The use of near infrared reflectance spectroscopy (NIRS) for the chemical analysis of meat and feedstuffs

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MASTER OF SCIENCE IN AGRICULTURE

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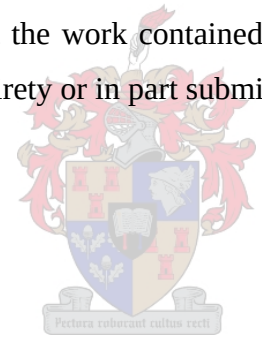
DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.



Signature:…….. …….. Date:…….

For my parents, Danie and Riana Viljoen

This thesis represents a compilation of manuscripts; each chapter is an individual entity and repetition between chapters is therefore unavoidable.

Parts of this thesis have been presented at:

1. ***GSSA/SASAS Joint Congress*, Christiana, May 2002, in the form of a presentation and two posters.**

Presentation

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Posters

Viljoen, M., Brand, T.S., Burger, W.W. & Hoffman, L.C., 2002. Differences in the chemical composition and digestibility of straw and hay of winter grain produced in a Mediterranean rainfall area.

Viljoen, M., Brand, T.S., Liebenberg, D. & Hoffman, L.C., 2002. Variation in the chemical composition of different types of winter grain produced in the Western Cape region of South Africa.

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Viljoen, M., Hoffman, L.C. & Brand, T.S., 2003. Prediction of the chemical composition of mutton with near infrared reflectance spectroscopy.

Abstract

Title:	The use of near infrared reflectance spectroscopy (NIRS) for the chemical analysis of meat and feedstuffs
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Near infrared reflectance spectroscopy (NIRS) was evaluated as a tool to predict the chemical composition of ostrich meat, mutton and feedstuffs. Seventy-three calibrations were developed. NIRS analyses were conducted on an InfraAlyzer 500 spectrophotometer between 1100 and 2500 nm wavelengths.

Near infrared reflectance calibrations were developed for (i) the proximate chemical composition of ostrich meat; (ii) the proximate chemical and mineral composition of mutton; (iii) the chemical composition and digestibility of lupins, full-fat canola and the determination of alkaloids in lupins; (iv) the chemical composition and digestibility of winter grains and maize; (v) the chemical composition and digestibility of cereal hay, cereal straw, wheat stubble and alfalfa-grass/hay mixtures. The chemical composition of different types of winter grain produced in the Western Cape area of South Africa, as well as the chemical composition and digestibility of winter grain hay and straw produced in a Mediterranean rainfall area were also determined.

Near infrared reflectance spectrometry proved to be successful for the prediction of crude protein (CP) and fat in both animal species, as well as for ash and dry matter (DM) in freeze-dried mutton. Accurate calibrations were also developed for certain minerals (K, P, Na, Mg, Fe and Zn).

Calibrations proved to be accurate for all the relevant chemical constituents in lupins, as well as the DM, CP and fat calibrations in full-fat canola. The alkaloid calibration showed potential although more samples should be included for a more accurate calibration. Winter grain calibrations were accurate for fat, acid detergent fibre (ADF), *in vitro* organic matter digestibility (IVOMD), lysine and methionine. All chemical composition calibrations of maize, except for ash, were accurate for future prediction. Chemical composition calibrations of wheat stubble and alfalfa-grass hay mixtures resulted in prediction with similar or slightly higher accuracy than calibrations reported in the literature. The calibrations developed for cereal hay and straw were not suitable for prediction purposes. Possible reasons were discussed to explain these inaccurate calibrations.

Variation shown between different types of cereal grain (2-row barley, 6-row barley, oats, wheat and triticale) accentuated the need for the analysis of different batches of grain produced under different conditions for use in animal feed. The chemical composition and digestibility of winter grain straw and hay were also obtained. These values provide a database for calculation of inclusion levels of these feedstuffs in animal diets.

These investigations showed the NIRS to be a successful and rapid tool for the prediction of the chemical composition of ostrich and lamb meat and locally produced feedstuffs.

Opsomming

Titel:	Die gebruik van naby infrarooi refleksie spektroskopie (NIRS) vir die chemiese ontleding van vleis en voedingsgewasse
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Naby infrarooi refleksie spektroskopie (NIRS) is geëvalueer as 'n metode om die chemiese samestelling van volstruis- en skaapvleis, asook voedingsgewasse te voorspel. Drie en sewentig kalibrasies is ontwikkel. NIRS ontledings is gedoen met ‘n InfraAlyzer 500 spektrofotometer tussen die golflengtes 1100 en 2500 nm.

Naby infrarooi refleksie kalibrasies is ontwikkel vir (i) die basiese chemiese samestelling van volstruisvleis; (ii) die basiese chemiese en minerale samestelling van lamsvleis; (iii) die chemiese samestelling en verteerbaarheid van lupiene en volvet kanola en die bepaling van alkaloïede in lupiene; (iv) die chemiese samestelling en verteerbaarheid van wintergrane en mielies; (v) die chemiese samestelling en verteerbaarheid van graanhooie, graanstrooie, koringstoppels and lusern-gras/hooi mengsels. Die chemiese samestelling van verskillende tipes wintergrane wat in die Wes-Kaap omgewing van Suid-Afrika geproduseer word, sowel as die chemiese samestelling en verteerbaarheid van wintergraanhooi en –strooi wat geproduseer word in ‘n Mediterreënse reënval omgewing, is ook bepaal.

Naby infrarooi refleksie spektrofotometrie was suksesvol in die voorspelling van ru-proteïen (RP) en vet in beide dierspesies, sowel as vir die bepaling van as en droë materiaal (DM) in gevriesdroogde lamsvleis. Akkurate kalibrasies is ook ontwikkel vir sekere minerale (K, P, Na, Mg, Fe en Zn).

Kalibrasies ontwikkel vir die chemiese samestellings komponente in lupiene is reg deur die bank akkuraat, sowel as die DM, RP en vet kalibrasies vir volvet kanola. Die alkaloïed kalibrasie het goeie potensiaal getoon, alhoewel meer alkaloïedmonsters benodig word vir ‘n akkurate kalibrasie. Wintergraan kalibrasies was akkuraat vir vet, suurbestande vesels (SBV), *in vitro* organiese materiaal verteerbaarheid (IVOMV), lisien en metionien. Al die chemiese komponent kalibrasies vir mielies, behalwe die een vir as, was akkuraat vir toekomstige bepalings. Chemiese komponent kalibrasies vir koring stoppels en lusern-gras/hooi mengsels het akkuraatheid getoon wat gelyk, of effe beter, is as kalibrasies wat in die literatuur opgeteken is. Kalibrasies ontwikkel vir graanhooie en graanstrooie was nie geskik vir voorspellingsdoeleindes nie. Moontlike redes vir die onakkurate kalibrasies is volledig bespreek.

Variasie gevind tussen verskillende tipes wintergrane (2-ry gars, 6-ry gars, hawer, koring en korog) het die behoefte beklemtoon vir ontledings van grane geproduseer onder verskillende omgewingstoestande vir gebruik in dierevoeding. Die chemiese samestelling en verteerbaarheid van wintergraanstrooi en –hooi is ook bepaal. Hierdie waardes verskaf ‘n databasis vir berekening by insluiting van hierdie voergewasse in diererantsoene.

Hierdie ondersoek het bewys dat NIRS ‘n suksesvolle en vinnige metode is vir die voorspelling van die chemiese samestelling van volstruis- en lamsvleis en plaaslik geproduseerde voergewasse.

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Abbreviations

ADF	Acid detergent fibre
CF	Crude fibre
CP	Crude protein
DM	Dry matter
IVOMD	*In vitro* organic matter digestibility
IVDMD	*In vitro* dry matter digestibility
NDF	Neutral detergent fibre
NIRS	Near infrared reflectance spectroscopy
PLSR	Partial least square regression
r	Multiple correlation coefficient
SEC	Standard error of calibration
SEL	Standard error of laboratory
SEP	Standard error of performance
TDN	Total digestible nutrients

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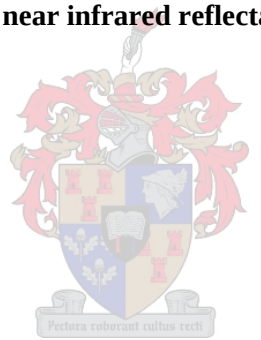

Chapter 5

Prediction of the chemical composition of winter grain and maize with near infrared reflectance spectroscopy

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Chapter 1

LITERATURE REVIEW

1.1 General introduction

The analysis of animal feedstuffs for major nutrients is one of the most important considerations in animal nutrition, since feeding costs normally comprise 75 - 80% of the cost of intensive commercial animal production enterprises. Whilst *in vivo* animal production studies will always remain the ultimate means of assessing the nutritive value of a feed, it is too time-consuming and expensive, particularly when a large number of feeds is to be evaluated. Inevitably, the increase in demand for information on feed composition, due to competitiveness in the animal feed industry, puts pressure on the analytical procedures, some of which have changed little in 130 years (Flinn, 1991).

The application of near infrared reflectance spectroscopy (NIRS) to determine the composition of feed is not a new concept. This physical, non-destructive technique represents the most significant development in feed analysis for many years. Since its introduction for the measurement of moisture and protein in grains (Hymowitz *et al.*, 1974; Williams, 1975; Norris *et al.*, 1976) the number of analytical applications of NIRS has expanded dramatically, not only in agriculture, but also in other industries. In the last decade, tremendous progress in computer technology and its commercial accessibility took place, which allowed for the use of large databanks for universal calibrations. Chemometricians can now develop more acceptable and sound applications. Near infrared reflectance spectroscopy, however, remains a secondary method and its accuracy still depends on the accuracy of the primary laboratory techniques. The NIRS method can still not be used to analyse every possible analyte in a biological product. This method is intended to analyse the major constituents in biological products and minor associated organic and inorganic analytes (Nelson, 2001).

The meat industry routinely determines meat composition (fat, water, protein) for quality monitoring and processing product formulation. Meat, as a raw material, is extremely variable and may range from 1 - 65% fat, 25 - 80% water and 5 - 25% protein. Compositional analysis on a batch-by-batch basis is essential (King-Brink *et al.*, 1996). The traditional methods (e.g. AOAC methods) of greatest accuracy and precision are typically time-consuming and involve separate methods for each component (fat, water, protein). Consequently, numerous rapid methods have been developed for use in meat composition measurements. However, these methods usually measure only one component at a time. Near infrared technology has unique potential for applications in the meat industry, because it is both rapid and capable of measuring several components simultaneously. Near infrared spectroscopy has shown promise as a rapid and effective tool for predicting the meat composition of different animal species, either in the laboratory (Kruggel *et al*., 1981) or with on-line determinations (Isaksson *et al*., 1996) using reflectance, transmittance or fiber optic technology (Mitsumoto *et al.*, 1991). Some of the applications of NIRS in the meat industry include analysis

of proximate values, discriminating between species or muscle groups, measuring sensory characteristics and determining colour and salt. NIRS is also used for on-line analysis in the meat industry.

The major advantage of NIRS is that it is a non-destructive analytical method requiring no chemical reagents. Once calibrations are in place, it is a rapid and less expensive technique compared to conventional analytical methods.

1.2 Theory of NIRS

Infrared is the region of the electromagnetic spectrum located next to the visible region. Near infrared owes its name to being the "near" part of the infrared region in relation to the visible region and is usually defined by the wavelength range 700 to 3000 nanometers (nm) (Norris, 1989). It is common to divide the near infrared region into two parts. Light in the range 1200 to 2500 nm is absorbed heavily by water and is therefore used for reflectance measurements, while the range 700 to 1200 nm is suitable also for transmission measurements since the water absorption is significantly less. The near infrared region is characterised by absorption bands caused by stretching vibrations of hydrogen (H) bonds with carbon (C), oxygen (O) or nitrogen (N) atoms. Infrared light contains less energy (per photon) than visible light and much less energy than ultraviolet light and X-rays. As a result, infrared light is relatively safe, its photons being unable to break down organic molecules. It can, however, increase the temperature of a material and is therefore used as a heat source (e.g. in cooking). Even though infrared light contains relatively little energy, it still interacts with substances and can be absorbed by materials, leading to an increase in the vibrations between atoms within a molecule and consequently to an increase in the temperature of the material. Molecular bonds are not static connections between atoms, although they are typically present as such. The easiest way to visualise a molecular bond is by imagining that the bonds between atoms are like springs (instead of bars). Whenever the molecule gets disturbed, these springs allow the atoms to vibrate (Figure 1). Materials can also emit infrared light when the vibrations return to their original state (Van Kempen, 2001).

The light frequency required to increase a molecule's vibrations is dependent on the molecule's configuration (Figure 2). For example, to increase the vibrations between the hydrogen and carbon atoms in glucose, the atoms require light of a different frequency from that required for the same bond in lysine. It is this characteristic of infrared light that can be utilised for identifying and quantifying materials as each molecule has its own infrared profile or fingerprint. The amount of light absorbed is a direct function of the concentration of the molecules present (Van Kempen, 2001).

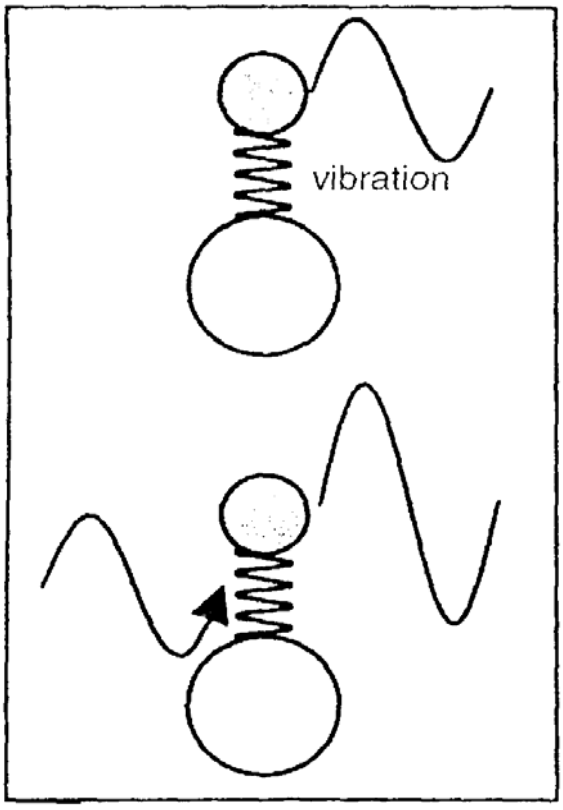

Figure 1 Vibrations within molecules (Van Kempen, 2001).

