter through their linkage with organic complexes, chelates, and pigments such as chlorophyll in forages (Osborne *et al.*, 1993; Ruano-Ramos *et al.*, 1999; Cozzolino & Moron, 2004).

Chloride (Cl), followed by the ash content, showed the highest RPD ratio (3.74) and r^2 (0.94). This is somewhat surprising and difficult to explain as Cl is mostly found in linkage with inorganic complexes. The SECV (1.02) and r^2 (0.93) values for ash were similar to those reported by García & Cozzolino (2006) in legume forages. The RPD value (3.12) was also fair and applicable for screening (Table 3). It was however higher than the 2.5 value mentioned before and could be regarded as adequate for quality assurance. The successful calibration of lucerne hay ash content is well documented in the literature (Redshaw *et al.*, 1986; Windham *et al.*, 1991; Scholtz, 2001). The relationship is probably mainly indirect as previously mentioned. De Boever *et al.* (1996) described the effect of ash on the NIR spectrum. Mineral soil induces a baseline shift, particularly in the lower part of the NIR spectrum, reducing the specific NIR absorption signals of the organic constituents (Paul, 1987). The good ratios (RPD = 3.12) and high r^2 (0.93) but relatively high prediction error (SECV = 1.02) for ash has to be associated with the broad range of the samples.

The SEC and SECV obtained in the present study (Table 4) for the different macro minerals compared well with those reported in the literature (Clark *et al.*, 1987; Stoltz, 1990). Stoltz (1990) reported in comparison with the present study (Table 4) similar results for lucerne hay Ca (r^2 = 0.77 and SEC = 0.24) and K (r^2 = 0.80 and SEC = 0.25). On the other hand inferior results for P (r^2 = 0.41 and SEC = 0.05) and superior results for Mg (r^2 = 0.90 and SEC = 0.03) were found by Stoltz (1990) compared to the current study. In all the minerals analysed, the SEC values are lower than the SD, indicating that NIRS could be used to determine concentration changes (Cozzolino & Moron, 2004) for Ca, P, Mg, K, Na and Cl in lucerne hay.

The relatively high r^2 values of Ca (0.74) and P (0.78) can be attributed to their defined linkage with chelates and chlorophyll (Ruano-Ramos *et al.*, 1999). For Na ($r^2 = 0.76$), the correlation is predominantly related with O-H overtones (Cozzolino & Moron, 2004).

The wavelength correlations for S showed specific spectral patterns related with plant pigments, related with O-H overtones and S-H overtones (Clark *et al.*, 1989; Cozzolino & Moron, 2004). Hence, the poor correlation for S ($r^2 = 0.24$) in the present study can possibly be attributed to the denaturation of the lucerne plant pigments during sample preparation and storage. Similar calibration results on S were also reported by Clark *et al.* (1989) for lucerne hay.

Relationships between chemically determined and NIRS predicted values for S and the rest of the minerals are graphically illustrated in Figure 4 and Appendix B3, respectively.
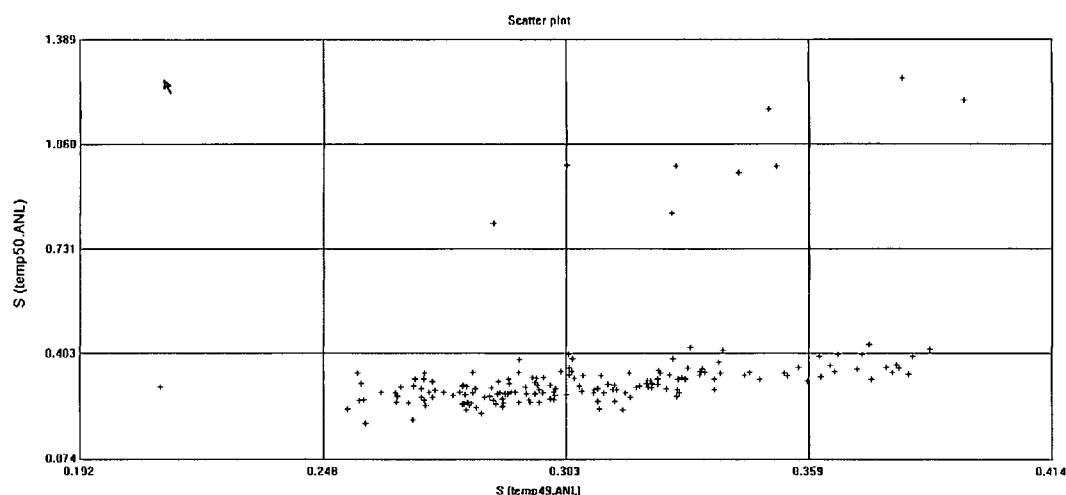
Figure 4   Near infrared reflectance spectroscopy (NIRS) predicted values versus reference values sulphur (S) for lucerne hay.

According to the r^2 values in Table 2 and Table 4 the ash and Cl calibration models were useable with caution for most applications including research. The r^2 of the rest of the mineral calibrations were only adequate for screening purposes. According to Edney *et al.* (1994) the RPD values calculated for ash and Cl calibrations met the requirements (>3.0) for quality assurance, whereas calibration models for the rest of the minerals were not recommended by all the authors for NIRS use. The SECV value of S was almost equal to the SD (0.06) (RPD = 1.08), which indicated that the instrument was not predicting the reference

values at all (Williams, 2007). Thus, prediction of the ash and Cl content of lucerne hay by NIRS were the only mineral parameters that gave acceptable accuracy to be used for routine analysis.

4   CONCLUSIONS

The r^2, SECV and RDP results recorded in the present study indicated that the NIRS procedure can be used with acceptable accuracy to predict DM, CP, ADF, NDF, lignin, ash and Cl for quality assurance. A possible explanation for the high calibration performance of these parameters in the current study might be due to the pre selection of samples based on their H distances with the SELECT algorithm even when the cut off point was set as 1.0, and by the wide range observed in the concentration of these constituents.

Intermediate NIRS calibration models were found for NDF-CP, sugar, IVOMD$_{24}$ and IVOMD$_{48}$ that could be considered adequate for screening purposes. Poor calibration results for the remaining parameters, namely SP, ADF-CP, fat, starch, NDFD and the macro minerals (Ca, P, Mg, P, Na and S), with the exception of Cl, indicated that the NIRS technique is less accurate than chemical methods.

Ultimately, precision of NDFD and IVOMD in lucerne hay by NIRS would be preferred, because unlike laboratories, NIRS prediction systems can be easily standardized. Additional research would be of value to pursue improving NIRS prediction of NDFD values of lucerne hay. The NIRS procedure entails ease, rapidity and economy of lucerne hay analysis however, there is a very real need to find ways of obtaining improved calibration data for digestibility procedure. Improve understanding of the causes of variation in the digestibility of lucerne hay is essential in realizing this goal.

However, RPD values for calibration models investigated in the present study are regarded by Williams & Norris (2001) as fair to very poor and thus not recommended for quality control. Hence, according to these criteria further work needs to be done to build robust NIRS calibration models for South African lucerne hay, to be able to detect minor changes in parameters such as SP, ADF-CP, fat, starch, NDFD and the minerals with the objective to use in modern nutritional models. However, the usefulness of applying NIRS in these cases will largely depend on the accuracy that is required. On the other hand, the variance for

ADF-CP, fat, starch and minerals in the current study are so low (SD<0.68) that accurate analyses, for use in current models or screening purposes, might not be necessary.

CHAPTER 6

A MODEL FOR ASSEING *MEDICAGO SATIVA* L. HAY QUALITY

1.   INTRODUCTION

Lucerne (*Medicago sativa* L.) hay is a very important roughage source for livestock in South Africa. Since forages are predominantly used by ruminants as source of nutrition, forage quality is an expression of the characteristics that affect consumption, nutrient utilisation and resulting animal performance, therefore, production potential. Currently adopted models for assessing forage quality addresses only the chemical composition of the feed without considering interaction among other feeds (associated effects), rate of passage and physical characteristics. For this reason, forages are often referred to as functional feeds (Zinn & Ware, 2007). This stresses the necessity of considering the animal. One method available to evaluate the nutrition of dairy cows, and not so far exploited to assess the quality of forages, is the Cornell Net Carbohydrate and Protein System (CNCPS) (Tylutki *et al.*, 2007).

The dairy feed industry is the largest consumers of lucerne hay. Consequently, its nutritive value and/or nutrient contribution for especially dairy cattle are important and needs to be incorporated into lucerne hay quality assessment. The CNCPS is widely acknowledged as one of the most advanced ruminant feed evaluation models presently developed. The non-linear nature of the CNCPS model integrates nutrient intake, ruminal fermentation, intestinal digestion, absorption, and metabolism of chemical analyses and mathematical models with cattle requirements for each production situation (Knowlton *et al.*, 1992; Fox *et al.*, 2000; Fox *et al.*, 2004). Fox *et al.* (2004) stated that the utility of the CNCPS is supported by the observation that various components have been adapted by the NRC (2000, 2001).

Several researchers assessed the ability of the CNCPS to predict lactation performance in dairy cows (Stone, 1996; Ruiz *et al.*, 2001; Ruiz *et al.*, 2002; Fox *et al.*, 2004; Recktenwald & Van Amburg, 2006; Tylutki *et al.*, 2007). In an evaluation with individual fed dairy cows, the CNCPS accounted for 90% of the variation in actual milk production with a 1.3% bias

(Fox *et al.*, 2004). The CNCPS is therefore a valuable aid to assess the quality of lucerne hay accurately according to animal performance. As previously mentioned, lucerne hay is mostly used for dairy nutrition and should lucerne hay quality accordingly be related to its milk production potential.

Two important feeding values used in feed formulation are energy (digestible energy; metabolisable energy) and protein (rumen degradability). A method which allows rapid and accurate estimates of energy value and degradability of the nitrogen fraction of lucerne is needed. Any determination method involving surgically treated animals is not acceptable owing to problems of labour, housing, technical resources, expense and speed. The assessment of energy value can be made more precisely if the feed is subjected to a chemical analysis (McDonald *et al.*, 1995). The analytical data can either be used to "type" the feed more accurately, or it could be fitted into equations that have been devised to predict energy value from chemical composition. McDonald *et al.* (2002) mentioned that the determination of ADF is particularly useful for forages as there is a good statistical correlation between it and the extent to which the food is digested (digestibility). In addition neutral detergent fibre (NDF) has been related to intake regulation, digestibility, and chewing activity in various dairy cow experiments (Mertens, 1982). Recently much emphasis has been placed on lignin and digestibility of NDF (NDFD) by dairy nutritionist. Evaluation of forages for NDFD is being conducted to aid prediction of total forage digestibility (Hoffman *et al.*, 2003), whereas lignin impacts the digestibility of the forage directly as indigestible material and indirectly as it inhibits digestion of chemically associated fibre (Van Soest, 1994).

Scholtz (2001) is of opinion that more complete models are needed to integrate chemical information collected in the laboratory with animal and feeding situation characteristics to predict, digestibility and animal performance more accurately. Such models do not need to be sophisticated mathematically, but must be realistic in terms of their dependence on, and correspondence to, biological principles and theories. These models derived from chemical analysis should provide the basis for more efficient forage utilisation by ruminants through more accurate determination of forage nutritive value and optimising diet characteristics.

The usage of near infrared reflectance spectroscopy (NIRS) for the rapid and economical nutritional evaluation of lucerne hay is a very real need for a practical lucerne hay evaluation model. Thus, the usefulness of applying NIRS in lucerne quality assessment will largely depend on the accurate prediction of the selected parameter's calibration equations, used in the quality model. The most robust NIRS calibration equations were identified and discussed in Chapter 5 and these should, however, be carefully consider when developing (modelling) and/or identifying an appropriate model for South African lucerne hay quality grading.

The objective of this study was to identify chemical parameters and/or models for assessing lucerne hay quality, using NIRS analysis and CNCPS milk prediction as criterion of accuracy.

2   MATERIALS AND METHODS

2.1   Chemical analysis and *in vitro* digestibility

As described in Chapter 3, six hundred lucerne (*Medicago sativa* L.) hay samples were collected from several commercial irrigation farms in the main lucerne producing areas in South Africa, which varied in location, soil characteristics (texture, organic matter, N content, pH) and farm management. Hundred and sixty eight samples that represented the South African lucerne hay population were selected, analysed (chemical and *in vitro*) and calibration equations were developed as described in Chapter 5.

2.2   Parameter calculation

The calculated energy and protein parameters used in the current study were described in Chapter 3.

2.3   Assumptions

The current study was based on the milk production level, feed ingredients and dry matter intake (DMI) of a typical high producing South African commercial Holstein herd. The age of cows was assumed to be 42 months, body weight 700 kg, days pregnant 0, days since calving 60, body condition scoring (BCS) 3.00, average daily gain (ADG) 0.079 kg/d, milk

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production 45 l/d, milk fat 3.55%, and milk protein 2.88%. Ambient temperature was 20°C and relative humidity (RH) was 50%, with no wind or other sources of environmental stress.

The basal diet consisted of: hominy chop, ground maize, wheat bran, molasses, whole cotton seed, cotton oil-cake (OC), soy OC, sunflower OC, lucerne hay and a mineral-vitamin pre-mix. A physical and chemical composition of the basal diet used in the current study is presented in Table 1. Chemical composition of the concentrates, minerals and vitamins used in the basal diet were selected from the AMTS.Cattle (AMTS.Cattle version 1.1.0.1, AMTS, LLC, 418 Davis RD, Cortland, NY, 13045, USA) feed library. The lucerne hay used in the basal diet was selected from the average chemical values of 168 selected lucerne hay samples, previously discussed in Chapter 3. These samples represented the chemical variation for the South African lucerne hay population. The chemical, physical and biological characteristics of feeds in the basal diet are set out in Table 2.

2.4   Diet formulation

As presently implemented, the CNCPS does not automatically balance or optimise diets (Knowlton *et al.*, 1993; Tylutki, 2007b). The basal diet formulation was based on the following criterion namely: metabolisable protein (MP) from bacteria >50% of total MP supply; non fibre carbohydrate (NFC) <40%; physical effective NDF (peNDF) and predicted ruminal pH above the minimum requirement of 22% and 6.4, respectively; rumen ammonia- and peptide-balance >100%, respectively; milk urea nitrogen (MUN) between the allowable 12 – 18mg/dL; methionine (Met) and lysine (Lys) retained >2.2% and >6.8% of MP supply, respectively whereas the Lys:Met ratio was maintained around 3:1; metabolisable energy (ME) and MP allowable milk within 2 l of each other.

The lucerne in the basal diet was replaced with the rest of the South African lucerne hay population described in Chapter 3. A diet with lucerne hay as sole roughage source was formulated to evaluate the effect of lucerne hay quality on ME and MP allowable milk. Hence, a total of 168 simulations were run to obtain milk yield (MY) values. A DMI of 25kg/d were maintained during the simulation to cancel the effect of DMI. The effect of

replacing lucerne hay in the basal diet on the protein fractions and MUN were addressed by using the lowest ME or MP allowable milk yield (kg/d) for each lucerne hay sample.

Table 1   **Physical and chemical composition of the basal diet on a dry matter basis**

Diet constituents	Content (%DM)	DMI (kg/d)
Hominy chop	13.96	3.49
Ground maize	21.95	5.49
Wheat Bran	2.00	0.50
Molasses	2.00	0.50
Whole cottonseed	6.26	1.57
Cotton oil-cake	1.19	0.30
Soya oil-cake	7.99	2.00
Sunflower oil-cake	1.19	0.30
Min/Vit. [a]	3.46	0.87
Lucerne hay [b]	40.00	10.00
Chemical composition		
Dry matter	91.10	
Forage	40.00	
Crude protein	18.80	
Rumen degradable protein	11.32	
RDP (%CP) [c]	60.21	
Soluble protein	24.45	
Acid detergent fibre	19.39	
Neutral detergent fibre	30.19	
Forage neutral detergent fibre	17.60	
Forage NDF (%NDF) [d]	58.30	
peNDF [e]	19.25	
Lignin	8.70	
Non fibre carbohydrates	36.08	
Sugar	6.56	
Starch	23.26	
Soluble fibre	6.26	
Ether extract	4.70	
Ash	10.40	

[a] Mineral-vitamin (Min/Vit) pre-mix

[b] Average of the South Africa population

[c] Rumen degradable protein (RDP); expressed as a percentage of the basal diet crude protein (CP) content

[d] Forage neutral detergent fibre (NDF); expressed as a percentage of the basal diet NDF content

[e] Physical effective neutral detergent fibre (peNDF)

Table 2   Chemical, physical and biological characteristics of feeds in the basal diet.

Chemical analysis [a]	Hominy chop[1]	Ground maize[1]	Wheat Bran[1]	Molasses[1]	Whole cottonseed[1]	Cotton oil-cake[1]	Soya oil-cake[1]	Sunflower oil-cake[1]	Min/Vit[1]	Lucerne hay[2]
DM	89.96	89.40	88.60	78.00	93.35	90.73	90.11	92.41	98.00	92.69
Crude protein (%DM)	11.03	8.40	19.00	4.50	22.45	42.63	53.32	40.37		20.74
Soluble protein (%CP)	30.44	11.00	32.61	100.00	29.66	22.24	12.91	29.71	0.00	30.00
NPN (%SP)	35.00	70.00	45.00	92.00	2.50	40.00	55.00	37.00	0.00	50.00
ADIP (%CP)	5.40	5.00	4.46	0.00	8.87	3.41	1.56	2.30	0.00	8.20
NDIP (%CP)	13.84	15.00	18.43	2.50	24.34	8.14	4.90	7.65	0.00	20.64
NFC (%DM)	41.76	76.30	28.66	82.78	5.45	14.80	28.12	15.58	0.00	20.37
Sugar (%DM)	4.60	1.55	5.80	78.85	5.10	9.30	13.50	7.80	0.00	5.71
Starch (%DM)	37.00	75.23	23.25	0.37	1.33	2.32	1.99	1.37	0.00	2.03
Soluble fibre (%DM)	0.16	0.00	0.00	3.56	0.00	3.18	12.63	6.41	0.00	12.63
ADF (%DM)	11.29	4.00	2.24	0.00	37.23	23.87	5.62	29.25	0.00	33.22
NDF (%DM)	35.70	9.40	43.30	0.50	49.38	34.49	9.11	37.24	0.00	44.06
pef (%)	25.00	25.00	33.30	0.00	85.00	36.00	23.00	40.00	0.00	80.00
Lignin (%NDF)	3.34	2.90	3.93	0.00	9.65	6.55	0.88	8.17	0.00	16.68
Ash (%DM)	1.36	1.60	5.26	12.12	3.00	5.73	6.77	5.15	100.00	12.97
Ether extract (%DM)	10.15	4.30	4.72	0.10	19.72	2.35	2.68	1.66	0.00	1.86
NDIP (%DM)	1.53	1.26	3.51	0.11	5.46	3.47	2.61	3.09	0.00	4.28
ADIP (%DM)	0.60	0.42	0.85	0.00	1.99	1.45	0.83	0.93	0.00	1.70
Lignin (%DM)	1.19	0.27	1.66	0.00	4.77	2.26	0.08	3.04	0.00	7.35

[1] Feed chemical composition values adapted from the AMTS.Cattle feed library (AMTS.Cattle version 1.1.0.1, AMTS, LLC, 418 Davis RD, Cortland, NY, 13045, USA)

[2] Average of the South African population

[a] Abbreviations for chemical analysis: Dry matter (DM); non-protein nitrogen (NPN); acid detergent insoluble protein (ADIP); neutral detergent insoluble protein (NDIP); non-fibre carbohydrate (NFC); acid detergent fibre (ADF), neutral detergent fibre (NDF); physical effective factor (pef)

Replacing luceme hay in the basal diet with the rest of the South African population also resulted in a variation in nutritional factors like peNDF, NFC and pH that could influence milk composition (milk fat) and the occurrence of sub-acute ruminal acidosis (SARA). Rectifying changes in these nutritional factors when replacing luceme hay in the basal diet seemed to be unnecessary, as the maximum NFC values and minimum pH values were acceptable and most of the luceme hay used resulted in diets that generally satisfied the peNDF needs of dairy cows (Mertens, 1997; Fox *et al.*, 2004; Tylutki *et al.*, 2007). Furthermore, the buffering capacity of luceme hay and its high pectin content (McBumey *et al.*, 1983; Fox *et al.*, 2000; Calberry *et al.*, 2003) would induce a smaller effect of peNDF on ruminal pH. Considering changes in milk fat when evaluating the quality of luceme hay is also not so important as many milk buyers in South Africa have no prerequisite regarding milk fat content. Therefore rectifying changes in these nutritional factors by adding feeds in the diet were unnecessary and would result in confounded effects.

The same procedures were followed to formulate diets for low milk producing Holsteins (25kg/d) and Jerseys (15kg/d) as well as high producing Jerseys (32kg/d).

2.5   Statistical analysis

Statistical analyses were performed using SAS 9.1.3 Service Pack 4 (2002-2003). Descriptive statistics namely the mean, standard deviation, coefficient of variation, median, range, minimum and maximum values were calculated for the quantitative variables. The Pearson correlation coefficient was used to perform correlation analyses on the entire dataset. Simple and multiple regression analyses were done separately for individual sources of variation. In the multiple regression analyses, forward selection was used in the stepwise regression calculations. This method starts with no variables in the model and adds variables. Variables were only retained in the model when they significantly ($P < 0.05$) contributed to the accountable variances. Expressions of the proportion of the sum of squares for the dependent variable explained by these regressions were calculated as r^2.

3. RESULTS AND DISCUSSION

3.1 Animals

In an attempt to eliminate the possible confounding effects of different dairy cow breeds and production groups, similar simulation models as described for high producing Holsteins (45kg/d) were performed on low producing Holsteins (25kg/d) and Jerseys (15kg/d) as well as high producing Jerseys (32kg/d). The results revealed a non-significant (P>0.05) difference between the ranking of lucerne hay samples with regards to potential allowable milk production. Hence, for practical reasons results and discussions were focused on high producing Holsteins.

3.2 Diets

According to Tylutki (2007b) CNCPS models to predict cattle performance are based upon inputted animal and feed descriptions and that variables which represents CNCPS theory should be used as constraints. These variables are set out in par. 2.4. The theory behind the selected variables is that ME and MP allowable milk are highly aggregated variables (Tylutki, 2007a). Thus, maintenance, growth and pregnancy requirements need to be satisfied first for ME and MP allowable milk to resort within acceptable levels.

The ability to predict forage quality, particularly with regards to its energy content and the amount of feed that the animal will consume voluntarily, is fundamental to any forage evaluation programme or model. Because of the complexity of factors controlling feed consumption (Roseler et al., 1997), accurate prediction of diet DMI is problematic. However, the close relationship between DMI and milk yield (MY) of dairy cows is well defined by research (Roseler et al., 1997; Voelker et al., 2002; Hristov et al., 2004). Hristov et al. (2004) postulated that DMI is driven by milk production when lactation diets are fed. This concept was supported by the lag between peak milk yield (MY) and peak DMI during the early stages of lactation (NRC, 2001). As previously mentioned the DMI of all simulations in the present study, were kept a constant, notwithstanding the changes in milk production when replacing the lucerne hay in the basal diet. A constant DMI means that

differences in hay quality was manifested in a higher or lower ME/MP allowable milk production.

The nutritional indicators and milk production (ME and MP allowable milk) response of replacing lucerne hay in the basal diet with the rest (n = 168) in the South African population are presented in Table 3.

Table 3   Nutritional indicators and milk response of 168 SA lucerne hay based diets

	Minimum	Mean	Median	Maximum	Range	SD[1]	CV[2]
Milk yield [a] (kg/d)	35.72	43.01	43.58	48.69	12.97	2.78	6.46
Metabolisable energy milk (kg/d)	35.72	43.01	43.58	48.69	12.97	2.78	6.46
Metabolisable protein milk (kg/d)	38.42	45.74	46.02	52.76	14.34	2.88	6.30
peNDF [b] (%DM)	14.40	19.26	19.20	26.20	11.80	2.17	11.27
pH	6.04	6.25	6.24	6.55	0.51	0.09	1.48
Non fibre carbohydrates (%DM)	29.20	36.12	36.55	41.60	12.40	2.85	7.88
Rumen NH3 (%Rqd)	144.00	221.79	219.00	335.00	191.00	32.82	14.80
Metabilisable protein from bacteria (%)	37.50	45.60	45.70	51.20	13.70	2.52	5.53
Milk urea nitrogen (mg/d)	9.20	17.09	16.90	24.40	15.20	2.94	17.17
Diet ME (MJ/kg)	8.63	10.71	10.78	11.73	3.10	0.49	4.55
Lysine (%MP)	6.22	6.40	6.40	6.51	0.29	0.06	0.91
Methionine (% MP)	1.58	1.72	1.72	1.83	0.25	0.05	2.68

[1] Standard deviation

[2] Coefficient of variation

[a] First limiting (metabolisable energy or metabolisable protein) allowable milk production

[b] Physically effective neutral detergent fibre (peNDF)

Variation in lucerne hay quality according to ME and/or MP milk yield (MY) range from 35.72 to 48.69kg/d. Thus, normal variance of MP and ME in South African lucerne hay alone can vary milk production of high producing Holstein cows by 12.97kg per cow per day (Table 3).

3.3   Parameters

The correlation of milk yield (MY) with chemical, digestibility and calculated parameters are given in Table 4. Selected linear regression equations to predict milk yield (MY) are presented in Table 5.

Table 4 **Correlation (r) between milk yield (MY) and nutritional parameters of lucerne hay**

Parameter	r
Chemical	
Acid detergent fibre	-0.82
Neutral detergent fibre	-0.78
Sugar	0.69
Lignin	-0.62
Ash	-0.55
Acid detergent fibre-crude protein (ADF-CP)	-0.51
Fat	0.50
Neutral detergent fibre-crude protein (NDF-CP)	-0.45
Soluble protein	0.34
Crude protein	0.19
Starch*	0.11
Digestibility	
In vitro organic matter digestibility at 24 hours	0.72
In vitro organic matter digestibility at 48 hours	0.60
In vitro Neutral detergent fibre digestibility at 24 hours	0.46
In vitro Neutral detergent fibre digestibility at 48 hours	0.32
Calculated [a]	
Non fibre carbohydrate	0.91
CB2 (soluble fibre)	0.86
In vitro dry matter digestibility at 48 hours (IVDMD48)	0.81
In vitro dry matter digestibility using lignin in the equation (IVDMDlig)	0.77
Cellulose	-0.76
Neutral detergent fibre minus neutral detergent fibre-crude protein (NDFn)	-0.74
CA4 (sugars)	0.69
CC (indigestible NDF)	-0.62
Adjusted crude protein	0.62
Organic matter	0.55
PB2 (soluble protein)	0.53
PC (acid detergent fibre-crude protein)	-0.51
Neutral detergent fibre digestibility, estimated from lignin (NDFDlig)	-0.49
Hemicelluloses	-0.48
Truly digestible crude protein	0.42
PA (non-protein nitrogen)	0.34
PB1 (soluble protein)	0.34
CB3 (available neutral detergent fibre)	-0.34
PB3 (neutral detergent fibre-crude protein)	-0.31
CHO (carbohydrate)	0.30
Soluble protein/Crude protein	0.28
Lignin/(neutral detergent fibre minus neutral detergent fibre-crude protein)	-0.21
Lignin/cellulose	-0.20
CB1 (starch) *	0.11
Lignin/neutral detergent fibre *	-0.08

* $p > 0.05$ non significant values

[a] Calculated parameters and CNCPS carbohydrate and protein nutrient pools (Tylutki *et al.*, 2007) as described in Chapter 3 (Table 1).

Table 5   **Simple linear regression equations for predicting milk yield (MY) and the coefficient of determination (r^2) between dependent and independent variables in lucerne hay**

Independent variate (X)	Dependent variate (Y)	r^2	Regression equation $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6$
Chemical and digestibility			
Acid detergent fibre (X_1)	Milk Yield	0.67	$Y = 60.72 - 0.54X_1$
Ash (X_2)		0.87	$Y = 64.40 - 0.48X_1 - 0.38X_2$
Lignin (X_3)		0.96	$Y = 64.18 - 0.23X_1 - 0.53X_2 - 0.90X_3$
NDFD24 [a] (X_4)		0.97	$Y = 64.16 - 0.21X_1 - 0.53X_2 - 0.85X_3 - 0.09X_4$
Acid detergent-crude protein (X_5)		0.98	$Y = 60.72 - 0.21X_1 - 0.12X_2 - 0.51X_3 - 0.73X_4 + 0.07X_5$
Chemical, digestibility and Calculated			
Non fibre carbohydrate (X_1)	Milk Yield	0.82	$Y = 31.20 + 0.47X_1$
Lignin (X_2)		0.92	$Y = 37.22 + 0.40X_1 - 0.60X_2$
Ash (X_3)		0.96	$Y = 48.89 + 0.22X_1 - 0.99X_2 - 0.33X_3$
NDFD24 [a] (X_4)		0.97	$Y = 53.76 + 0.08X_1 - 1.67X_2 - 0.46X_3 + 29.20X_4$
Acid detergent-crude protein (X_5)		0.98	$Y = 48.40 + 0.13X_1 - 1.42X_2 - 0.39X_3 + 24.51X_4 + 0.07X_5$
Fat (X_6)		0.99	$Y = 45.85 + 0.09X_1 - 1.38X_2 - 0.38X_3 + 38.13X_4 + 0.09X_5 - 0.50X_6$

[a] *In vitro* neutral detergent fibre digestibility at 24 hours

All the variables included in the regression equations have a significant (P<0.05) contribution to the coefficients of determination (r^2). The best equations based on chemical, digestibility and calculated parameters are presented.

3.3.1   Chemical and digestibility parameters

The highest correlation of a chemical or digestibility parameter with MY was obtained with ADF. The ADF concentration in lucerne hay explained 67% of the MY variance (Figure 1) followed by NDF with 61% (Table 4). According to the regression equations, MY decreases by 5.4% (Table 5) and 3.4% units for each % DM increase in ADF and NDF content of lucerne hay, respectively. The other chemical and digestibility parameters had moderate to low impact on MY.
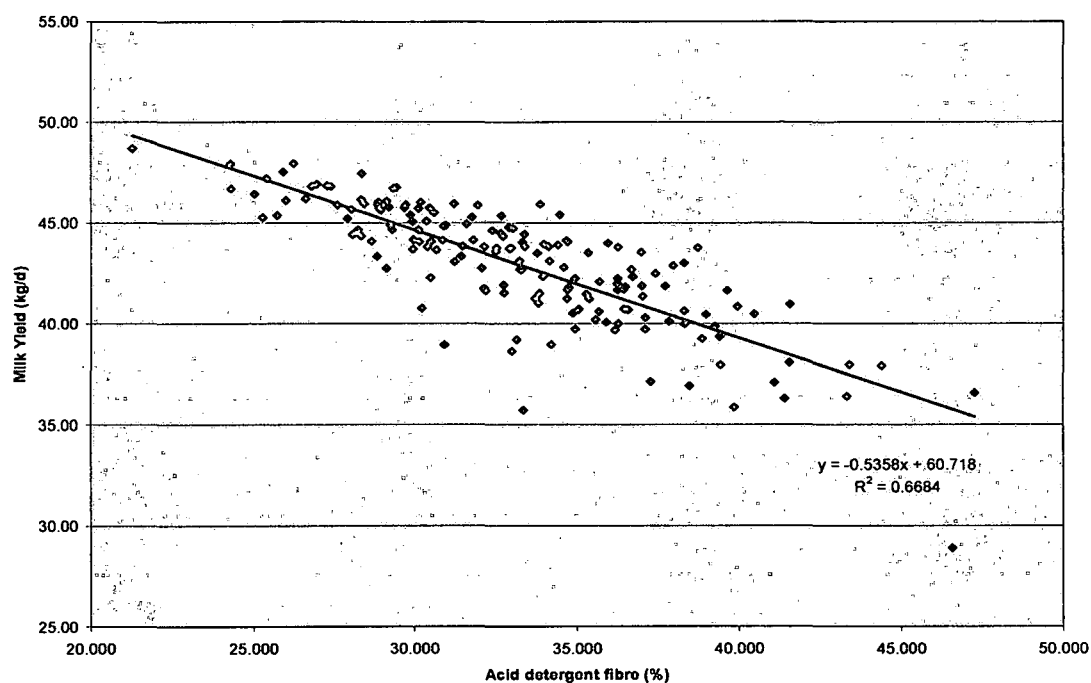
Figure 1   Relationship between predicted milk yield and acid detergent fibre

It is well documented that potential MY is highly dependable of the energy content of a feed (Van Soest, 2004; Tylutki *et al.*, 2007). Thus, lucerne hay ADF showed the best relationship with ME in the diet. The contribution of ADF in predicting MY was somewhat surprising because of all the discrepancies in the literature around ADF, as a commendable indicator of digestibility and/or energy value of a feed. On the other hand Undersander & Moore (2004) stated that ADF has long been used to estimate the energy content of forages. Many studies had shown that the concentration of ADF in forages was correlated negatively with digestibility (Harlan *et al.*, 1991; Minson, 1982). Most energy prediction models used in the past by commercial testing laboratories have been based on this relationship (Weiss, 1998). Van Soest (1964) described a highly significant (P<0.0001) negative relationship between ADF content and extend to which legumes are digested. This is in agreement with the findings of Cilliers & Van der Merwe (1993) (P<0.0001; r^2 = 0.74) using veld herbage. Rohweder *et al.* (1976) also stated that ADF is the chemical assay of choice to estimate the IVOMD of a wide range of forages including lucerne hay. The historical ADF-TDN prediction, as described in Chapter 3 (par. 3.1.7), currently used in California and several western states in the US (Robinson & De Peters, 2002; Putnam, 2004) are based on the linear

relationship observed between ADF level of luceme hay and its TDN value (Bath & Marble, 1989)

In contrast with these results, Scholtz (2001) reported that ADF compared to NDF predicted IVOMD of luceme hay less accurately ($r^2 = 0.47$ vs. $r^2 = 0.57$). According to Van Soest *et al.* (1991) ADF is not a valid fibre fraction for nutritional use or for the prediction of digestibility. Because ADF does not contain hemicelluloses it is not an accurate estimate of fibre in feeds (Mertens, 2002). It was developed as a preparatory step for the determination of lignin (Van Soest, 1963) and was never intended to be a measure of fibre in feeds. The NRC (2001) recommendation for dairy cows did not mentioned ADF at all as a tool for diet balancing, but focuses on the use of NDF in the summative TDN equation. Van Soest *et al.* (1978) demonstrated that ADF accounted for only 56% of the variability in digestible dry matter (DDM). Accordingly, Moore *et al.* (2002) reported significant differences between observed and predicted digestible dry matter (DDM). It was furthermore noted that neither ADF nor NDF were related to DDM in aftermath cuttings of luceme hay ($r^2 = 0.04$). The reason for the discrepancy in the literature could be attributed to the use of different populations and species in development of these empirical equations. According to Combs *et al.* (1998) and Weiss (1998) empirical equations are specific to a given population and species.