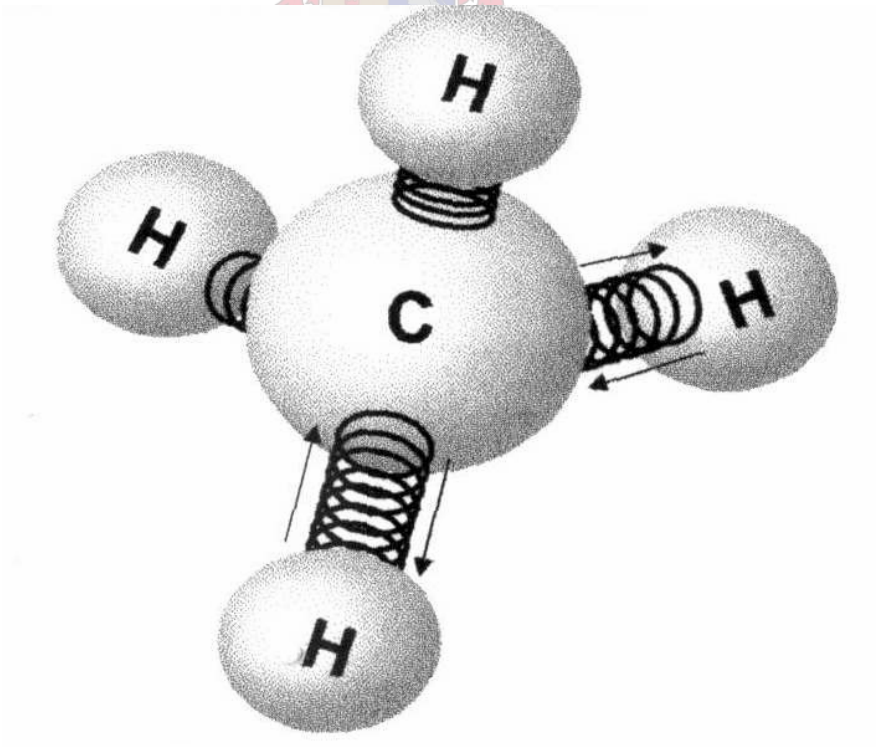

Figure 2 Vibrating bonds in a methane molecule (Shadow, 2000).

Near infrared spectrophotometers were traditionally based on filters (up to 19) for obtaining different wavelengths of light. Modern equipment typically uses monochromators, specially constructed mirrors and concave defraction gratings that reflect light in a wavelength-dependent manner, thus allowing exposure of the sample to a single wavelength at a time (Van Kempen, 2001). This allows samples to be scanned over the entire near infrared region (they are also referred to as scanning instruments). Constituents PRESENT IN small concentrations, which were normally too small to be detected with filter instruments, can now be predicted with scanning instruments. However, as this monochromator relies on mechanical positioning of the mirror for its wavelength accuracy, the spectra generated are less reproducible from machine to machine. This makes the sharing of calibrations between different laboratories more difficult (Van Kempen, 2001).

When a sample is irradiated with monochromatic light, some energy is absorbed by the chemical constituents present and some is diffusely reflected. Therefore, the near infrared diffuse reflectance signal contains information about the composition of the sample. The difference between the energy entering the sample and the diffuse reflectance escaping, is measured by the NIRS instrument and is related to the concentration of the constituent (Norris, 1989). Examples of this phenomenon are given in Figures 3 - 5.

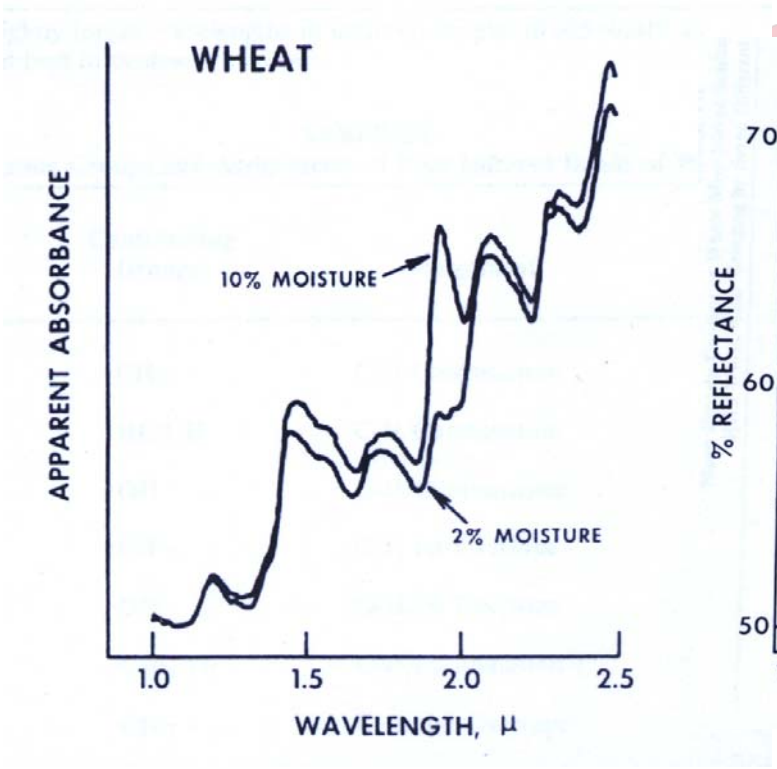

Figure 3 Reflectance spectra for ground red spring wheat containing 2% and 10% moisture (Law & Tkachuk, 1977).

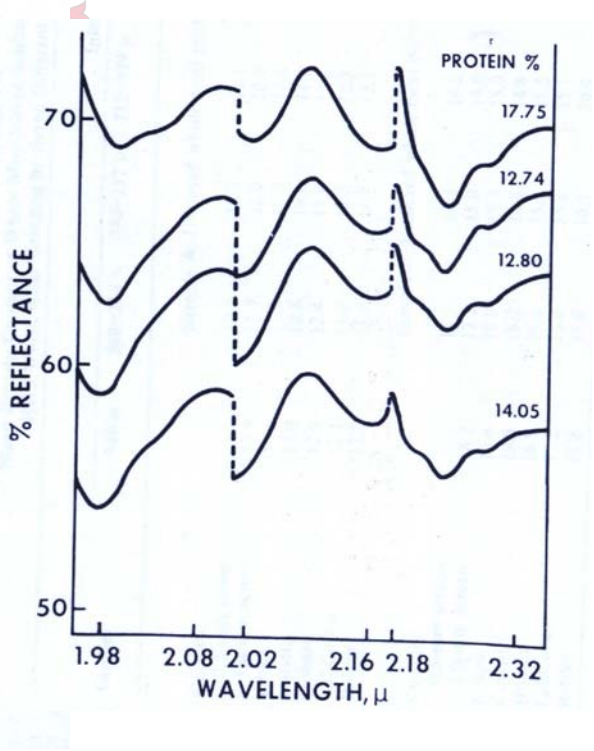

Figure 4 Reflectance spectra of ground red spring wheat of different protein contents (Williams & Thompson, 1978).

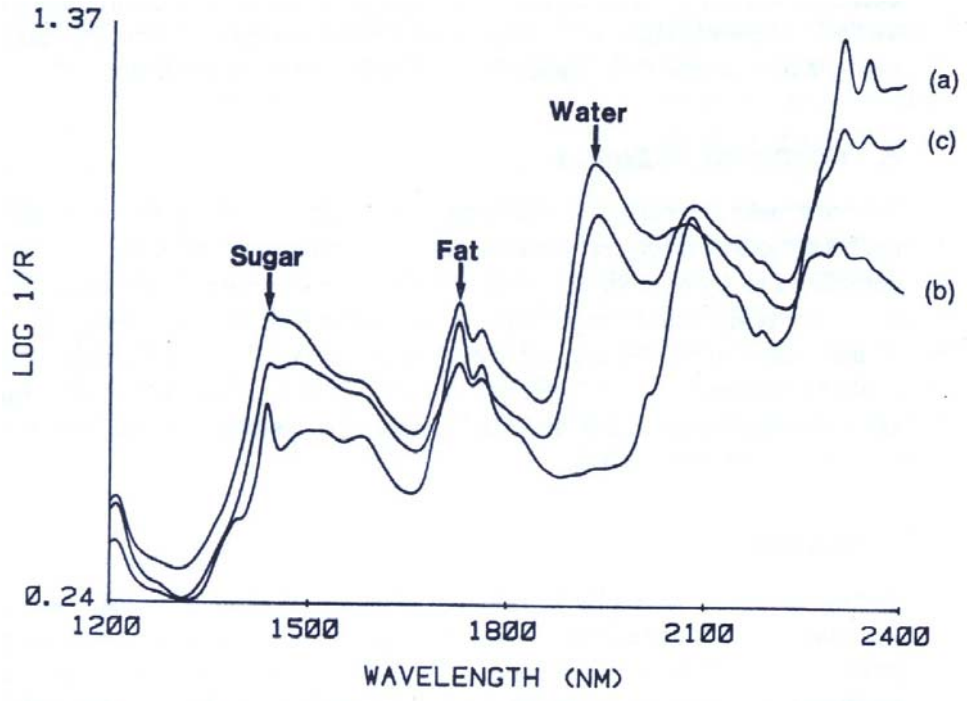

Figure 5 NIRS spectra of mixtures of sugar/fat (a), sugar/fat/water (b) and sugar/fat/flour/water (c) (Osborne, 1992).

In transmission spectroscopy, the Beer-Lambert law states that absorbance (A) is directly proportional to concentration:

$$A = \log I_o/I = \log 1/T = kcl$$

Where I_o = intensity of the incident radiation; I = intensity of the transmittance radiation; T = transmittance; c = concentration of absorbing molecules; l = path length; and k = a constant of proportion.

This relationship is fundamental to spectroscopy and can equally be applied to the diffuse reflectance of light scattering materials. In the case of NIRS, reflectance (R) is analogous to transmittance, so

$$A = \log 1/R = kcl.$$

However, path length in the reflectance mode cannot be a constant as it is in transmission measurements, so it becomes an extra unknown as well as concentration. This means that NIRS measurements must be made at several wavelengths, with the use of complex mathematical procedures (Flinn, 1991).

Consequently NIRS spectra are plots of log 1/R against wavelength and a typical near infrared scanning monochromator will yield 700 readings for every sample between 1100 and 2500 nm. The spectra appear as smooth, rolling lines with few well-defined features, but consist of many overlapping bands, since the reflectance spectrum of an intact feed, for example, is the summation of the spectra of its major chemical components (Norris, 1989). The challenge to the chemist is to extract analytically useful information on the chemical composition from the reflectance data (Flinn, 1991).

It is the shape of the NIR spectral line, or the rate of change in slope with wavelength, that conveys chemical information (Barton, 1989). First or second derivative plots are therefore useful as they can resolve overlapping peaks into component absorptions and to a large extent remove baseline variations (Barton, 1989).

1.3 Transmittance vs. Reflectance

Originally, the sample presentation method used in near infrared spectrometers was transmittance where the sample was introduced into a narrow, wedge-shaped cell (Van Kempen, 2001). However, even when working with finely ground samples, it is very difficult to fill these cells in such a manner that the material is distributed evenly. As a result, near infrared transmission is not very popular for the use in animal feeds (Van Kempen, 2001). Transmittance is successfully used in mid infrared spectroscopy where solid samples are diluted with potassium bromide (transparent in the mid-infrared region), a procedure which adds to the time taken to process the sample.

An alternative method of sample presentation is to use reflectance. For this technique the sample is placed in a cup and illuminated with near infrared light. This light can have two different fates: it can be reflected off the surface of the sample or it can enter the sample where it may be absorbed if it encounters a bond that matches the light's frequency. The percentage of light absorbed varies with wavelength, according to the chemical and physical structure of the sample. The amount of light absorbed may be determined by comparing the intensity of the original light with that of the reflected light (Shadow, 2000).

The near infrared reflectance technique presents several problems as light reflection is strongly affected by the characteristics (e.g. size) of the particles. Light reflection off the surface of particles confounds the measurement, and spectral data have to be corrected mathematically for this, thereby introducing an additional error (Van Kempen, 2001).

According to Holman (personal communication, 2003, LabWorld (Pty.) Ltd., Unit 6, Sunninghill Office Park, Peltier str, Sunninghill, 2157, South Africa), most instruments employed in SA, both for feeds and grain, are reflectance instruments. Holman also states that the advantages of reflectance vs transmittance is that reflectance: (a) does not need a critical sample thickness/lightpath; (b) reflected energy is measured more accurately than transmitted energy through a sample; and (c) optically dense samples (sunflower and canola) can only be analysed using reflectance (light cannot pass through this sort of sample unless the thickness/light path is very narrow rendering transmittance machines ineffective).

1.4 Calibration

Routine use of the NIRS technique first requires that the instrument be calibrated against a standard reference method. Many different calibration methods have been proposed, but there are basic principles, which have been shown to be vital to the success of NIRS calibrations. The first major step involves the selection of a set of calibration samples from a larger population. These samples must represent all sources of variation likely to be found in future unknown samples of similar material, including sample preservation and processing methods (Shenk *et al.*, 1979; Windham *et al*., 1989; Cozzolino & Murray, 2002). Samples can be selected from a population in a random or structured fashion, or on the basis of spectral characteristics.