The correlation matrix of the chemically and digestibility measured parameters are presented in Table 6. The correlation coefficients in Table 6 revealed a moderate coefficient of determination ($r^2 = 0.56$) between ADF and $IVOMD_{48}$. In fact a better prediction was observed between ADF and $IVOMD_{24}$ ($r^2 = 0.62$). The possible reasons for the better prediction ability of $IVOMD_{24}$ versus $IVOMD_{48}$ were previously discussed in Chapter 3 (par. 3.1.6). Despite its low level, lignin explained about 71% of the $IVOMD_{48}$ variance (Table 3). The stronger correlation observed with lignin and $IVOMD_{48}$ ($r = -0.84$) versus $IVOMD_{24}$ ($r = -0.72$) was expected because the lignin component, which is indigestible, would always increase relative to the other cell wall fractions as fermentation time increases. Morrison (1972) also reported lignin to be the best single chemical predictor of *in vivo* digestibility for grass silage. Although a strong relationship exist between lignin content and digestibility

(IVOMD$_{48}$), similar but poor relationships were found between lignin or IVOMD$_{48}$ and MY (Table 4). Thus, while *in vitro* and *in situ* estimates of digestibility have long been recognised as being more closely related to animal performance than chemical values (Undersander, 2002), the opposite was found for luceme hay in the present study. The approach followed in the current study could have contributed to these discrepancies. It is the first time that chemical parameters in luceme hay were related to variation in MY caused by including the luceme hay in a complete diet.

The NDF method was designed initially to isolate the insoluble dietary fibre components in plant cell walls namely; cellulose, hemicellulose and lignin (Van Soest & Wine, 1967). While NDF residue is often considered to represent these cell wall fractions, NDF is actually defined as a nutritional entity describing the variable and incompletely digestible portion of forages (Van Soest, 1994). In contrast with the above results Mertens (1980) proposed the use of NDF to estimate fibre content and energy value of feeds. Robinson & De Peters (1999) and Putnam (2004) recommended the substitution of ADF with NDF as NDF are considered to be the more clearly defined cell-wall fraction which captures all the structural fibre. This was confirmed by Scholtz (2001) who reported ADF to predict IVOMD of luceme hay less accurate ($r^2 = 0.47$) than NDF ($r^2 = 0.57$). Even though ADF only captures 70 to 85% of the structural fibre, ADF ($r^2 = 0.56$) marginally outperformed NDF ($r^2 = 0.52$) in the current study. The high coefficient of correlation ($r = 0.91$) observed in Table 6 between ADF and NDF was expected. The hemicellulose fraction in NDF is the primary difference between these two fibres analyses (Elizalde *et al.* (1999). A similar correlation ($r = 0.91$) was reported by Robinson & De Peters (2002) for Californian luceme hay. Recently Putnam (2004) evaluated luceme hay data from multiple states in the US and found ADF and NDF to be highly correlated ($r = 0.96$) in pure (grass free) luceme hay. Therefore, the slightly lower correlation observed in the current study could be explained by the possible grass contamination in the South African luceme hay population, as discussed in Chapter 3 (par. 3.1). Accordingly, the differences in hemicellulose concentration of grass and luceme hay are primary responsible for the discrepancy, as mentioned above.

Table 6   Correlation matrix for chemical and digestibility parameters [a]

	CP	SP	ADF-CP	NDF-CP	ADF	NDF	lignin	fat	starch	sugar	ash	NDFD24	NDFD48	IVOMD24	IVOMD48
CP	1														
SP	0.55	1													
ADF-CP	0.30		1												
NDF-CP	0.35	-0.23	0.65	1											
ADF	-0.42	-0.54	0.43	0.42	1										
NDF	-0.33	-0.59	0.44	0.64	**0.91**	1									
lignin		-0.39	0.71	0.63	0.74	0.73	1								
fat		0.23	-0.24	-0.23	-0.50	-0.44	-0.42	1							
starch		-0.20							1						
sugar		0.26	-0.47	-0.57	-0.64	-0.65	-0.66	0.35	0.28	1					
ash							-0.25			-0.16	1				
NDFD24		0.26	-0.17		-0.39	-0.27	-0.49	0.37		0.42		1			
NDFD48	0.29	0.21		0.22	-0.31		-0.32	0.30		0.19		**0.80**	1		
IVOMD24	0.31	0.55	-0.35	-0.35	**-0.79**	-0.73	-0.72	0.48		0.63		0.69	0.60	1	
IVOMD48	0.16	0.49	-0.48	-0.48	-0.75	-0.72	**-0.84**	0.41		0.69	0.18	0.59	0.47	**0.86**	1

[a] Only significant (P<0.05) correlations are given

Abbreviations: Crude protein (CP); soluble protein (SP); acid detergent fibre-crude protein (ADF-CP); neutral detergent fibre-crude protein (NDF-CP); acid detergent fibre (ADF); neutral detergent fibre (NDF); neutral detergent fibre digestibility at 24 (NDFD24) and 48 hours (NDFD48); *in vitro* organic matter digestibility at 24 (IVOMD24) and 48 hours (IVOMD48)

When using any fibre determination to estimate digestibility, alias energy content, the assumption is made that there is a constant linear relationship between fibre concentration and digestibility (Undersander & Moore, 2002). From the available literature it is evident that considerable variation exists in the digestibility of dry matter relative to the ADF content. Nevertheless, in the current study it would seem that, compared to the other single chemical and digestibility parameters, ADF in lucerne hay was the most accurate estimator of energy value or milk yield in dairy diets (Table 4).

The determination coefficient of the regression equation to predict MY was appreciably improved to a level of 87% by including ash (Table 5). The occurrence of ash as the second significant and negative contributor to MY is probably associated with the lower energy concentration of soil contaminated and/or above-normal mineral concentration of South African lucerne hay, as discussed in Chapter 3 (par. 3.1.2). Hoffman (2003) stressed the importance of ash measurement in predicting energy value of forages. This hypothesis stems from the negative effect of ash on total digestible nutrients alias energy content of a feed. Ash has no energy value (Weiss, 1998), thus, the high ash concentration of lucerne hay found in the present study (Chapter 3, par. 3.1.2) could have a profound effect on the potential available energy of the diet intended for milk production.

It is evident from Table 5 that the combination of the three pure chemical parameters (ADF, ash and lignin) formed the best three term equation. It explained almost all (96%) of the variance in MY. Despite the low level, ash and lignin accounted for 30% and 38%, respectively, of the variance in MY (Table 4 and Figure 2). Furthermore, MY was decreased by 5.3%- and 9.0%-units for an 10 g/kg DM increase in ash and lignin content of lucerne hay, respectively, when using the proposed three-term regression equation (Table 5). The relationship of lignin with digestibility and other chemical parameters were previously discussed in this chapter and Chapter 3 (par. 3.1.4.1c). From the correlation coefficients in Table 6 it seems that ADF, ash and lignin showed moderate ($r = 0.74$; $P<0.0001$) to very weak ($r = 0.13$) inter-correlations among each other. Therefore, even though lignin is a component of ADF, the alleged auto-correlation ($r = 0.74$) was relatively low enough not to

hamper their independence. These results confirmed the added dimension of ADF, ash and lignin to the regression equation in terms of differentiating between different qualities of lucerne hay. This means that the three-term regression equation accurately predict lucerne hay quality in a dairy diet, alias potential milk yield.
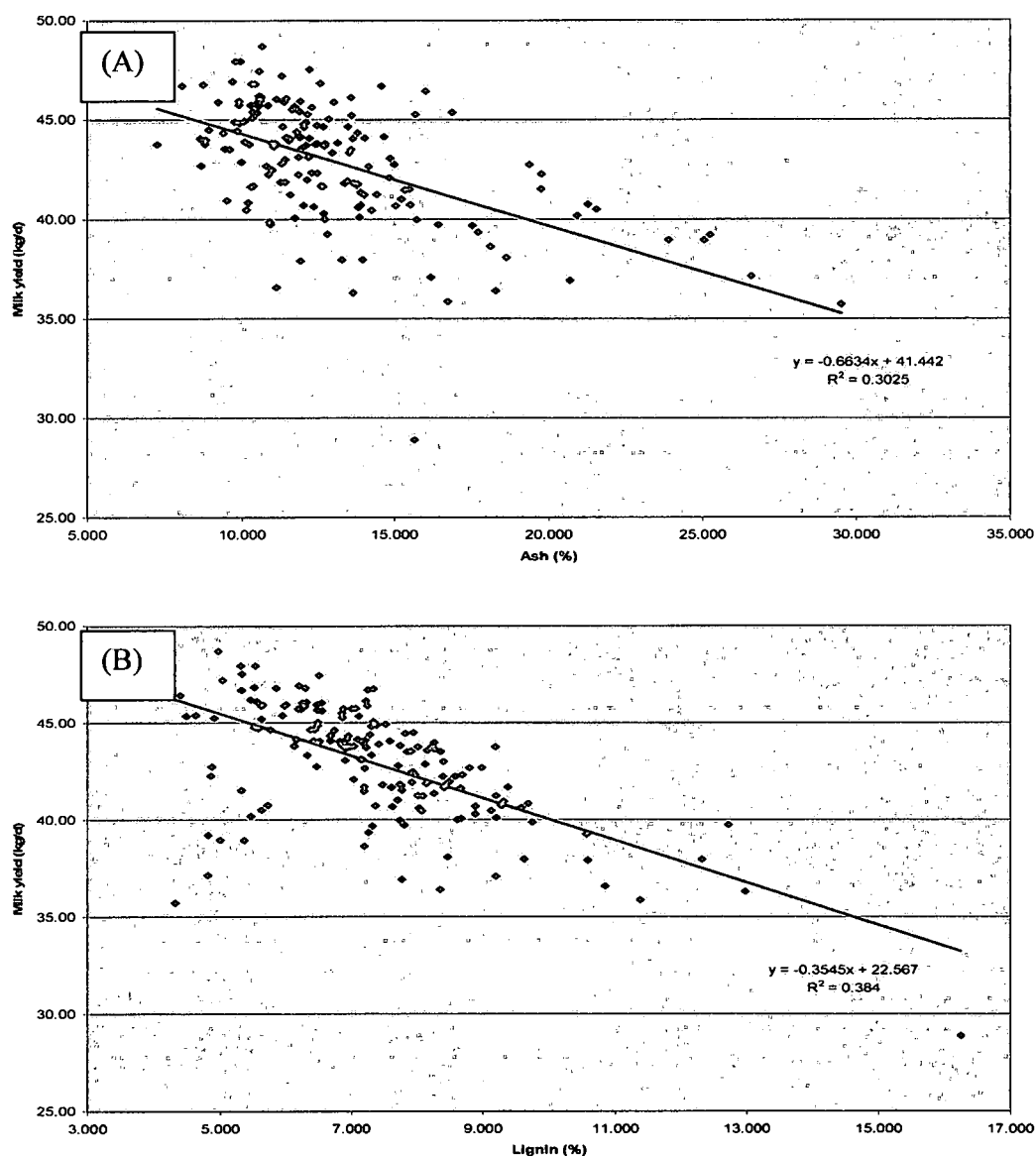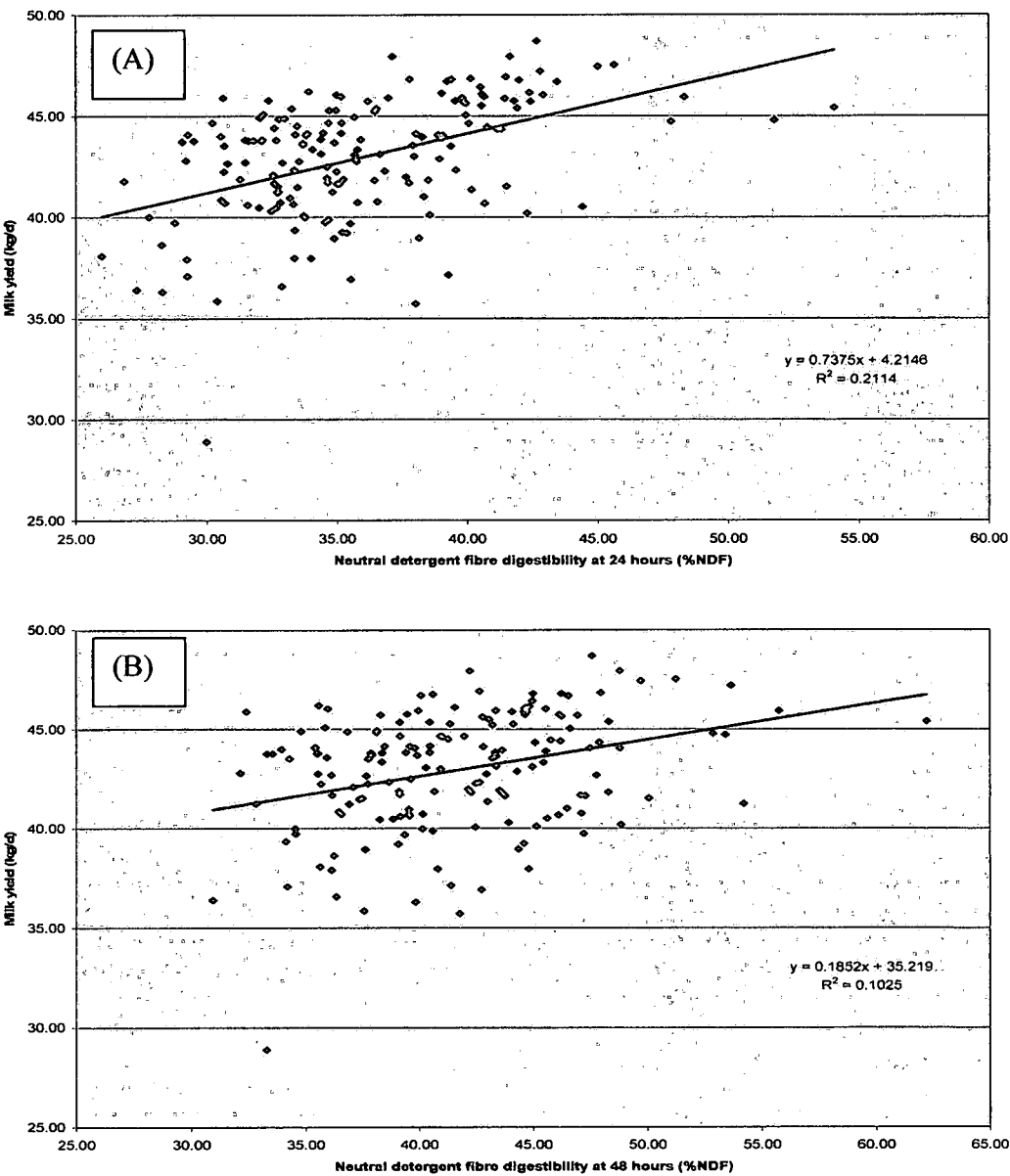

Figure 2   **Relationship between predicted milk yield and: (A) ash and (B) neutral detergent fibre digestibility at 48 hours (NDFD48)**

The accountable variance of 96%, based on pure chemical parameters (ADF, ash and lignin), could only be marginally improved to 97% by the inclusion of a digestibility parameter (NDFD24). The dramatic impact of NDFD content of forage on the energy value of a diet is well documented by research (Ruiz *et al.*, 1995; Oba & Allen, 1999; Hoffman & Bauman, 2003). According to Ruiz *et al.* (1995) digestibility of fibre has a significant influence on DMI and milk production of dairy cows. In a study conducted by Oba & Allen (1999) they reported that a one unit rise in NDFD (*in vitro*) was associated with a 0.17kg/day rise in DMI (digestibility and intake are related) and a 0.25 kg rise in 4% fat corrected milk (FCM) yield. In addition, Ivan *et al.* (2005) reported increased DMI and 4% FCM production when silage with higher NDF content and -NDF digestibility was substituted for silage with a lower NDF content and –NDF digestibility. According to Oba & Allen (2000) increased NDFD may result in increased energy density of and microbial N production. These results indicated that forages with high NDFD improved MY by increasing energy intake. Tylutki (2008, Pers. Comm.) suggested that high producing dairy cows are more sensitive to changes in NDFD than lower producers. This could be explained by its effect on rumen fill and energy concentration of the diet. According to Allen & Mertens (1988) NDFD is a function of the potentially digestible fraction, its rate of digestion and rate of passage.

As NDFD content evaluates the available energy in a feed, as stated above, it was expected to correlate highly with MY. In contrast with these expectations, NDFD24 and NDFD48 explained only 21 and 10%, respectively, of the variance observed in MY (Table 4 and Figure 3). Again, as with IVOMD, the better values observed in shorter incubation period (24 vs. 48hr) could be explained by the rational that shorter incubation times better describe the digestion potential of NDF, because feed is not retained in the rumen of high producing dairy cows for 48hr (Hoffman & Combs, 2004). Zinn & Ware (2007) also suggested that retention time of fibre in the rumen or length of time that fibre is exposed to the fibrolytic process is influenced by initial fibre particle size, rate of particle size reduction (chewing, rumination), particle density and rate of digestion. Zinn & Ware (2007) supported by Alvarez *et al.* (2000) and Ware *et al.* (2004) noted that when forages are substituted into a diet on a equal NDF basis, differences among forages in feeding value becomes small, or non-substantial. In this regard Placencia *et al.* (2003) as cited by Zinn & Ware (2007)

demonstrated that when either sudangrass or elephant grass replaced 45% of the NDF provided by lucerne hay in a lactation diet for Holstein cows, there were no forage source effects on DMI, milk yield and milk efficiency, although percentage milk fat was greater when grass hays contributed a portion of the forage NDF (Zinn & Ware, 2007). In contrary with other research already mentioned, the results of the present study imply that NDFD might have a marginal effect on animal performance.

Figure 3   Relationship between predicted milk yield and: (A) neutral detergent fibre digestibility at 24 hours (NDFD24) and (B) 48 hours (NDFD48)

The reason for the discrepancy recorded in the current study and available literature could possibly be explained by the theory of Oba & Allen (1999). They suggested that although many experiments have reported NDFD data, interpretation of results is difficult due to a diversity of confounding factors. Experiments comparing forages that differ only in NDFD are exceptional. In addition, Oba & Allen (1999) stated that it is difficult to confirm that other factors that could potentially affect animal performance did not vary. Therefore, in contrast to the chemical analysis (ADF, ash and lignin), ruminal NDFD is not an intrinsic function of the forage, per se, but rather a complex function of the complete diet (Zinn & Ware, 2007). They also noted that ruminal fibre digestion may be more limited by ruminal fibrolytic capacity (condition of ruminal pH) than by the digestive quality of the fibre.

The positive contribution of ADF-CP in the five-term regression equation (Table 5) is difficult to explain due to its negative effect (Table 4) on MY (r = -0.51). Krishnamoorthy *et al.* (1983) stated that ADF-CP is highly resistance to rumen and lower intestinal track digestion. ADF-CP contains proteins closely bound to lignin, Maillard products and tannin-protein complexes (Sniffen *et al.*, 1992; Van Soest, 1994). ADF-CP shows a moderate positive relationship with lignin (r = 0.71) and very poor correlations with the rest of the chemical and digestibility parameters (Table 6).

According to the four- and five-term regression equations, MY decreases only by 0.9 and 0.7%-units for each 10 g/kg DM increase in of NDFD$_{24}$ and ADF-CP of lucerne hay, respectively (Table 5). NDFD has low reproducibility between laboratories and development of an accurate NIRS equation has proven to be problematic due to its non-linear kinetics (Chapter 5; par. 3.2.2). Accordingly, its calibration performance was regarded as intermediate and not recommended for accurate routine analysis. The poor NIRS prediction results of ADF-CP also hampered the rapidity and economy of its analysis for routine use in regression equations. Therefore, the marginal contribution of NDFD and ADF-CP on predicting MY would not justify its inclusion due to economy and the lack of robust NIRS calibration equations, as described in Chapter 5 (par. 3.2.1). On the other hand, the acceptable accuracy to predict ADF, ash and lignin content (Chapter 5; par. 3.2.1) of lucerne

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hay, for quality assurance, ensures a rapid, economical and accurate estimate of MY potential for lucerne hay.

3.3.2 Calculated parameters

Table 7 contains the results obtained from correlation analysis between selected parameters and -models. The highest correlation (r = 0.91) of a calculated parameter with MY was obtained with non fibre carbohydrates (NFC) (Table 4). The slightly weaker relationships of CB2 (r = 0.86), IVDMD48 (r = 0.81) and IVDMDlig (r = 0.77) with MY (Table 4) and high inter-correlation among these parameters (CB2, IVDMD48 and IVDMDlig; r > 0.80; Table 7) was expected as these parameters all included NFC to a large extend in its calculation (Chapter 3; par. 3.1.4.2). According to Hall (2005) the balance between fibre and NFC especially the profile of NFC (sugar, starch and pectin) has drawn more concern due to their influence on animal performance and digestion of other feeds. These influences are discussed in Chapter 3 (par. 3.1.4.2). An even poorer relationship was found between the *in vitro* digestibility parameters and NFC (r^2 < 0.28; Table 7) than was previously reported between *in vitro* digestibility and ADF (r^2 < 0.62; Table 6). Again, this confirms the possible incapability of the *in vitro* digestibility parameters of lucerne hay to quantify available energy and/or production potential.

Table 7 **Results of correlation analysis (coefficient of correlation) of selected parameters**

	IVDMD48	IVDMDlig	NFC
CB2	0.8	0.85	
IVDMD48		0.87	
IVOMD24			0.53
IVOMD48			0.4
NDFD24			0.2
NDFD48			0.07
ADF			-0.71

Abbreviations: Soluble fibre fraction (CB2) as described by Tylutki *et al.* (2007); *in vitro* dry matter digestibility at 48 hours (IVDMD48); *in vitro* dry matter digestibility using lignin in the equation (IVDMDlig); non fibre carbohydrate (NFC); *in vitro* organic matter digestibility at 24 (IVOMD24) and 48 hours (IVOMD48); neutral detergent fibre digestibility at 24 (NDFD24) and 48 hours (NDFD48); acid detergent fibre (ADF)

The combination of NFC, lignin and ash formed the best three-term equation when investigating available parameters (chemical, digestibility and calculated). Using NFC means analysing additional chemical parameters. These additional parameters includes: NDF-CP, fat, CP and NDF of which only the latter two produced accurate NIRS calibration equations, as discussed in Chapter 5 (par. 3.2.1). The absence of CP in any of the regression equations revealed that CP content in lucerne hay was irrelevant in assessing lucerne hay quality or production potential. This was confirmed by its very poor correlation with MY (r = 0.19; Table 4). The fat content in lucerne hay was expected to have a minor, if any, effect on milk yield due to its low concentration reported in lucerne hay (Chapter 3; par 3.1.5). The appearance of NFC as the first preference contributor, when using all parameters (chemical, digestibility and calculated), means that NFC and ADF are interchangeable parameters in predicting MY with similar accuracy (r = 0.96) using multiple regression equations (Table 5). However, only a moderate relationship was found between NFC and ADF (r^2 = 0.50; Table 7). This is in agreement with the findings of Scholtz (2001) for South African lucerne hay (r^2 = 0.56). From the results in Table 5 it is evident that a similar accuracy (r^2 = 0.96) was obtained with only pure chemical analysis (ADF, ash and lignin), previously discussed in par. 3.3.1. This proposed regression equation (model), named lucerne milk value (LMV), can be used with great accuracy to determine the quality of lucerne hay in practice. Furthermore, as already mentioned, the rapid and accurate analysis of ADF, ash and lignin with a NIRS are according to Chapter 5 possible. This simple but accurate lucerne hay quality evaluation model requires minimum costs to maintain robust NIRS calibrations. As previously stated, it is the opinion of the author that the accountable variance of 96% could not be appreciably improved in practice by adding more parameters to the regression equation.

4.   CONCLUSIONS

The evaluation of different parameters for assessing lucerne hay quality revealed large differences in the accuracy of prediction as measured by MY. From the results of the present study it seems that the ADF of lucerne hay in a dairy diet is the single pure chemical parameter most closely related to milk yield production potential of dairy cattle. The regression equation based on ADF explained more of the variance in MY than *in vitro*

digestibility parameters. A marked improvement in the accuracy of milk yield prediction and therefore luceme hay quality occur by including ADF, ash and lignin in a multiple linear equation. Furthermore, accurate NIRS calibration equations for these parameters (ADF, ash and lignin) has proven to successfully assist in producing a accurate, rapid and economical estimate of MY potential for luceme hay. Further approaches to enhance the accuracy of the regression equation by adding additional parameters ($NDFD_{24}$ and ADF-CP) to the regression equation would not be viable in practice due to economy and poor calibration equations for these parameters.

The relative poor performance of the protein parameters (ACP, ADF-CP,NDF-CP and CP) in predicting MY suggests that protein content of luceme hay, in general, is a unreliable indicator of production potential (MY) and/or luceme hay quality.

It is clear that milk yield derived from the CNCPS model by replacing luceme hay in a basal diet with others in the South African luceme hay population can be significantly predicted with high accuracy by the developed empirical model (LMV) consisting of only ADF, ash and lignin.

CHAPTER 7

EVALUATION OF MODELS FOR ASSESSING *MEDICAGO SATIVA* L. HAY QUALITY

1. INTRODUCTION

The results in Chapter 6 indicated that the lucerne milk value (LMV) and therefore the quality of lucerne hay can accurately be predicted from a model including acid detergent fibre (ADF), ash and lignin. Several quality evaluation models have been developed over the history of forage quality evaluation research (Moore & Undersander, 2002) namely; Relative Feed Value (RFV; Rohweder *et al.*, 1976), Total Forage Index (TFI; Hutjens, 1995), Adjusted Total Forage Index (ATFI; Erasmus, 2000), Forage Quality Index (FQI; Moore *et al.*, 1984), Relative Forage Quality (RFQ; Moore & Undersander, 2002). The fundamental characteristic of diets for ruminants, around which all other nutrients are structured, is energy content (Robinson, 2005). Therefore, the majority of existing models for lucerne hay quality assessment are based on its digestible energy intake potential. Some models also include protein parameters. The results in Chapter 6 revealed, however, that protein parameters are an unreliable indicator of lucerne hay quality. Furthermore, although several international hay grading systems exist, much uniformity exists between the systems.

As indicated in Chapter 6, it is important to consider the animal to evaluate the different available models for assessing lucerne hay quality. In this regard the Cornell Net Carbohydrate and Protein System (CNCPS), as proposed by Tylutki *et al.* (2007), could be an important and valuable tool to evaluate the accuracy of different models to determine the quality of lucerne hay for milk production. Accordingly, the usage of near infrared reflectance spectroscopy (NIRS) is essential for the rapid analysis of lucerne hay and should the accuracy of calibration equations be considered when selecting the appropriate model for lucerne hay quality grading.

The objective of this study was to evaluate current proposed models for assessing lucerne hay quality, using NIRS analyses and CNCPS milk production prediction as a criterion of accuracy.

2. MATERIALS AND METHODS

2.1 Chemical analysis

Chemical and *in vitro* analyses were carried on the 168 samples selected from the 600 lucerne hay (*Medicago Sativa* L.) samples as described in Chapter 3.

2.2 Quality models

Several models to determine the quality of lucerne were evaluated.

2.2.1 Relative feed value (RFV)

Relative feed value (RFV) was estimated from digestible dry matter (DDM) (eq. 10) and dry matter intake (DMI) (eq. 9) as follows:

$$RFV = (\%DDM) \times (DMI \text{ as } \% \text{ of body weight}) \times (0.775)$$

(Rohweder *et al.*, 1976). (eq. 25)

2.2.2 Forage Quality Index (FQI)

The Florida Extension Forage Testing Program recommended forage Quality Index (FQI) from 1982 to 2002 (Moore *et al.*, 1984). FQI (eq. 11) combine voluntary forage intake and TDN content into one value (Moore & Sollenberger, 2002) where voluntary total digestible nutrients (TDN) intake is used as a multiple of the TDN requirement for maintenance as illustrated below. According to Moore & Undersander (2002) it is assumed that TDN is equivalent to *in vitro* organic matter digestibility (IVOMD) (Tilley & Terry, 1963), when digestible ether extract in forages is negligible.

2.2.3 Total Forage Index (TFI)

Hutjens (1995) uses the term Total Forage Index (TFI) (eq. 12) to describe an index which builds on RFV, but adds a protein value and a physical value to the RFV model.

2.2.4 Adjusted Total Forage Index (ATFI)

The effect of heat damage on protein availability was calculated from the crude protein (CP) and acid detergent fibre-nitrogen (ADF-N) content (Erasmus, 2000). This adjusted crude protein (ACP) was used in the TFI formula:

$$ATFI = RFV + (ACP\% \times x) \tag{eq. 26}$$

where: x = a multiplier of 6

2.2.5 Relative Forage Quality (RFQ)

Moore & Undersander (2002) kept the same concept and format as for RFV except that TDN replaced DDM. Furthermore, NDF digestibility is part of the TDN and DMI calculation in the RFQ model (eq. 13).

DMI calculations for lucerne, clover and legume/grass mixtures (DMI_{legume}) (eq. 14) are calculated from the equation proposed by Mertens (1987).

Total digestible nutrients for lucerne, clovers and legume/grass mixtures (TDN_{legume}) (eq. 16) are calculated based on the latest NRC recommendations (NRC, 2001) using *in vitro* estimates of digestible NDF (not those calculated from lignin) as follows:

2.2.6 Lucerne Quality Index (LQI)

LQI was based on the same concept and format than RFV except that IVOMD, rather than ADF, was used to estimate digestible organic matter (DOM) of lucerne hay, and CP was also incorporated into the equation (Scholtz & Van der Merwe, 2006 –unpublished data). LQI was calculated from the following formula and expressed as an index:

$$LQI = DMI \times (IVOMD \times (CP \times 3.8))/3.95) \tag{eq. 27}$$

where: DMI = dry matter intake (eq. 9)

IVOMD = *In vitro* OM digestion (Tilley & Terry, 1963)

3.8 = multiplier, developed from data on CP economical values of 4 different oil cakes compared to the energy value of maize grain over a period of 6 years. This gave more weight to CP in the model.

3.95 = devisor used to adjust the equation to have a index mean of 100

2.2.7 Total Digestible Nutrients (TDN)

The National Research Council in the last revision of Nutrient Requirements for Dairy Cattle (2001) revised the historical TDN equation based on the summative equation of Weiss *et al.* (1992), assuming intake at one times maintenance or 1x (eq. 15).

2.4 Milk yield calculation

A modified version of CNCPS (CNCPSv6) (AMTS.Cattle version 1.1.0.1, AMTS, LLC, 418 Davis RD, Cortland, NY, 13045, USA) was used to calculate the effect of lucerne hay quality on metabilisable energy (ME) and metabolisable protein (MP) allowable milk, as described in Chapter 6 (par. 2.3 and 2.4). The lowest ME or MP allowable milk, known as milk yield (MY), for each lucerne hay was used as an evaluation criterion for the different quality models.

2.5 Statistical analysis

Statistical analyses were performed using SAS 9.1.3 Service Pack 4 (2002-2003). The Pearson correlation coefficient was used to perform correlation analyses on the entire dataset.

3. RESULTS AND DISCUSSION

3.1 Models

Table 1 include the results obtained from the correlation matrix for quality models and milk yield.

**Table 1**   **Correlation matrix quality models and milk yield (MY)[a]**

	MY	TDNlig	TDN48	RFV	TFI	ATFI	FQI	RFQ	LQI
MY	1								
TDNlig	0.99	1							
TDN48	0.86	0.84	1						
RFV	0.78	0.78	0.71	1					
TFI	0.67	0.68	0.74	0.93	1				
ATFI	0.77	0.77	0.70	0.91	0.88	1			
FQI	0.91	0.90	0.87	0.90	0.83	0.82	1		
RFQ	0.87	0.86	0.92	0.91	0.89	0.83	0.95	1	
LQI	0.70	0.70	0.73	0.96	0.98	0.90	0.87	0.91	1

[a] All correlations were significant (P<0.05)

Abbreviations: Milk yield (MY); total digestible nutrients calculated by using NDFDlig, calculated from lignin, to calculate TDN (TDNlig); total digestible nutrients calculated by using 48 hour *in vitro* NDF digestibility procedure (NDFD48) to calculate TDN (TDN48); relative feed value (RFV); total forage index (TFI); forage quality index (FQI); relative forage quality (RFQ); lucerne quality index (LQI).

The highest correlation of a quality model with MY was obtained with the summative TDN equation of Weiss *et al.* (1992), using lignin to determine truly digestible NDF (NDFDlig) (Chapter 3; par. 3.1.7). The NRC (2001), however, recommends the use of either neutral detergent fibre digestibility at 48 hours (NDFD48) or NDFDlig to determine truly digestible NDF. The large difference obtained in the accuracy of predicting MY from TDN using NDFDlig (TDNlig; $r^2 = 0.98$) and TDN using NDFD48 (TDN48; $r^2 = 0.74$), respectively in the current study (Figure 1), are in disagreement with the above recommendations of the NRC (2001). Although highly significant (P<0.0001), the relationship ($r^2 = 0.2$; Table 2) between NDFDlig and NDFD48 was not good enough to be used interchangeably for the estimation of the summative TDN equation. Similar results were obtained by Robinson *et al.* (2004) who also evaluated the NRC (2001) lignin model (NDFDlig) to estimate NDFD. They found little relationship between NDFD as estimated by lignin (NDFDlig) and *in vitro* NDFD at 48 hours (NDFD48) in barley and distillers grain. They reported a superior relationship using the *in vitro* digestibility (NDFD48) measurements as comparing to using lignin (NDFDlig) to estimate NDFD. Robinson *et al.* (2004) stated that "the primary reason for this occurrence was the absolute failure of the lignin-based procedure to predict in vitro digestion of NDF at 48 hr". In addition, Linn (2003) suggested that the NDFDlig equation does not consider feed type. Lignin does affect digestibility of NDF, however, the effect is variable with different forages, cuttings and/or environments. Consequently, a universal NDFD equation for all

types of forages is highly unlikely. Hoffman *et al.* (2003) suggested that the influence of NDFD on total TDN prediction of forages is reasonably small in relationship to all the factors that influence energy status in ruminant animals. However, in the current study the replacement of NDFD48 with NDFDlig in the TDN equation, resulted TDNlig to explain 24% more of the variance in MY than TDN48.
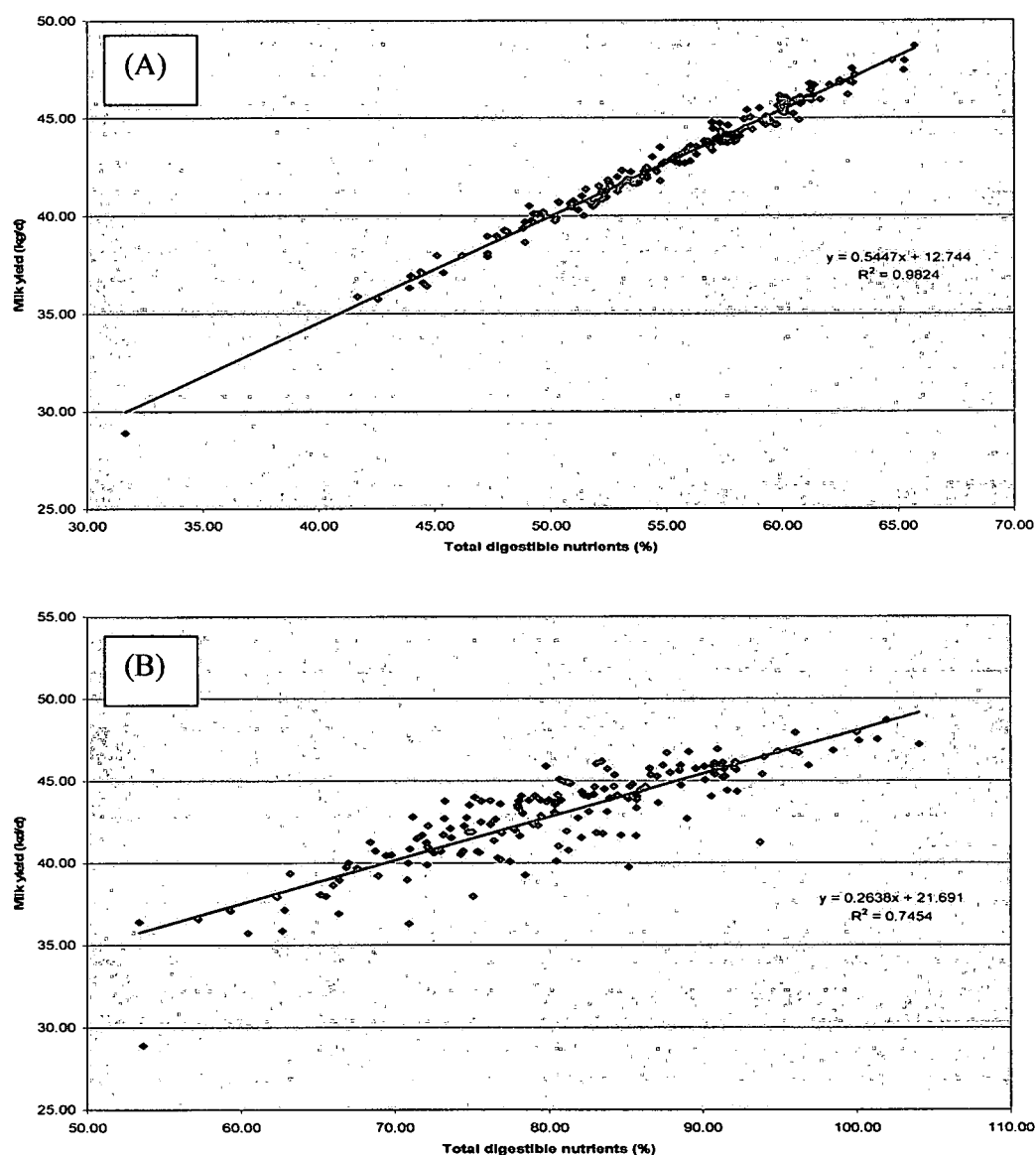
Figure 1   **Relationship between predicted milk yield and: (A) TDNlig and (B) TDN48**

Table 2   **Results of correlation analysis (coefficient of correlation) of selected parameters**

	NDFD48	TDNlig	RFV
NDFDlig	0.45		
ADF		-0.81	
NDF		-0.76	
CP		0.19	0.63
NFC		0.91	
ACP			0.82

Abbreviations: *In vitro* neutral detergent fibre digestibility at 48 hours (NDFD48); total digestible nutrients calculated by using NDFDlig, calculated from lignin, to calculate TDN (TDNlig); relative feed value (RFV); acid detergent fibre (ADF); neutral detergent fibre (NDF); crude protein (CP); non fibre carbohydrates (NFC); adjusted crude protein (ACP)

In addition the MY prediction ability of NDFD48 and NDFDlig were weak, but noticeably different namely $r^2 = 0.10$ and $r^2 = 0.25$, respectively (Chapter 6; Table 4). Accordingly NDFDlig seems to be the better estimate in the current study. Even though a strong relationship ($r^2 = 0.64$; Chapter 6; Table 6) existed between NDFD48 and NDFD24, NDFD24 showed a better relationship to MY ($r^2 = 0.20$ and $r^2 = 0.10$, respectively), as previously discussed in Chapter 6 (par. 3.3.1; Figure 3). However, the *in vitro* NDF digestibility assay becomes less repeatable when shorter incubation times are used.

Even though NDFDlig had a dramatic impact on the ability of TDNlig to predict MY, a relative poor negative but significant ($P<0.0001$) correlation was found between lignin and MY ($r = -0.62$; Chapter 6; Table 4 and Figure 2-B). Similar low correlations were also found between these two (TDNlig and MY) and IVOMD48 ($r = 0.58$ and $r = 0.60$, respectively; $P<0.0001$). Analysis of correlation between TDNlig and NFC ($r = 0.91$), ADF ($r = -0.81$) and NDF ($r = -0.76$) showed strong to moderate significant ($P<0.0001$) relationships (Table 2). NFC showed the highest ($r = 0.91$), whereas CP the lowest ($r = 0.19$) correlation with TDNlig (Table 2). This was expected due to the high and low contribution of NFC and CP, respectively to the TDNlig equation (eq. 15).

The almost perfect relationship ($r^2 = 0.98$; Table 1) between TDNlig of lucerne hay and MY, predicted from the complete diet seems to be the ultimate theoretically lucerne hay quality

model. However, the use of this model from a practical and economical view could be problematic. The following chemical parameters are needed to estimate TDN, namely, NDF, NDF-CP, ADF-CP, lignin, fat, ash and CP (eq. 15). As observed in Chapter 5 only CP, NDF, ash and lignin produced accurate NIRS calibration equations for quality assurance. The remaining NIRS calibrations (NDF-CP, ADF-CP and fat) were less accurate than chemical analyses. On the other hand, the variance for ADF-CP and fat reported in Chapter 5 were low (SD<0.68) and might accurate analysis, for use in TDN$_{lig}$, not be essential.

According to Zinn & Ware (2007) the most popular models for assessing the comparative feeding value of forages include: Forage Quality Index (FQI), Relative Feed Value (RFV) and Relative Forage Quality (RFQ). The development and shortcomings of these models are well documented and defined in literature (Moore & Undersander, 2002; Zinn *et al.*, 2004; Zinn & Ware, 2007). According to Zinn & Ware (2007) all three approaches assess quality differences among forages according to DMI and digestibility and tend to rank forages similarly (Moore & Undersander, 2002). A positive strong significant (P<0.0001) correlation existed between these models (r>0.90; Table 1). The highest correlation was observed between FQI and RFQ (r = 0.95). Thus, the relative performance of these quality models was expected to be similar. In terms of correlations with MY, FQI marginally outperformed RFQ (r = 0.91 and r = 0.87, respectively).

Figure 2 and 3 graphically illustrates the relationships between predicted MY and the most popular models used for assessing lucerne hay quality.
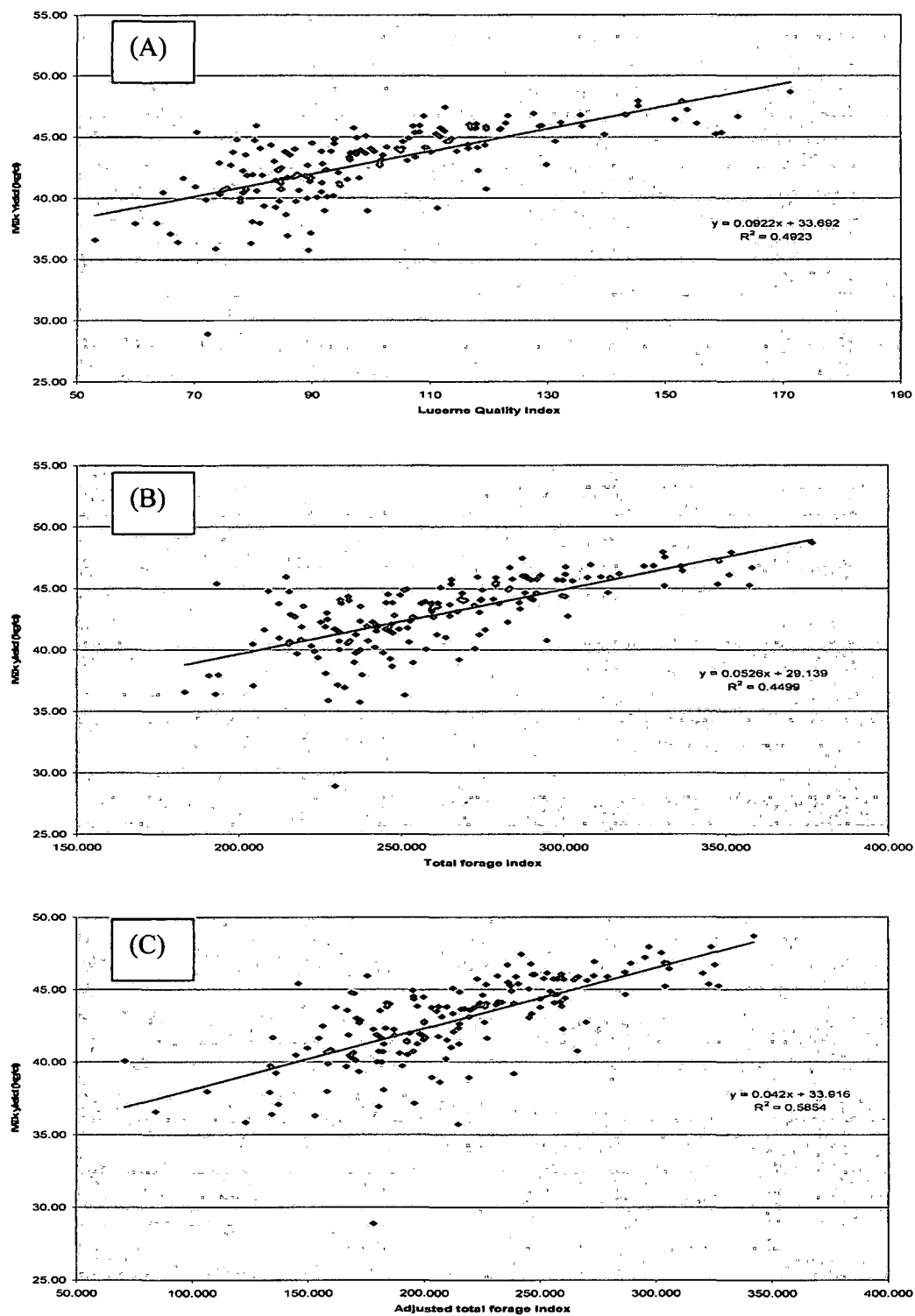
Figure 2   Relationship between predicted milk yield and: (A) lucerne quality index, (B) total forage index and (C) adjusted total forage index
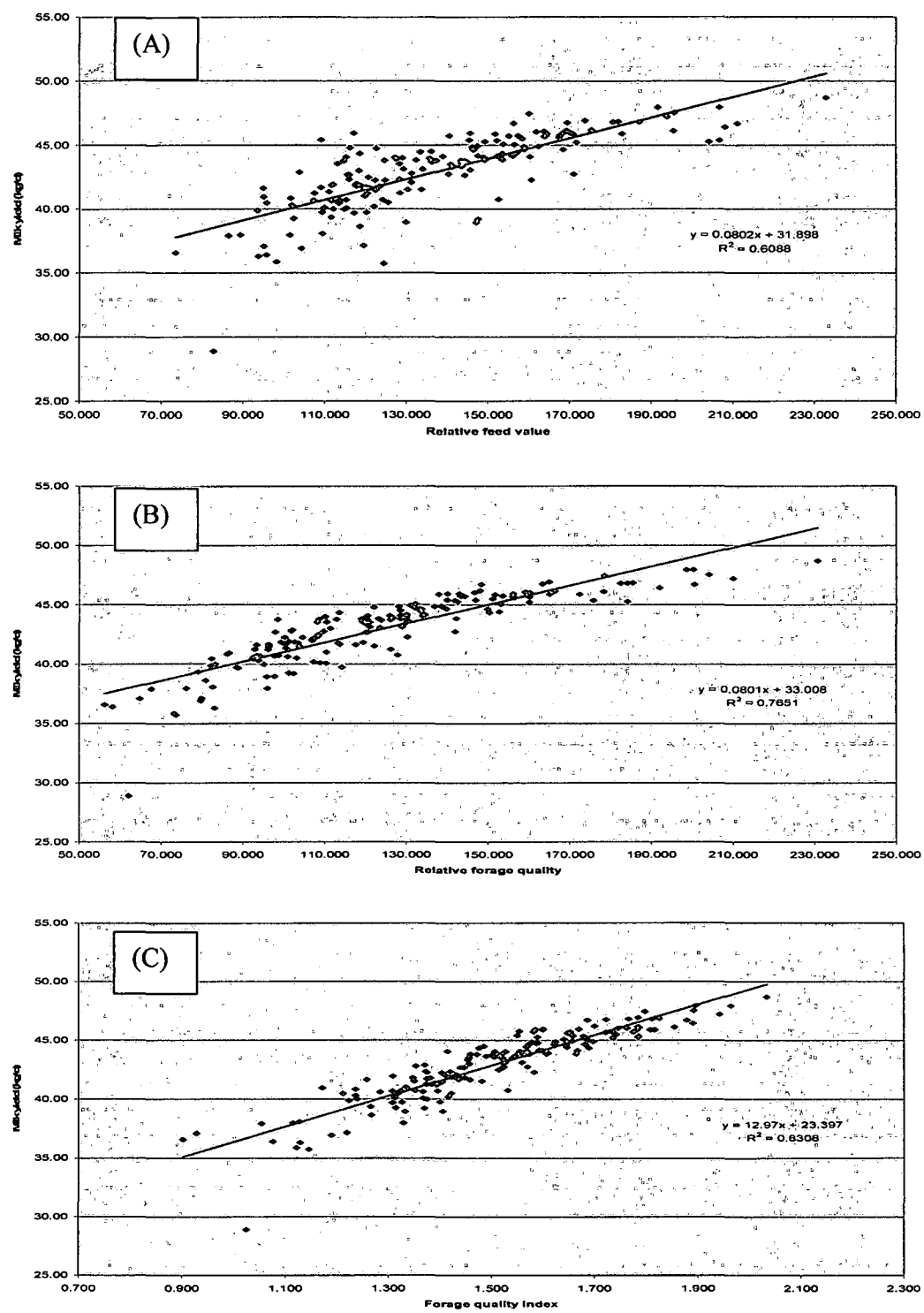
Figure 3   Relationship between predicted milk yield and: (A) relative feed value and (B) relative forage quality and (C) forage quality index

However, according to the relationship with MY ($r^2 = 0.61$), RFV as a practical model to determine lucerne hay quality was still inferior to the use of an individual chemical parameter like ADF ($r^2 = 0.67$), and similar to NDF ($r^2 = 0.61$) as observed in Chapter 6 (Table 4). The r^2 and significance of estimating RFV from either NDF ($r^2 = 0.94$; P<0.0001) or ADF ($r^2 = 0.87$; P<0.0001), suggest that the advantage to be gained from the use of RFV as an indication of lucerne hay quality are small (Figure 4). Figure 4 also shows the exponential relationship between RFV and the fibre fractions ADF and NDF. The graphs suggest that these exponential equations explained even more of the variation in RFV than the linear equations mentioned above. Similarly, Putnam (2004) demonstrated that the relationship between the NDF level of lucerne hay and RFV was virtually perfect ($r^2 = 0.99$).

FQI and RFV does not account for the CP content in the forage. It is based only on fibre levels and is therefore an index of forage digestibility and an estimate of energy value or energy intake potential (Taylor, 1997). The models TFI and LQI were developed and based on the same concept and format as RFV, but with the incorporation of CP as suggested by Hutjens (1995), to reflect more completely the nutritive value of the forage. The ATFI was developed from TFI by Erasmus (2000) by replacing CP with adjusted crude protein (ACP) to compensate for unavailable CP (ADF-CP). Failure to obtain improved MY predictions using LQI ($r^2 = 0.45$; Figure 2A), TFI ($r^2 = 0.49$; Figure 2B) and ATFI ($r^2 = 0.59$, Figure 2C) rather than RFV ($r^2 = 0.61$, Figure 3A), however, suggested that there were no benefit to be gained by using quality models that include CP or ACP. Therefore, the benefit of lucerne hay protein could possibly be overvalued by several conventional grading models (RFQ, LQI, TFI and ATFI) due to its (CP) low relationship with MY ($r^2 = 0.04$; Chapter 6; Table 4) and poor utilisation by ruminants (Martin & Mertens, 2005). Even though a strong significant (P<0.0001) relationship existed between these quality models, the inclusion of chemical components (CP and ACP) in TFI and ATFI showed a significant (P<0.0001) but relative poor and moderate relationship with RFV ($r^2 = 0.40$ and $r^2 = 67$; respectively) (Table 2). The significant (P<0.0001) stronger correlation observe between ACP and MY ($r = 0.62$; Chapter 6, Table 4), compared to CP and MY ($r = 0.19$; Chapter 6; Table 4), was manifested in a stronger correlation of ATFI ($r = 0.77$) with MY, compared to LQI ($r = 0.70$) and TFI (r = 0.67) (Table 1).
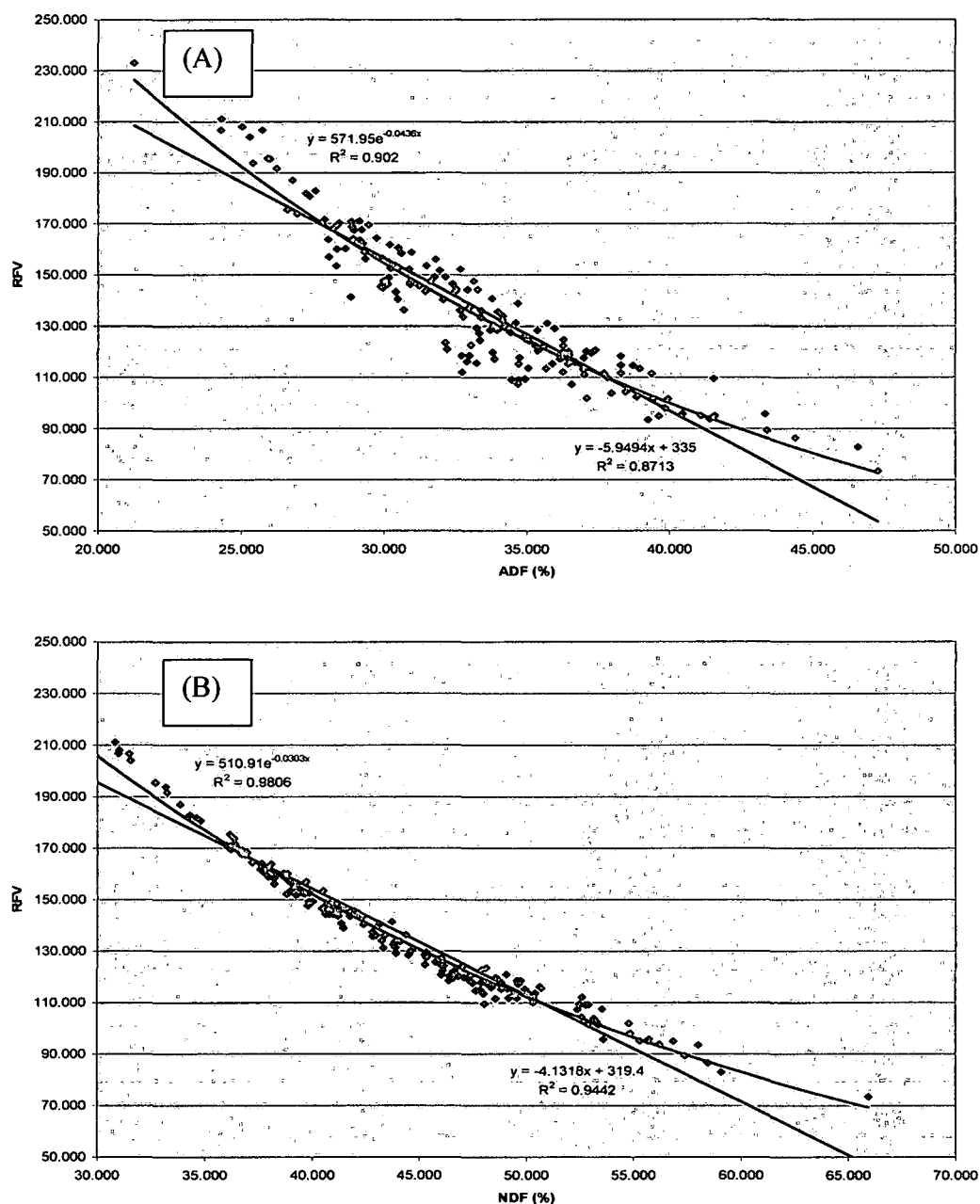
Figure 4 **Relationship of relative feed value with: (A) acid detergent fibre and (B) neutral detergent fibre**

The negative effect of CP on MY was further confirmed by the stronger significant relationship of FQI to MY ($r^2 = 0.82$; P<0.0001) compared to LQI ($r^2 = 0.49$; P<0.0001). The FQI models consist of the same chemical components (IVOMD and NDF) as LQI,

except for the exclusion of CP. Accordingly it might be speculated that the inferior relationship of RFQ ($r^2 = 0.76$) with MY, compared to FQI ($r^2 = 0.82$), could also be assign to the presence of CP, although low, in its calculation. A possible explanation for the negative effect of lucerne hay-CP on CP-containing quality models (LQI, TFI, ATFI) in predicting MY, could be due to its highly fermentable characteristic in the rumen. The rapid degradation in the rumen by microbes results in excessive excretion of nitrogenous waste by the animal (Martin & Mertens, 2005). These excessive levels of nitrogen in the rumen that are absorbed into the cow circulatory system, increases milk urea nitrogen (MUN) levels in the milk and filtering load placed on the kidneys (Van Soest, 1994). This process calls for additional energy usage that could have otherwise been used for milk production. Evidence from several experiments indicates that the protein in lucerne hay is utilised inefficiently by lactating dairy cows (Broderick *et al.*, 1992). The CNCPS takes this into account, hence the possible decrease in available energy for milk production. Energy is the nutrient most limiting in diets of high producing dairy cows, as previously discussed in Chapter 6, and probably also the nutrient most apt to need supplementation in diets containing high levels of lucerne hay. Therefore, the importance of energy to animal production and its impact on limiting the level of lucerne hay that can be fed to high producers, would suggest that energy may be the most important criteria to use in evaluating the quality of lucerne hay.

Comparisons between the most popular and/or accurate models for ranking 18 randomly selected lucerne hay samples are illustrated in Table 3 and Appendix C. The superior accuracy of TDNlig and the proposed LMV model in Chapter 6, over currently adopted laboratory approaches (FQI, RFQ, RFV, LQI and TFI) are clearly verified in Table 3. These results contrasts somewhat with the findings of Moore & Undersander (2002) who reported similar ranking of forages with FQI, RFQ and RFV. Thus, for the purpose of accurate and practical implementation of a uniform grading system in SA, the LMV model seems to be the most appropriate.

Table 3   **Ranking of 18 randomly selected lucerne hay samples according to different models, in order of decreasing accuracy**

MY	TDNlig	LMV	FQI	RFQ	RFV	LQI	TFI
1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2
3	4	3	3	3	3	4	4
4	3	4	7	4	8	9	8
5	5	7	5	5	5	5	5
6	8	5	6	6	12	15	17
7	6	6	4	7	6	7	7
8	9	9	8	8	9	6	6
9	7	10	12	14	15	16	18
10	10	8	10	11	11	10	10
11	11	11	11	12	7	8	9
12	12	12	14	13	14	14	14
13	13	13	13	10	10	11	11
14	15	15	9	9	4	3	3
15	14	14	16	15	17	17	16
16	16	16	15	17	16	13	15
17	17	17	17	16	13	12	12
18	18	18	18	18	18	18	13

Abbreviations: Milk yield (MY); total digestible nutrients calculated by using NDFDlig, calculated from lignin, to calculate TDN (TDNlig); lucerne milk value (LMV); forage quality index (FQI); relative forage quality (RFQ); relative feed value (RFV); lucerne quality index (LQI); total forage index (TFI)

4.   CONCLUSIONS

From the evaluation results of the present study it seems that models for assessing lucerne hay quality revealed large differences in the accuracy of prediction as measured by MY. The best results were obtained with the summative TDN equation used by NRC (2001). However this model involves high analysis costs to develop and maintain NIRS calibrations. Accordingly, the NIRS calibration equations for several chemical parameters necessary to calculate TDN were unacceptable to maintain the high accuracy of predicting MY and therefore lucerne hay quality

The relative poor performance of ACP and CP containing quality models suggest that there is no benefit to be gained by including ACP or CP in quality models for assessing lucerne hay quality.

CHAPTER 8

GENERAL CONCLUSIONS

The purpose of this study was to develop a national uniform grading system for lucerne hay in South Africa. Development of a grading system entitles different facets that are, as one might anticipate, inherently dependant on producing a desirable evaluation system that reflects the animal performance potential of the lucerne hay fed. These facets include the identification of the most appropriate sampling, as well as sample handling and preparation procedures, accurate NIRS-nutrient calibration equations and an accurate, simplistic cost effective quality model predicting animal performance. Lucerne hay is mostly used in dairy cow diets and an evaluation system should, in the first place, as in the present study, be directed at dairy cows (milk production). Such an evaluation system would probably also be applicable for other ruminants such as beef cattle and sheep. It should be verified in further studies for all types of animals including horses and especially ostriches.

The chemical and digestibility characteristics of lucerne hay have been comprehensively determined by analytical procedures in the present study. In order to validate the potential parameters for quality assessment, the nutritional characteristics and variation in nutritive value of South African lucerne hay is of utmost importance and was accordingly investigated. The overall variation that occurred in energy and protein composition, as well as in the utilisation of nutrients in lucerne hay emphasises the need for a rapid and accurate quality evaluation system (model) for South African lucerne hay. This was supported by the observed variation in milk yield (MY) as predicted by the Cornell Net Carbohydrate and Protein System (CNCPS) for the South African lucerne hay population (range = 12.97 l).

The highest coefficient of variation (CV = 66.2%) was observed for proteins closely bound to lignin (acid detergent fibre-crude protein; ADF-CP (%DM)), which is usually associated with heat damage caused by a high moisture content (Van Soest, 1994). It seems from the ADIP (%CP) results of the present study that heat damage possibly occurred only in a small percentage (6%) of the samples. Accordingly, the low ADF-CP content confirmed that lucerne hay moisture content was within the desired range of effective storage. On the other hand, the relatively high dry matter content observed for the lucerne hay in the current study could be explained by moisture losses during sample preparation (grinding) and storage.

From the results of the present study it seems that moisture losses during the grinding of lucerne hay ranged from 14.7% up to 41.1% (of the original moisture content) for the lowest and highest containing moisture sample, respectively. It is clear that moisture losses were more profound in the higher than in the lower moisture samples. These results suggested that analytical moisture standards for lucerne hay should be based on the original moisture content of samples before grinding. Furthermore, the degree of hydration may influence the optimum area of near infrared (NIR) spectrum where the absorbers of specific constituents occur, thus affecting the whole NIR wavelength region (De Boever *et al.*, 1996; Williams & Norris, 2001). Thus, it seems that moisture content of a sample could affect the prediction results of all the other parameters (CP, ADF, NDF, etc.). Therefore, further investigation and improvement on developing useful NIRS calibrations for lucerne hay quality parameters should be directed at scanning of unground samples to eliminate the effect of grinding (moisture loss) on the NIR spectrum. This will facilitate the more accurate prediction of baled lucerne hay, with regard to nutritive value as fed to the animal. According to Williams (2007, Pers. Comm.) this would be viable for the majority of NIRS instruments. Even though the moisture content of the original unground sample was calculated with high accuracy ($r^2 = 0.88$) from the moisture content of the ground sample using an exponential equation ($y = 1.9795e^{0.2019x}$), it should be used with caution due to population specificity.

Electronic moisture testers (probes) provide an instantaneous moisture concentration reading; however, the results from the present study have shown that these predicted moisture values failed to accurately predict moisture content around the critical moisture level (16% and higher).