The optimum number of samples to be selected for calibration has been the subject of some debate. Windham *et al.* (1989) concluded that in narrow-based or "closed" populations, less than 100 samples (minimum 50) were usually adequate, whereas in broad-based or "open" populations, 150 or more samples were necessary. In a closed population, all samples (and hence all variation) are available at the time of calibration. In an open population, new samples may have to be periodically added in order that the calibration remains robust indefinitely (Barton *et al.*, 1986; Flinn, 1991). Calibration equations developed from closed populations often yield better statistics but have limited value beyond those populations, compared with robust, broad-based equations from open populations (Shenk & Westerhaus, 1991).

Once the calibration set has been selected, the samples must be analysed for the constituents of interest by conventional reference methods. This step is the most difficult, but essential, in developing an NIRS calibration. Poor calibration accuracy has often been incorrectly blamed on NIRS instruments, while in most cases the laboratory reference method (and associated factors, such as wrong numbering of samples and transcription errors) was wrong (Williams & Sobering, 1996). The major drawback with NIRS is that wet chemistry is both the basis for developing and evaluating a calibration, or both "judge and jury" (Flinn, 1991).

The mathematical treatment of data necessary in relating log 1/R values to chemical components is a study in itself. The technique partial least square regression (PLSR) is most commonly used for the calibrations of feedstuffs and meat (King-Brink *et al.*, 1996; Berardo, 1997; Bruno-Soares *et al.*, 1998; Park, *et al.*, 1999; Alomar *et al*., 2003; Cozzolino & Murray, 2002).

When the "best" calibration equation has been selected, it must be validated with samples not included in the original calibration (Windham *et al*., 1989). When applied to open populations, such as when feed samples are routinely tested in a feed mill, the selected equations also need to be monitored on a regular basis. The performance of a calibration equation on sets of validation samples will depend on the degree to which all sources of variation in the validation samples are encompassed in the calibration set.

1.5 Use of NIRS for the prediction of the composition of meat samples

There have been a few reports on the proximate analysis of different types of meat by NIRS. Kruggel *et al.* (1981), Lanza (1983), Mitsumtoto *et al.* (1991), King-Brink *et al.* (1996), Alomar *et al.* (2003) and

Cozzolino & Murray (2002) have reported NIRS calibrations for moisture, protein and fat content in beef. Lanza (1983) and King-Brink *et al.* (1996) also did work on the proximate analysis of pork. Calibrations have also been developed for the proximate analysis of lamb (Kruggel *et al.*, 1981; Cozzolino & Murray, 2002), chicken (McElhinney *et al.*, 1999; Cozzolino & Murray, 2002) and turkey (McElhinney *et al.*, 1999). Standard error of performance (SEP) values ranged from 0.57 - 1.92%, 0.48 - 0.79% and 0.28 - 2.49% for moisture, protein and fat content, respectively between the different types of meat. The differences between the SEP values showed the diversity between different types of meat, especially the fat content. Alomar *et al.* (2003) reported that a lack of strong predicting ability for protein in meat exists. Most of the calibrations were developed with minced meat. Cozzolino & Murray (2002) developed calibrations for intact meat samples and SEP values ranged from 1.52 - 1.59% for moisture, 0.69 - 2.39% for protein and 0.81 - 4.69% for fat content. The obtained values were less accurate than those of minced samples, but the practical application for analysis of intact samples is of more use in the meat industry.

Discriminating analysis:

It is not easy to distinguish visually between different cuts and species of meat, especially when they are deboned and frozen in large blocks (McElhinney *et al.*, 1999). This could lead to adulteration where more expensive cuts are replaced with cheaper ones. Given also the particularly high value of certain meats, adulteration with cheaper species has the potential to yield considerable financial rewards. Additionally, consumers may desire to avoid certain meat species for religious (for example, pork) or perceived health (for example, beef) reasons. As a result of the increased incidence of bovine spongiform encephalitis (BSE) in beef cattle, especially in the UK, there is greater consumer interest in the avoidance of such meat (McElhinney *et al.*, 1999). In order to detect such fraud and protect consumers and traders, rapid screening methods for meat species identification are needed.

Ding & Xu (1999) conducted a study with NIRS on kangaroo and beef meat and they showed that beef could be differentiated from kangaroo meat with classification accuracy up to 100%. Thyholt *et al.* (1997) and McElhinney *et al.* (1999) conducted NIRS studies on dry extract and raw homogenised meats, respectively, and in both studies single-species meat samples were classified 90 - 100% correctly. In the group classification, to discriminate between different types of meat, their best models produced between 85 and 100% accuracy. NIRS proved thus to be an effective screening method for classification of different types of meat and to detect species adulteration.

Another area where discriminating spectroscopy has been used is to distinguish between fresh and frozen-then-thawed meat (Downey & Beauchêne, 1997; Thyholt & Isaksson, 1997). Meat is increasingly consumed as part of the ready-to-eat products of the food processing industry. As a result of its high value, the opportunity presents itself for the fraudulent replacement of premium quality material with grades which are inferior. Fresh meat is, indeed, understood as being meat that has been chilled post-slaughter and stored at normal refrigeration temperatures prior to purchase or use (Downey & Beauchêne, 1997). For storage over longer periods (months up to years) freezing is normally utilised. However, while frozen storage is

effective in protecting against microbiological deterioration of meat, the meat's organoleptic properties suffer. The consumer perception of such meat is inferior to that of the fresh material and this is reflected in the price which it realises (Downey & Beauchêne, 1997). Both studies (Downey & Beauchêne, 1997; Thyholt & Isaksson, 1997) concluded that NIRS can be used as a screening method to distinguish between fresh and frozen-then-thawed meats, although the composition of the meat juice should be used, rather than the analytical values of the meat itself.

Sensory characteristics, colour measurement and salt determination:

Studies were also conducted for the measurement of hardness, tenderness and juiciness in raw, intact meat samples with NIRS(Hildrum *et al.*, 1994; Park *et al.*, 1998). Lack of consistency in meat tenderness has been identified as one of the major problems facing the beef industry (Park *et al.*, 1998). NIRS technology to accurately classify beef for tenderness based on cooked *longissimus* sheer force has been developed (Shackelford *et al.*, 1997), but some beef processors are reluctant to implement the technology because it is destructive. NIRS calibrations for tenderness were conducted, using Warner-Bratzler shear force as reference values. NIRS was able to predict the *longissimus* Warner-Bratzler shear force with correlation coefficients (r) in the range 0.80 - 0.90. Juiciness, however, was not well predicted (Hildrum *et al.*, 1994; Park *et al.*, 1998). The authors suggested that the level of accuracy obtained would likely be less than that reported, because of the variability in the aging response.

McCaig (2002) used visible/near-infrared reflectance spectroscopy (VNIR) to measure the L**a***b** colour characteristics for 50 food and agricultural products. The relationship with VNIR and the tristimulus colorimeter was very high with correlation coefficients of 0.97 - 0.98, 0.94 - 0.96 and 0.98 - 0.99 for L*, *a** and *b**, respectively.

Begley *et al.* (1984) used NIRS to measure the amount of salt (NaCl) in canned cured hams. They reported a high correlation (r = 0.96) and a SEP value of 0.17%. They explained that the ability of NIRS to measure salt is due to the shift in the water spectrum caused by salt-induced changes in the amount of hydrogen bonding.

On-line analysis:

Both Isaksson *et al.* (1996) and Tøgersen *et al.* (1999) reported the use of NIRS for on-line measurements in meat processing plants. SEP values were reported that ranged from 0.82 - 1.49% for fat, from 0.94 - 1.33% for water and from 0.35 - 0.70% for protein, depending on the sample set and species of animal. The values were slightly higher than off-line predictions. However, because of the labour-saving effects of on-line analysis, and the uncertainty of manual sampling methods for off-line analysis, the prediction errors are acceptable to the meat processing trade, and in fact put to regular use.

1.6 Chemical composition of animal feed samples

Cereal grains are important agricultural crops in the Western Cape region of South Africa. The protein content of grains can vary from 8 - 21% (barley), 10 - 22% (wheat), 8 - 21% (triticale) and 8 - 21% (oats) as described by Brandt *et al.* (unpublished data). Most grain types grown on farms, for the purpose of producing grains for human and livestock consumption, yield considerable amounts of crop by-products, which are normally consumed by ruminant animals. Such by-products usually contain high amounts of fibrous substances (Kosilla, 1984). The chemical composition of different types of hay and straw produced at different locations may, however, differ due to different climatic and soil conditions. Ley cropping can also influence soil conditions. The variation in composition of grain and grain by-products could therefore have a noticeable effect on animal performance if the diet is formulated on mean table values.

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1.7 Use of NIRS for the prediction of forage quality

Conventional analytical methods and animal feeding trials have been used to evaluate feed and forage materials (Flinn, 1991). However, the cost and labour requirements are excessive resulting in difficulties in performing cost analysis for research or advisory purposes. Analysing a sample for ash, dry matter (DM), crude protein (CP), fat, crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), *in vitro* organic matter digestibility (IVOMD) and true digestible nutrients (TDN) could take up to three weeks and cost as much as R690.00 per sample (December 2002, LNR Irene, Private Bag X2, Irene, 0062).

Most reports attribute the first successful use of NIRS for the predicting of the *in vitro* and *in vivo* digestibility of forages to Norris *et al.* (1976). Using a scanning monochromator instrument, Norris *et al.* (1976) found r values of 0.78 and 0.90 and SEP values of 3.58% and 2.64% for the prediction of *in vivo* and *in vitro* DM digestibility, respectively. Norris *et al.* (1976) were also the first to demonstrate, for forages, the enhanced predictive ability of NIR spectra transformed by a second-order derivative of the basic log 1/R spectral data.

In the years following the work of Norris *et al*. (1976), there were a large number of reports on the use of NIRS to predict many aspects of forage composition. A large number were devoted to the estimation of chemical fractions, such as CP and NDF (Shenk *et al.*, 1981; Shenk & Westerhaus, 1985; Redshaw *et al.*, 1986; Flinn & Murray, 1991; Orman & Schumann Jr., 1991; Smith & Flinn, 1991; Hardy *et al.*, 1995; Rippke *et al.*, 1995; Berardo, 1997; Bruno-Soares *et al.*, 1998). Depending on the instrument, calibration procedure employed and the forage species investigated, standard errors of performance have been found to range from 0.8 - 4.1% (ash), 0.19 - 2.33% (DM), 0.26 - 7.2% (CP) 1.45 - 11.5% (NDF), 1.26 - 7.1% (ADF), 1.51 - 3.74% (*in vitro* dry matter digestibility - IVDMD), 2.51 - 4.3% (IVOMD), 0.18 - 0.27% (fat) and 1.23 - 6.3% (CF) (Shenk *et al.*, 1981; Shenk & Westerhaus, 1985; Redshaw *et al.*, 1986; Flinn & Murray, 1991; Orman & Schumann Jr., 1991; Smith & Flinn, 1991; Hardy *et al.*, 1995; Rippke *et al.*, 1995; Berardo, 1997; Bruno-Soares *et al.*, 1998; Park *et al.*, 1998). In 1988 the Association of Official Analytical Chemists accepted NIRS as an official method for the determination of CP and ADF content in forages (Barton & Windham, 1988).

Although the estimation of the protein content of cereal was one of the earliest applications of NIRS, more recent work has highlighted other possibilities, which proved the NIRS method capable of measuring other nutritional qualities. Edney *et al.* (1994) attempted to predict kernel plumpness (related to quality for rolling) of whole barley by NIRS, although the high SEP (11.5%) was disappointing. NIRS was investigated in a study by Ridgway & Chambers (1996) as a non-destructive detection method of insect stored-grain pests found externally or internally in wheat kernels. They reported that for both external and internal infestation there was substantial evidence that insect protein and/or chitin and moisture were being detected by NIRS (Ridgway & Chambers, 1996).

The determination of amino acids, such as lysine and methionine, in cereals has been limited by methods that are slow and relatively expensive (Van Kempen & Simmins, 1997). The feed industry still uses the measurement of nitrogen as the quality control tool for routine feedstuff evaluation, even though nitrogen does not always correlate well with the digestible amino acid content of feed samples (Van Kempen & Simmins, 1997). Reports on the use of NIRS as a rapid method for amino acid prediction (Rubenthaler & Bruinsma, 1978; Van Kempen & Simmins, 1997; Van Kempen & Bodin, 1998) proved NIRS to be an attractive choice for routine quality control in feed mills. Rubenthaler & Bruinsma (1978) reported a multiple correlation coefficient (r) of 0.98 and standard error of 0.049 mg lysine/100g protein in wheat. Van Kempen & Bodin (1998) compared NIRS with nitrogen-based regression and found that nitrogen-based regression worked equally well for wheat samples. However, for soybean meal and animal meals NIRS was more accurate than nitrogen-based regression. Variation with nitrogen-based regression observed in the prediction of amino acid in feedstuffs ranged from 14 - 81% (Van Kempen & Bodin, 1998).

Since minerals are by definition inorganic feed components, the value of NIRS would seem limited. However, Valdes *et al.* (1985) found comparable errors for Ca (SEP = 0.40; 0.24; 0.07) and P (SEP = 0.05; 0.04; 0.04) in hay, haylage and maize silage samples, but they reported low r values (possibly due to the narrow range of chemical values) and high variability in the calibrations. In a study involving a wide range of minerals in various forages, Clark *et al.* (1987a) concluded that accurate NIRS predictions were limited to calcium (Ca), phosphorus (P), potassium (Na) and magnesium (Mg). Clark *et al.* (1989) found that only aluminum and silicon could be determined by NIRS in the forage studied. Smith *et al.* (1991) obtained a satisfactory calibration for Mg in perennial ryegrass and suggested that NIRS may be a useful tool for the preliminary screening of ryegrass lines in a breeding program aimed at increasing the Mg content. In relation to reducing the mineral output by pigs, De Boever *et al.* (1994) showed that NIRS has the potential to predict total and phytate phosphorus content in plant-based feedstuffs. It would seem that prediction of mineral contents of forages by NIRS may be possible for some minerals, due to their association with organic components. At present NIRS does not seem to be the method of choice (Givens & Deaville, 1999).