The best single predictor of MY was the acid detergent fibre (ADF) content, which explained 67% of the measured MY variation. An improvement ($r = 0.96$) has, however, been accomplished by combining ADF, ash and lignin in a multiple regression and/or model named lucerne milk value (LMV) ($Y = 64.18 - 0.23ADF - 0.53ash - 0.90lignin$). This model has been proven to be the most accurate and acceptable NIRS calibration equations ($r^2 > 0.92$; ratio of prediction to deviation > 3.0; standard error of cross validation < 1.10).

Even though ADF captures less of the structural fibre, it (ADF) out-performed neutral detergent fibre (NDF) ($r^2 = 0.61$) in estimating available energy for milk production (MY). Accordingly, the regression equation based on ADF explained more of the variance in MY

than *in vitro* digestibility parameters ($r^2 < 0.52$). Therefore, it seems from the results of the present study that chemical extractions could be more closely related to animal performance than *in vitro* estimates of digestion. However, the shorter incubation period (24 hr) used for *in vitro* organic matter digestibility (IVOMD) ($r^2 = 0.52$) and neutral detergent fibre digestibility (NDFD) ($r^2 = 0.21$) both out-performed the 48 hr incubation time ($r^2 = 0.36$ and $r^2 = 0.10$, respectively) in predicting MY. According to these findings a shorter incubation period is more representative of the digestibility of high producing dairy cows.

The incidence of ash as the second most significant and most negative contributor to MY was most likely associated with the lower energy concentration of ash and the high occurrence of soil contamination indicated by the high and variable ash content (CV = 27.39%), of South African lucerne hay. From the results of the present study it was evident that 24% of the samples had an ash content above 14 %. This is probably an indication of soil and dust that settled on the lucerne hay before storing. Accordingly, the high mean ash value (12.97%) of lucerne hay in the current study could have had a detrimental effect on the energy value of the composite diet due to the zero energy contribution of ash. Furthermore, the contribution of the prominent high calcium (Ca) and potassium (K) content of lucerne hay in this study to the dietary cation-anion difference (DCAD) of dairy diets could increase the probability of milk fever in close-up dry cows. It is also important to note the high level of iron (Fe) in South African lucerne hay. This might interfere with the absorption of copper (Cu) and zinc (Zn) in the diet which could lead to depletion of these minerals in high producing dairy cattle.

The influence of lignin on the quality of lucerne hay was to be expected. Lignin has long been recognised as the chemical component most commonly associated with the impairment of forage digestion. Similar to ash, lignin has also theoretically, no digestible energy. Therefore, the variation in lignin, and its role as a nutritional entity especially in forages, should be acknowledged in the quest for developing an accurate quality model for assessing animal performance. Variability in lignin was found to be the second highest (CV = 23%) of the fibre fractions. This was expected due to the lower and higher lignin and NDF content, respectively, found in grasses compared to lucerne hay. The probable overall effect of grass contamination in lucerne hay in the current study was manifested in the majority of the energy related parameters (ADF, NDF, lignin, NDFD, etc.). Furthermore, the relatively low lignin/NDF relation of the samples in the present study was enough evidence to claim that

the majority of lucerne hay grown in South Africa is not from pure strands but is a mixture of lucerne and grass. This phenomenon produced a dataset for South African lucerne hay with generally more variation than found in the available literature for this species. It also stresses the importance of further development of the NIRS calibrations by means of validation, ring tests and identification of outliers.

The high NDF content of several lucerne hay samples in the current study proposed possible intake restriction thereof in high yielding dairy diets. Allen (1991) and Zinn *et al.* (2004) suggested that the lucerne hay in high producing dairy diets should contain a NDF content of 40% or less. Accordingly, only 33% of the South African population would have fitted this criterion. On the other hand, 8% of these samples contain less than 35% NDF; therefore, nutritionally disqualifying it as a roughage.

Interestingly, although the digestibility of NDF (NDFD) is commonly used to estimate energy value of diets it did not feature in the regression equation developed for MY. The reason for this discrepancy could possibly be explained by the theory of Oba & Allen (1999). They suggested that although many experiments have reported NDFD data, interpretation of results is difficult due to a diversity of confounding factors (particle fragility, rumen retention time, etc.) found in individual experiments. According to Zinn & Ware (2004) ruminal fibre digestion might be more limited by ruminal fibrolitic capacity than by the native degradability of the fibre. Furthermore, the results of the present study did not support the proposal of NRC (2001) that NDFD using the 48 hr *in vitro* procedure ($NDFD_{48}$) and NDFD calculated from lignin ($NDFD_{lig}$) can be used interchangeably in estimating the summative TDN equation, as proposed by Weiss *et al.* (1992). The significant difference between these two parameters could severely hamper the utility of the summative energy equation in predicting accurate TDN values for lucerne hay.

The *in vitro* digestibility parameters on organic matter (IVOMD) showed surprisingly low CVs. Thus, the mean IVOMD at 24hr ($IVOMD_{24}$) (69%) and 48hr ($IVOMD_{48}$) (73%) values in the current study seem to be relatively similar, irrespective of incubation time, and are accordingly representative of the South African lucerne hay population. From these results it is evident that *in vitro* incubation time had a minor effect on digestibility of lucerne hay organic matter (OM). Moreover, it can be hypothesised that the application of the IVOMD

procedure to evaluate lucerne hay forage could produce unsatisfactory results because of its poor relation to available energy and/or production potential.

According to the results of the present study the mean crude protein (CP) (20.07% DM) and soluble protein (SP) (6.2% CP) content of lucerne hay was higher and lower, respectively, than those found in the literature. Therefore, it seems that the South African lucerne hay population contains a lower concentration of rapidly degradable protein as reported by the NRC (2001). Furthermore, the observed variation in CP and other CP-fractions content stress the importance of analysis to ensure accurate diet formulation. Sample loss due to dust generated during the grinding process was non-significant related (r = 0.01; P>0.05) to moisture content of the unground sample. Therefore, even though the CP content of dust generated in the grinding process was significantly (P<0.0001) lower than that of the original sample, a non-significant (P>0.05) difference was recorded between CP content of the original and end product due to the grinding process. However, further studies are warranted to assess the effects of grinding on other chemical parameters.

Much emphasis has been placed on CP content of lucerne hay in the past as the ultimate predictor of quality. The relatively poor performance of CP (r^2 = 0.04) and other protein related parameters (r^2< 0.25; ACP, ADF-CP, NDF-CP and SP) in predicting MY suggested that the protein content of lucerne hay, as such, is an unreliable indicator of production potential, therefore of lucerne hay quality. In fact, the accuracy of models for assessing lucerne hay quality was reduced by the incorporation of CP. Thus, from the results of the present study it seems that only energy related parameters were relevant in modelling MY.

Accurate NIRS calibration equations of a nutritional parameter are inevitable for accurate diet formulation. From the results of the current study the potential of NIRS to predict the chemical composition of South African lucerne hay is accordingly, to the guidelines of several authors (Williams & Sobering, 1993; Edney et al., 1994; Mahison et al., 1999) as follows: good (RPD>3.0; SECV<3%) for DM, CP, ADF, NDF, lignin, ash and chloride (Cl), moderate (RPD=2.5-3.0) for NDF-CP, sugar, IVOMD24 and IVOMD48, and poor (RPD<2.5) for SP, ADF-CP, fat, starch, NDFD and the macro minerals (Ca, P, Mg, P, Na and S). However, according to the criteria of Williams & Norris (2001) further work needs to be done to build robust NIRS calibration models for the majority of the parameters to be used effectively in modern nutritional models. Even though the NIRS procedure entails the ease,

rapidity and economy of lucerne hay analysis, the calibration process is an ongoing process to familiarise calibration equations with the continual changing lucerne hay matrix. From these results it is evident that several quality parameters (IVOMD, SP, ADF-CP, starch and NDFD), although important nutritional entities, would fail when applied to mathematical models for accurate and economical lucerne hay quality prediction or diet formulation. This failure may also be ascribed to the variable degree of soil contamination which dilutes energy content. Mineral soil induces baseline shift in the lower part of the NIR spectrum, reducing the specific NIR signals of organic constituents (Paul, 1987). Ultimately, accurate NDFD and IVOMD analysis of lucerne hay by NIRS would be preferred as NIRS prediction systems are less vulnerable to experimental error.

From the evaluation results of the present study it seems that currently adopted approaches (models) (FOI, RFQ, RFV, LQI and TFI) for assessing lucerne hay quality revealed large differences in predicting production potential (MY). The theoretically-based summative TDN equation of Weis *et al.* (1992), using lignin to determine truly digestible NDF, almost fully described MY variation ($r^2 = 0.98$). The data suggested that the currently adopted quality models evaluated did not seem to improve the prediction of ME or MP allowable milk production compared to TDN (NRC, 2001), and the simple chemical parameter model (LMV; ADF, ash and lignin) proposed in the present study. Nevertheless, with regard to costs of laboratory analysis and maintaining robust NIRS calibration equations, the use of the TDN$_{lig}$ model in predicting MY, although less population-specific (robust), would not be practical and economically viable compared to the accurate more simplistic LMV empirical equation. Thus, for commercial application, this model (LMV) will provide a means to make rapid, simplistic and accurate assessment of milk production potential, alias quality, of South African lucerne hay in practice.

The question of defining forage quality with a single value or number has been raised by several researchers (Coppock, 1979; Putnam, 2004; Hall, 2005). However, the best single measure of forage quality is animal productivity. Thus, for the purpose of accurate and practical implementation of a uniform grading system in South Africa, the LMV model seems to be the most appropriate single value to assess lucerne hay quality.

The results of this study regarding sample preparation, NIRS calibrations developed and evaluated, and model development provides a valuable guideline for the implementation of a

national lucerne hay grading system in South Africa. A proposed application procedure of such a grading system is briefly outlined in Appendix D.

ABSTRACT

The purpose of this study was to develop a national grading system for luceme hay in South Africa by identifying the most appropriate sampling, as well as sample handling and preparation procedures, the most accurate NIRS – nutrient calibrations and an accurate, cost effective quality model. Six hundred luceme hay samples were obtained from different cuttings at different times in the seasons and from different commercial irrigation areas (sites) in South Africa. The 600 samples were scanned and screened through a NIR Systems Model 5000 monochromator (Foss). One hundred and sixty- eight samples representing the spectral characteristics of the South African luceme hay population were selected and chemical analysed.

The variation in nutritive value of South African luceme hay was evaluated as an initial study. The highest moisture content recorded (13.54%) was safely below the critical moisture level of 16% for effective storage. The coefficient of variation (CV) ranged from 1.2% for dry matter (DM) up to 66.2% for acid detergent fibre-crude protein (ADF-CP). The average ash content was 12.97% (7.3 to 29.5%), indicating soil contamination. Relatively high average values were recorded for calcium (Ca) (1.35%), potassium (K) (2.53%) and iron (Fe) (874 ppm). The fibre fractions varied as follows: acid detergent fibre (ADF) (21.26 to 47.28%), neutral detergent fibre (NDF) (28.89 to 65.93%), lignin (4.32 to 16.25%), cellulose (16.29 to 36.44%) and hemicellulose (5.26 to 19.86%). The mean IVOMD for both 24 and 48hr (69.26 and 73.19% DM, respectively), was representative (CV = ± 8%) of the luceme hay population. Crude protein (CP) (average = 20.7%DM) consists of 76.9% true protein. According to ADF-CP, 6% of the samples were heat damaged.

A second study was conducted to determine the effect of the grinding procedure on the moisture and CP content of the ground sample. Variance of analyses revealed significant ($P<0.0001$) differences in moisture concentration between ground (CV = 16.1%) and unground (CV = 27.4%) samples ranging from 14.7 up to 41.1% (of the unground sample). However, the grinding process had a non-significant ($P>0.05$) influence on the CP content of the final ground product. Even though r^2 between measured moisture results on unground samples and

values predicted by electronic moisture tester (EMT) seems to be significantly high (r = 0.79; P<0.0001), individual predicted values for higher moisture samples (>10%) failed to accurately predict moisture content around the moisture area of critical concern (16%) and higher.

In a third study, the accuracy of near infrared reflectance spectroscopy (NIRS) to predict chemical and digestibility parameters was investigated. Values for r^2 and ratio of prediction to deviation (RPD) used as estimates of calibration accuracy for chemical and digestibility parameters were considered as follows: good for DM (r^2 = 0.97; RPD = 4.84), CP (r^2 = 0.97; RPD = 4.57), ADF (r^2 = 0.95; RPD = 3.97), NDF (r^2 = 0.0.95; RPD = 3.99), lignin (r^2 = 0.94; RPD = 3.61), ash (r^2 = 0.93; RPD = 3.12) and chloride (Cl) (r^2 = 0.95; RPD = 3.74); intermediate for neutral detergent fibre-crude protein (NDF-CP) (r^2 = 0.91; RPD = 2.96), sugar (r^2 = 0.91; RPD = 2.82), *in vitro* organic matter digestibility at 24 hr (IVOMD$_{24}$) (r^2 = 0.90; RPD = 2.84) and IVOMD at 48hr (IVOMD$_{48}$) (r^2 = 0.89; RPD = 2.70); and low (RPD<2.31) for soluble protein (SP), acid detergent fibre-crude protein (ADF-CP), fat, starch, neutral detergent fibre digestibility (NDFD) and the macro minerals (Ca, P, Mg, P, Na and S). The results recorded in the present study indicated that the NIRS technique is acceptable for DM, CP, ADF, NDF, lignin, ash and Cl analysis and for inclusion in quality models.

Milk yield (MY) derived from the CNCPS model, by replacing the average lucerne hay in a complete diet with the rest of the 168 samples, was used as a criterion to evaluate and/or develop models for lucerne hay quality grading. The best single predictor of MY was the ADF content of lucerne hay, which explained 67% of the measured variation. The relatively poor performance of CP (r^2 = 0.04) and other protein related parameters (r^2< 0.25; ACP, ADF-CP, NDF-CP and SP) in predicting MY suggest that protein content of lucerne hay is an unreliable indicator of lucerne hay quality.

The developed empirical equation named lucerne milk value (LMV), including ADF, ash and lignin (Y = 64.18 – 0.23ADF – 0.53ash – 0.90lignin), accurately predict MY. Application of the theoretically-based summative TDN$_{lig}$ model of Weiss *et al.* (1992), using lignin to determine truly digestible NDF, explained almost all of the variation in MY (r^2 = 0.98). However, several of its components were poorly predicted by NIRS and therefore, not suited

for quality assessment. Current available models for assessing lucerne hay quality revealed lower accuracies, especially when protein was included in the model.

The results of the present study clearly indicated that large variations occur in the energy and protein composition as well as the utilisation of nutrients in South African lucerne hay. This emphasises the need for a rapid and accurate quality evaluation system for lucerne hay in practice. The developed LMV proved to be a practical, simplistic, economical and accurate quality evaluation model for commercial application.

OPSOMMING

Die studie het ten doel gehad om 'n nasionale graderingstelsel vir lusernhooi in Suid-Afrika te ontwikkel deur die mees toepaslike monsterneming-, sowel as monsterhanterings en - voorbereidingsprosedures, die mees akkurate NIRS-kalibrasies en 'n akkurate, koste-effektiewe kwaliteitsmodel te identifiseer. Seshonderd lusernhooi monsters is vanaf verskillende snysels gedurende verskillende tye van die seisoen vanaf verskillende kommersiële besproeiingsgebiede in Suid-Afrika ingesamel. Hierdie 600 monsters is met die hulp van 'n Naby-infrarooispektrofotometer (NIRS), Model 5000 monochromator (Foss) geskandeer. Eenhonderd agt-en-sestig monsters verteenwoordigend van die spektrale karakter van die Suid-Afrikaanse lusernhooi populasie is geselekteer en chemies ontleed.

Die variasie in die voedingswaarde van Suid-Afrikaanse lusernhhooi is in 'n eerste studie geevalueer. Die hoogste verkreë voginhoud, (13.54%), was veilig onder die kritiese vogvlak van 16% wat nodig is vir effektiewe opberging. Die koëffisiënt van variasie (KV) het vanaf 1.2% vir droëmateriaal (DM) tot 66.2% vir suurbestandevesel-protein (SBV-RP) gewissel. Die gemiddelde asinhoud was 12.97% (7.3 tot 29.5%) wat grondbesoedeling aangedui het. Relatiewe hoë gemiddelde waardes is vir kalsium (Ca) (1.35%), kalium (K) (2.53%) en yster (Fe) (874 ppm) verkry. Die veselfraksies het as volg gevarieer: suurbestandevesel (SBV) (21.26 tot 47.28%), neutraalbestande vesel (NBV) (28.89 tot 65.93%), lignien (4.32 tot 16.25%), sellulose (16.29 tot 36.44%) en hemisellulose (5.26 tot 19.86%). Die gemiddelde *in vitro* organiesemateriaalverteerbaarheid (IVOMV) vir beide 24 en 48 uur (69.26 en 73,19% DM, respektiewelik) was verteenwoordigend (KV = 8%) van die lusernhooi populasie. Ru-protein (RP) (gemiddeld = 20.7% DM) het uit 76.9% ware protein bestaan. Volgens suurbestande-onoplosbare-protein, was 6% van die monsters aan hitte beskadiging blootgestel.

'n Tweede studie is uitgevoer om die effek van maalprosedure op die vog en die RP inhoud van die gemaalde monster te bepaal. 'n Variansie-analise het betekenisvolle (P<0.0001) verskille in vogkonsentrasie tussen gemaalde (KV = 16.1%) en ongemaalde (KV = 27.4%) monsters aangetoon wat van 14.7 tot so hoog as 41.1% (van die ongemaalde monster) gewissel het. Daarenteen het die maalproses 'n nie-betekenisvolle (P>0.05) invloed op die RP inhoud van die

finale gemaalde produk gehad. Alhoewel r^2 tussen die voginhoud van die ongemaalde monsters, en die waardes deur die elektroniese vogmeter (EVM) voorspel beduidend hoog was ($r = 0.79$; P<0.0001), was die voorspelling vir hoër vogmonsters (>10%) rondom die van kritiese voginhoud (16%) onakkuraat.

In 'n derde studie is die akkuraatheidondersoek van 'n NIRS om die chemiese en verteerbaarheids parameters te voorspel. Waardes vir r^2 en die verhouding van voorspelling teenoor afwyking (RPD), as beramers van die akkuraatheid van kalibrasies vir chemiese en verteerbaarheidsparameters gebruik en was as volg goed vir DM ($r^2 = 0.97$; RPD = 4.84), RP ($r^2 = 0.97$; RPD = 4.57), SBV ($r^2 = 0.95$; RPD = 3.97), NBV ($r^2 = 0.0.95$; RPD = 3.99), lignien ($r^2 = 0.94$; RPD = 3.61), as ($r^2 = 0.93$; RPD = 3.12) en chloried (Cl) ($r^2 = 0.95$; RPD = 3.74); gemiddelde vir neutraalbestandevesel-ruprotein (NBV-RP) ($r^2 = 0.91$; RPD = 2.96), suiker ($r^2 = 0.91$; RPD = 2.82), *in vitro* organiesemateriaalverteerbaarheid op 24 uur ($IVOMV_{24}$) ($r^2 = 0.90$; RPD = 2.84) en IVOMV op 48uur ($IVOMV_{48}$) ($r^2 = 0.89$; RPD = 2.70); en laag (RPD<2.31) vir oplosbare protein (OP), SBV-RP, vet, stysel, neutraalbestandevesel verteerbaarheid (NBVV) en die makro minerale (Ca, P, Mg, P, Na and S). Die resultate van die huidige studie dui daarop dat die NIRS tegniek aanvaarbaar is vir DM, RP, ADF, NVB, lignien, as en Cl -analise sowel as vir insluiting in kwaliteitsmodelle.

Melkproduksie (MP) voorspel deur die "Cornell Net Carbohydrate and Protein System" (CNCPS) model, deur lusern met gemiddelde waardes in 'n volledige dieët met die res van die 168 monsters te vervang, is as kriteria gebruik vir die evaluasie en/of ontwikkeling van modelle vir lusernhooi kwaliteitsbepaling. Die enkele beste voorspeller van MP was SBV-inhoud van lusernhooi, wat 67% van die gemete variasie verklaar het. Die relatiewe swak vertoning van RP ($r^2 = 0.04$) en ander protein verwante parameters (r^2<0.25; ACP, SBV-RP, NBV-RP EN OP) in die voorspelling van MP dui daarop dat proteininhoud van lusernhooi 'n onbetroubare aanduider van lusernhooikwaliteit is.

Die empiriese ontwikkelde vergelyking genaamd lusernmelkwaarde (LMW), wat insluit SBV, as en lignien (Y=64.18 - 0.23SBV - 0.53as - 0.90lignien), het MP akkuraat voorspel. Die toepassing van die teoreties-gebaseerde "summative" TDNlig model van Weiss *et al.* (1992),

deur van lignien gebruik te maak om ware verteerbare NBV te bepaal, het byna al die variasie in MP ($r^2 = 0.98$) verklaar. Verskeie van die komponente word egter swak deur NIRS voorspel en is nie geskik vir kwaliteitbepaling nie. Huidige beskikbare modelle vir die bepaling van lusernhooikwaliteit toon swak akkuraatheid veral wanneer protein by die model ingesluit word.

Die resultaat van die huidige studie het duidelik aangetoon dat daar groot variasie in die energie- en proteïnsamestelling, asook benutting van voedingstowwe in Suid-Afrikaanse lusernhooi voorkom. Dit beklemtoon die noodsaaklikheid van 'n vinnige en akkurate kwaliteitsevalueringstelsel vir lusernhooi in die praktyk. Die ontwikkelde LMW blyk 'n praktiese, eenvoudige, ekonomiese en akkurate kwaliteitevalueringsmodel te wees, vir kommersiële gebruik.

REFERENCES

Abrams, S.M., 1988. Sources of error in predicting digestible dry matter from the acid-detergent fibre content in forages. *Anim. Feed Sci. Technol.* 21:205-208.

Abrams, S.M., Shenk, J.S., Westerhaus, M.O. & Barton, F.E., 1987. Determination of forage quality by near infrared reflectance spectroscopy: Efficacy of broad-based calibration equations. *J. Dairy Sci.* 70:806-813.

Abreu, J.M.F. & Bruno-Soares, A.M., 1996. Soil intake by sheep - Effects in digestibility of roughage material. *An. Inst. Sup. Agron.* 45:523-532.

ADAS, 1998. On farm validation of the Cornell Net Carbohydrate and Protein System. ADAS Bridgets Research Centre Research Report. Martyr Worthy, Winchester, Hampshire, England.

AFRC, 1992. Technical committee on responses to nutrients, Report no. 9. Agricultural and food research council. Nutritive requirements of ruminant animals: protein. Farnham Royal, Commonwealth Agricultural Bureaux.

Alexandrov, A.N., Atanassova, S.L., Peltekova, V.D., Broderick, G.A. & Todorov, N.A., 1995. The use of near infrared spectroscopy for estimation of microbial contamination of *in sacco* forage residues and protein and dry matter degradability in the rumen. In Proc: 7th Intern. Symposium on protein metabolism and nutrition, 24-27 May, Vale de Santarem, Portugal. pp. 133-139.

Allen, M.S., 1991. Carbohydrate nutrition. *Vet. Clin. North America.* 7(2):327.

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

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

Allen, E. & Caddel, J., 1990. Collecting Forage Samples for Analysis. F-2589, Oklahoma State University.

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

Alvarez, E.G., Calderon, J.F., Montano, M.F., Ware, R.A. & Zinn, R.A., 2004. Influence of dietary forage level on digestive function and growth performance in cattle fed steam-flaked corn-based growing-finishing diets. *J. Anim. Vet. Adv.* 3:503-509.

Alvarez, E.G., Montano, M.F. & Zinn, R.A., 2000. Comparative feeding value of elephant grass in growing diets for feedlot cattle. In Proc: West. Sec. Am. Soc. Anim. Sci. 51:477-480.

Anderson, M.J. & Thacker, D.R., 1970. Elevation effects on nutritive characteristics of alfalfa. *J. Dairy Sci.* 53:676.

Andres, S., Calleja, A., Lopez, S., Gonzalez, J.S., Rodriguez, P.L. & Giraldez, F.J., 2005. Prediction of gas production kinetic parameters of forages by chemical composition and near infrared reflectance spectroscopy. *Anim. Feed Sci. Technol.* 123-124:487-499.

Andrews, S.M,. Tyrrell, H.F., Reynolds, C.K. & Erdman, R.A., 1991. Net energy for lactation of calcium soaps of long-chain fatty acids for cows fed silage-based diets. *J. Anim. Sci.* 74:2588.

Antoniewicz, A.M., Kowalczyk, J., Kanski, J., Gorska-Matusiak, Z. & Nalepka, M., 1995. Rumen degradability of crude protein of dried grass and lucerne forage measured by *in sacco* incubation and predicted by near infrared spectroscopy. *Anim. Feed Sci Technol.* 54:203-216.

AOAC, 1979. Official methods of analysis. 12th ed. Association of Official Analytical Chemists, Washington DC,USA.

AOAC, 1990. Official methods of analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

AOAC, 2000. Official methods of analysis. 17th ed. Association of Official Analytical Chemists International, Washington DC, USA.

AOAC, 2006. Official methods of analysis. 18th ed. Association of Official Analytical Chemists International, Washington DC, USA.

Arana, M., 1997. Why forage fibre quality is important. In Proc: 27th California Alfalfa Symposium, University of California, Davis, CA.

ARC, 1980. The Nutrient Requirements of Ruminant Livestock. Agricultural Research Council. Commonwealth Agricultural Bureau, Slough, Berkshire, UK.

ARC, 1984. The Nutrient Requirements of Ruminant Livestock. Supplement No. 1. Report of the Protein Group of the ARC Working Party. Farnham Royal, Commonwealth Agricultural Bureau, Slough, Berkshire, UK.

Atanassova, S., Pavlov, D. & Todorov, N., 1994. Estimation of the chemical composition, the net energy and the protein value of lucerne by NIRS and by regression equation on the basis of the days of vegetation or the accumulated temperature. *J. Agric. Sci.(Sofia)* 3:325-335.

Aufrere, J., & Michalet-Doreau, B., 1988. Comparison of methods for predicting the digestibility of feeds. *Anim. Feed Sci. Technol.* 20:203-218.

Baker, R.D. & Ball, S.T., 2001. Variations in Alfalfa hay grading. Extension paper. New Mexico State University, USA.

Baker, C.W., Givens, D.I., Deaville, E.R., 1994. Prediction of organic matter digestibility *in vivo* of grass silage by near infrared reflectance spectroscopy: effect of calibration method, residual moisture and particle size. *Anim. Feed Sci. Technol.* 50:17-26.

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.

Ball, D.M., Collins, M., Lacefield, G.D., Martin, N.P., Mertens, D.A., Olson, K.E., Putnam, D.H., Undersander, D.J. & Wolf, M.W., 2001. Understanding forage quality. American Farm Bureau Federation Publication 1-01, Park Ridge, IL.

Ball, S.T. & Lauriault, L., 1999. Alfalfa quality analysis. Extension paper. New Mexico State University, USA.

Balliette, J. & Torell, R., 1998. Alfalfa for beef cows. Cattle Producer's library, CL 319. http://www.unce.unr.edu/publications/Spec%20Pubs/CL-319.htm

Barnes, R.J., Dhanoa, M.S. & Lister, S.J., 1989. Standard normal variate transformation and de-trending of near infrared diffuse reflectance spectra. *Applied spectroscopy* 43:772-777.

Bath, D.L. & Marble V.L., 1989. Testing Alfalfa for its Feeding Value. Leaflet 21457. WREP 109. University of California Cooperative Extension, Agriculture and Natural Resources. 6701 San Pablo Ave., Oakland, CA 94608.

Batten, G.D., 1998. Plant analysis using near infrared reflectance spectroscopy: the potential and the limitations. *Aus. J. Exp. Agric.* 38:697-706.

Beauchemin, K.A., 1996. Using ADF and NDF in dairy cattle diet formulation - a western Canadian perspective. *Anim. Feed Sci. Technol.* 58:101-111.

Beede, D., 2003. Optimum dietary cation-anion difference for lactating dairy cows. In Proc: 4 State Applied Nutr. & Management Conf., La Crosse, WI. pp. 109-122.

Beede, D.K., Sanches, W.K. & Wang, C., 1992. Macrominerals. In: Large Dairy Herd Management. Eds. Van Horn, H.H. & Wilcox, C.J., American Dairy Science Association, Champaign, IL. pp. 272-286.

Beever, D.E. & Mould, F.L., 2000. Forage evaluation for efficient ruminant livestock production. In: Forage evaluation in ruminant nutrition. Eds. Givens, D.I., Owen, E., Axford, R.E.F. & Omed, H.M., CAB International, Wallingford, UK. pp. 15-42.

Beever, D.E. & Siddons, R.C., 1986. Digestion and metabolism in the grazing ruminant. In: Control of Digestion and Metabolism in Ruminants. Eds. Milligan, L.P., Grovum, W.L. & Dobson, A., Prentice-Hall, Englewood Cliffs, NJ. pp. 479-497.

Berardo, N., Dzowela, B.H., Hove, L., Odoardi, M., 1997. Near infrared calibration of chemical constituents of *Cajanus cajan* pigeon pea used as forage. *Anim. Feed Sci. Technol.* 69:201-206.

Berger, L.L., 1990. Comparison of National research council feedstuff material composition data with values from commercial laboratories. In Proc: MN Nutr. Conf. Univ. MN. pp. 158.

Bertrand, D., Lila, M., Furtoss, V., Robert, P. & Downey, G., 1987. Application of principal component analysis to the prediction of lucerne forage protein content and *in vitro* dry matter digestibility by NIR spectroscopy. *J. Sci. Food Agric.* 41:299-307.

Bethard, G., 1997. Estimating rumen available and non-structural carbohydrates in dairy cattle diets. Dairy Science graduate, Dairy Management, Virginia Tech., USA.

Bethard, G., 2002. Forage management from a nutritionists perspective. G&R Dairy Consulting, Inc., Wytheville, Virginia.

Bezeau, L.M. & Sonmor, L.G., 1964. The influence of levels of irrigation on the nutritive value of alfalfa. *Can. J. Plant Sci.* 44:505-508.

Biston, R., Dardenne, P. & Demarquilly, C., 1989. Determination of forage *in vivo* digestibility by NIRS. In Proc: 16th Intern. Grasslands Congr., Nice, France, vol. 2. pp. 7:895-896.

Blaxter, K.L., 1964. Utilisation of the metabolisable energy of grass. In Proc: Nutr. Soc. p. 62.

Bolton, J.L., 1962. Alfalfa Botany, cultivation and utilisation. World Crops Books, Leonard Hill, London.

Bowden, D.D., Taylor, K. & Davis, W.E.P., 1968. Water soluble carbohydrates in orchardgrass and mixed forages. *Can. J. Plant Sci.* 48:9-15.

Brimmer, P.J., De Thomas, F.A. & Hall, J.W., 2001. Method Development and Implementation of Near Infrared Spectroscopy in Industrial Manufacturing Processes. In: Near infrared technology in the agricultural and food industries. Eds. Williams, P.C. & Norris, K.H., American Association of Cereal Chemist, St. Paul, Minnesota, USA. pp. 199-214.

Brink, G.E. & Fairbrother, T.E., 1994. Cell wall composition of diverse clovers during primary spring growth. *Crop Sci.* 34:1666-1671.

Broderick, G.A., 1995. Desirable characteristics of forage legumes for improving protein utilisation in ruminants. *J. Anim. Sci.* 73:2760-2773.

Broderick, G.A., Abrams, S.M. & Rotz, C.A., 1992. Ruminal *in vitro* degradability of protein in alfalfa harvested as standing forage or baled hay. *J. Dairy Sci.* 75:2440-2446.

Broderick, G.A., Luchini, N.D., Smith, W.J., Reynal, S., Varga, G.A. & Ishler, V.A., 2000. Effect of replacing dietary starch with sucrose on milk production in lactating dairy cows. *J. Dairy Sci.* 83(Suppl. 1):248.

Broderick, G.A. & Satter, L.D., 1998. Balancing forage proteins. In Proc: 4-State Forage and Feeding and Management Conf., Wisconsin Dells, WI. pp. 19-34.

Bruno-Soares, A.M., Murray, I., Paterson, R.M. & Abreu, J.M.F., 1998. Use of near infrared reflectance spectroscopy (NIRS) for the prediction of the chemical composition and nutritional attributes of green crop cereals. *Anim. Feed Sci. Technol.* 75:15-25.

Burns, J.C., Mayland, H.F. & Fisher, D.S., 2005. Dry matter intake and digestion of alfalfa harvested at sunset and sunrise. *J. Anim. Sci.* 83:262-270.

Buxton, D.R. & Russell, J.R., 1988. Lignin constituents and cell-wall digestibility of grass and legume stems. *Crop Sci.* 28:553-558.

Calberry, J.M., Plaizier, J.C., Einarson, M.S. & McBride, B.W., 2003. Effects of replacing chopped alfalfa hay with alfalfa silage in a total mixed ration on production and rumen conditions of lactating dairy cows. *J. Dairy Sci.* 86:3611-3619.

Casper, D.P., Schingoethe, D.J. & Eisenbeisz, W.A., 1990. Response of early lactating cows to diets that vary in ruminal degradability of carbohydrates and amount of fat. *J. Dairy Sci.* 73:425.

Cherney, D.J.R., Cherney, J.H., Davidson, A.H., 1997. Characterisation of legume and grass residues following *in vitro* and *in sacco* ruminal digestion. In Proc: Int. Grassl. Congr. pp. 17:23-24.

Cherney, J.H. & Hall, M.H., 1997. Putting forage quality in perspective. Department of Soil, Crop, and Atmospheric Sciences, Cornell University, Ithaca.

Cilliers, J.W. & Van der Merwe, H.J., 1993. Relationships between chemical components of veld herbage and *in vitro* digestible dry matter by sheep and cattle. *Anim. Feed Sci. Tech.* 43:151-163.

Clark, D.H., Cary, E.E. & Mayland, H.F., 1989. Analysis of trace elements in forages by near infrared reflectance spectroscopy. *Agron. J.* 81:91-95.

Clark, D.H., Mayland, H.F., Lamb, R.C., 1987. Mineral analysis of forages with near IR reflectance spectroscopy. *Agron. J.* 79:485-490.

Coates, D.B., 2002. Is near infrared spectroscopy only as good as the laboratory reference values? An empirical approach. *Spectrosc. Eur.* 14(4):24-26.

Coblentz, W.K., Fritz, J.O., Fick, W.H., Crochran, R.C. & Shirley, J.E., 1998. *In situ* dry matter, nitrogen, and fibre digestion of Alfalfa, Red clover, and Eastern Gamagrass at four maturities. *J. Dairy Sci.* 81:150-161.

Coblentz, W.K., Jennings, J.A. & Coffey, K.P., 2004. Biology and effects of spontaneous heating in hay. In Proc: 34th California Alfalfa Symposium, 13-15 Dec., San Diego, CA.

Coleman, S.W. & Murray, I., 1993. The use of near infrared reflectance spectroscopy to define nutrients digestion of hay by cattle. *Anim Feed Sci. Technol.* 44:237-249.

Collins, M., 1988. Composition of fibre digestion in morphological components of an alfalfa-timothy sward. *Anim. Feed Sci. Technol.* 19:135-143.

Combs, D.K., Beyer-Neumann, E.P., Rodriques, M.T., Undersander, D.J. & Hoffman, P.C., 1998. Current research in forage utilisation at the UW-Dairy Science Department. Departments of Dairy Science and Agronomy, University of Wisconsin-Madison.

Cone, J.W., Cline-Theil, W., Malestein, A. & Van't Klooster, A.T., 1989. Degradation of starch by incubation with rumen fluid. A comparison of different starch sources. *J. Sci. Food Agric.* 49:173-183.

Conrad, H.R., Weiss, W.P., Odwongo, W.O. & Shockey, W.L., 1984. Estimating net energy lactation from components of cell solubles and cell walls. *J. Dairy Sci.* 67:427-436.

Coppock., C.E., 1997. Balancing rations for forage quality. Coppock nutritional services. Dellwood, Leredo. TX, USA.

Cozzolino, D. & Moron, A., 2004. Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes. *Anim Feed Sci. Tech.* 111:161-173.

Crampton, E.W., Donefer, E. & Lloyd, L.E., 1960. A nutritive value index for forages. *J. Anim. Sci.* 19:538.

Cruywagen, C.W., 2001. Latest developments in measuring and monitoring feed quality for high-producing ruminants. Department of Animal Sciences. University of Stellenbosch, Stellenbosch.

Currier, C.G., Malton, B.A., Arledge, J.S. & Marble, V.L., 1984. Sampling alfalfa hay. In Proc: National alfalfa hay quality testing workshop, Chicago, IL. pp. 8-16.

Daccord, R., Arrigo, Y. & Vogel, R., 1996. Nutritional value of maize silage. *Rev. Suisse Agric.* 28:17.

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

Davies, A.M.C. & Grant, A., 1987. Air-conditioning-generated noise in a near-infrared spectrometer caused by fluctuations in atmospheric water-vapour. *Appl. Spectrosc.* 41:1248-1250.

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De Boever, J.L., Cottyn, B.G., De Brabander, D.L., Vanacker, J.M. & Boucque, Ch. V., 1996. Prediction of the feeding value of grass silages by chemical parameters, *in vitro* digestibility and near-infrared reflectance spectroscopy. *Anim. Feed Sci. Technol.* 60:103-111.

De Boever, J.L., Cottyn, B.G., Bruysse, F.X., Wainman, F.W. & Vanaker, J.M., 1986. The use of an enzymatic technique to predict digestibility, metabolisable and net energy of compound feedstuffs for ruminants. *Anim. Feed Sci. Technol.* 14:203-214.

De Boever, J.L., Cottyn, B.G., Vanacker, J.M. & Boucque, Ch. V., 1995. The use of NIRS to predict the chemical composition and the energy value of compound feeds for cattle. *Anim. Feed Sci. Technol.* 51:243-253.

Deaville, E.R. & Flinn, P.C., 2000. Near infrared (NIR) spectroscopy: an alternative approach for the estimation of forage quality and voluntary intake. Eds. Givens, D.I., Owen, E., Axford, R.F.E. & Omedi, H.M., CAB International, Reading, UK.

Dowman, M.G. & Collins, F.C., 1982. The use of enzymes to predict the digestibility of animal feeds. *J. Sci. Food Agric.* 33:689-696.

Dryden, G. McL., 2003. Near infrared reflectance spectroscopy: Applications in deer nutrition. Publication No. W03/007, School of Animal Studies, Queensland, Australia.

Dulphy, J.P. & Demarquilly, C., 1983. Voluntary feed consumption as an attribute of feeds. In: Feed Information and Animal Production. Eds. Robards, G.E. & Packhampp, R.G. Commonwealth Agricultural Bureaux, Slough. pp. 135-156.

Eastridge, M.L., 2002. Energy in the new dairy NRC. *Feedstuffs* p.11.

Edney, M.J., Morgan, J.E., Williams, P.C. & Campbell, L.D., 1994. Analysis of feed barley by near infrared reflectance spectroscopy. *J. Near Infrared Spectrosc.* 2:33-41.

Elizalde, J.C., Merchen, N.R. & Faulkner, D.B., 1999. Fractionation of fibre and crude protein in fresh forages during the spring growth. *J. Anim. Sci.* 77:476-484.

Ellis, W.C., Wylie, M.J. & Matis, J.H., 1988. Dietary-digestive interactions determining the feeding value of forages and roughages. In: Feed Science. Eds. Orskov, E.R., pp. 177-225. Elsevier, Amsterdam

Erasmus, L.J., 2000. Associative effects in dairy feeds – are they real. Part 2. *Afma Matrix.* 9(2):17-23.

Erasmus, L.J., Prinsloo, J., Botha, P.M. & Meissner, H.H., 1990. Establishment of a ruminal protein degradation database for dairy cattle using the *in situ* polyester bag technique. 3. Roughages. *S. Afr. J. Anim. Sci.* 20:124-134.

Felton, E.E.D. & Kerley, M.S., 2002. Intrinsic factors affecting forage intake and digestibility: Evaluation of current and future methods of determination. In Proc: NFTA Workshop, 11 July, Demoines, IA.

Fisher, D.S., Mayland, H.F. & Burns, J.C., 2002. Variation in ruminant preference for alfalfa hays cut at sunup and sundown. *Crop Sci.* 42:231-237.

Fox, D.G., Barry, M.C., Pitt, R.E., Roseler, D.K. & Stone, W.C., 1995. Application of the Cornell Net Carbohydrate and Protein model for cattle consuming forages. *J. Anim. Sci.* 73:267.

Fox, D.G., Sniffen, C.J., O'Conner, J.D., Russel, J.B. & Van Soest, P.J., 1992. A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. *J. Anim. Sci.* 75:3578-3596.

Fox, D.G. & Tedeschi, L.O., 2002. Application of physically effective fibre in diets for feedlot cattle. Animal Science Department, Cornell University Ithaca, NY.

Fox, D.G., Tedeschi, L.O., Tylutki, T.P., Russel, J.B., Van Amburgh, M.E., Chase, L.E., Pell, A.N. & Overton, T.R., 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Anim. Feed Sci. and Technol.* 112:29-78.

Fox, D.G., Tylutki, T.P., Tedeschi, L.O., Van Amburgh, M.E., Chase, L.E., Pell, A.N., Overton, T.R. & Russel, J.B., 2003. The Net Carbohydrate and Protein System for evaluating herd nutrition and nutrient excretion: model documentation. Mimeo No. 213, Animal Science Dept., Cornell University, Ithaca, NY.

Fox, D.G., Tylutki, T.P., Van Amburgh, M.E., Chase, L.E., Pell, A.N., Overton, T.R., Tedeschi, L.O., Rasmussen, C.N. & Durbal, V.M., 2000. The Net Carbohydrate and Protein System for evaluating herd nutrition and nutrient excretion: Model documentation. Mimeo No. 213. Animal Science Dept., Cornell University, Ithaca, NY.

Garcia, J. & Cozzolino, D., 2006. Use of near infrared reflectance (NIR) spectroscopy to predict chemical composition of forages in broad-based calibration models. *Agricultura Tecnica (Chile). 66(1):41-47.*

Garcia, A. & Kalscheur, K., 2005. Particle size and effective fibre in dairy cow diets. College of Agriculture & Biological science. South Dakota State University.

Garcia, A.D., Olson, W.G., Otterby, D.E., Linn, J.G. & Hansen, W.P., 1989. Effects of temperature, moisture, and aeration on fermentation of alfalfa silage. *J. Dairy Sci.* 72, 93-103.

Girard, V. & Dupuis, G., 1988. Effect of structural and chemical factors of forages on potentially digestible fibre, intake, and true digestibility by ruminants. *Can. J. Anim. Sci.* 68:787-799.

Givens, D.I., 1993. The role of NIRS for forage evaluation - the present and future. In Proc: NIR Spectroscopy: Developments in Agriculture and Food Int. Conf. 12 Oct., Birmingham. ADAS Drayton Research Centre. pp. 68.

Givens, D.I., Baker, C.W. & Zamime, B., 1992a. Regions for normalised near infrared reflectance difference spectra related to the rumen digestion of straws. *Anim. Feed Sci. Technol.* 36:1-12.

Givens, D.I. & Deaville, E.R., 1999. The current and future role of near infrared reflectance spectroscopy in animal nutrition: a review. *Aus. J. Agric. Res.* 50:1131-1145.

Givens, D.I., De Boever, J.L. & Deaville, E.R., 1997. The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans. *Nutrition Research Reviews.* 10:83-114.

Givens, D.I., Everington, J.M. & Adamson, A.H., 1992b. The chemical composition and energy value of high temperature dried grass produced in England. *Anim. Feed Sci. Technol.* 36:215-518.

Goering, H.K., Gordon, C.H., Hemken, R.W., Waldo, D.R., Van Soest, P.J. & Smith, L.W., 1972. Analytical estimates of nitrogen digestibility in heat damaged forages. *J. Dairy Sci.* 55:1275.

Goering, H.K. & Van Soest, P.J., 1970. Forage fibre analyses (Apparatus, reagents, procedures and some applications). Agricultural Handbook No. 379. A.R.S., U.S. Dept. of Agric.

Goff, J.P., 1999. Physiologic factors to consider in order to prevent periparturient disease in the dairy cow, with special emphasis on milk fever. In Proc: 3rd Western Dairy Management conference. pp.99-112.

Goff, J.P., 2000. Pathophysiology of calcium and phosphorus disorders. *Vet. Clin. North Am. Food Anim. Pract.* 16:319-337.

Goff, J.P. & Horst, R.L., 1997. Effects of the addition of potassium or sodium but not calcium to prepartum rations on milk fever in dairy cows. *J. Dairy Sci.* 80:176-186.

Goings, R.L., Jacobson, N.L., Beitz, D.C., Littledike, E.T. & Wiggers, K.D., 1974. Prevention of parturient paresis by a prepartum calcium deficient diet. *J. Dairy Sci.* 57:1184-1188.

Gordon, C.H., Decker, A.M., Ewiseman, H.G., 1962. Some effects of nitrogen fertiliser, maturity and light on the composition of orchard grass. *Agron. J.* 54:376.

Grant, R., 1994. Feeding and Nutrition. University of Nebraska-Lincoln, Institute of Agriculture and Natural Resources, Coop. Ext. Serv., USA.

Grant, R.J., 1997. Interactions among forage and nonforage sources of fibre. *J. Dairy Sci.* 80:1438-1446.

Grant, R.J., 2001. Formulating dairy rations for fibre and starch to optimise animal health and productivity. Department of Animal Science, Univ. of Nebraska.

Grant, R.J., Colenbrander, V.F. & Mertens, D.R., 1990. Milk fat depression in dairy cows: role of particle size of alfalfa hay. *J. Dairy Sci.* 73:1823.

Green, H.B., Horst, R.L., Beitz, D.C. & Littledike, E.T., 1981. Vitamin D metabolites in plasma of cows fed a prepartum low- calcium diet for prevention of parturient hypocalcemia. *J. Dairy Sci.* 64:217-226.

Griffin, T.S., Cassida, K.A., Hesterman, O.B. & Rust, S.R., 1994. Alfalfa maturity and cultivar effects on chemical and *in situ* estimates of protein degradability. *Crop Sci.* 34:1654-1661.

Griggs, T.C., Lobos, K.B. & Kingery, P.E., 1999. Digestibility analysis of undried, unground, and dry ground herbage by near-infrared reflectance spectroscopy. *Crop Sci.* 39:1164-1170.

Groenewald, T. & Köster, H., 2005. Near infrared (NIR) spectroscopy - The rapid analyses technique of the future. *Afma Matrix* 27:18-27.

Grönum, C.F., Van Schalkwyk, H.D., Jooste, A., Louw, D.B., Du Plessis, J.H., Geldenhuys, F.I. & Geldenhuys, J., 2000. Lucerne production in South Africa. Chair in International Agricultural Marketing & Development, UFS, Bloemfontein.

Hall, M.B., 1994. Pectin: The structural non-structural carbohydrate. In Proc: Cornell Nutr. Conf. For Feed Manuf., Ithaca, NY. pp. 1-18.

Hall, M.B., 1998. Making nutritional sense of metabolisable carbohydrates. In Proc: 9th Ann. Florida ruminant nutr. Symp. Gainesville, FL. pp. 108-121.

Hall, M.B., 2000. Neutral detergent soluble carbohydrates - Nutritional Relevance and Analysis. Univ. of Florida, bulletin number 339.

Hall, M.B., 2002. Working with non-NDF carbohydrates with manure evaluation and environmental considerations. Department of Animal Sciences, University of Florida, Gainesville.

Hall, M.B., 2005. Beyond RFV/Q: What are you missing with your hay quality tests? In Proc: California Alfalfa and Forage Symposium, 12-14 Dec., Visalia, CA, University of California, Davis.

Hall, M.B. & Herejk, C., 2001. Differences in yields of microbial crude protein from *in vitro* fermentation of carbohydrates. *J. Dairy Sci.* 84:2486-2493.

Hannaway, D.B. & Ballerstedt, P.J., 1988. Testing the quality of alfalfa hay. Extensions Service. PNW 223. Oregon State University, USA.

Hannaway, D.B., Roldn-Modr k, E., DelCurto, T. & Ballerstedt, P.J., 1992. Calibration of near infrared reflectance spectroscopy (NIRS) for determination of forage quality of oregon-grown grass seed straw.

Hanson, C.H., 1972. Alfalfa science and technology. Agronomy series nr. 15. American Society of Agronomy. Madison Wisconsin, USA.

Hanson, A.A., Barnes, D.K. & Hill, R.R., 1988. Alfalfa and alfalfa improvement. Academic Press, Inc., 111 Fifth Avenue, NY.

Harlan, D.W., Holter, J.B. & Hayes, H.H., 1991. Detergent fibre traits to predict productive energy of forages fed free choice to non-lactating dairy cattle. *J. Dairy Sci.* 74:1337-1353.

Hartnell, G.F., Hatfield, R.D., Mertens, D.R. & Martin, N.P., 2005. Potential benefits of plant modification of alfalfa and corn silage to dairy diets. In Proc: Southwest Nutr. Conf. pp. 156-172.

Hatfield, R.D., Jung, H.G., Ralph, J., Buxton, D.R. & Weimer, P.J., 1994. A comparison of the insoluble residues produced by the Klason lignin and acid detergent lignin procedures. *J. Sci. Food Agric.* 65:51-58.

Hatfield, R.D., Ralph, J. & Grabber, J.H., 1999. Cell wall structural foundations: Molecular basis for improving forage digestibilities. *Crop Sci.* 39 (1):27-37.

Hatfield, R.D. & Weimer, P.J., 1995. Degradation characteristics of isolated and *in situ* cell wall lucerne pectic polysaccharides by mixed ruminal microbes. *J. Sci. Food Agric.* 69:185-196.

Heldt, J.S., Cochran, R.C., Stokka, G.L., Farmer, C.G., Mathis, C.P., Titgemeyer, E.C. & Nagaraja, T.G., 1999. Effects of different supplemental sugars and starch fed in

combination with degradable intake protein on low-quality forage use in beef steers. *J. Anim. Sci.* 77:2793-2802.

Herrero, M., Murray, I., Fawcett, R.H. & Dent, J.B., 1996. Prediction of the *in vitro* gas production and chemical composition of kikuyu grass by near-infrared reflectance spectroscopy. *Anim. Feed Sci. Technol* 60:51-67.

Hindrichsen, I.K., Kreuzer, M., Madsen, J. & Bach Knudsen, K.E., 2006. Fibre and lignin analysis in concentrate, forage, and feces: Detergent versus enzymatic-chemical method. *J. Dairy Sci.* 89:2168-2176.

Hoffman, P.C., 2003. New developments in analytical evaluation of forages and total mixed rations. Department of Dairy Science, University of Wisconsin-Madison.

Hoffman, P.C., 2004. Impact of NDF digestibility on energy content of forages and dairy rations. Department of Dairy Science, University of Wisconsin-Madison.

Hoffman, P.C. & Bauman, L.M., 2003. Strategies to improve milk yield of lactating dairy cows fed red clover silage. *Prof. Anim. Sci.* 19:178-187.

Hoffman, P.C., Brehm, N.M., Hasler, J.J., Bauman, L.M., Peters, J.B., Combs, D.K., Shaver, R.D. & Undersander, D.J., 1999. Development of a novel system to estimate protein degradability in legume and grass silages. *J. Dairy Sci.* 82:771-779.

Hoffman, P.C. & Combs, D., 2004. Using NDF digestibility in ration formulation. Focus on Forage. Vol. 6: No. 3.

Hoffman, P.C., Combs, D.K. & Casler, M.D., 1998. Performance of lactating dairy cows fed alfalfa silage or perennial ryegrass silage. *J. Dairy Sci.* 81:162-168.

Hoffman, P.C., Lundberg, K.M., Bauman, L.M. & Shaver, R.D., 2003. *In vitro* NDF Digestibility of forages: The 30 vs. 48 hour debate. Focus on Forage. Vol. 5: No. 16.

Hoffman, P.C., Shaver, R.D., Combs, D.K., Undersander, D.J., Bauman, L.M. & Seeger, T.K., 2001. Understanding NDF digestibility of forages. Focus on Forage, Vol. 3: No. 10.

Hoffman, P.C., Sievert, S.J., Shaver, R.D., Welch, D.A. & Combs, D.K., 1993. *In situ* dry matter, protein, and fibre degradation of perennial forages. *J. Dairy Sci.* 76:2632-2643.

Holechek, J.L., Shenk, J.S., Vavra, M. & Arthun, D., 1982. Prediction of forage quality using near infrared reflectance spectroscopy on esophageal fistula samples from cattle on mountain range. *J. Anim. Sci.* 55:971-975.

Holt, D.A. & Hilst, A.R., 1969. Daily variation in carbohydrate content of selected forage crops. *Agron. J.* 61:239-242.

Holter, J.B., West, J.W. & McGilliard, M.L., 1997. Predicting *ad libitum* dry matter intake and yield of Holstein cows. *J. Dairy Sci.* 80:2188-2199.

Hovell, F.D.De B., Ngambi, J.W.W., Barber, W.P. & Kyle, D.J., 1986. The voluntary intake of hay by sheep in relation to its degradability in the rumen as measured in nylon bags. *Anim. Production* 42:111-118.

Hristov, A.N., Price, W.J. & Shafii, B., 2004. A meta-analysis examining the relationship among dietary factors, dry matter intake, and milk yield and milk protein yield in dairy cows. *J. Dairy Sci.* 87:2184-2196.

Hristov, A.N., Price, W.J. & Shafii, B., 2005. A meta-analysis on the relationship between intake of nutrients and body weight with milk volume and milk protein yield in dairy cows. *J. Dairy Sci.* 88:2860-2869.

Hruschka, W.R., 1987. Data analysis: wavelength selection methods. In: Near-infrared technology in the agricultural and food industries. Eds. Williams, P. & Norris, K., St. Paul, MN. American Association of Cereal Chemists Inc., pp. 35-55.

Hsu, H., McNeil, A., Okine, E.K., Mathison, G.W. & Soofi-Siawash, R., 1998. NIRS for measuring *in situ* degradability in barley forages. *J. Near Infrared Spec.* 6:129-143.

Hunt, W.H. & Pixton, S.W., 1974. Storage of cereal grains and their products. American Association of Cereal Chemists, St. Paul, MN. pp. 1-55.

Hutjens, M.F., 1995. Time for TFI. Dairy Today. 10(2), 20.

Hutjens, M.F., 1998. Purchasing and valuing feed. In: Hoards Dairyman Feeding guide. pp. 50-53.

Hvelplund, T., Weisbjerg, M.R. & Søegaard, K., 1997. Use of *in vitro* digestibility methods to estimate *in vivo* digestibility of straws. Danish Institute of Agricultural Sciences, Research Centre Foulum. Tjele, Denmark.

Illius, A.W. & Jessop, N.S., 1996. Metabolic constraints on voluntary intake in ruminants. *J. Anim. Sci.* 74:3052-3062.

INRA, 1978. Alimentation des ruminants. INRA Publication, Versailles.

INRA, 1981. Prevision de la Valeur Nutritive des Aliments des ruminants. INRA Publication, Versailles.

INRA, 1988. Alimentation des Bovins, Ovins et Caprins. INRA, Paris.

Ivan, S.K., Grant, R.J., Weakley, D. & Beck, J., 2005. Comparison of a corn silage hybrid with high cell-wall content and digestibility with a hybrid of lower cell-wall content on performance of Holstein cows. *J. Dairy Sci.* 88:244-254.

Jagusch, K.T., Clarke, V.R. & Jay, N.P., 1970. Lamb production from animals weaned at 3 to 5 weeks of age on to lucerne. *N.Z.Jl.. Agric.Res.* 13:808-814.

Janicki, F.J. & Stallings, C.C., 1988. Degradation of crude protein in forages determined by *in vitro* and *in situ* procedures. *J. Dairy Sci.* 71:2440.

Jeffries, T.W., 1990. Biodegradation of lignin-carbohydrate complexes. Biodegradation 1. pp. 163-176.

Jung, H.G. & Engels, F.M., 2002. Alfalfa stem tissues: cell wall deposition, composition, and degradability. *Crop Sci.* 42:524-534.

Jung, H.G. & Lamb, J.F.S., 2004. Prediction of cell wall polysaccharide and lignin concentrations of alfalfa stems from detergent fibre analysis. *Biomass and Bioenergy* 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.

Jung, H.G., Varel, V.H., Weimer, P.J. & Ralph, J., 1999. Accuracy of Klason lignin and acid detergent lignin methods as assessed by bomb calorimetry. *J. Agric Food Chemistry* 47:2005-2008.

Jung, H.G. & Vogel, K.P., 1986. Influence of lignin on digestibility of forage cell wall material. *J. Anim. Sci.* 62:1703-1712.

Kalu, B.A. & Fick, G.W., 1981. Quantifying morphological development of alfalfa for studies of herbage quality. *Crop Sci.* 21:267-272.

Kalu, B.A. & Fick, G.W., 1983. Morphological stage of development as a predictor of alfalfa herbage quality. *Crop Sci.* 6:1167-1172.

Kelling, K., Peters, J., Rankin, M. & Undersander, D., 2002. Potassium in forages. Focus on Forage, Vol 4: No. 4.

Kirilov, A., 2002. Lucerne quality and possibilities for its estimation. Institute of Forage Crops, Gen. Pleven, Bulgaria.

Klinkowski, M. 1933. Lucerne: its ecological position and distribution in the world. Bull. Imp. Bur. Pl. Genet. Herb. Pl., 12. pp. 61

Knowlton, K.F., Pitt, R.E. & Fox, D.G., 1992. Dynamic model prediction of the value of reduced solubility of alfalfa silage protein for lactating dairy cows. *J. Dairy Sci.* 75:1507-1516.

Knowlton, K.F., Pitt, R.E. & Fox, D.G., 1993. Model-predicted value of enzyme-treated alfalfa silage for lactating dairy cows. *J. Prod. Agric.*, 6:280-286.

Kolver, E.S., Muller, L.D., Barry, M.C. & Penno, J.W., 1998. Evaluation and application of the Cornell net carbohydrate and protein system for dairy cows fed diets based on pasture. *J. Dairy Sci.* 81:2029-2039.

Krachunov, I., 1998. Estimation on near infrared reflectance spectroscopy (NIRS) for estimation of lucerne energy nutritive value for ruminants. *Anim. Sci. (Sofia)* 35(2):36-41.

Krishnamoorthy, U., Muscato, T.V., Sniffen, C.J. & Van Soest, P.J., 1982. Nitrogen fractions in selected feedstuffs. *J. Dairy Sci.* 65:217-225.

Krishnamoorthy, U.C., Sniffen, C.J., Stern, M.D. & Van Soest, P.J., 1983. Evaluation of a mathematical model of digesta and *in vitro* simulation of rumen proteolysis to estimate the rumen undegraded nitrogen content of feedstuffs. *Br. J. Nutr.* 50:555.

Lacefield, G.D., 2004. Alfalfa Quality: What is it? What can we do about it? And, will it pay? In Proc: National Alfalfa Symposium, San Diego, CA.

Landau, S., Nitzan, R., Barkai, D. & Dvash, L., 2006. Excretal near infrared reflectance spectrometry to monitor the nutrient content of diets of grazing young ostriches (*Struthio camelus*). *S. Afric. J. Anim. Sci.* 36(4):248-256.

Lanyon, L.E., 1980. Pennsylvania alfalfa growers program alfalfa mineral relationships. In Proc: Annual conf. Of Penn. Forage and Grasslands Council, 24-25 Nov. pp. 46-52.

Lauriault, L.M., Ball, S.T., Flynn, R.P., Glover, C.R., Gomez, B. & Ray, I.M., 1998. Sampling Guidelines for Hay Testing. Guide A-328, College of Agriculture and Home Economics, New Mexico State University.

Lean, I.J., DeGaris, P.J., McNeil, D.M. & Block, E., 2006. Hypocalcemia in dairy cows: Meta-analysis and dietary cation anion difference theory revisited. *J. Dairy Sci.* 89:669-684.

Licitra, G., Hernandezb, T.M. & Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.* 57:347-358.

Linn, J.G., 1992. Coping with changing feed quality. In: Large dairy herd management. Eds. Van Horn, H.H. & Wilcox, C.J., American Dairy Science Association. Champaign, IL. pp. 318-325.

Linn, J.G., 2003. Energy in the 2001 dairy NRC: Understanding the System. In Proc: Minnesota Dairy Health Conf., College of Veterinary Medicine, May 2003. pp. 102-110.

Linn, J.G., & Martin, N.P., 1999. Forage quality tests and interpretations. College of Agricultural, Food and Environmental Sciences. University of Minnesota, USA.

Lippke, H. & Herd, D.B., 1990. Foragval: Intake and gain estimated from acid detergent fibre and crude protein of the forage diet. *Tex. Agric. Expt. Sta. MP*-1708.

Llamas-Lamas, G. & Combs, D.K., 1990. Effect of alfalfa maturity on fibre utilisations by high producing dairy cows. *J. Dairy Sci.* 73:1069-1080.

Lofgren, P.A. & Warner, R.G., 1970. Influence of various fibre sources and fractions on milk fat percentage. *J. Dairy Sci.* 53:296.

Lyons, R.K. & Stuth, J.W., 1992. Fecal NIRS equations for predicting diet quality of free-ranging cattle. *J. Range Manage.* 45:238-244.

MacRae, J.C. & Armstrong, D.G., 1969. Studies on intestinal digestion in the sheep. 2. Digestion of some carbohydrate constituents in cereal and hay-cereal. *Br. J. Nutr.* 23:377.

Makoni, N.F., Broderick, G.A. & Muck, R.E., 1997. Effect of modified atmospheres on proteolysis and fermentation of ensiled alfalfa. *J. Dairy Sci.* 80:912-920.

Malley, D.F., Yesmin, L., Wray, D. & Edwards, S., 1999. Application of near infrared spectroscopy in analysis of soil mineral nutrients. *Comm. Soil Sci. Plant Anal.* 30:999-1012.

Mansfield, S.D., Mooney, C. & Saddler, J.N., 1999. Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnology Progress* 15:804-816.

Mark, H., Ritchie, G.E., Roller, R.W., Ciurszak, E.W., Tso, C. & MacDonald, S.A., 2002. Validation of a near-infrared transmission spectroscopic procedure, part A: validation protocols. *J. Pharmaceutical and Biomedical Analysis.* 28:251-260.

Marten, G.C., Brink, G.E., Buxton, D.R. Halgerson, J.L. & Hornstein, J.S., 1984. Near infrared reflectance spectroscopy analysis of forage quality in four legume species. *Crop Sci.* 24:1179-1182.

Martin, N.P., Ellingboe, R.L., Peterson, P.R., Matteson, M., Stucker, R.E. & Linn, J.G., 1992. Sampling evaluation of alfalfa hay. In Proc: Am. Forage Grassl. Conf. pp. 1:66-71.

Martin, N.P. & Mertens, D.R., 2005. Reinventing alfalfa for dairy cattle and novel uses. In Proc: California Alfalfa and Forage Symposium, 12-14 December, Visalia, CA.

Marum, A.H. & Aastveit, P., 1990. The precision of the Tilley and Terry method compared to the NIRS method in estimating digestibility in breeding programs. In Proc: 3rd Intern. Conf. On Near Infrared Spectroscopy. pp. 566-569.

Mathews, M.C. & Putnam, D.H., 1998. The 'California recognised' program and California hay guidelines. In Proc: 28th California Alfalfa Symposium, 3-4 Dec., Reno, NV.

Mathison, G.W., 1990. New methods of forage evaluation. In Proc: 11th Western Nutr. Conf., Sept. 13-12, University of Alberta, Edmonton. pp. 59-69.

Mathison, G.W., Hsu, H., Soofi-Siawash, R., Recinos-Diaz, G., Okine, E.K., Helm, J. & Juskiw, P., 1999a. Prediction of composition and ruminal degradability characteristics of barley straw by near infrared reflectance spectroscopy. *Can. J. Anim. Sci.* 79:519-523.

Mathison, G.W., Soofi-Siawash, R., Okine, E.K., Helm, J. & Juskiw, P., 1999b. Factors influencing composition and ruminal degradability of barley straw. *Can. J. Anim. Sci.* 97:343-351.

Mayland, H., Mertens, D., Taylor, B., Burns, J., Fisher, D., Gregorini, P., Ciavarella, T., Smith, K., Shewmaker, G. & Griggs, T., 2005. Diurnal changes in forage quality and their effects on animal preference, intake, and performance. In Proc: California Alfalfa Symposium, 12-14 Dec., Visalia, CA, University of California, Davis.

McBeth, L.J., Coffey, K.P., Coblentz, W.K., Turner, J.E., Scarbrough, D.A., Bailey, C.R. & Stivarius, M.R., 2001. Impact of heating-degree-day accumulation during bermudagrass hay storage on nutrient utilization by lambs. *J. Anim. Sci.* 79:2698-2703.

McBurney, M.I., Van Soest, P.J. & Chase, L.E., 1983. Cation exchange capacity and buffering capacity of neutral detergent fibres. *J. Sci. Food Agric.* 34:910-916.

McCullough, M.E., 1994. Total Mixed Rations and supercows. 2nd ed. Hoards Dairyman. W.D. Hoard & Sons Co. Wisconsin, USA.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 1995. Animal nutrition, 5th ed., Longman. Singapore.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 2002. Animal nutrition, 6th ed., Longman. Singapore.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. & Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro. J. Agric. Sci. (Camb.)* 93:217.