There have also been suggestions that NIRS may play a role in screening various forage species for anti-quality factors (Clark *et al.*, 1987b). These are normally present in small concentrates but may be detectable if they affect NIRS spectra. The first report of this nature was made by Clark *et al.* (1987b), who used NIR to measure the total alkaloid concentration in tall larkspur and velvet lupin samples. They found r

values to exceed 0.90 and SEC values of 0.10% for alkaloids in larkspur (alkaloid range 0.26 - 1.72%) and 0.04% for alkaloids in lupins (alkaloid range 0.09 - 0.60%). The spectral comparison of plant material and alkaloids showed strong relationships between wavelengths selected in the equations and the alkaloid peaks. They suggested that NIRS could have great potential for the rapid screening of forage species for toxicity, particularly in breeding programmes, but in the case of total alkaloid concentration, NIRS was limited by the accuracy of the chemical methods. Windham *et al.* (1988) successfully used NIRS to determine tannin concentration in *Sericea lespedeza*, and found the accuracy to be similar to that of the reference method.

1.8 Transfer of calibrations between instruments

With NIRS now widely adopted for routine analysis of feedstuffs the problem of transferability of calibrations between instruments, either within or between laboratories remains. Transferability is particularly difficult for heterogeneous and high moisture samples when differing types of equipment are being used. It would therefore be advantageous if calibrations developed on one NIRS instrument could be successfully transferred to another NIRS instrument, irrespective of manufacturer. Unfortunately spectral differences exist even between instruments of the same make and model (Park *et al.*, 1999). These differences in spectra are due to differences in the optical and electronic characteristics of the instruments and even very small differences will affect predictions of parameters when using the same equation (Van Kempen, 2001).

Transfer of NIRS calibrations across instruments is commonly achieved by adjusting the equations for slope and bias. This technique may achieve the desired result by matching spectra from different instruments. An alternative approach is to standardise the instruments so that they produce identical spectra (Shenk *et al.*, 1985) and this 'cloning' approach has been refined by Shenk & Westerhaus (1985). This method has proved successful when homogeneous material such as dried milled samples (Shenk *et al.*, 1985) or whole grain samples (Dardenne *et al.*, 1992) are tested. However, difficulties arise when fresh samples, with more than 20% moisture or high log 1/R values, are involved because at high log 1/R values the reference data become non-linear due to stray light (Shenk & Westerhaus, 1996). Park *et al.* (1999) conducted a study on the transferring of grass silage (moisture ranging between 55 - 88%) calibrations between different types of monochromators. They cloned the two scanning monochromators using the ISI (Intrasoft International) cloning software. The software produces standardisation files which when applied to spectra for the slave instruments make them 'look like' spectra from the master instrument and so allow accurate prediction by equations developed on the master instrument. The standardised spectra predictions were highly correlated to the master predictions, thus proving that this backward method of calibration transfer worked very successfully. In comparison, the common method of sloping and biasing equations for use in other instruments was examined (Park *et al.*, 1999). These researchers found that calibrations for biological parameters transferred more successfully when the cloning technique was employed. An alternative approach also used by these authors (Park *et al.*, 1999) was the forward method of calibration transfer, where the calibration set of spectra were standardised to look as if they were scanned on the slave

instrument and then the equations were rerun. These equations were then applied directly to the slave spectra. This method also proved successful, but slightly less accurate than the backward calibration transfer. The study by Park *et al.* (1999) consequently demonstrated that the method of cloning monochromators of two different types and without the use of sealed sample sets or generic standards, has proven very successful even with forages with a high moisture content.

1.9 The use of NIRS in South Africa

There are in excess of 500 fixed filter NIRS systems in South Africa which are primarily used for the grading at intake of wheat, maize, sunflower, barley, canola, soya and sorghum (M Holman, 2003, personal communication, LabWorld (Pty.) Ltd., Unit 6, Sunninghill Office Park, Peltier str, Sunninghill, 2157, South Africa). Together with these instruments there are also instruments calibrated for quality and process control of the final milled products at milling companies. Most of these fixed filter instruments use only 6 specific wavelengths. There are also instruments fitted with visible light silicon detectors which enable the accurate determination of ash and colour variances. The constituents which are most commonly analysed for include protein, fat/oil, moisture, starch, ash and fibre. In the past the animal feed industry frowned on using NIRS as a means of quality control for raw and/or final products. The problem is that different suppliers of certain raw products supply a different type of product with varying colour, texture and finish. This leads to multitudes of matrix variations in the NIRS analysis which need to be incorporated in final calibrations, whereas wheat, maize, soya and other small grains, because of their uniformity, were never a problem for the raw product analysis on NIRS.

On the final product analysis, there were hundreds of different final products produced by the different feed mills. These products would all have to be calibrated for and no feed mill produces exactly the same product as the next. Once again too many matrix variations. What was done to assist in this scenario was to develop a management software which allowed each user to develop his own calibrations and analyse each different product (raw or final) on the same instrument (M Holman, 2003, personal communication, LabWorld (Pty.) Ltd., Unit 6, Sunninghill Office Park, Peltier str, Sunninghill, 2157, South Africa). The instruments were operated from a computer that has the ability to store all the different calibrations as well as to run the regression/calibration software. This took some time to get off the ground but within a year all the major feed companies were successfully using fixed filter instruments for analysis on both raw and final products. These instruments were configured with 20 and 44 wavelengths respectively to handle the different and varying matrices inherent in the feeds. With all fixed filter instruments there is a certain degree of sample preparation. This preparation normally only involves grinding the sample through a hammermill (preferred) or a grinding mill when moisture loss in milling is a major concern (M Holman, 2003, personal communication, LabWorld (Pty.) Ltd., Unit 6, Sunninghill Office Park, Peltier str, Sunninghill, 2157, South Africa). Keeping this grinding process in mind as well as the fact that contamination of samples can take place due to dirty mills, it could already be seen that there were potential problems looming. Correct sample packing and compression also play a role in ensuring accurate results and there is a moisture loss in the

sample preparation (M Holman, 2003, personal communication, LabWorld (Pty.) Ltd., Unit 6, Sunninghill Office Park, Peltier str, Sunninghill, 2157, South Africa).

All this led to the development of 'whole grain' instruments working on Fourier Transform Infrared (FTIR), monochromator (moving) and spinning filter technology. Whole grain instruments which are used for basic grain analysis have found most of their success in spinning filter technology. This is faster than FTIR and has fewer mechanical drawbacks compared to monochromators. In the feed industry, however, monochromators would have worked better that any other NIRS principle because they can analyse samples across the NIRS spectrum. This allows for all the small nuances and matrix variations within feed products to be observed and calibrated for. However, monochromators, especially those with the moving grating, require a lot of maintenance, regular re-alignment and have problems with dust (M Holman, 2003, personal communication, LabWorld (Pty.) Ltd., Unit 6, Sunninghill Office Park, Peltier str, Sunninghill, 2157, South Africa). These are factors which prevented these instruments from being employed successfully in the feed industry. The feed industry has now begun moving towards Diode Array instruments which have a fixed grating and tremendous ease and speed of use. Diode arrays allow for 2-3 second analysis across 350-2500nm wavelengths with no sample preparation. This makes these instruments ideal for the analysis of feeds. This has led to the acquisition of scanning DA instruments around the world. The inherent disadvantages of the DA's are their high cost, initial calibration which takes time and the cost of maintenance (M Holman, 2003, personal communication, LabWorld (Pty.) Ltd., Unit 6, Sunninghill Office Park, Peltier str, Sunninghill, 2157, South Africa).

In South Africa there are not many 'on-line' applications running. The South African sugar industry has successfully used the DA instruments to monitor cane intake on-line as have some users of DA's in the cotton/sunflower and soya industry. There are also online applications where NIRS is incorporated in harvesters to analyse wheat, maize, etc coming off the field. Most users will take a sample before processing either off the line or at final stages for analysis on a freestanding instrument. As on-line analysis requires speed of analysis, this means DA technology. Other technologies will not be easy to use for online applications on moving/changing samples, as their analysis time is too long (M Holman, 2003, personal communication, LabWorld (Pty.) Ltd., Unit 6, Sunninghill Office Park, Peltier str, Sunninghill, 2157, South Africa).

Near infrared reflectance spectroscopy is considered a critical factor in the process- and quality control of all processes in the agricultural and food industry. NIRS is on the increase. However, markets are saturated and the demand for NIRS is more toward new NIRS technology to replace existing old systems. The exchange rate is a limiting factor for South Africa and Africa as these instruments all originate in Europe or the USA (M Holman, 2003, personal communication, LabWorld (Pty.) Ltd., Unit 6, Sunninghill Office Park, Peltier str, Sunninghill, 2157, South Africa).

1.10 Aim of the study

NIRS has been described as the most exciting technique to hit the agricultural and feed industries since the introduction of the Kjeldahl test, and can rapidly test the quality of agricultural (and other) products on a scale not previously imagined, with proven benefits to industry and research (Flinn, 1991). This thesis is a combined presentation of seven independent studies conducted over a two-year period. The aim of the work presented was: (i) to develop NIRS calibration models for the proximate chemical composition of ostrich meat; (ii) to develop NIRS calibration models for the proximate chemical and mineral composition of mutton; (iii) to develop NIRS calibration models for chemical composition and digestibility of lupins, full-fat canola and the determination of alkaloids in lupins; (iv) to develop NIRS calibration models for chemical composition and digestibility of winter grains and maize; (v) to develop NIRS calibration models for chemical composition and digestibility of cereal hay, cereal straw, wheat stubble and alfalfa-grass/hay mixture; (vi) to determine the differences between the chemical composition of different types of winter grain produced in the Western Cape area of South Africa; and (vii) to determine the differences between the chemical composition and digestibility of winter grain hay and straw produced in a Mediterranean rainfall area. In the Western Cape region of South Africa a lack of calibrations for locally-produced feedstuffs and meats exists. This study was therefore conducted to try and fill that void.

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Chapter 2

Prediction of the chemical composition of ostrich meat with near infrared reflectance spectroscopy

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Abstract

Near infrared reflectance spectroscopy (NIRS) was used to predict the chemical composition of freeze-dried ostrich meat samples. Tenderloin (*M. ambiens*), big drum (*M. iliofibularis*) and fan fillet (*M. gastrocnemius*) samples (n = 160) were included in the study. Samples were minced, freeze-dried and analysed accordingly to standard laboratory procedures for ash, dry matter (DM), crude protein (CP) and fat content. Samples were then scanned (1100-2500 nm) and partial least-square regression (PLSR) was used to predict the chemical composition. Multiple correlation coefficients (r) and standard errors of calibration (SEC) for the chemical analysis of freeze-dried ostrich meat were: ash (0.72; 0.29%); DM (0.72; 1.01%); CP (0.98; 0.55%); and fat (0.99; 0.29%). The r values for the validation set and the standard error of performance (SEP) for the different constituents were: ash (0.71; 0.23%); DM (0.84; 0.72%); CP (0.97; 0.64%); and fat (0.99; 0.18%). Calibrations were accurate for CP and fat.

Keywords: chemical composition, near infrared reflectance spectroscopy, ostrich meat

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Introduction

Ostrich meat is perceived and marketed as a healthy alternative to other types of red meat due to a favourable fatty acid profile and a low intramuscular fat content (Sales, 1994). The high ultimate pH value of ostrich meat makes it an ideal processing meat, since the natural water binding capacity is high, which in turn can reduce the use of moisture retaining agents such as phosphates when being processed (Fisher & Hoffman, 1998). Fat and fat substitutes can be used to formulate products of nutritional composition that can successfully compete against similar products in other red meat markets. Frequent analysis of the chemical composition is important in the processing of ostrich meat.

Near infrared reflectance spectroscopy (NIRS) has been shown to be successful as a rapid tool for predicting meat composition of different animal species either in the laboratory (Kruggel *et al.*, 1981; Lanza, 1983) or during on-line determinations (Isaksson *et al.*, 1996; Tøgersen *et al.*, 1999) using reflectance, transmittance or fibre optic technology (Mitsumoto *et al.*, 1991). NIRS have been used successfully for prediction of the composition of beef (King-Brink *et al.*, 1995; Kruggel *et al.*, 1981), pork (Lanza, 1983;

McElhinney *et al.*, 1999), lamb (Kruggel *et al.*, 1981; Cozzolino & Murray, 2002), chicken (Cozzolino & Murray, 2002; McElhinney *et al.*, 1999), turkey (McElhinney *et al.*, 1999) and kangaroo (Ding & Xu, 1999). NIRS analysis is an empirical method requiring reference methods to firstly develop the calibrations and secondly to establish periodical control checks (Alomar *et al.*, 2003). It could, however, provide rapid and accurate results of muscle chemical composition, often in conditions unsuitable for chemical analysis, such as on-line analysis in the industrial meat processing industry (Tøgersen *et al.*, 1999). Consequently, the objective of this work was to evaluate NIRS as a potential tool to predict the proximate chemical composition of ostrich meat.