Menke, K.H. & Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Research Development.* 28:7-55.

Mertens, D.R., 1973. Application of theoretical mathematical models to cell_wall digestion and forage intake in ruminants. Ph.D. Dissertation, Cornell University, Ithaca, NY.

Mertens, D.R., 1979. Adjusting heat-damaged protein to a crude protein basis. *J. Anim. Sci.* 42:259.

Mertens, D.R., 1980. Fibre content and nutrient density in dairy rations. In Proc: Dist. Feed Conf., Cincinnati, OH. p. 35.

Mertens, D.R., 1982. Using neutral detergent fibre to formulate dairy rations. In Proc: Ga. Nutr. Conf. For the feed industry. Athens, University of Georgia. pp. 116-126.

Mertens, D.R., 1986. Effect of physical characteristics, forage particle size and density on forage utilisation. In Proc: Nutrition Symp. Am. Feed Industry Assoc., St. Louis, MO. pp. 91.

Mertens, D.R., 1987. Predicting intake and digestibility using mathematical models of ruminal function. *J. Anim. Sci.* 64:1548-1558.

Mertens, D.R., 1988. Balancing carbohydrates in dairy rations. Mimeo No. 109. Animal Science Dept., Cornell University, Ithaca, NY.

Mertens, D.R., 1992. Nonstructural and Structural Carbohydrates. In: Large Dairy Herd Management. Eds. Van Horn, H.H. & Wilcox, C.J., American Dairy Science Association, Champaign, IL. pp. 219-235.

Mertens, D.R., 1994. Regulation of forage intake. In: Forage Quality, evaluation and utilisation. Eds. Fahey, G.C., Collins, M. & Mertens, D.R., ASA, CSSA and SSSA Madison, WI. pp. 450-493.

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

Mertens, D.R., 2002. Nutritional implications of fibre and carbohydrate characteristics of corn silage and alfalfa hay. In Proc: California animal nutrition conf., Fresno, CA. p. 94-107.

Mertens, D.R., 2003. Challenges in measuring insoluble dietary fibre. *J. Anim. Sci.* 81:3233-3249.

Michalet-Doreau, B. & Cerneau, P., 1991. Influence of foodstuff particle size on *in situ* degradation of nitrogen in the rumen. *Anim. Feed Sci. Technol.* 35:69-81.

Michaud, R., Lehman, W.F. & Rumbaugh 1988. World Distribution and Historical Development. In: Alfalfa And Alfalfa Improvement. Eds. Hanson, A.A., Barnes, D.K. & Hill, R.R, JR, American Society of Agronomy, Inc, Madison, Wisconsin, USA. pp. 25-91.

Miller, W.J., 1979. Dairy Cattle Feeding and Nutrition. Animal and Dairy Science Department. Academic Press Inc., NY.

Miller, W.J. & Oddoye, E.O.K., 1989. Prediction of voluntary intake of conserved forages by cattle and sheep from degradability characteristics determined using synthetic fibre bags in sheep. *Animal production* 48:637-638 (Abstr.)

Minson, D.J., 1982. Effect of chemical composition on feed digestibility and Metabolizable energy. *Nutr. Abstr. Rev. Ser.* B52(10):591-615.

Minson, D.J., 1990. Forage in ruminant nutrition. Academic Press, San Diego. pp. 483.

Moe, P.W., Reid, J.T. & Tyrrell, H.F., 1965. Effect of level of intake on digestibility of dietary energy by high-producing cows. *J. Dairy Sci.* 48:1053-1061.

Mooney, R., 2002. Kick some ash. Hay and forage grower, 1 Sept., Minneapolis, MN.

Moore, J.E., Brant, M.H., Kunkle, W.E. & Hopkins, D.I., 1999. Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance. *J. Anim. Sci.* 77(Suppl.2):122-135.

Moore, J.E., Brown, W.F. & Hall, M.B., 1998. Evaluation of equations for estimating total digestible nutrient concentrations in forage grasses. In Proc: Am. For. Grsld. Coun., Indianapolis, IN. AFGC, Georgetown, TX. pp. 117-121.

Moore, J.E., Burns, J.C. & Fisher, D.S., 1996. Multiple regression equations for predicting relative feed value of grass hays. In Proc: Am. For. Grsld. Coun., Vancouver, BC. AFGC, Georgetown, TX. pp. 135-139.

Moore, J.E. & Coleman, S.W., 2001. Forage intake, digestibility, NDF and ADF: How well are they related? In Proc: 13th Annual Florida Ruminant Nutrition Symposium, University of Florida, Gainsville. pp. 16-32.

Moore, J.E. & Kunkle, W.E., 1999. Evaluation of equations for estimating voluntary intake of forages and forage-based diets. *J. Anim. Sci.* (Suppl. 1)204.

Moore, J.E., Kunkle, W.E., Bjorndal, K.A., Sand, R.S., Chambliss, C.G. & Mislevy, P., 1984. Extension forage testing program utilising near infrared reflectance spectroscopy. In Proc: Am. For. Grsld. Coun., Houston, TX. AFGC, Georgetown, TX. pp. 41-52.

Moore, J.E., Kunkle, W.E. & Brown, W.F., 1991. Forage quality and the need for protein and energy supplements. In Proc: 40th Annual Florida Beef Cattle Short Course, May, 13, Gainesville, FL.

Moore, J.E. & Sollenberger, L.E., 2002. Forage Quality. Institute of Food and Agricultural Sciences. University of Florida.

Moore, J.E. & Undersander, D.J., 2002. Relative forage quality: An alternative to relative feed value and quality index. In Proc: 13th Annual Florida Ruminant Nutr. Symposium. University of Florida, Gainsville. pp. 16-32.

Morrison, F.B., 1961. Feeds and Feeding abridged. Vail-Ballou Press, Inc., Binghamton, NY.

Morrison, I.M., 1972. A semi-micro method for the determination of lignin and its use in predicting the digestibility of forage crops. *J. Sci. Food Agric.* 23:455-463.

Morrison, I.M., 1980. Hemicellulosic contamination of acid detergent residues and their replacement by cellulosic residues in cell wall analysis. *J. Sci. Food Agric.* 31:639-645.

Morse, D. & Sedivec, I.C., 1990. Know your forage. NDSU Ext. Serv. North Dakota State University, USA.

Moss, A.R. & Givens, D.I., 1990. Chemical composition and *in vitro* digestion to predict digestibility of field-cured and barn-cured grass hays. *Anim. Feed Sci. Technol.* 29:73-87.

Mould, F.L., 2003. Predicting feed quality - chemical analysis and *in vitro* evaluation. *Field Crop Research.* 84:31-44.

Murray, I. & Williams, P.C., 1987. Chemical principles of near-infrared technology. In: Near Infrared technology in the Agricultural and Food Industries. Eds. Williams, P. & Norris, K., American Association of Cereal Chemists, St. Paul, MN. pp. 17-34.

Murray, I., 1986. Near infrared reflectance analysis of forages. In: Recent advances in animal nutrition. Eds. Haresign, W. & Cole, D.J.A., Studies in the agricultural and food sciences, Butterworths, UK. pp. 141-156.

Murray, I., 1993. Forage analysis by near infrared spectroscopy. In: Sward measurement handbook. Eds. Davies, A., Baker, R.D., Grant, S.A. & Laidlaw, A.S., 2nd Ed. The British Grassland Society, Reading, UK.

Murray, I., Kridis, M.S., Barber, G.D., Givens, D.I. & Offer, N.W., 1987. Grass silage *in vivo* digestibility prediction by NIR. In: Forage quality analysis by near infrared reflectance spectroscopy. Eds. Paul, C., Commission of the European Communities, Brussels. pp. 46.

Neter, J. & Wasserman, W., 1974. Applied linear statistical models. Richard D. Irving, Inc., Homewood, IL.

NIRS 2, 1995. Routine analysis manual (NIRSystem User's Manual). NIRSystem, Infrasoft International, Port Matilda, USA.

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

Norris, K.H., Barnes, R.F., Moore, J.E. & Shenk, J.S., 1976. Predicting forage quality by near infrared reflectance spectroscopy. *J. Anim. Sci.* 43:889-897.

NRC, 1966. Nutrient requirements of domestic animals. Nutrient requirements of dairy cattle. No. 3. Washington, D.C.

NRC, 1984. Nutrient requirements of beef cattle. 6th rev. ed. National Academy Press, 2101 Constitution Ave., NW, Washington, DC 20418.

NRC, 1985. Nutrient requirements of sheep. National Academy Press, 2101 Constitution Ave., NW, Washington, DC 20418.

NRC. 1989. Nutrient requirements of dairy cattle. 6th rev. ed. National Academy Press. Washington. DC.

NRC, 1996. Nutrient requirements of beef cattle 7th rev. ed. National Academy Press. Washington. DC.

NRC, 2000. Nutrient requirements of Beef Cattle, 7th ed. (updated). National Academy Press, Washington, DC.

NRC, 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Academy Press, Washington, DC.

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: 3. Digestibility and microbial efficiency. *J. Dairy Sci.* 1350-1358.

O'Conner, J.D., Sniffen, C.J., Fox, D.G. & Chalupa, W., 1993. A net carbohydrate and protein system for evaluating cattle diets. IV. Predicting amino acid adequacy. *J. Anim. Sci.* 71:1298-1311.

Oetzel, G.R., 2000. Management of dry cows for the prevention of milk fever and other mineral disorders. *Vet. Clin. North Am. Food Anim. Pract.* 16:369-386.

O'Keeffe, M., Downey, G. & Brogan, J.C., 1987. The use of near infrared reflectance spectroscopy for predicting the quality of grass silage. *J. Sci. Food Agric.* 38:209-216.

Orloff, S.B. & Marble, V.L., 1997. Quality and quality testing. In: Intermountain alfalfa management. Eds. Orloff, S.B., Carlson, H.L. & Teuber, L.R., Division of Agriculture and Natural Resources Communication Services, Publication 6701, San Pablo Ave. Oakland, CA 94608-1239. pp. 117-126.

Osborne, B.G., Fearn, T. & Hindle, P.H., 1993. Practical near infrared spectroscopy with applications in food and beverage analysis, Longman Scientific and Technical, UK.

Palmquist, D.L., 1991. Influence of source and amount of dietary fat on digestibility in lactating cows. *J. Dairy Sci.* 74:1354-1360.

Paul, C., 1987. Effect of soil contamination on NIRS analysis of grass silage. In: Forage quality analysis by near infrared reflectance spectroscopy. Eds. Paul, C., Commission of the European Communities, Brussels. pp 151-171.

Park, R.S., Agnew, R.E., Gordon, F.J., Steen, R.W.J., 1998. The use of near infrared reflectance spectroscopy (NIRS) on undried samples of grass silage to predict chemical composition and digestibility parameters. *Anim. Feed Sci. Technol.* 72:155-167.

Parker, D.S., 2001. Amino acid nutrition in beef cattle. Product development manager. Novus Europe s.a/nv. Brussels.

Pell, A.N. & Schofield, P., 1993. Computerised monitoring of gas production to measure forage digestion *in vitro. J. Dairy Sci.* 76:1063.

Peterson, P.R., 2002. The relative forage quality (RFQ) index replaces RFV. CPM Short Course, Nov. 26. Extension forage specialist, Dept. of Agronomy & Plant Genetics, Univ. of Minnesota.

Pichard, D.G. & Van Soest, P.J., 1977. Protein solubility of ruminant feeds. In Proc: Cornell Nutr. Conf., Ithaca, NY. pp. 91.

Pichard, D.G., 1977. Forage nutritive value. Continuous and batch *in vitro* fermentations and nitrogen solubility. Ph.D. Dissertation. Cornell Univ., Ithaca, NY.

Piper, C.V., 1935. Forage Plants and their Culture. Macmillan Co., New York. pp 671.

Pitt, R.E., Van Kessel, J.S., Fox, D.G., Barry, M.C. & Van Soest, P.J., 1996. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. *J. Anim. Sci.* 74:226-244.

Polan, C.E., 1992. Protein and amino acids for lactating cows. In: Large Dairy Herd Management, Eds. Van Horn, H.H. & Wilcox, C.J., American Dairy Science Association. Champaign, IL. pp. 236-246.

Putnam, D.H., 1998. Recommended principles for proper hay sampling. University of California, Davis.

Putnam, D.H., 2000. Producing high quality alfalfa: Factors that influence alfalfa forage quality. In Proc: CA Plant and Soil Conference, 19-20 Jan., Stockton, CA.

Putnam, D.H., 2004. Forage quality testing and markets; where are we going? In Proc: 34th California Alfalfa Symposium, 13-15 Dec., San Diego, California.

Putnam, D.H., Lamb, C., Peterson, G., Orloff, S. & Kirby, D., 1997. 1995 Alfalfa cultivar forage quality trial result. Agronomy progress report No. 256. University of California, Davis.

Putnam, D.H. & Undersander, D., 2006. The future of alfalfa forage quality testing in hay markets. In Proc: 2006 Western Alfalfa Symposium, 11-13 Dec., Reno, NV.

Rayburn, E.B. & Fox, D.G., 1993. Variation in neutral detergent fibre intake of Holstein cows. *J. Dairy Sci.* 76:544-554

Raymond, W.F., 1969. The Nutritive value of Forage Crops. *Adv. Agron.* 21:1-108.

Rebole, A., Trevino, J., Caballero, R & Alzueta, C., 2001. Effect of maturity on the amino acid profiles of total and nitrogen fractions in common vetch forage. *J. Sci. Food Agric.* 81:455-461.

Recktenwald, E.B. & Van Amburgh, M.E., 2006. Examining nitrogen deficiencies in lactating dairy cattle using corn silage based diets. In Proc: Cornell. Nutr. Conf. Feed Manuf. New York State College of Agriculture & Life Science, Cornell University. pp. 205-217.

Redshaw, E.S., Mathison, G.W., Milligan, L.P. & Weisenburger, R.D., 1986. Near IR reflectance spectroscopy for predicting forage composition and voluntary consumption and digestibility in cattle and sheep. *Can. J. Anim. Sci.* 66:103-116.

Reeves III, J.B., 1988a. Near IR reflectance spectroscopic analysis of sodium chlorite-treated forages and other plant materials. *J. Dairy Sci.* 71:143-151.

Reeves III, J.B., 1988b. Chemical assays for fibre, lignin, and lignin components: interrelationships and near infrared reflectance spectroscopic analysis. *J. Dairy Sci.* 71: 2976-2985.

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

Reuss, S., 2001. Enhanced forage evaluation: NDF digestibility. UW-Extension, Marinette County.

Richards, C.J., Pedersen, J.F., Britton, R.A., Stock, R.A. & Krehbiel, C.R., 1995. *In vitro* starch disappearance procedure modifications. *Anim. Feed Sci. Technol.* 55:35-45.

Robert, P., Bertrand, D. & Demarquilly, C., 1986. Prediction of forage digestibility by principal component analysis of near infrared reflectance spectra. *Anim. Feed Sci. Technol.* 16:215-224.

Roberts, C.A., Stuth, J, & Finn, P.C., 2003. NIRS application in forages and feedstuffs. In: Near infra-spectroscopy in agriculture. Eds. Roberts, C.A. & Reeves, *J., Agron. Monogr.* 321. ASA, CSSA, and SSSA, Madison, WI.

Roberts, C.A., Stuth, J. & Finn, P.C., 2004. Analysis of forages and feedstuffs. In: Near infrared spectroscopy in agriculture. Eds. Roberts, C.A. & Workman, J. & Reeves, J.B., American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, USA. pp. 231-269.

Robinson, P.H., 1998. What are dairy nutritionists looking for in alfalfa hay? In Proc: 28th California alfalfa symposium, 3-4 Dec., Reno, NV.

Robinson, P.H., 1999. Neutral detergent fibre (NDF) and its role in alfalfa analysis. In Proc: 29th California alfalfa symposium, Fresno, CA. University of California, Davis. pp. 60-67.

Robinson, P.H., 2005. The changing role of forage fibre in dairy rations. In Proc: California alfalfa and forage symposium, 12-14 Dec., Visalia, CA.

Robinson, P.H., Campbell Mathews, M. & Fadel, J.G., 1999. Influence of storage time and temperature on *in vitro* digestion of neutral detergent fibre at 48 h, and comparison to 48 h *in sacco* neutral detergent fibre digestion. *Anim. Feed Sci. Technol.* 80:257-266.

Robinson, P.H. & De Peters, E.J., 2002. Why does the dollar value of alfalfa hay not continue to increase as its TDN increases? In Proc: 32nd California alfalfa symposium, 11-13 Dec., Reno, NV.

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

Robinson, P.H. & McQueen, R.E., 1992. Influence of rumen fermentable neutral detergent fibre levels on feed intake and milk production. J. Dairy Sci. 80:520-532.

Robinson, P.H., Putnam, D.J. & Mueller, S., 1998. Interpreting your forage test report. California Alfalfa & Forage Review. Alfalfa Workgroup, University of California, Vol. 1, Nr. 2.

Rohweder, D.A., Barnes, R.F. & Jorgensen, N.A., 1978. Proposed hay grading standards based on laboratory analyses for evaluating quality. J. Anim. Sci. 47:747-759.

Rohweder, D.A., Jorgensen, N. & Barnes, R.F., 1976. Using chemical analysis to provide guidelines in evaluating forages and establishing hay standards. Feedstuffs. 48:22.

Rominger, R.S.D., Smith, D. & Peterson, L.A., 1975. Yields and elemental composition of alfalfa plant parts in late bud under two fertility levels. Can. J. Plant Sci. 55:69-75.

Roseler, D.K., Fox, D.G., Pell, A.N. & Chase, L.E., 1997. Evaluation of alternative equations for prediction of intake for Holstein dairy cows. J. Dairy Sci. 80:864-877.

Ross, D.E. & Van Amburgh, M.E., 2003. Amino acid composition of ruminant feeds and feed fractions. J. Anim. Sci. 81(Suppl.1),217(Abstr.)

Ruano-Ramos, A., Garcia-Ciudad, A. & Garcia-Criado, B, 1999. Near infrared spectroscopy predictions of mineral content in botanical fractions form semi-arid grasslands. *Anim. Feed Sci. Techn.* 77:331-343.

Ruiz, R., Albrecht, G.L., Tedeschi, L.O., Jarvis, G., Russell, J.B. & Fox, D.G., 2001. Effect of monensin on the performance and nitrogen utilization of lactating dairy cows consuming fresh forage. *J. Dairy Sci.* 84:1717-1727.

Ruiz, T.M., Bernal, E., Staples, C.R., Sollenberger, L.E. & Gallaher, R.N., 1995. Effect of dietary neutral detergent fibre concentration and forage source on performance of lactating cows. *J. Dairy Sci.* 78:305-319.

Ruiz, R., Tedeschi, L.O., Marini, J.C., Fox, D.G., Pell, A.N., Jarvis, G. & Russel, J.B., 2002. The effect of a ruminal nitrogen (N) deficiency in dairy cows: evaluation of the Cornell net carbohydrate and protein system ruminal N deficiency adjustment. *J. Dairy Sci.* 85:2986-2999.

Russell, J., Murray, I. & Fraser, A.R., 1989. Near and mid-infrared studies of the cell wall structure of cereal straw in relation to its rumen degradability. In: Physico-chemical characterisation of plant residues for industrial and feed use. Eds. Chesson, A. & Orskov, E.R., Elsevier, London. pp. 13-24.

Russel, J.B., O'Conner, J.D., Fox, D.G., Van Soest, P.J. & Sniffen, C.J., 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70:3551-3561.

Russell, J.B. & Wilson, D.B., 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *J. Dairy Sci.* 79:1503-1509.

Sanderson, M.A., Hornstein, J.S. & Wedin, W.F., 1989. Alfalfa morphological stage and its relation to *in situ* digestion of detergent fibre fractions of stems. *Crop Sci.* 29:1315-1319.

Sanson, D.W. & Kercher, C.J., 1996. Validation of equations used to estimate relative feed value of alfalfa hay. *Prof. Anim. Sci.* 12:162-166.

Sarwar, M., Firkins, J.L. & Eastridge, M.L., 1992. Effects of varying forage and concentrate carbohydrates on nutrient digestibility and milk production by dairy cows. *J. Dairy Sci.* 75:1533-1542.

SAS, 2003. SAS 9.1.3 Service Pack 4, Cary NC: SAS Institute Inc.

Sauvant, D., Dulphy, J.P. & Michalet-Doreau, B., 1990. The concept of fibrosity index of ruminant feeds. *Productions Animales.* 3:309.

Scholtz, G.D.J., 2001. Models for lucerne quality grading. M.Sc. (Agric) Dissertation, UFS, Bloemfontein.

Schroeder, J.W., 2006. Forage nutrition for ruminants. Extension Service, North Dakota State University. AS-1250.

Selvendran, R.R., 1984. The plant cell wall as a source of dietary fibre: Chemistry and structure. *Am. J. Clin. Nutr.* 39:320-337.

Seo, S., Tedeschi, L.O., Schwab, C.G. & Fox, D.G., 2006. Development and evaluation of empirical equations to predict feed passage rate in cattle. *Anim. Feed Sci. Technol.* 128:67-83.

Sheaffer, C.C., Martin, N.P., Jewett, J.G., Halgerson, J., Moon, R.D. & Cuomo, G.R., 2000. Sampling requirements for forage quality characterization of rectangular hay bales. *Agron. J.* 92:64-68.

Shenk, J.S. & Westerhaus, M.O., 1985. Accuracy of NIRS instruments to analyse forage and grain. *Crop Sci.* 25:1120-1122.

Shenk, J.S. & Westerhaus, M.O., 1991. Population structuring of near infrared spectra and modified partial least square regression. *Crop Sci.* 31:1548-1555.

Shenk, J.S. & Westerhaus, M.O., 1993. Analysis of agriculture and food products by near infrared reflectance spectroscopy. Infrasoft International (ISI), Silver Spring, MD, USA.

Shenk, J.S. & Westerhaus, M.O., 1994. The application of near infrared reflectance spectroscopy (NIRS) to forage analysis. In: Forage quality, evaluation and utilisation. Eds. Fahey, G.C., Crop Science Society of America, Lincoln, Nebraska. pp. 406-449.

Shenk, J.S. & Westerhaus, M.O., 1996. Calibration the ISI way. In: near infrared spectroscopy: The future waves. Eds. Davies, A.M.C. & Williams, P. NIR Publications, West Sussex, UK. pp. 198-202.

Shenk, J.S., Westerhaus, M.O. & Hoover, M.R., 1979. Analysis of forages by infrared reflectance. *J. Dairy Sci.* 62:807-812.

Shenk, J.S., Westerhaus, M.O. & Templeton, W.C. Jnr., 1985. Calibration transfer between near infrared reflectance spectrophotometers. *Crop Sci.* 25:159-161.

Shewmaker, G.E. & Thaemert, R., 2004. Measuring moisture in hay. In Proc: National Alfalfa Symposium, 13-15 Dec., San Diego, CA. pp. 313-320.

Siciliano-Jones, J. & Murphy, M.R., 1991. Specific gravity of various feedstuffs as affected by particle size and *in vitro* fermentation. *J. Dairy Sci.* 74:896-901.

Smith, D., 1969. Influence of temperature on the yield and chemical composition of "Vernal" alfalfa at first flower. *Agron. J.* 61:470-472.

Smith, D., 1981. Removing and analysing carbohydrates from plant tissue. Wisconsin Agric. Exp. Stn. Rep. No. R2107, Madison, USA.

Smith, J.A., Grisso, R.D., Van Bargen, K. & Anderson, B., 1996. Management tips for round bale hay harvesting, moving and storage. Institute of Agriculture and Natural Resources, University of Nebraska, USA.

Smith, L.W. & Waldo, D.R., 1969. Method for sizing forage cell wall particles. *J. Dairy Sci.*, 52:2051.

Smith, W., 2000. New developments in dairy cattle nutrition. *Afma Matrix.* 9(4):16.

Smith, W.A., 1992. Recent advances in dairy cattle nutrition. Department of Animal Science, University of Stellenbosch, Stellenbosch.

Sniffen, C.J., O'Conner, J.D., Van Soest, P.J., Fox, D.G. & Russel, J.B., 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70:3562-3577.

Snyman, L.D. & Joubert, H.W., 1992. Prediction of the chemical composition and *in vitro* dry matter digestibility of a number of forages by near infrared reflectance spectroscopy. *S. Afr. J. Anim. Sci.* 23:20-23.

Staples, C.R., 1992. Forage Selection, Harvesting, Storing, and Feeding. In: Large Dairy Herd Management. Eds. Van Horn, H.H. & Wilcox, C.J., American Dairy Science Association. Champaign, IL. pp. 347-357.

Stern, M.D., Bach, A. & Calsamiglia, S., 1997. Alternative techniques for measuring nutrient digestion in ruminants. *J. Anim. Sci.* 75:2256-2276.

Stokes, S., 2002. The importance of forage quality for milk production and health. TAMU Research & Extension Center, A&M University, Texas.

Stolz, M.A., 1990. Provisional assessment of quality components in luceme (*Medicago sativa*) and white clover (*Trifolium repens*) using a near infrared reflectance spectrophotometer. *S. Afr. J. Plant* 7(2):105-112.

Stone, W.C., 1996. Applied topics in dairy cattle nutrition: 1. Soyhulls as either a forage of concentrate replacement in early lactation Holstein dairy cattle, 2. Evaluations of the Cornell net carbohydrate and protein system's metabolizable protein requirement as supply in Holstein dairy cattle, 3. *In vitro* effects of lipids on fermentation systems. Ph.D. Dissertation, Cornell University, Ithaca, NY.

St-Pierre, N.R. & Weiss, W.P., 2007. Application of analytical and statistical tools in the feed industry: Controlling diet variation. In proc: AFMA Forum, 6th Intern. Congr. for the Animal Feed Industry, 13-16 March, Sun City, South Africa.

Strobel, H.J. & Russell, J.B., 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. *J. Dairy Sci.* 69:2941-2947.

Stuth, J., Jama, A. & Tolleson, D., 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. *Field Crops Research* 84:45-56.

Stuth, J.W., Freer, M., Dove, M. & Lyons, H., 1999. Nutritional management for free-ranging livestock. In: Nutrition of herbivores. Eds. Jung, H., ASAC. pp. 696-751.

Sudweeks, E.M., Ely, E.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.

Sullivan, J.T., 1966. Studies of the hemicelluloses of forage plants. *J. Anim. Sci.* 25:83-86.

Taylor, R.W., 1997. How to judge hay quality visually. Agronomy Facts Series: AF-15, 16. Soil fertility and crop production. Department of Plant & Soil Sciences. University of Dolaware, USA.

Temme, D.G., Harvey, R.G., Fawcett, R.S. & Young, A.W., 1979. Effects of annual weed control on alfalfa forage quality. *Agron. J.* 71:51-54.

Theander, O., & Aman, P., 1980. Chemical composition of some forages and various residues from feeding value determinations. *J. Sci. Food Agric.* 31:31-37.

Thiex, N. & Richardson, C.R., 2003. Challenges in measuring moisture content of feeds. *J. Anim. Sci.* 81:3255-3266.

Thilsing-Hansen, T., Jorgensen, R.J. & Ostergaard, S., 2002. Milk fever control principles: A review. *Acta Vet. Scand.* 43:1-19.

Thomas, J.W., Yu, Y., Middleton, T. & Stallings, C., 1982. Estimations of protein damage. In: Protein requirements for cattle: Symposium, Oklahoma State University, Stillwater, OK., Eds. Owens, F.N. pp. 81-98.

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

Traxler, M.J., Fox, D.G., Van Soest, P.J., Pell, A.N., Lascano, C.E., Lanna, D.P.D., Moore, J.E., Lana, R.P., Velez, M. & Flores, A., 1998. Predicting forage indigestible NDF from lignin concentration. *J. Anim. Sci.* 76:1469-1480.

Tylutki, T.P., 2002. Improving herd nutrient management on dairy farms: 1) Individual cow milk production variance. 2) Developing a quality management program on a commercial dairy farm: a six sigma approach. 3) Variation in nutrient content of feeds on a commercial dairy farm. 4) Predicting phosphorus excretion by dairy cattle. 5) Incorporating risk in managing dairy cattle nutrition. Ph.D. Dissertation, Cornell Univ., Ithaca, NY.

Tylutki, T.P., 2007a. Study guide, AMTS.Cattle version 1.1.0.1, AMTS, LLC, 418 Davis RD, Cortland, NY. 13045, USA.

Tylutki, T.P., 2007b. Ruminants - Protein and Carbohydrate optimisation in ruminants. In Proc: AFMA Forum, 6th Intern. Congr. for the Animal Feed Industry, 13-16 March, Sun City, South Africa.

Tylutki, T.P., Fox, D.G., Durbal, V.M, Tedeschi, L.O., Russel, J.B., Van Amburgh, M.E., Overton, T.R., Chase, L.E. & Pell, A.N., 2007. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. *Anim. Feed Sci. Technol.*: (In press).

Tylutki, T.P., Fox, D.G. & McMahon, M., 2004. Implementation of nutrient management planning on a dairy farm. *Professional Anim. Scientist.* 20:58-65.

Undersander, D.J., 2002. Determination of digestible NDF. In Proc: NFTA Workshop, 11 July, Demoines, IA.

Undersander, D.J., Martin, N. & Mertens, D., 2005. What are the differences between forage testing laboratories and the impact on your operation? In Proc: 2005 Annual Conference volume 14 (cd-rom). American forage and grassland council, p.5.

Undersander, D., Mertens, D.R. & Thiex, N., 1993. Forage analyses procedures. In Proc: National Forage Testing Association Conf., Omaha, NE. pp. 73-91.

Undersander, D.J. & Moore, J.E., 2002. Relative forage quality. Focus on Forage Vol.4: No. 5.

Undersander, D.J. & Moore, J.E., 2004. Relative forage quality (RFQ) - Indexing legumes and grasses for forage quality. In Proc: National Alfalfa Symposium, 13-15 Dec., San Diego, CA. University of California, Davis.

USDA-Market News, 2004. California Market Summary. USDA-Market News Service, Moses Lake, WA. http://www.ams.usda.gov/lsmnpubs/PDF Nomthly/CALHAY2003.pdf

Valdes, E.V., Hunter, R.B. & Pinter, L., 1987. Determination of quality parameters by near infrared reflectance spectroscopy in whole-plant corn silage. *Can. J Plant Sci.* 67:747-754.

Van der Merwe, F.J. & Smith, W.A., 1991. Dierevoeding, Anim. Sci. (Pty) Ltd.

Van der Merwe, F.J., 1970. Dierevoeding. Kosmo-uitgewery Edms Bpk., Stellenbosch.

Van Saun, R.J., 1991. Dry cow nutrition - the key to improving fresh cow performance. Veterinary clinics of North America; *Food Anim. Practice* 7:599.

Van Soest, P.J., 1963. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency. A review. *J. Dairy Sci.* 46:204.

Van Soest, P.J., 1964. Symposium on nutrition and forage and pastures: New chemical procedures for evaluating forages. *J. Anim. Sci.* 23:838-845.

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., 1967. Development of a comprehensive system of feed analysis and its application to forages. *J. Anim. Sci.* 26:119-128.

Van Soest, P.J., 1968. Chemical estimate of the nutritional value of feeds. In Proc: Cornell Nutrition Conf. Ithaca, NY. pp. 38-48.

Van Soest, P.J., 1973. The uniformity and nutritive availability of cellulose. Fed. Proc. 32:1804-1808.

Van Soest, P.J., 1975. Physio-chemical aspects of fibre digestion. In: Digestion and Metabolism in the ruminant. Eds. McDonald, I.W. & Warner, A.C.I., Univ. of New England Publ. Unit, Armidale, N.S.W., Aust. pp. 352-365.

Van Soest, P.J., 1981. Limiting factors in plant residues of low biodegradability. *Agric. Environ.* 6:135-143.

Van Soest, P.J., 1982. Nutritional ecology of the ruminant. Comstock, Cornell Univ. Press, Ithaca, NY.

Van Soest, P.J., 1987. Practical aspects of forage quality. In Proc: SW Nutr. & Mgmt. Conf., Tempe, AZ. pp. 90-98.

Van Soest, P.J., 1993. Cell wall matrix interactions and degradation - session synopsis. In: Forage cell wall structure and digestibility. ASA, CSSA and SSSA. Eds. Jung, H.G., Hatfield, R.D., Buxton, D.R. & Ralph, J. Madison, WI. pp. 377-395.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, 2nd Ed. Cornell Univ. press, Ithaca, NY.

Van Soest, P.J., Mertens, D.R. & Deinum, B., 1978. Preharvest factors influencing quality of conserved forage. *J. Anim. Sci.* 47:712-720.

Van Soest, P.J. & Robertson, J.B., 1985. Analysis of forages and fibrous foods. Lab Manual for Animal Science 613. Dept. Animal Sci., Cornell University, Ithaca, NY.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. Symposium: Carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. *J. Dairy Sci.* 74:3583-3597.