Materials and methods

Samples used for calibrations (n = 160) were obtained from an unpublished study done by S.J. van Schalkwyk, 2000 (Klein Karoo Agricultural Development Centre, PO Box 313, Oudtshoorn, 6620, RSA), and consisted of tenderloin (*M. ambiens*; n = 53), big drum (*M. iliofibularis*; n = 53) and fan fillet (*M. gastrocnemius*; n = 54) samples. The samples were minced, freeze-dried, ground with a Knifetec 1095 Sample Mill (Tecator, Box 70, S-263 21 Hoganäs, Sweden) using a 1 mm sieve and analysed for chemical composition. The crude protein (CP) was measured by a FP-428 Nitrogen and Protein Determinator (Leco Corporation, 3000 Lakeview Avenue, St Joseph, MI 49085-2396). Lipid (petroleum ether extraction) was measured according to AOAC (1984) (Method number 7.061). Dry matter (DM) was determined by drying a sample (*ca*. 1.0 g) at 100°C to a constant weight and ash content by placing the sample in a furnace at 500°C overnight (AOAC, 1984) (Method numbers 7.003 and 7.009, respectively). Each reference sample was analysed once and the standard error of laboratory (SEL) was calculated with the equation:

$$SEL = s.d. / n^{0.5} \quad \text{(Snedecor \& Cochran, 1980),}$$

where standard deviation (s.d) can be described as:

$$s.d. = (\sum (x_i - x)^2 / n-1)^{0.5} \quad \text{(Snedecor \& Cochran, 1980).}$$

The SEC is defined as $[\sum(X_i - Y_j)^2/(n-p-1)]^{0.5}$ (Windham *et al.*, 1989), where X_i is the value determined by conventional analytical methods, Y_j is the value determined by NIRS, n is the number of samples and p is the number of terms in the calibration equation. The SEP is defined as $[\sum(X_i - Y_j)^2/(n-1)]^{0.5}$ where X_i, Y_j and n are as previously defined (except that X_i and Y_i are from a different population) (Windham *et al.*, 1989). Multiple correlation coefficient (r) can be described as $[1-(SEC^2(n-k-1)/s.d._{prop}(n-1)]^{0.5}$ where n is the number of calibration set spectra, k is the number of factors and $s.d._{prop}$ is the standard deviation of the reference property values (Westerhaus, 1989).

The samples were divided into two sets for each constituent: a larger set (calibration set) for development of the calibrations and a smaller set (validation set) to test the accuracy of the calibrations (n values are shown in Tables 1 and 2, respectively). Outliers were removed according to suggestions by the software (Bran+Luebbe SESAME Version 2.00-software, BRAN+LUEBBE GmbH, Norderstedt, Germany). Outliers listed as 'T'- and 'H'-values were taken into consideration. The 'T'-value measures how closely the reference value matches the predicted value. The spectrum is listed and flagged with an asterisk (*) if the 'T'-value is greater than 2.5 times the standard error of calibration. These values can be potential outliers, because they do not fit the calibration equation as well as the other samples. The 'H'-value is a measure of leverage. It places a numerical value on the influence of a particular spectrum in determining the regression line. It is a measure of multidimensional distance of a spectrum to the regression line. If a spectrum with a large 'H'-value has a small 'T' value, it is likely to be valuable for the calibration. If both the 'H' and the 'T' values are large, it is more likely to be a true outlier. Equations of best fit were chosen for each constituent based on statistical analysis. After removal of the outliers, every fifth sample was selected for the validation sets. Samples were stored in a freezer before analysis with the NIRS. Analyses were, however, performed once samples reached room temperature. Cross testing were done on the samples to test the accuracy of the reference values before the calibrations were derived.

NIRS analyses were done with an InfraAlyzer 500 Near Infrared Reflectance Analyser (IA-500) using Bran+Luebbe SESAME Version 2.00-software (BRAN+LUEBBE GmbH, Norderstedt, Germany). Approximately 6 g of each sample was packed into an open sample cup. Spectra were measured over the wavelength range 1100-2500 nm, recorded as log 1/R at 2 nm intervals. Calibration equations were developed for each constituent following the recommended protocol of Windham *et al.* (1989). Calibrations were developed by means of partial least-square regression (PLSR) on normalised spectra for ash and on second derivative spectra (segment = 1; gap = 0) for DM, CP and fat content.

Results and discussion

The range, mean values, standard deviations (s.d.) and coefficients of variation (c.v.) for the different constituents for the calibration and validation sets are shown in Table 1 and Table 2, respectively. The variation in the chemical composition of the samples used seem to cover the whole spectrum of variation found for different types of meat as reported in the literature (Morris, *et al.*, 1995; Sales, 1996; Cilliers *et al.*, 1998; Paleari *et al.*, 1998). The distribution of the reference samples for the calibration sets of freeze-dried ostrich meat are shown in Figure 1. The most favourable distribution for a calibration set would be to have an even distribution of samples throughout the sample range. Calibration sets with insufficient distribution of the samples could lead to inaccurate calibrations. DM can possibly be an example of such a calibration set, because the numbers of samples at the outer limits of the constituent range were very low. An increase in the number of samples at these points should improve the accuracy of the calibration.

Table 1 Summary of chemical composition (%) of freeze-dried ostrich meat used in the calibration set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	141	4.97	4.31	6.07	0.41	8.25
DM	142	97.11	94.07	100.42	1.43	1.47
CP	150	90.40	84.33	94.63	2.53	2.80
Fat	153	4.04	1.41	8.98	1.78	44.06

Table 2 Summary of chemical composition (%) of freeze-dried ostrich meat used in the validation set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	30	4.84	4.31	5.50	0.29	5.99
DM	28	97.01	94.53	99.37	1.42	1.46
CP	32	90.59	85.45	93.93	2.69	2.97
Fat	32	3.83	1.41	8.33	1.65	43.08

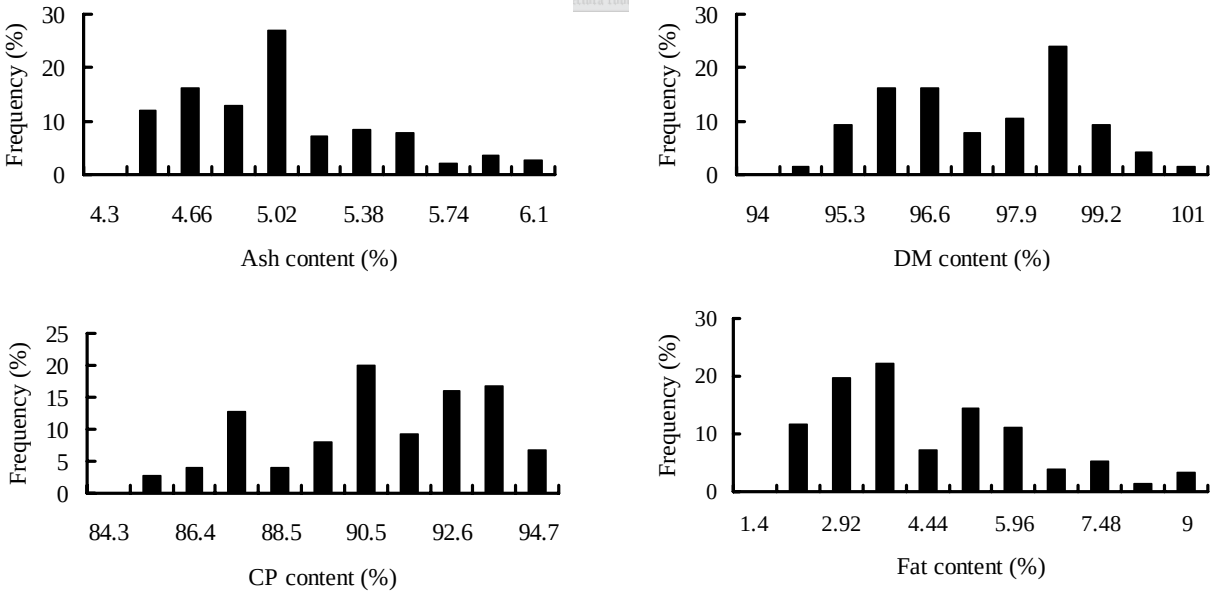

Figure 1 The distribution of the calibration sets for ash, DM, CP and fat content in freeze-dried ostrich meat.

Table 3 shows the standard error of calibration (SEC) and multiple correlation coefficient (r) values for the equations of best fit obtained for each of the constituents. The r values for the validation sets and standard error of performance (SEP) are also shown in Table 3, as well as the SEL, predicted and laboratory mean values.

If the SEP for the validation is within two multiplications of the SEL for the primary reference method analysis, the final NIRS equation can be accepted for use, and the SEP for validation can be used as a reliable indication of the accuracy of the final NIRS equation (Windham *et al.*, 1989). Standard error of performance values for CP (0.64%) and fat (0.18%) were within those limits and can therefore successfully be used to predict the chemical composition of ostrich meat. Due to the absence of literature on freeze-dried meat calibrations, values were compared with raw meat calibrations. The SEP values calculated were similar to values reported in previous studies on other species. Alomar *et al.* (2003) reported SEP values of 0.48% (CP) and 0.44% (fat) for beef. Cozzolino & Murray (2002) obtained values of 0.55% (CP) and 0.47% (fat) for lamb meat. Tøgersen *et al.* (1999) reported SEP values for on-line prediction of industrial scale ground meat batches that range from 0.94-1.33% for moisture, 0.35-0.70% for CP and 0.82-1.49% for fat.

The SEP value of DM (0.75%) was more than double the SEL value (0.27%). Ash had a low r value, together with a high SEP value. NIRS is therefore not suitable for predicting the moisture and ash values of freeze-dried ostrich meat. Alomar *et al.* (2003) also found ash in beef to be poorly predicted (r = 0.66) by NIRS. This is probably due to the fact that near infrared radiation does not interact with pure minerals or inorganic compounds in their ionic forms and salts. However, minerals can sometimes be measured with NIRS if they are related to the organic fraction, either through associations with organic acids, chelates or forming salts which affects hydrogen bonding in moist samples (Shenk & Westerhaus, 1995). Freeze-drying is an expensive and timely method, but the advantage of freeze-drying before calibrating is that freeze-dried samples are more homogeneous than raw meat samples. Kruggel *et al.* (1981) reported that the fat content influenced the particle size of the samples. Ostrich meat, however, have a very low fat content (3.83%, Table 3) and the fat globules would not have a big influence on the characteristics of the samples. Fat and CP calibrations of freeze-dried ostrich meat did not seem to be more accurate than calibrations reported on raw meat samples (Kruggel *et al.*, 1981; Alomar *et al.*, 2003). It is therefore probably not worth the cost to freeze-dry samples for NIRS calibrations and predictions. The correlation between the NIRS predicted and the laboratory determined values are shown in Figure 2.

Table 3 Statistics of the calibration equations (for freeze-dried ostrich meat) of best fit and validation, including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL).

Chemical Component	Number of PLSR factors	Calibration set r	Calibration set SEC (%)	Validation set r	Validation set SEP (%)	SEL (%)	Laboratory Mean Values (%)	Predicted Mean Values (%)
Ash	10	0.72	0.29	0.71	0.23	0.05	4.84	4.89
DM	4	0.72	1.01	0.85	0.75	0.27	97.01	97.05
CP	7	0.98	0.55	0.97	0.64	0.47	90.59	90.67
Fat	3	0.99	0.29	0.99	0.18	0.29	3.83	3.83

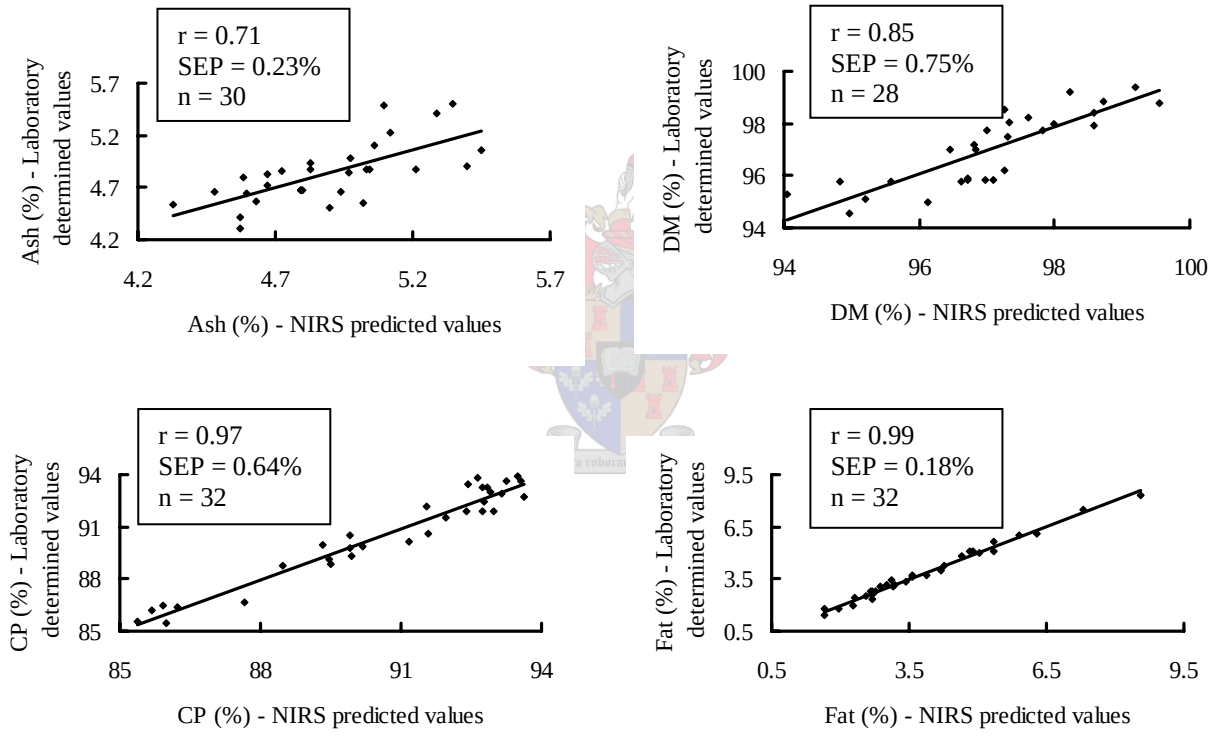

Figure 2 Relationship between laboratory determined and NIRS predicted values for ash, DM, CP and fat content of ostrich muscle (freeze-dried) using between 28 and 32 samples for each validation.

The variance plots for the different constituents are shown in Figure 3. The number of factors chosen for each calibration are marked with a dotted line. The chosen factors represent the optimum relationship between the SEC values and the SEP value, which resulted in the equation of best fit for each constituent. It is unusual for the SEP values to be lower than the SEC values as seen for the ash and DM validation plots. It should, however, be taken into consideration that the calibrations were developed for a closed population. As described in the materials and methods, the calibraiton and validation sets were separated after removal of the outliers, leaving samples with similar composition and variation.

Normalisation and second derivative spectra for freeze-dried ostrich meat are shown in Figure 4. The most important wavelengths for water, protein and fat prediction are marked alphabetically and with different colours. The bands of pure water in the 1100 to 2500 nm region consists of three bands and are marked with blue lines (A = 1190 nm, C = 1450 nm; H = 1940 nm) (Osborne *et al.*, 1993). The five protein bands in this region are marked with green lines (D = 1510; E = 1680; I = 1980; J = 2050; K = 2180) (Osborne *et al.*, 1993). The red lines represent the five bands for fat in the near infrared region (B = 1200; F = 1734; G = 1765; L = 2310; M = 2345) (Osborne *et al.*, 1993). In order to use the information contained in the spectra for the determination of fat, for example, is it necessary to correct the absorption due to overlapping absorptions. A good example is the overlapping of water at 1190 nm (position A in Figure 4) with fat at 1200 nm (position B in Figure 4). The peak for fat is not clearly noticeable in the normalised transformation, but when the transformation was changed to second derivative, the peak for fat was clearly noticeable next to the water peak. The same occurrence was found at wavelengths 1940 nm (H) and 1980 nm (I) where the peaks for water and protein overlapped in the normalised transformation. The peaks, however, is again clearly noticeable in the second derivative transformation. Another example was the overlapping of two broad peaks at wavelengths 2310 nm (L) and 2345 nm (M), where the intensity of the two peaks was reduced by one another in the normalised transformation (Osborne *et al.*, 1993). The intensity of the two peaks, however, was clearly visible in the second derivative transformation. When compared to a peak of similar intensity in the second derivative transformation, for example the protein peak at 2050 nm (J), the overlapping of the two fat peaks in the normalised transformation was emphasised.

Conclusion

The NIRS calibrations developed in this study showed high correlation coefficients and SEP values relative to analogous AOAC methods, particularly for fat and CP. The calibrations for ash and DM were not that accurate on freeze-dried ostrich meat. The accurate correlation for CP and fat indicated that applications to the meat industry could be very useful. The advantages of time savings and multi-component analysis offered by the NIRS measurement are major considerations for the meat industry. It is, however, suggested that calibrations should be developed on raw meat samples, due to the time and cost implications of freeze-drying.

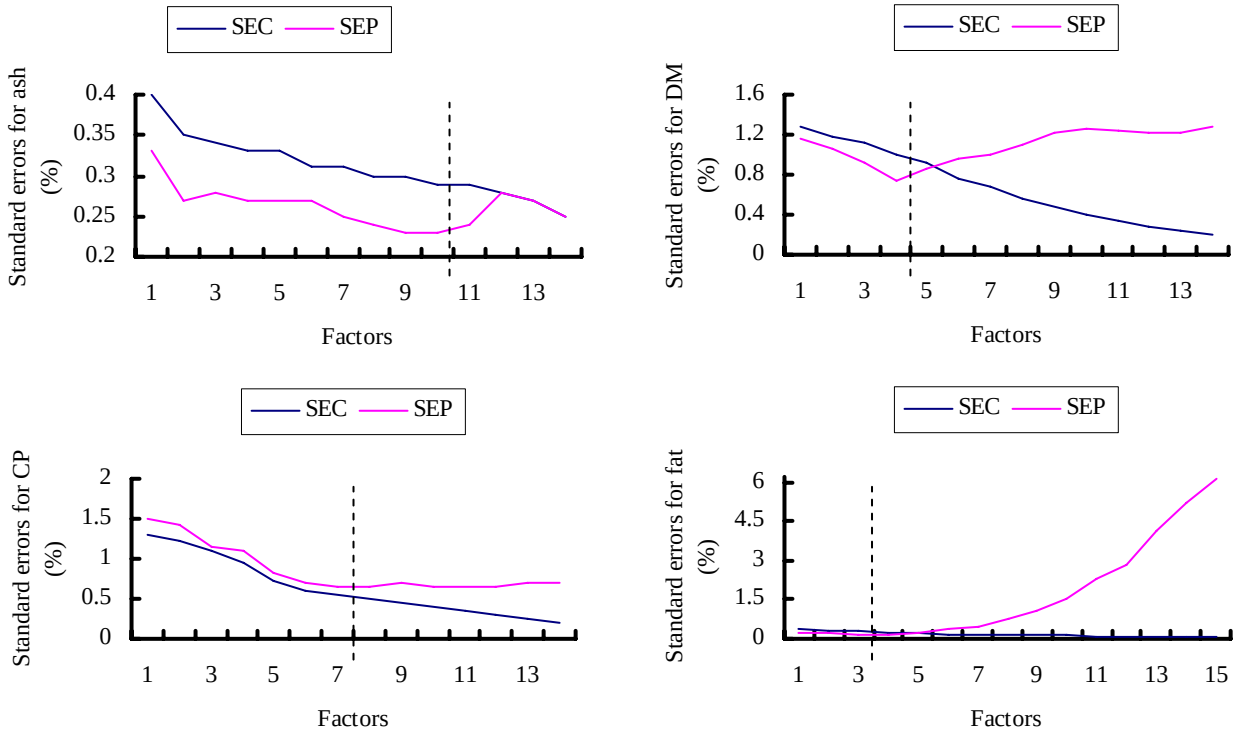

Figure 3 Variance plots for ash, DM, CP and fat in freeze-dried ostrich meat.

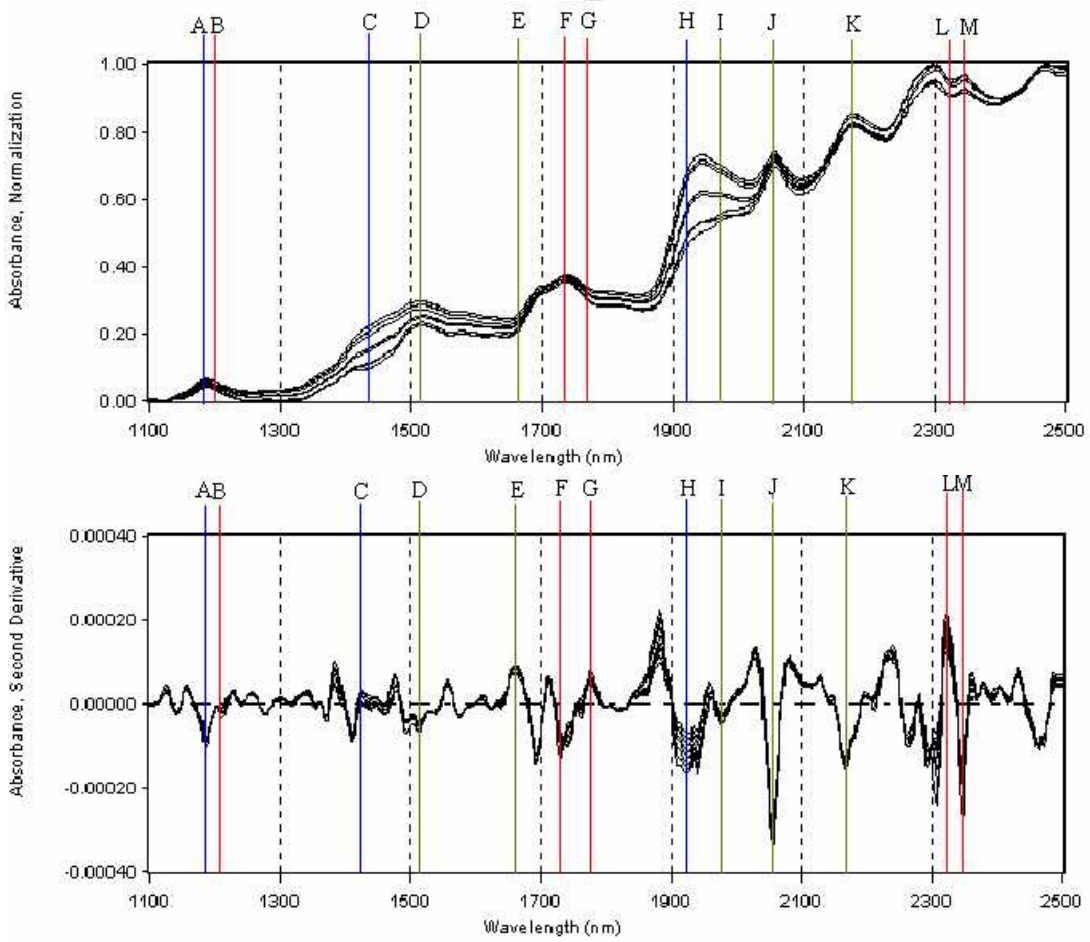

Figure 4 Absorbance and second derivative spectra of freeze-dried ostrich meat samples, showing the most important wavelengths for water, protein and fat prediction.

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Chapter 3

Prediction of the chemical composition of mutton with near infrared reflectance spectroscopy

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Abstract

Near infrared reflectance spectroscopy (NIRS) was evaluated as a tool to predict the chemical composition of freeze-dried mutton. Samples used for the ash, dry matter (DM), crude protein (CP) and fat calibrations consisted of *M. longissimus dorsi* (eye muscle) from 19-month-old Merino sheep, while mineral calibrations were developed with *M. semimembranosus* from Merino crossbreed lambs slaughtered at a live weight of 40 kg. Samples were minced, freeze-dried and analysed according to standard laboratory procedures. Samples were scanned (1100-2500 nm) and partial least-square regression (PLSR) was used to predict the chemical and mineral composition. Multiple correlation coefficients (r) and standard error of performance (SEP) for chemical composition constituents were: ash (0.97; 0.15%), DM (0.96; 0.38%), CP (1.00; 0.92%) and fat (1.00; 0.43%), respectively. K, P, Na, Mg, Fe and Zn showed acceptable SEP values of 600 mg/kg, 900 mg/kg, 77.89 mg/kg, 40 mg/kg, 3.15 mg/kg and 3.59 mg/kg, respectively. The r values ranged from 0.86 for Zn and K to 0.92 for Mg. Very low r-values (0.26 - 0.49) were obtained for Cu, B, Mn, Ca and Al. It was concluded that NIRS could be used as a rapid tool for predicting proximate chemical composition and certain minerals in freeze-dried mutton.

Keywords: chemical composition, mineral composition, mutton, near infrared reflectance spectroscopy

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Introduction

Near infrared reflectance spectroscopy (NIRS) has been developed as a rapid tool for estimation of chemical composition of foods (Osborne, 1992). It has been used for the determination of moisture and protein content in cereal grains (Williams, 1975; Shenk & Westerhaus, 1985; Reeves, 1994), moisture, protein and oil contents of oilseeds (Hymowitz *et al.*, 1974; Krishnan *et al.*, 1994) and major constituents in forages (Norris *et al.*, 1976). This spectropic technique has been developed to replace the laborious, time-consuming and expensive conventional methods, i.e. Kjeldahl method for protein, various solvent extraction methods for fat and oven-drying methods for moisture (Lanza *et al.*, 1983). Ben-Gera & Norris (1968) used transmission spectroscopy in the NIR range to determine the fat and moisture contents of meat products. Kruggel *et al.* (1981) estimated fat, moisture and protein contents in fresh emulsified beef and ground lamb by NIR reflectance, while Lanza (1983) determined moisture, protein, fat and calorie contents in raw

emulsified pork and beef by NIR reflectance and transmittance. All these studies, however, were conducted on fresh meat. The energy absorbed by water is temperature dependent. This is due to the presence of hydrogen bonds between the molecules, which alter the force constant for the covalent O-H bond and the frequency of the O-H absorption band. The hydrogen bonds may also give a distribution of O-H bond lengths, which give rise to the broad area of absorption (Thyholt & Isaksson, 1997). An increase in temperature causes disruption of hydrogen bonds by thermal collisions, giving a change in the absorption profile. Thus, there will be an increase in absorption at the higher frequency area of the O-H absorption region. A temperature shift will also affect hydrophilic components such as protein and carbohydrates, which form hydrogen bonds with the water molecules. Therefore temperature fluctuations reduce the accuracy of NIR analysis of several compounds if water is present (Thyholt & Isaksson, 1997). Consequently, removing water (e.g. by freeze-drying) means removing the hydrogen bonding interference and giving small molecules, such as sugars, amino acids and minerals, more characteristic spectra.

Minerals do not have reflectance spectra in the infrared region. If some form of correlative relationship can be found, it would be in association with some organic constituent(s) that varies as the mineral varies in the sample (Shenk *et al.*, 1979). Minerals in agricultural products probably exist in both organic and inorganic complexes. The possibility that NIRS could be used for determining mineral concentrations would therefore seem remote (Clark *et al.*, 1987). Shenk *et al*. (1979; 1981), Valdes *et al.* (1985) and Clark *et al.* (1987; 1989), however, reported the use of NIRS for determining mineral composition in forages. Shenk *et al.* (1979) and Valdes *et al.* (1985) reported accurate calibrations for K, Mg, Ca and P. Clark *et al.* (1987) reported successful calibrations for Ca, P, K and Mg and suggested that NIRS is indirectly measuring these minerals by their association with organic acids. They did not, however, find any similarities in wavelengths chosen for P and those highlighted in phytate or phosphate spectra.