Van Soest, P.J. & Sniffen, C.J., 1984. Nitrogen fractions in NDF and ADF. In Proc: Dist. Feed Conf. pp. 39-73.

Van Soest, P.J., Sniffen, C.J., Mertens, D.R., Fox, D.G., Robinson, P.H. & Krishnamoorthy, U.C., 1981. A net protein system for cattle: The rumen submodel for nitrogen. In: Protein Requirements for Cattle. Ed. Owens, F.N., Div. Of Agric., Oklahoma State Univ., Stillwater. pp.265.

Van Soest, P.J., Van Amburgh, M.E., Robertson, J.B. & Knaus, 2005. Validation of the 2.4 times lignin factor for ultimate extent of NDF digestion, and curve peeling rate of fermentation curves into pools. Department of Animal Science, Cornell University and Department of Livestock Sciences, Vienna, Austria.

Van Soest, P.J. & Wine, R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *J. AOAC* 50:50-55.

Van Waes, C., Kirilov, A., Krachounov, I., Petkov, P. & Carlier, L., 1998. Prediction of *in vitro* digestibility and chemical composition of lucerne by NIRS. *Plant Sci. (Sofia)* 35(9):708-711.

Van Wyk, H.P.D., Oosthuizen, S.A. & Meyer, E.E., 1955. Nutritive value of South African feeds. III. Hay and pasture crops, silage, cereals, tubers and pods. *Science bulletin.* 354:19-23.

Varga, G.A. & Kononoff, P., 1999. Dairy rationing using structural and non structural carbohydrates: from theory to practice. In Proc: Southwest Nutr. Management Conf., Dept. Anim Sci., Univesity of Arizona, Jucson, AZ. pp. 77.

Villalobos, J.L., Burns, J.C., Fisher, D.S. & Pond, K.R., 1991. *In vitro* dry matter disappearance and cell-wall concentrations of flacidgrass masticates predicted by near infrared reflectance spectroscopy. *Crop Sci.* 31:1571-1574.

Voelker, J.A., Burato, G.M. & Allen, M.S., 2002. Effects of pretrial milk yield on responses of feed intake, digestion, and production to dietary forage concentration. *J. Dairy Sci.* 85:2650-2661.

Volkers, K.C., Wachendorf, M., Loges, R., Jovanovic, N.J. & Taube, F., 2003. Prediction of the quality of forage maize by near-infrared reflectance spectroscopy. *Anim. Feed Sci. Technol.* 109:183-194.

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

Waldo, D.R. & Jorgensen, N.A., 1981. Forages for high animal production: Nutritional factors and effects of conservation. *J. Dairy Sci.* 64:1207-1229.

Wang, Z., Eastridge, M.L. & Qiu, X., 2001. Effects of forage neutral detergent fibre yeast culture on performance of cows during early lactation. *J. Dairy Sci.* 84:204-212.

Ward, J.K., Tefft, C.W., Sirny, R.J., Edwards, H.W. & Tillman, A.D., 1957. Further studies concerning the effect of alfalfa ash upon the utilisation of low quality roughages by ruminant animals. *J. Anim. Sci.* 16:635-641.

Ward, R.T., 2005. Opportunities and limitations in the use of NDF fibre digestibility values. Cumberland Valley Analytical Services, Inc., Penn State Dairy Cattle Nutrition Workshop.

Wedin, W.F., Burger, A.W. & Ahlgren, H.L., 1956. Effect of soil type, fertilization and stage of growth on yield, chemical composition, and biological value of ladino clover *(Trifolium repens L.)* and alfalfa *(Medicago sativa). Agron. J.* 48:147-152.

Weiss, W.P., 1993. Symposium: prevailing concepts in energy utilisation by ruminants: predicting energy values of feeds. *J. Dairy Sci.* 76:1802-1811.

Weiss, W.P., 1994. Estimation of digestibility of forages by laboratory methods. In: Forage Quality, Evaluation and Utilisation. Eds. Fahey, G.C. Jr., American Soc. Agronomy, Madison, WI. pp. 644-681.

Weiss, W.P., 1998. Symposium: energy availability. Estimating available energy content of feeds for dairy cattle. *J. Dairy Sci.* 81:830-839.

Weiss, W.P., 2002. Overview and application of the 2001 NRC energy system. In Proc: Florida Ruminant Nutrition Symposium. pp.1-15.

Weiss, W.P., Conrad, H.R. & Shockey, W.L., 1983. Predicting digestible protein using acid detergent insoluble nitrogen. *J. Dairy Sci.* 66(suppl.):192(Abstract).

Weis, W.P., Conrad, H.R. & Shockey, W.L., 1986. Digestibility of nitrogen in heat damaged alfalfa. *J. Dairy Sci.* 69:2658.

Weiss, W.P., Conrad, H.R. & St. Pierre, N.R., 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. *Anim. Feed Sci. Technol.* 39:95-110.

Westgate, J.M., 1908. Alfalfa. Farmers' bulletin 339. Government Printing Office, Washington.

Williams, P.C., 1987. Variables affecting near-infrared reflectance spectroscopic analysis. In: Near-infrared technology in the agricultural and food industries. Eds. Williams, P. & Norris, A., American Association of Cereal Chemists, St. Paul, MN. pp. 143-167.

Williams, P.C., 2001. Implementation of near infrared technology. In: Near infrared technology in the agricultural and food industries. Eds. Williams, P.C. & Norris, K.H., American Association of Cereal Chemist, St. Paul, Minnesota, USA. pp. 145-169.

Williams, P.C., 2006. Near-infrared technology - Getting the best out of light. Edition 4.0, PDK Projects, Inc., Nanaimo B.C., Canada.

Williams, P.C., 2007. Near-infrared technology - Getting the best out of light. Edition 5.1, PDK Projects, Inc., Nanaimo B.C., Canada.

Williams, P.C. & Cordiero, H.M., 1985. Effect of calibration practice on correction of errors induced in near-infrared protein testing of hard red spring wheat by growing location and season. *J. Agric. Sci. Cambridge* 104:113-123.

Williams, P.C. & Krischenko, V.P., 1986. A comparative study of two computerised spectrophotometers for the near-infrared analysis of wheat for protein. *Applied Spectroscopy* 40:785-790.

Williams, P.C. & Norris, K.H., 2001. Variables affecting near-infrared spectroscopic analysis. In: Near infrared technology in the agricultural and food industries. Eds. Williams, P.C. & Norris, K.H., American Association of Cereal Chemist, St. Paul, Minnesota, USA. pp. 171-185.

Williams, P.C., Norris, K.H., & Zarowsky, W.S., 1982. Influence of temperature on estimation of protein and moisture in wheat by near-infrared reflectance. *Cereal Chem.* 59:473-477.

Williams, P.C. & Sobering, D.C., 1993. Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grain and seeds. *J. Near Infrared spectrosc.* 1:25-32.

Wilson, J.R. & Hatfield, R.D., 1997. Structural and chemical changes of cell wall types during stem development: consequences for fibre degradation by rumen microflora. *Aust. J. Agric. Res.* 48:165-180.

Wilson, J.R. & Mertens, D.R., 1995. Cell wall accessibility and cell structure limitations to microbial digestion of forage. *Crop Sci.* 35:251-259.

Winch, J.E. & Major, H., 1980. Predicting nitrogen and digestibility of forages using near infrared reflectance photometry. *Can. J. Plant Sci.* 61:45-51.

Yang, W.Z. & Beauchemin, K.A., 2005. Effects of physically effective fibre on digestion and milk production by dairy cows fed diets based on corn silage. *J. Dairy Sci.* 88:1090-1098.

Yang, W.Z. & Beauchemin, K.A., 2006. Physically effective fibre: Method of determination and effects on chewing, ruminal acidosis, and digestion by dairy cows. *J. Dairy Sci.* 90:2826-2838.

Yang, W.Z. & Beauchemin, K.A., 2007a. Altering physically effective fibre intake through forage proportion and particle length: Digestion and milk production. *J. Dairy Sci.* 90:3410-3421.

Yang, W.Z. & Beauchemin, K.A., 2007b. Altering physically effective fibre intake through forage proportion and particle length: chewing and ruminal pH. *J. Dairy Sci.* 90:2826-2838.

Yansari, A.T., Valizadeh, R., Naserian, A., Christensen, A., Yu, P. & Shahroodi, F.E., 2004. Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows. *J. Dairy Sci.* 87:3912-3924.

Yu, P., Christensen, D.A., McKinnon, J.J. & Markert, J.D., 2003. Effect of variety and maturity stage on chemical composition, carbohydrate and protein subfractions, *in vitro* rumen degradability and energy values of timothy and alfalfa. *Can. J. Anim. Sci.* 83:279-290.

Yu, Y., 1974. Comparisons of chemical, *in vitro* and *in vivo* methods for estimating nutritive values of normal and chemically treated haylages. Ph.D. Dissertation, Michigan State Univ., East Lansing, MI. pp. 270.

Zinn, R.A., Corona, L. & Ware, R.A. 2004. Forage Quality: Impacts on cattle performance and economics. In Proc: National Alfalfa Symposium, 13-15 Dec., San Diego, CA.

Zinn, R.A., & Ware, R.A., 2007. Forage quality: Digestive limitations and their relationships to performance of beef and dairy cattle. In Proc: 22nd Annual Southwest Nutrition & Management Conference, 22-23 Febr., Tempe, AZ.

APPENDIX A

Formulas from NRC (2001) and McDonald *et al.* (2002)

NRC (2001)

TDN_{1x} (%) $\quad = \text{tdCP} + (\text{tdFA} \times 2.25) + \text{tdNDF} + \text{tdNFC} - 7$

where: tdCP (%) $\quad$ = truly digestible CP for forages

$\quad\quad\quad\quad\quad\quad = \text{CP} \times \exp[-1.2 \times (\text{ADICP/CP})]$

tdFA (%) $\quad$ = truly digestible fatty acids

$\quad\quad\quad\quad\quad\quad = 1.0 \times \text{EE} - 1\%$

Note: If $\text{EE} < 1$, then $\text{FA} = 0$

tdNDF (%) $\quad$ = truly digestible neutral detergent fibre

$\quad\quad\quad\quad\quad\quad = 0.75 \times (\text{NDFn} - \text{ADL}) \times ((1 - (\text{ADL/NDFn})^{0.667}))$

or $= \text{NDFD}_{48}$

where: $\text{NDFn} = \text{NDF} - \text{NDFCP}$

tdNFC(%) $\quad$ = truly digestible non-fibre carbohydrates

$\quad\quad\quad\quad\quad\quad = \text{NFC}_{\text{NRC}} = 0.98 \, (100 - [(\text{NDF} - \text{NDICP}) +$

$\quad\quad\quad\quad\quad\quad\quad \text{CP} + \text{EE} + \text{ash}])$

7 $\quad\quad\quad$ = Metabolic fecal TDN

DE_{1x} (Mcal/kg) $\quad = (\text{tdNFC}/100) \times 4.2 + (\text{tdNDF}/100) \times 4.2 +$

$\quad\quad\quad (\text{tdCP}/100) \times 5.6 + \text{FA}/100) \times 9.4 - 0.3$

DE_p (Mcal/kg) $\quad = \text{DE}_{1x} \times$ discount factor

where: Discount factor

$\quad\quad\quad = \text{TDN1X} - (((0.18 \times \text{TDM1X}) - 10.3 \times$

$\quad\quad\quad\quad \text{Intake})/\text{TDN1X})$

Note: No discount was applied to lucerne hay

samples below 60 % TDN_{1x}

ME_p (Mcal/kg) $\quad = 1.01 \times \text{DE}_p - 0.45) + (0.0046 \times (\text{EE} - 3))$

NEl_p (Mcal/kg) $\quad = (0.703 \times \text{MEp} - 0.19) + (((0.097 \times \text{MEp} +$

$\quad\quad\quad 0.10)/0.97) \times (\text{EE} - 3))$

TTDOM $\quad\quad = \text{TDN} - ((\text{EE} - 1) \times 1.25)$

McDonald et al. (2002)

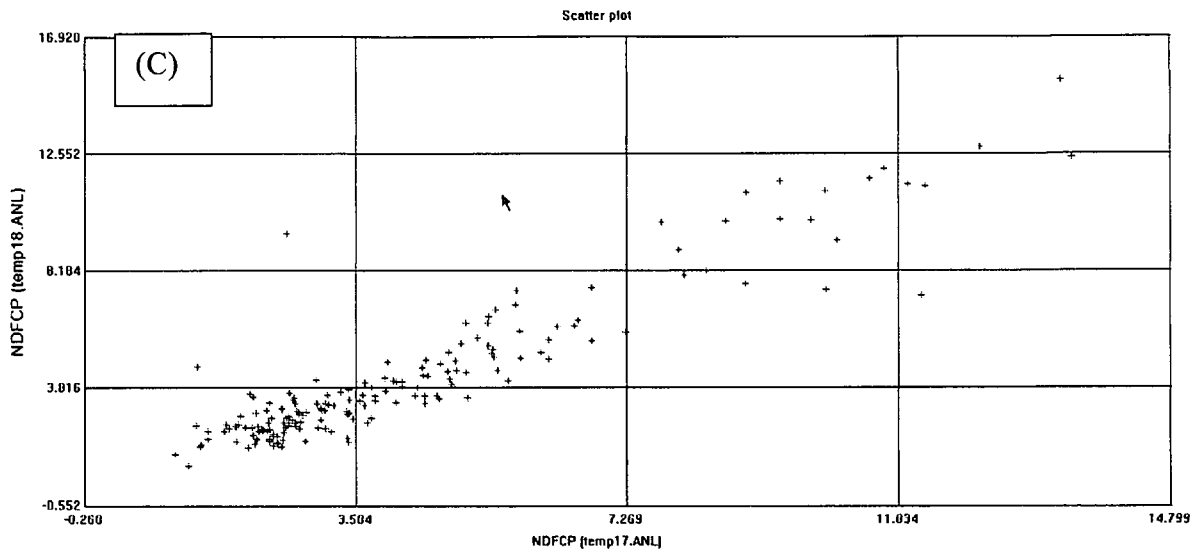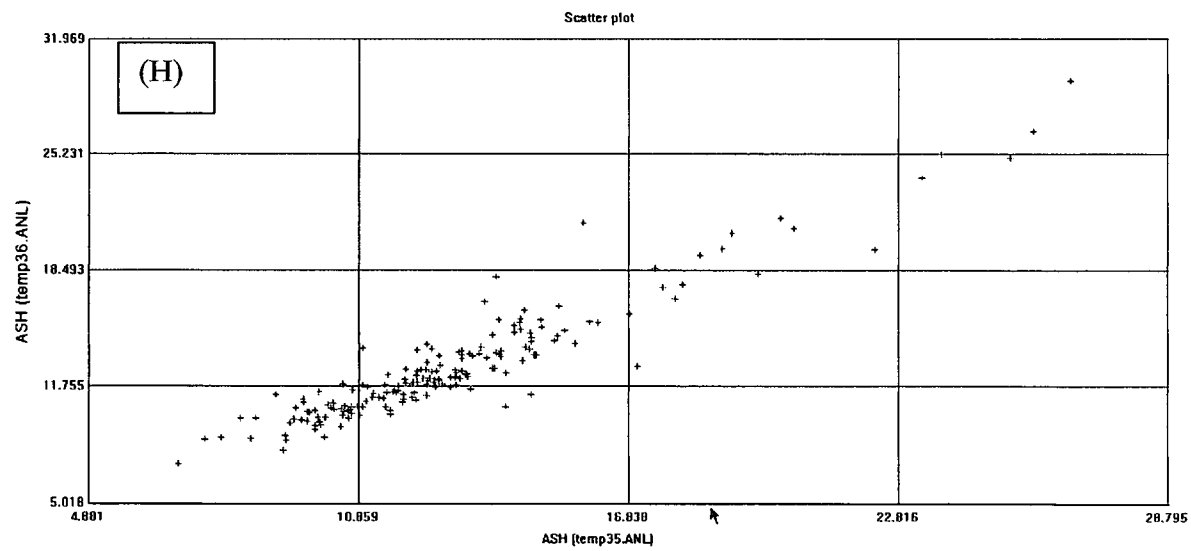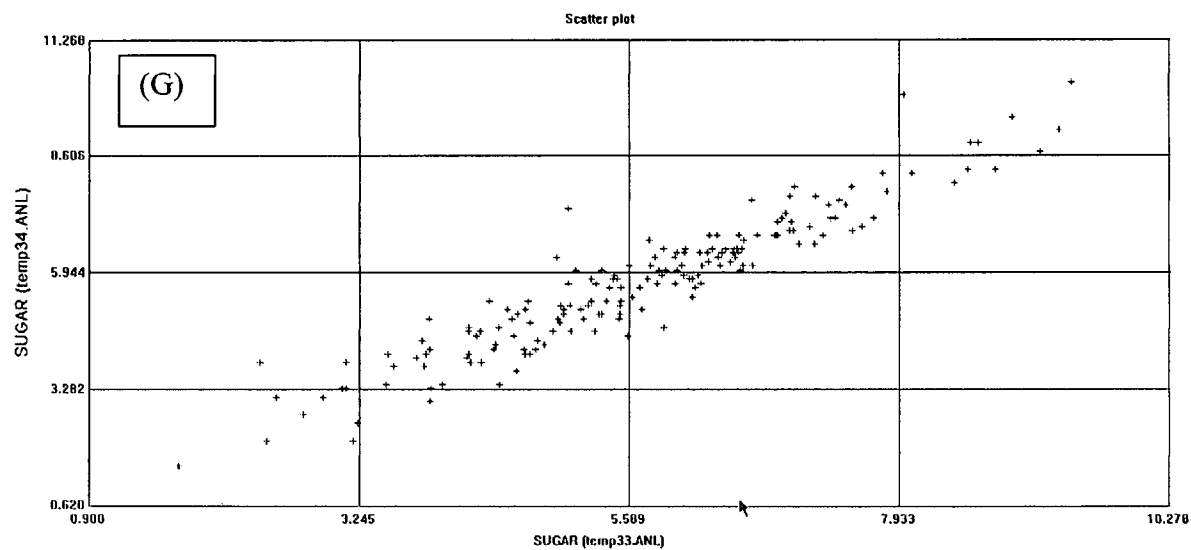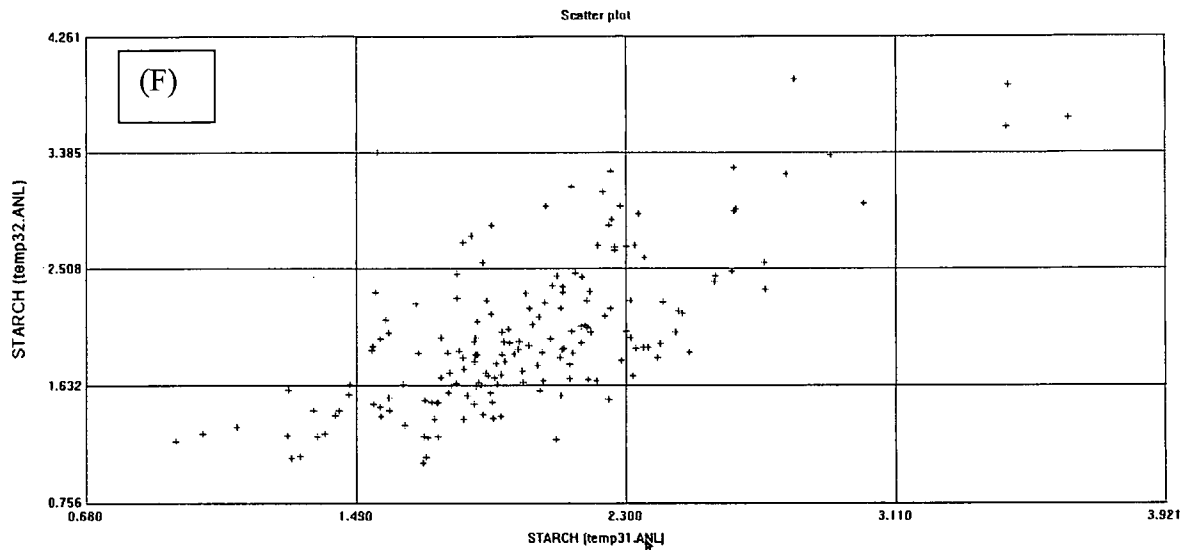|	
ME (MJ/kg DM)	$= 0.016 \text{ IVOMD}_{48}$
ME_{fat} (MJ/kg DM)	$= (35 \text{ MJ/kg} \times \text{EE})/100$
FME (MJ/kg DM)	$= \text{ME} - \text{ME}_{fat}$

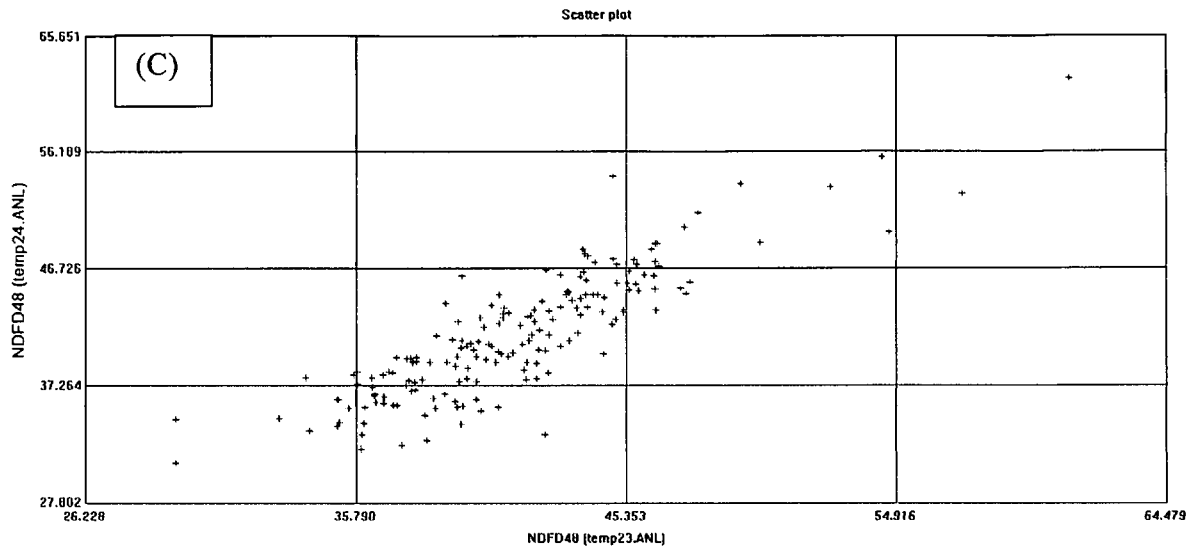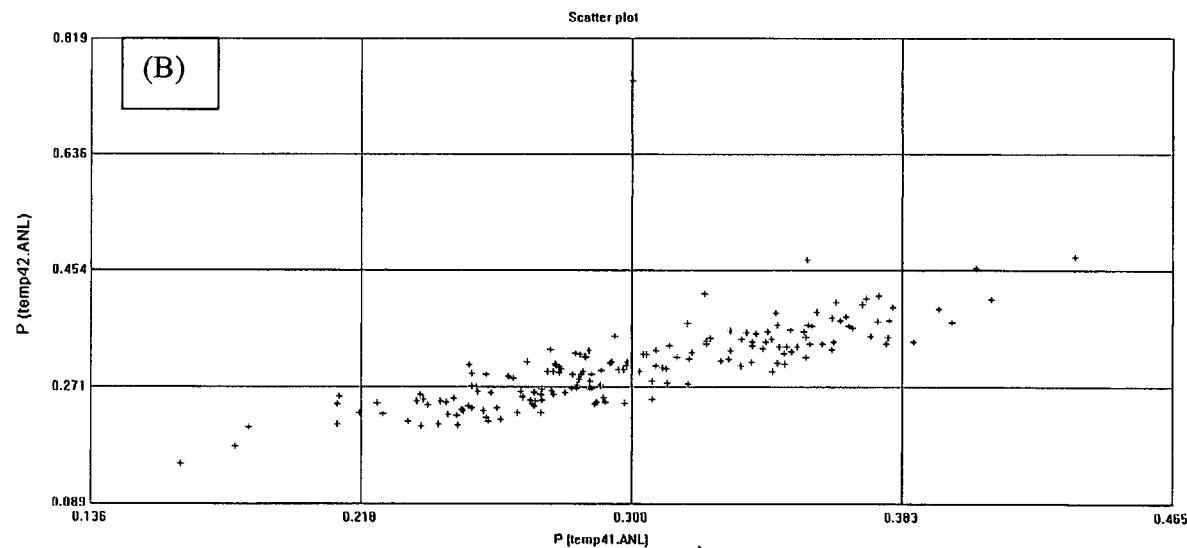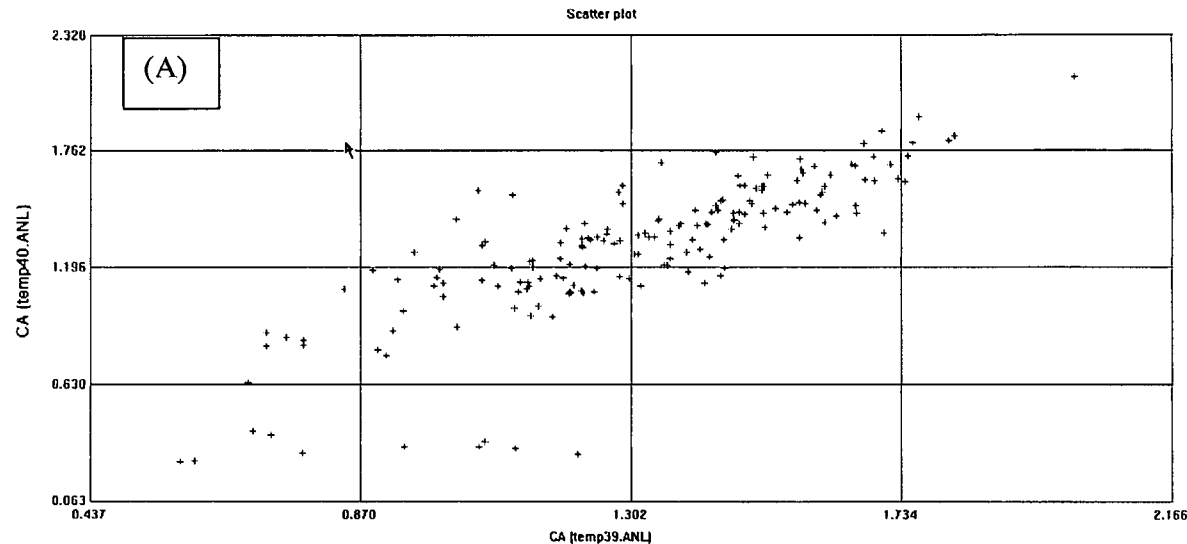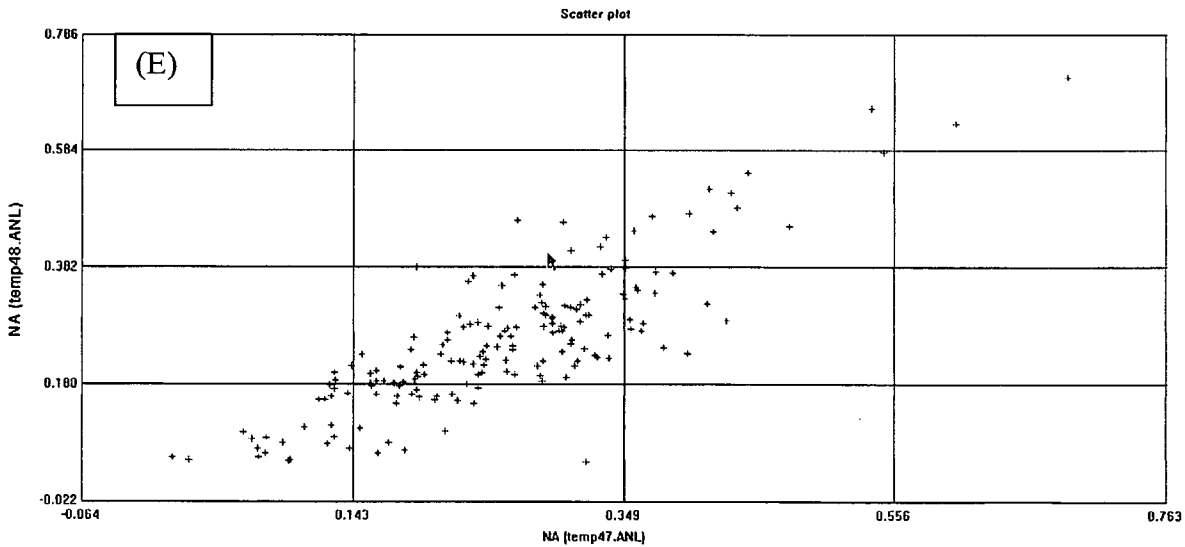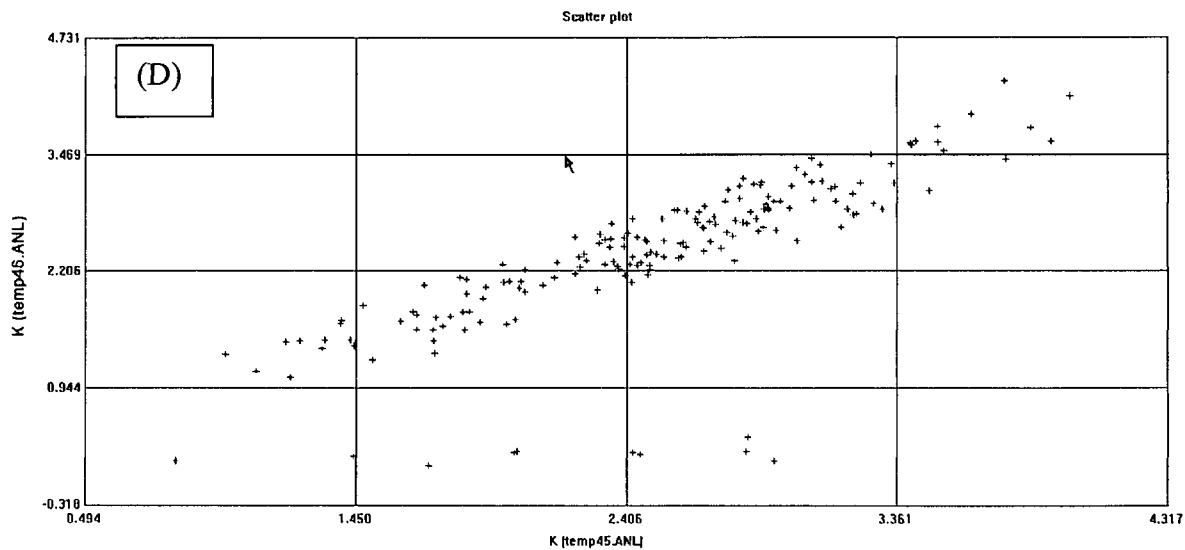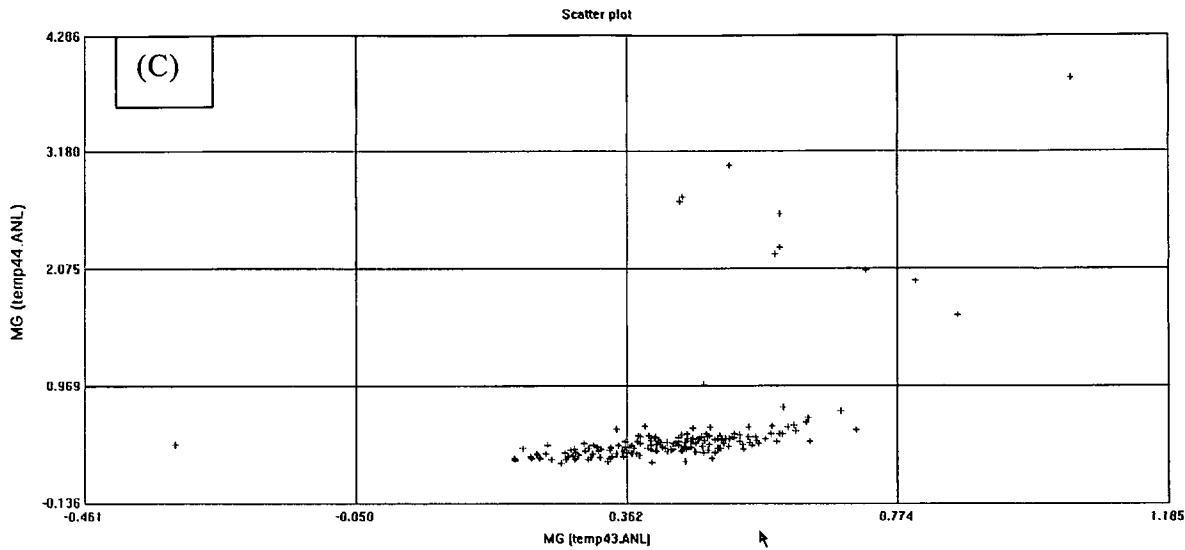# APPENDIX B1

Near infrared reflectance spectroscopy (NIRS) predicted chemical values versus reference values for lucerne hay quality: A = Soluble protein, B = Acid detergent fibre - crude protein, C = Neutral detergent fibre - crude protein, D = Lignin, E = Fat, F = Starch, G = Sugar, H = Ash.