The aim of this study was to develop NIRS calibrations for the proximate and mineral composition of freeze-dried mutton samples.

Materials and methods

Samples analysed for ash, dry matter (DM), crude protein (CP) and fat used for NIRS calibrations were the same as those used in a study by Cloete (2002) and consisted of *M. longissimus dorsi* from 19-month-old Merino sheep. Mineral calibrations were developed with *M. semimembranosus* samples from Merino crossbreed lambs slaughtered at a live weight of 40 kg. The samples were minced, freeze-dried, ground with a Knifetec 1095 Sample Mill (Tecator, Box 70, S-263 21 Hoganäs, Sweden) using a 1 mm sieve and analysed for chemical composition. The protein was measured by a FP-428 Nitrogen and Protein Determinator (Leco Corporation, 3000 Lakeview Avenue, St Joseph, MI 49085-2396). Lipid (petroleum ether extraction) was measured according to AOAC (1984) (Method number 7.061). Moisture was determined by drying a sample (*ca*. 1.0 g) at 100°C to a constant weight and ash content by placing the sample in a furnace at 500°C overnight (AOAC, 1984) (Method numbers 7.003 and 7.009, respectively).

Minerals were determined according to Watson (1994). Element concentrations were measured on an ICP-AES (Inductive Coupled Plasma Atomic Emission Spectrophotometer; Liberty Series AA Varian).

All samples were divided into two sets for each constituent: a larger set for the calibration equations (calibration set) and a smaller set for the validation (validation set) of the calibrations (n values are shown in Tables 1, 2, 4 & 5, respectively). Outliers were removed according to suggestions by the software (Bran+Luebbe SESAME Version 2.00-software, BRAN+LUEBBE GmbH, Norderstedt, Germany). Outliers listed as 'T'- and 'H'-values were taken into consideration. The 'T'-value measures how closely the reference value matches the predicted value. The spectrum is listed and flagged with an asterisk (*) if the 'T'-value is greater than 2.5 times the standard error of calibration. These values can be potential outliers, because they do not fit the calibration equation as well as the other samples. The 'H'-value is a measure of leverage. It puts a numerical value on the influence of a particular spectrum in determining the regression line. It is a measure of multidimensional distance of a spectrum to the regression line. If a spectrum with a large 'H'-value has a small 'T' value, it is likely to be valuable for the calibration. If both the 'H' and the 'T' values are large, it is more likely to be a true outlier. Equations of best fit were chosen for each constituent based on statistical analysis. After removal of the outliers, every fifth sample was selected for the validation sets. Wet chemistry and NIRS analyses were done simultaneously for all the samples.

NIRS analyses were done with an InfraAlyzer 500 Near Infrared Reflectance Analyser (IA-500) using Bran+Luebbe SESAME Version 2.00-software (BRAN+LUEBBE GmbH, Norderstedt, Germany). Approximately 6 g of each sample was packed into an open sample cup. Spectra were measured over the wavelength range 1100-2500 nm, recorded as log 1/R at 2 nm intervals. Calibration equations were developed for each constituent following the recommended protocol of Windham *et al.* (1989). Calibrations were developed by means of partial least-square regression (PLSR)on normalised spectra for Na, Fe, Zn and Mn, on first derivative spectra (segment = 1; gap = 0) for Al, Cu, Mg and P, and on second derivative spectra (segment = 1; gap = 0) for ash, DM, CP, fat, B, Ca and K content.

Results and discussion

The range, mean values, standard deviations (s.d.) and coefficients of variation (c.v.) of the calibration and validation sets for the chemical composition constituents are shown in Tables 1 and 2, respectively. The variation in the chemical coposition of the samples used seem to cover the whole spectrum found for mutton reported in the literature for mutton (Hopkins *et al.*, 1992; Teixeira *et al.*, 1996; Berg *et al.*, 1997). The fat content of the mutton (Table 1) showed a large variation (7.30% - 51.80%). Kruggel *et al.* (1981) suggested, in a study on ground lamb meat, that NIRS is not as suitable for the determination of protein compared to the determination of fat and moisture when used with lamb meat samples containing 17.8 to 26.2% fat. The study (Kruggel *et al*, 1981), however, was conducted on raw meat and it was found that the fat content influenced the particle size of the samples. This phenomenon was not observed in this investigation, possibly due to the use of freeze-dried samples.

Table 1 Summary of chemical composition (%) of freeze-dried mutton samples used in the calibration set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	128	3.81	2.09	5.17	0.70	18.37
DM	131	93.41	90.55	95.92	1.07	1.15
CP	118	73.92	52.94	86.95	8.97	12.13
Fat	120	19.20	7.30	51.80	11.02	57.40

Table 2 Summary of chemical composition (%) of freeze dried mutton used in the validation set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	26	3.87	2.27	4.67	0.68	17.57
DM	26	93.45	90.55	95.92	1.29	1.38
CP	23	71.74	53.49	84.33	10.48	14.61
Fat	27	19.76	7.30	51.80	12.24	61.94

Table 3 shows the statistics, including standard errors of calibration (SEC) and multiple correlation coefficient (r) values for the equations of best fit obtained for each of the constituents. The r values for the validation sets and standard errors of performance (SEP) are also shown in Table 3, as well as the standard error of laboratory (SEL) (Snedecor & Cochran, 1980) and predicted mean values. If the SEP for the validation is within two multiplications of the SEL for the primary reference method analysis, the final NIRS equation can be accepted for use, and the SEP for validation can be used as a reliable indication of the accuracy of the final NIRS equation (Windham *et al.*, 1989). All four of the chemical constituent calibrations fitted these limitations and could be accepted for rapid predictions of the constituents (ash = 0.15% (SEP) vs. 0.13% (SEL); DM = 0.38% (SEP) vs. 0.25% (SEL); CP = 0.92% (SEP) vs. 2.18% (SEL); fat = 0.43% (SEP) vs. 2.36% (SEL)). The SEC and r values for CP (1.42% and 0.99) and fat (0.66% and 1.00) were similar to that reported by Kruggel *et al.* (1981) who noted values of 0.61% and 0.77 for protein and 2.41% and 0.85 for fat in raw lamb. The reason for the difference between values obtained in the two studies could be due to the freeze-dried state of the samples used in this investigation. The physical appearance of freeze-dried meat is more homogenous than raw minced meat, due to the influence of the fat on the particle size of raw minced meat samples (Kruggel *et al.*, 1981). Water is extracted and cannot have any influence on possible temperature fluctuations. The SEP values from this investigation were similar to values obtained in studies with wet beef (DM = 0.59%; CP = 1.15%; fat = 0.27% - Lanza, 1983); (DM = 1.21%; CP = 0.45%; fat = 1.30% - Tøgersen *et al.*, 1999) and wet pork (DM = 0.66%; CP = 0.92%; fat = 0.28% - Lanza, 1983); (DM = 1.18%; CP = 0.57%; fat = 1.35% - Tøgersen *et al.*, 1999).

Table 3 Statistics of the calibration equations of best fit and validation including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL).

Chemical Component	Number of PLSR factors	Calibration set r	Calibration set SEC (%)	Validation set r	Validation set SEP (%)	SEL (%)	Laboratory Mean Values (%)	Predicted Mean Values (%)
Ash	1	0.93	0.25	0.97	0.15	0.13	3.87	3.87
DM	13	0.99	0.16	0.96	0.38	0.25	93.45	93.37
CP	3	0.99	1.42	1.00	0.92	2.18	71.74	71.58
Fat	2	1.00	0.66	1.00	0.43	2.36	19.76	19.76

The correlation between the NIRS predicted values and the laboratory determined values for the proximate composition are shown in Figure 1.

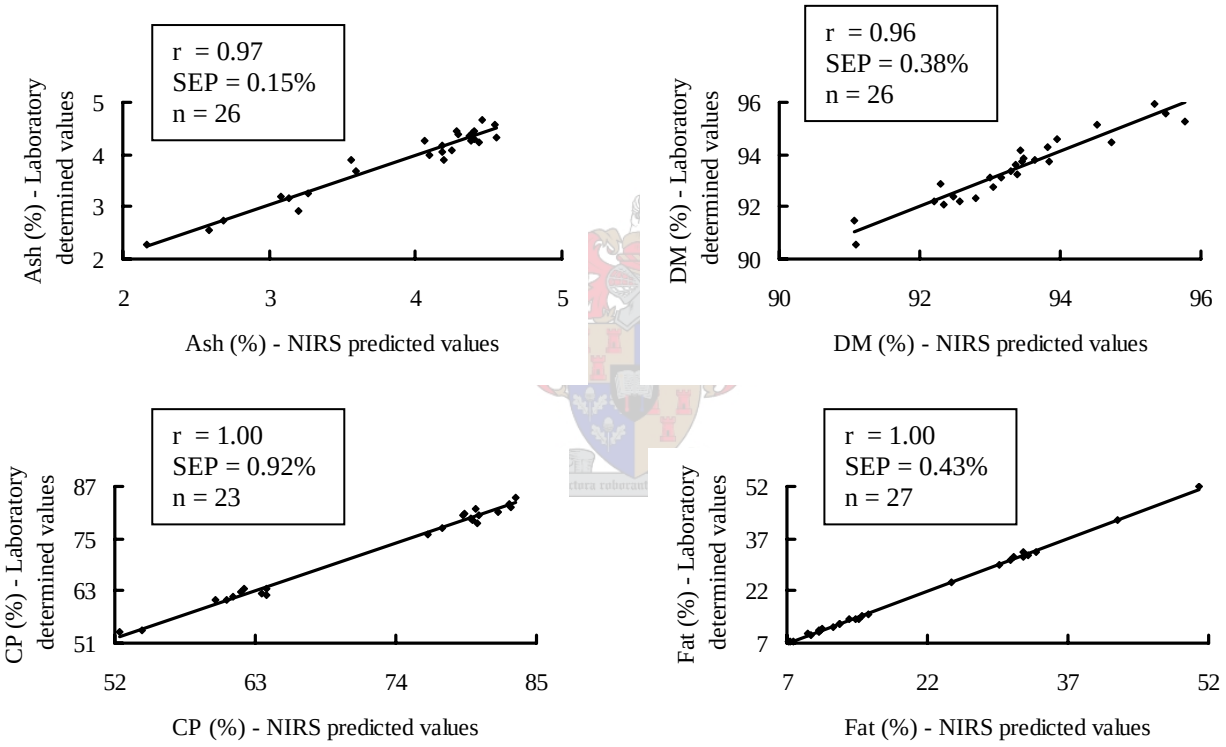

Figure 1 Relationship between laboratory determined and NIRS predicted values for ash, DM, CP and fat content in freeze-dried mutton, using between 23 and 27 samples for each validation.

Some of the important wavelengths for water (1190 nm; 1940 nm) (Osborne *et al.*, 1993), protein (1680 nm; 2050 nm; 2180 nm) (Osborne *et al.*, 1993) and fat (1200 nm; 1734 nm; 1765 nm; 2310 nm; 2345 nm) (Osborne *et al.*, 1993) in freeze-dried mutton corresponded with wavelengths noted for the same constituents in freeze-dried ostrich meat (Chapter 2), in spite of the differences in protein and fat composition of the different types of meat.

The range, mean values, standard deviations (s.d.) and coefficients of variation (c.v.) of the calibration and validation sets for the minerals are shown in Table 4 and Table 5, respectively. The number of samples used for the K, Na, B and Mn calibrations were less than the minimum number (50) of samples suggested for a narrow-based population (Windham *et al.*, 1989). This was, however, the only samples available and calibrations were attempted to test the accuracy of NIRS for the particular minerals. If the number of samples were to be increased, it would result in more robust, and probably more accurate, calibrations.

Table 4 Summary of the mineral composition (mg/kg freeze-dried) of the calibration set for freeze-dried mutton meat showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Mineral	n	Mean	Min	Max	s.d.	c.v.
K	49	9 100.00	7 400.00	11 500.00	1 100.00	12.08
P	51	8 200.00	5 000.00	10 600.00	1 900.00	23.17
Na	48	1 154.67	831.00	1 629.00	173.45	15.02
Mg	52	600.00	500.00	700.00	60.00	10.00
Cu	52	0.79	0.57	2.09	0.21	26.58
Fe	52	35.89	26.20	58.40	6.60	18.39
Zn	50	56.78	45.90	72.30	6.07	10.69
B	44	0.46	0.24	0.90	0.15	32.61
Mn	40	0.31	0.24	0.46	0.05	16.13
Ca	51	200.00	100.00	300.00	60.00	30.00
Al	51	4.62	2.72	8.31	1.41	30.52

Pectora roborant cultus recti

Table 5 Summary of the mineral composition (mg/kg freeze-dried) of the validation set for freeze-dried lamb meat showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Mineral	n	Mean	Min	Max	s.d.	c.v.
K	10	9 300.00	8 300.00	11 500.00	1 200.00	12.90
P	10	8 800.00	5 400.00	10 400.00	1 500.00	17.05
Na	10	1 170.00	960.00	1 629.00	179.44	15.34
Mg	11	600.00	500.00	700.00	80.00	13.33
Cu	10	0.75	0.58	0.94	0.12	16.00
Fe	10	35.12	26.20	47.90	6.73	19.16
Zn	10	58.93	51.50	72.30	7.05	11.96
B	10	0.45	0.30	0.67	0.14	31.11
Mn	10	0.30	0.24	0.37	0.04	13.33
Ca	10	200.00	100.00	300.00	60.00	30.00
Al	10	4.47	3.60	6.10	0.89	19.91

Table 6 shows the SEC and r values for the equations of best fit obtained for each of the constituents. The r values for the validation sets and SEP values are also shown in Table 6, as well as the SEL and predicted and laboratory mean values. Calibrations which were acceptable on account of their SEP values in comparison with the SEL values were: K (600 mg/kg vs. 400 mg/kg); P (900 mg/kg vs. 500 mg/kg); Na (77.89 mg/kg vs. 56.75 mg/kg), Mg (40 mg/kg vs. 20mg/kg); Fe (3.15 mg/kg vs. 2.13 mg/kg) and Zn (3.59

mg/kg vs. 2.23 mg/kg). Clark *et al.* (1987) suggested that NIRS is indirectly measuring these minerals by their association with organic constituents(s) that varies as the mineral varies in the sample.