Scatter plot (A)

Scatter plot (B)

Scatter plot (C) — NDFCP (temp18.ANL) vs NDFCP (temp17.ANL)

Scatter plot (D) — LIG (temp26.ANL) vs LIG (temp25.ANL)

Scatter plot (E) — FAT (temp30.ANL) vs FAT (temp29.ANL)

Scatter plot

(F)

STARCH (temp32.ANL) vs STARCH (temp31.ANL)

Scatter plot

(G)

SUGAR (temp34.ANL) vs SUGAR (temp33.ANL)

Scatter plot

(H)

ASH (temp36.ANL) vs ASH (temp35.ANL)
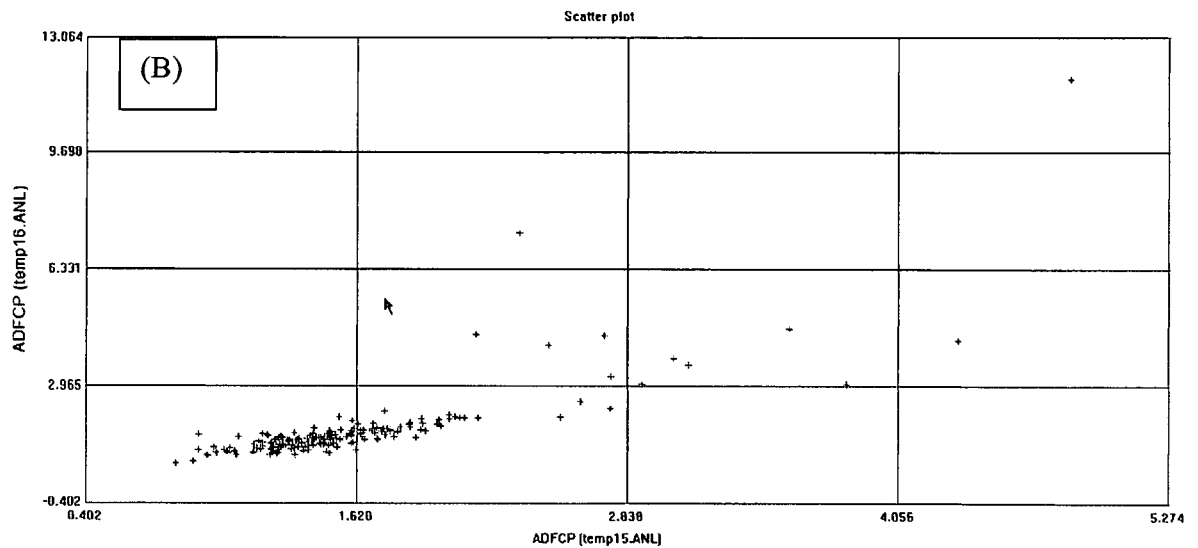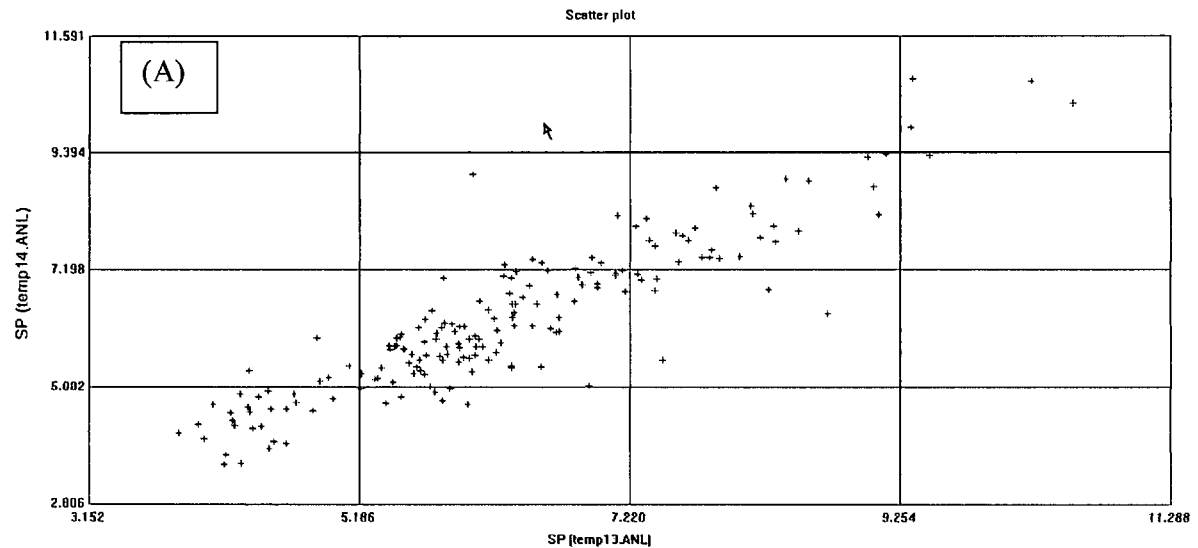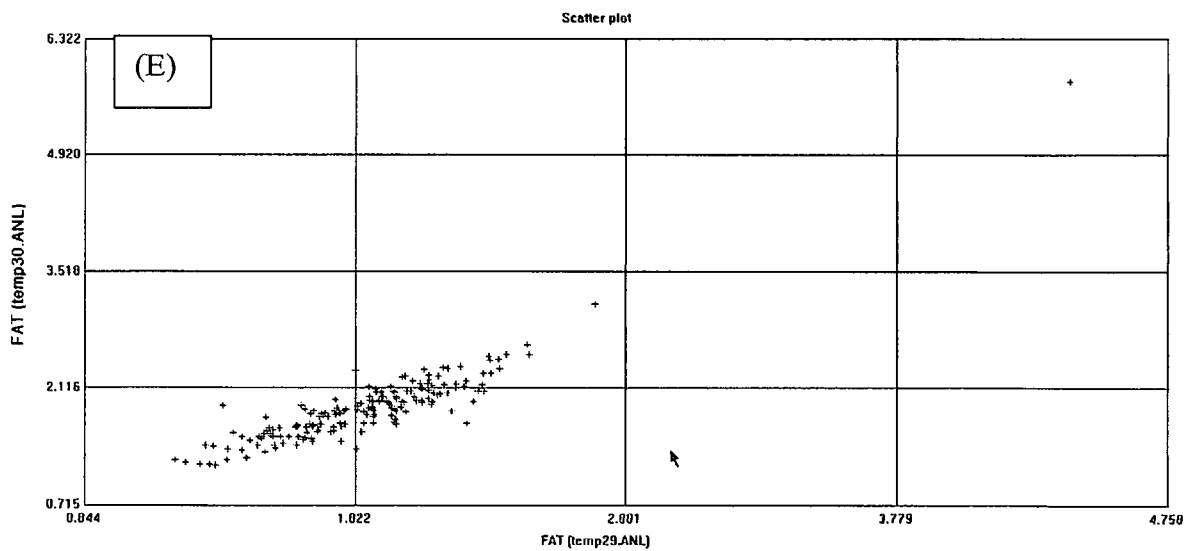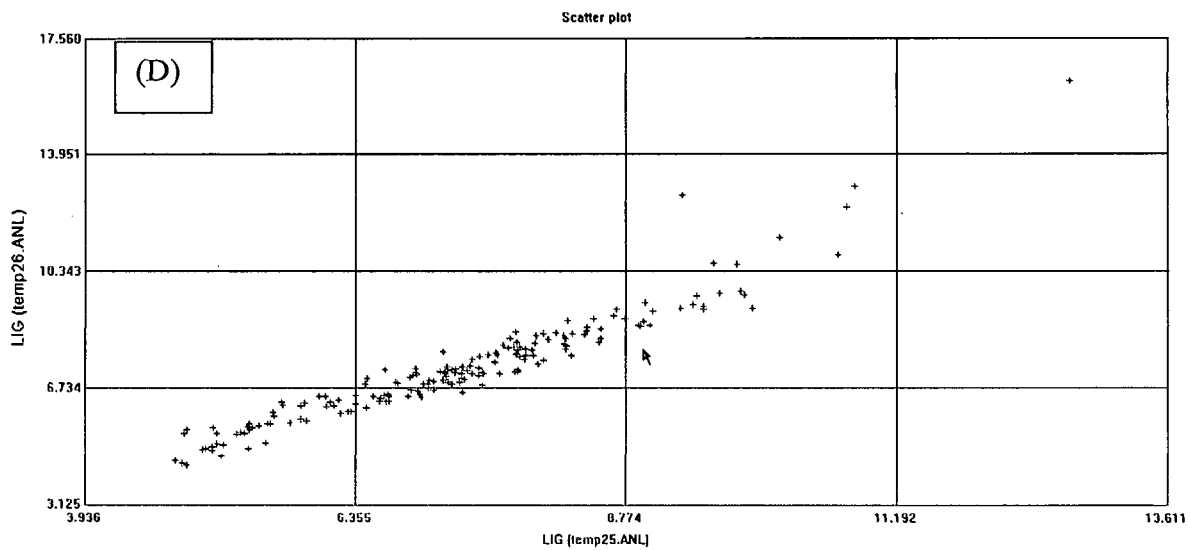
Appendix B2

Near infrared reflectance spectroscopy (NIRS) predicted *in vitro* values versus reference values for lucerne hay quality: A = In vitro organic matter digestibility at 48 hours, B = Neutral detergent fibre digestibility at 24 hours, C = Neutral detergent fibre digestibility at 48 hours.
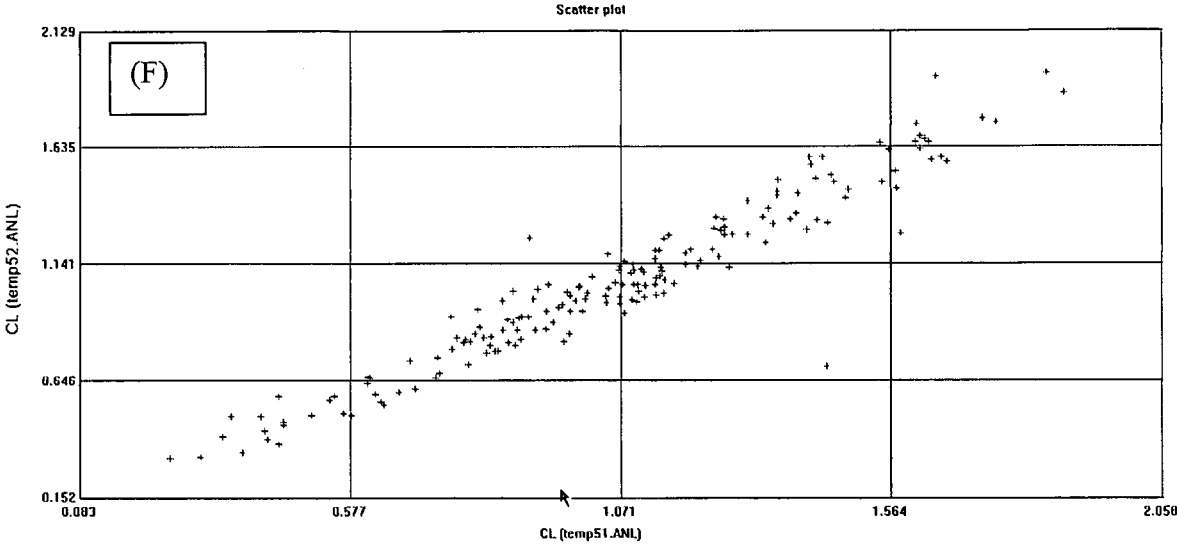
Scatter plot
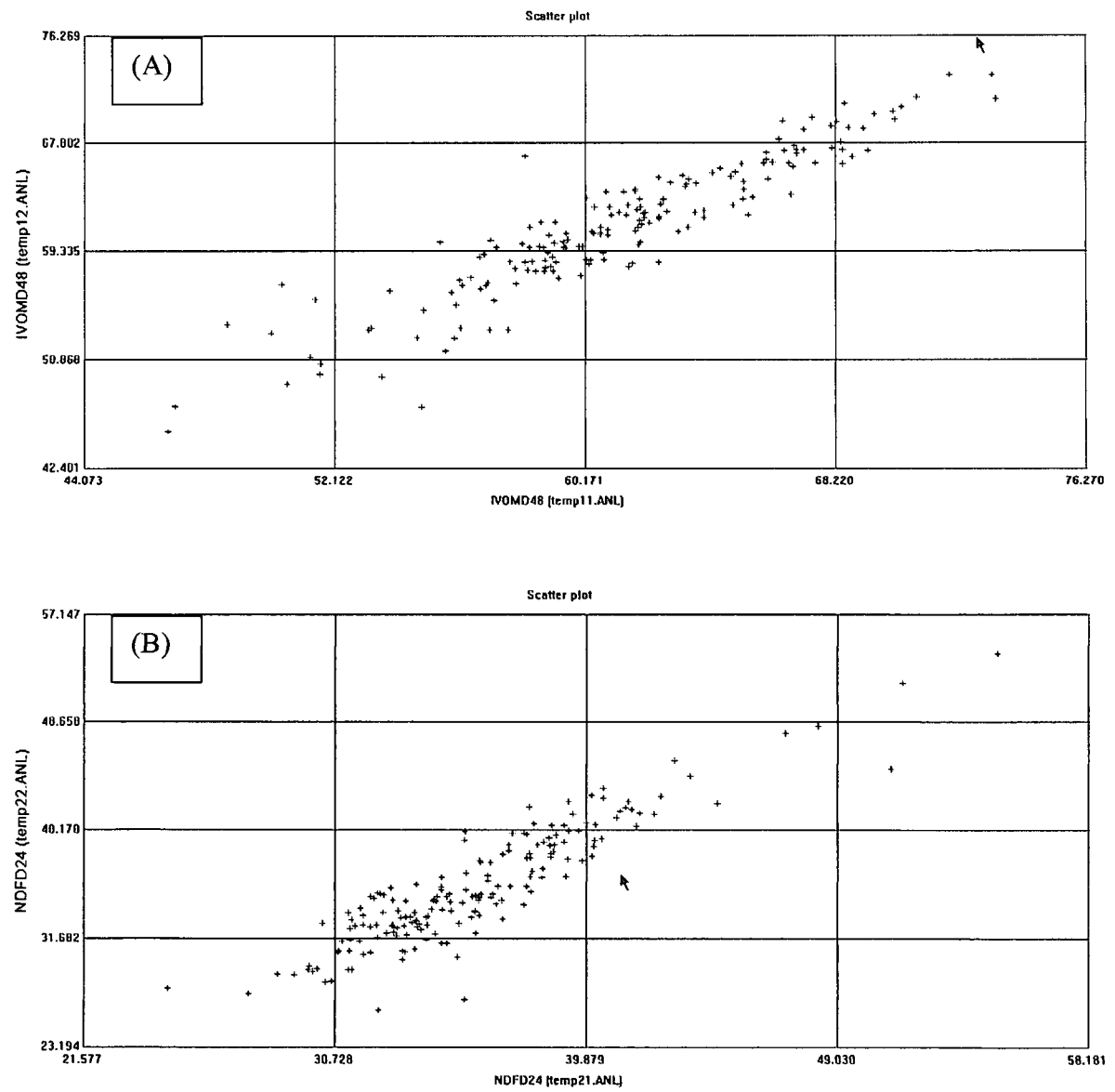
Appendix B3

Near infrared reflectance spectroscopy (NIRS) predicted mineral values versus reference values for lucerne hay: A = Calcium, B = Phosphorus, C = Magnesium, D = Potassium, E = Sodium, F = Chloride.
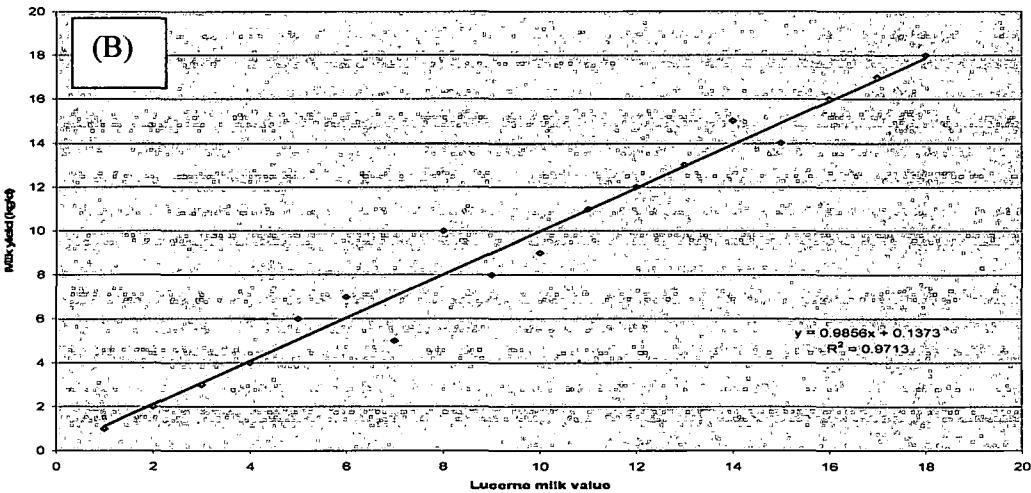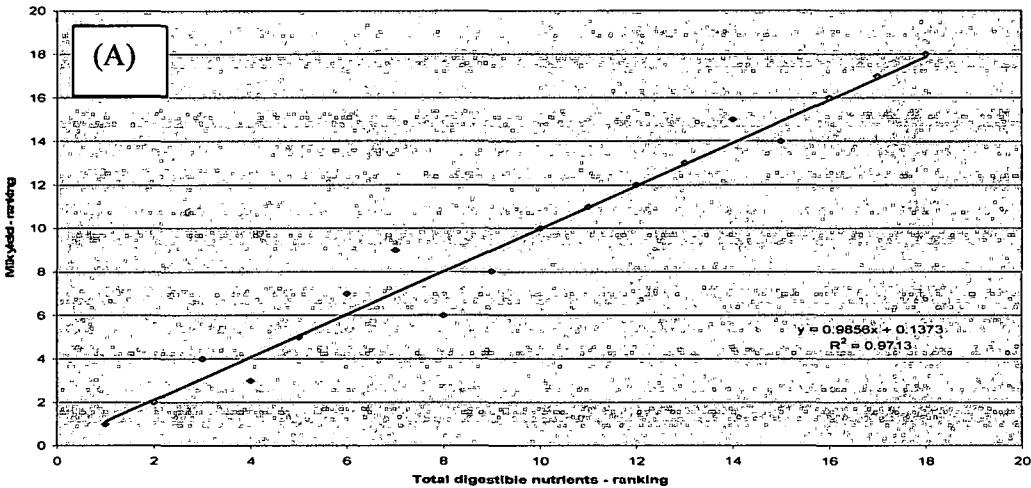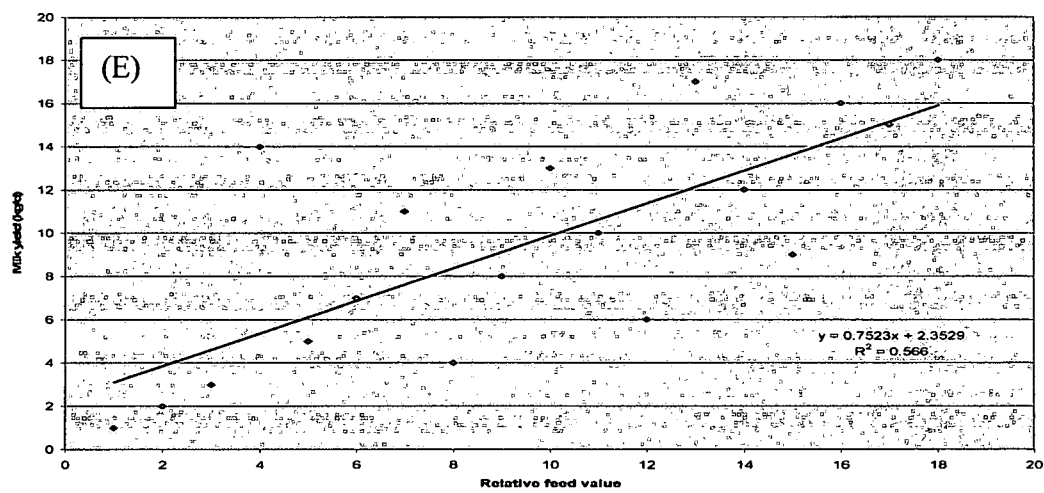

Scatter plot (A)

Scatter plot (B)

Scatter plot

(C)

Scatter plot
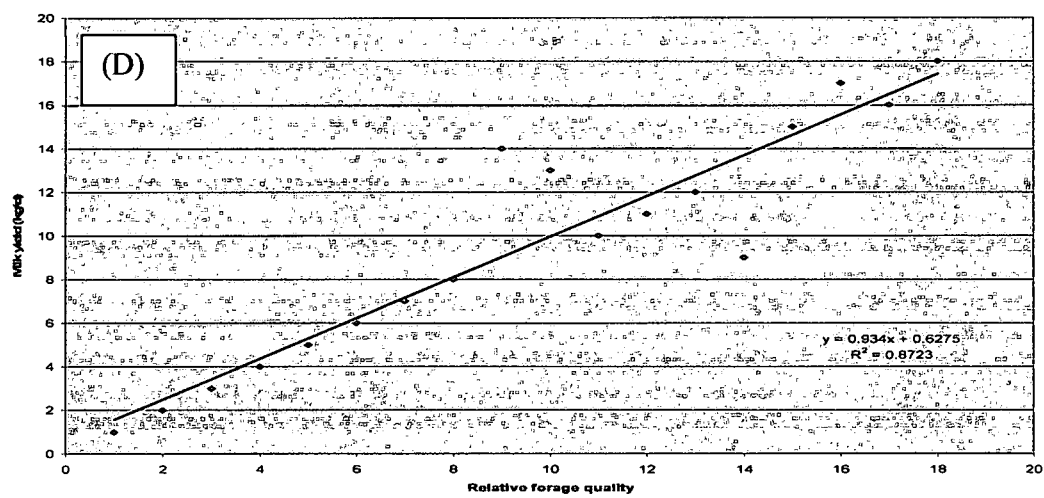
(D)

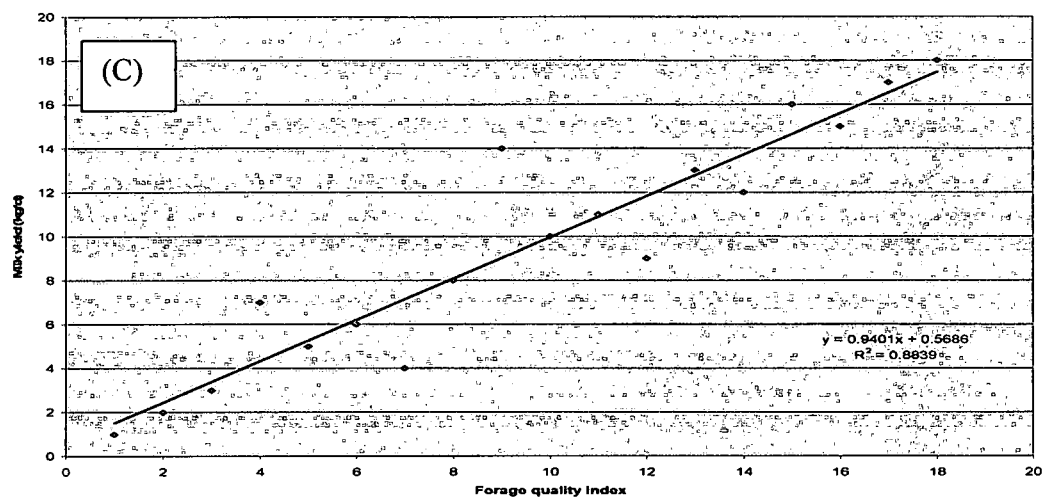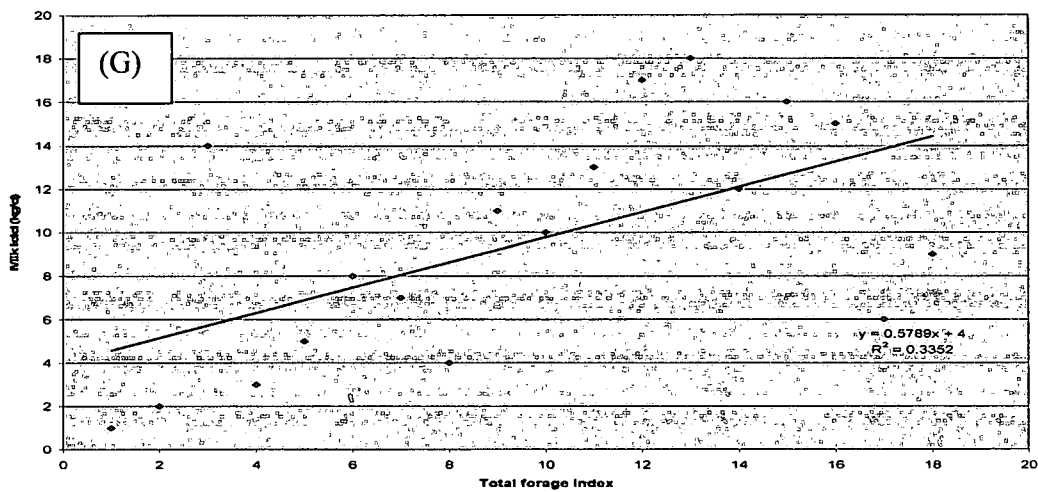

Scatter plot

(E)

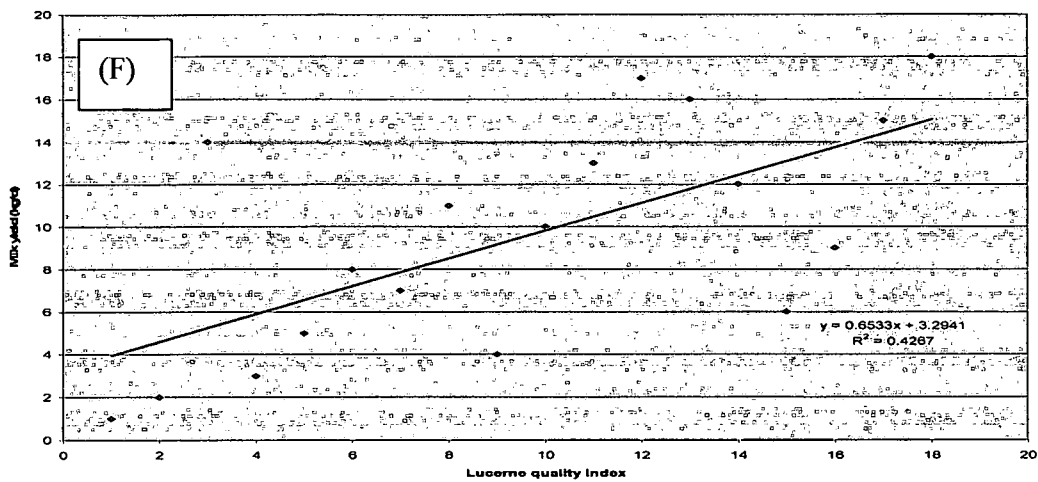Scatter plot (F)

APPENDIX C

Relationship between milk yield ranking and model ranking for 18 randomly selected samples from the South African lucerne hay population: A = Total digestible nutrients (TDN$_{lig}$), B = Lucerne milk value (LMV), C = Forage quality index (FQI), D = Relative forage quality(RFQ), E = Relative feed value (RFV), F = Lucerne quality index (LQI), G = Total forage index (TFI)

(C)

$y = 0.9401x + 0.5686$
$R^2 = 0.8839$

Forage quality index

(D)

$y = 0.934x + 0.6275$
$R^2 = 0.8723$

Relative forage quality

(E)

$y = 0.7523x + 2.3529$
$R^2 = 0.566$

Relative feed value

277

(F)

y = 0.6533x + 3.2941
R² = 0.4287

(G)

y = 0.5789x + 4
R² = 0.3352

APPENDIX D

APPLICATION

The purpose of this section is to supply according to the literature results of this study and consultation with the South African lucerne hay industry a set of guidelines to ensure a uniform and accurate national grading system. The proposed procedures for a practical grading system for lucerne (*Medicago sativa* L.) hay in South Africa is summarised in four parts namely: sampling and sample preparation, analytical analysis, a quality model and quality standards.

SAMPLING AND SAMPLR PREPARATION - Adopted from Bath & Marble (1989), Putnam (1998) and Scholtz and Van der Merwe (2006-unpulished data)

The following procedure must be followed in taking a representative sample:

1. Identify a single batch of lucerne hay that is homogenous in terms of:

- the same cut

- the same variety

- the same field

- the same physiological stage

- cut within a 48 hour period

2. Sampling must be done randomly

- That means that there must be no prior reason why a specific bale will be selected / not on the basis of colour, amount of leaves, etc.

3. Sampling apparatus specifications

• A specific standardised hollow bit sampler (probe) with a plastic collection bottle is prescribed to ensure easy and quick sampling (Lucerne Tech Consult CC., Hopetown, South Africa)

4. Drill technique

• drill in the middle of the top of the bale at a 90° angle or in the middle of the side of the bale at a 45° angle.

• the drill must penetrate the bale at least 325mm (as indicated on the drill)

• the drill bit must continuously be kept sharp

5. Take enough sub-samples randomly spread throughout the batch.

• a minimum of 20 are needed to limit variation

• number of sub-samples per bale: small bale = one

                                                              big and round bales = two

6. Sample size

• 180 to 250g is desirable

• if less than 180g, the sample is not representative

7. Sample handling

• Seal unground sample in an air-tight plastic bag or bottle and to the nearest NIR instrument registered at the National Lucerne Organisation (NLO).

• Samples must be protected against direct heat and direct sunlight.

- un-milled samples may under no circumstances be sub-divided.

ANLYSIS

- The unground sample of 180g -250g is analysed for moisture, acid detergent fibre (ADF), ash and lignin. Details regarding registered NIR instruments available at the NLO.

- Prior to the grinding of samples, the moisture content of the composite sample will be analysed by NIRS, equipped with a separate moisture calibration, developed on samples in the unground status.

    - All samples must be ground through a 1 mm sieve with a hammer mill type grinder (16 000 rpm 5Hp-motor).

Both apparatus mentioned above (probe and grinder) will be issued with serial numbers to ensure the use of approved equipment and therefore uniformity.

- Ring tests

The NLO will appoint an independent body to certify NIR instruments acceptably for Lucerne hay grading. To achieve this, one milled sample from each grade (four samples) will be sent to each instrument operator at the start of the season in September and again during December of each year. The instrument operator will scan these samples and submit the results to the appointed body for verification and certification.

- Calibration improvement

Each year the appointed body will collect 50 unground samples spread during the season from each of the four main producing areas. These samples will be grind under controlled conditions and sent to the network controllers for scanning and identification of spectral outliers. These outliers will be chemically analysed and add to the calibration.

- Validations

Network controllers will receive 10 unknown samples from the independent appointed body. The results will be sent to the appointed body for validation.

- Further improvement

In an attempt to accommodate the possible effects of moisture on the NIR spectra and therefore calibration equations, a similar study needs to be conducted as described in the present study. This will include the collection of 600 unground samples representative of the South African luceme hay population. The representative samples will be scanned with the NIRS in the unground status and then chemical analysed.

MODEL

Luceme hay quality is expressed as luceme milk value (LMV) and calculated as follows:
LMV = 64.18 − 0.23 ADF − 0.53 ash -.0.90 lignin

QUALITY STANDARDS

Currently the following quality standards are used in the South African luceme hay industry

Grade/Class	Lucerne quality index (LQI)	Foreign material (%)
Prima	> 116	Absent
1	116-93	Absent
2	92-65	Present
3	<65	Present

- The maximum moisture allowed as determined by the NIR instrument, = 16%.

- Harmful plants and proclaimed weeds will be sent back.

- Loads with funny smells, mould and signs of excessive heat, can also be sent back.

• If a load is obviously identified as of mixed quality, it will be classified as the lowest grading.

It should, however, be considered to do away with the classification of luceme hay in grades and simply rank it according to LMV. Grades fail to differentiate between different qualities within a specific grade. In addition, extreme high and low quality hays are not rewarded and/or discriminated against. Furthermore, the price of luceme hay could be related to the specific LMV.