The relative small numbers of samples, which were randomly selected for validation, could result in samples deviating a lot form the mean of the selected samples. In the validation set for Na, most of the samples ranged between 960 mg/kg and 1237 mg/kg, except for one sample with the concentration of 1629 mg/kg. It is conceded that the leverage caused by this particular data point could influence the outcome of the validation, but at present this is the only samples available. An increase in the number of samples for both the calibration and validation sets would probably solve this problem. Further research is necessary to conclude to the accuracy of NIRS for prediction of minerals in freeze-dried mutton.

Table 6 Statistics of the calibration equations of best fit and validation including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL).

Chemical Component	Number of PLSR factors	Calibration set r	Calibration set SEC(mg/kg)	Validation set r	Validation set SEP(mg/kg)	SEL(mg/kg)	Laboratory Mean Values (mg/kg)	Predicted Mean Values (mg/kg)
K	5	0.94	400.00	0.86	600.00	400.00	9 300.00	9 400.00
P	5	0.85	1 100.00	0.88	900.00	500.00	8 800.00	8 500.00
Na	5	0.84	100.17	0.89	77.89	56.75	1 170.00	1 153.63
Mg	5	0.82	40.00	0.92	40.00	20.00	600.00	600.00
Cu	5	0.73	0.15	0.47	0.14	0.04	0.75	0.80
Fe	2	0.70	4.80	0.88	3.15	2.13	35.12	35.86
Zn	3	0.67	4.68	0.86	3.59	2.23	58.93	57.83
B	3	0.60	0.13	0.39	0.12	0.04	0.45	0.44
Mn	4	0.59	0.04	0.29	0.04	0.01	0.30	0.30
Ca	3	0.58	50.00	0.49	50.00	20.00	180.00	190.00
Al	1	0.32	1.35	0.26	0.86	0.28	4.47	4.58

The correlation between the NIRS predicted values and the laboratory determined values for the mineral composition are shown in Figure 2 - 4.

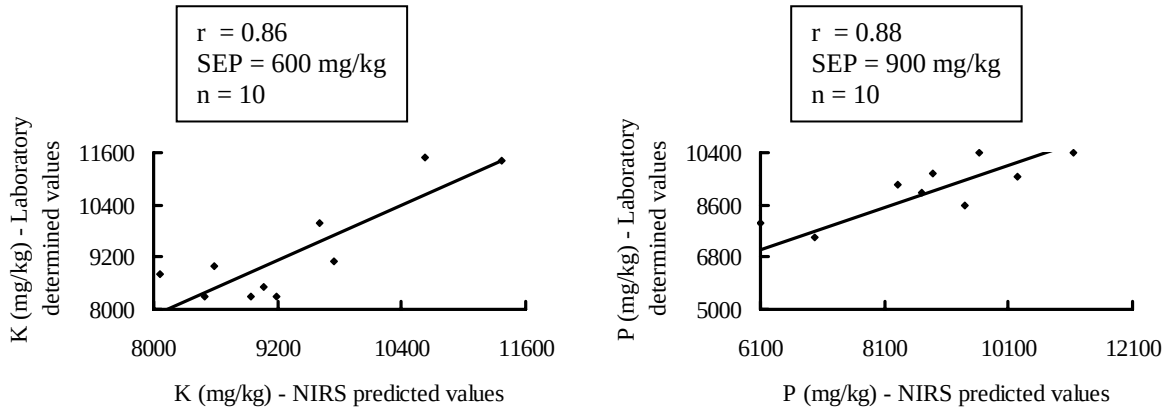

Figure 2 Relationship between laboratory determined and NIRS predicted values for K and P in freeze-dried mutton, using 10 samples for each validation.

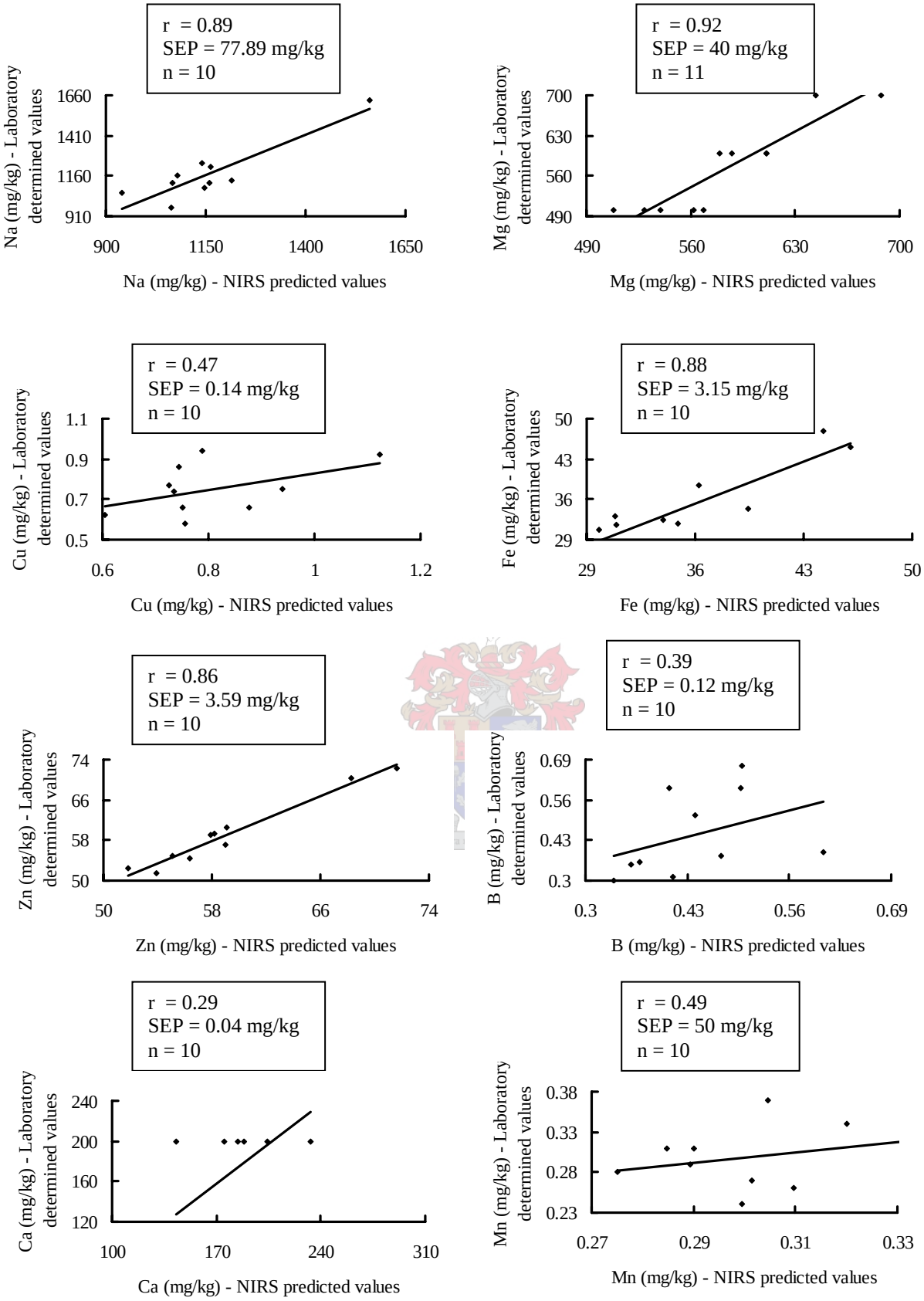

Figure 3 Relationship between laboratory determined and NIRS predicted values for Na, Mg, Cu, Fe, Zn, B, Ca and Mn in freeze-dried mutton, using 10 or 11 samples for each validation.

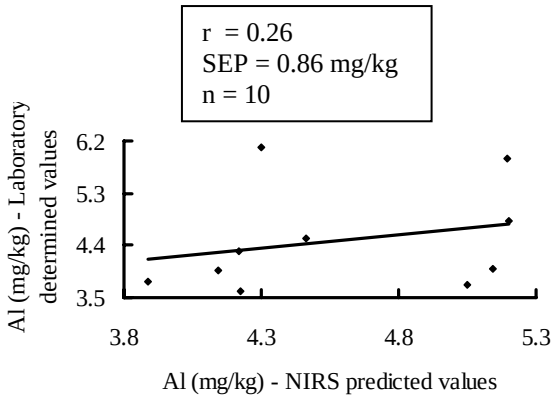

Figure 4 Relationship between laboratory determined and NIRS predicted values for Al in freeze-dried mutton, using 10 samples for the validation.

Conclusion

The major advantage of near infrared reflectance spectroscopy analysis is that once the instrument is calibrated, the results for protein and fat for freeze-dried mutton can be obtained within seconds. Freeze-dried samples led to more accurate calibrations than that noted in the literature, possibly due to the homogenous nature of the samples and the lack of moisture. The latter may change the chemical composition of the sample with fluctuations in temperature. Freeze-drying, however, can be expensive and timely. This led to the conclusion that if NIRS is to be used in the industry for quality control purposes, it would probably be more cost effective to have a less accurate calibration using raw meat than a more accurate calibration using freeze-dried meat. Accurate use of NIRS to determine mineral composition in mutton appears limited to certain minerals (K, P, Na, Mg, Fe and Zn) only.

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Chapter 4

Prediction of the chemical composition and digestibility of lupins and full-fat canola and the determination of alkaloids in lupins with near infrared reflectance spectroscopy

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Abstract

This investigation indicated that near infrared reflectance spectroscopy (NIRS) can be used for the rapid evaluation of the chemical composition of ground lupin and full-fat canola to a degree of accuracy comparable to that of conventional laboratory techniques. Random samples were selected from different lupin and canola cultivars produced at different localities over three years in the Western Cape region of South Africa. A total of 217 lupin samples and 263 full-fat canola samples were analysed to develop the different calibrations using an InfraAlyzer 500 spectrophotometer. Standard errors of performance (SEP) and multiple correlation coefficients (r) for validation were as follows: ash (0.16%; 0.93), dry matter (DM) (0.28%; 0.98), crude protein (CP) (1.07%; 0.98), fat (0.33%; 0.96), acid detergent fibre (ADF) (0.86%; 0.96), neutral detergent fibre (NDF) (1.57%; 0.88), *in vitro* organic matter digestibility (IVOMD) (1.38%; 0.94) and total digestible nutrients (TDN) (1.23%; 0.62) for lupin calibrations and ash (0.29%; 0.72), DM (0.25%; 0.97), CP (0.44%; 0.99) and fat (1.35%; 0.96) for full-fat canola calibrations. A calibration was also developed for the prediction of alkaloids in lupins (*Lupines albus*). Twelve lupin samples were collected throughout the Western Cape region of South Africa, analysed for total alkaloid concentration and mixed in different concentrations to create a larger variation. SEP and r values of 123.06 ppm and 0.95 gave an indication that NIRS could be successfully used for the prediction of total alkaloid concentration if a large enough calibration set was used to develop the calibration.

Keywords: alkaloids, chemical composition, full-fat canola, lupins, near infrared reflectance spectroscopy

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Introduction

The demand for protein in animal nutrition is increasing and protein is likely to become increasingly scarce and costly (Protein Advisory Committee, 1990). It is desirable that locally produced feeds be fully exploited as alternative protein sources (Brand *et al.,* 1995).

Both lupins and full-fat canola were found to be promising alternatives as protein sources (Brand *et al.*, 2002, Brand, 2001), as they are cultivated in the Western Cape area of South Africa as a ley crop. Full-fat canola and canola oilcake from recent released cultivars are high quality products and could be used to reduce feed costs for producers in this region (Brand, 2001). Lupins also contain a high concentration of protein (29.9-35.7%) (May *et al.*, 1993). Large variation occurs in the chemical composition of this grain legume and it is therefore important to determine the composition of lupins prior to inclusion in balanced diets for animals (Protein Research Trust, 2002).

Alkaloids are a principle toxic compound present in lupins. Alkaloids are a complex category of alkaline compounds with a nitrogen ring structure and pharmalogical activity. Plants may contain from one to several alkaloids. Total alkaloid concentration has also been shown to be negatively correlated with palatability in plants, as alkaloids are generally considered to be naturally bitter (Clark *et al.*, 1987). Livestock tends to reject forage containing high concentrations of alkaloids. However, some poisonous plants or plant parts with high concentrations of alkaloids are readily grazed by livestock, resulting in debilitation, abortions and death (Forbes & Burton, 1954; Marten *et al.*, 1976).

The determination of alkaloids is a lengthy procedure requiring several steps in liberating and extracting the alkaloid in organic solvents, purifying it, and quantifying it by either titration, gravimetric, chromatographic, colorimetric or precipitation methods (Clark *et al.*, 1987). The determination of total alkaloid concentration in plant samples with NIRS would save considerable time, labour and expense, and would allow evaluation of large numbers of samples, which is prohibited by standard chemical procedures.

Quality control of agricultural products is necessary in formulation of balanced diets in animal nutrition and is therefore an important field of interest in agricultural research (Flinn, 1991). The need to analyse a large number of samples rapidly has stimulated interest in alternative physical and chemical methods that require little sample preparation. The linking of modern instrumentation to computers greatly facilitates data evaluation and the output of results. To predict the feeding value of forages for animal performance, various indirect methods not involving the use of housed animals have been developed (NRC, 1984). Near infrared spectroscopy (NIRS) has been proposed as a method for the rapid evaluation of the chemical composition, associated quality attributes and physiological properties of forages (Norris *et al.*, 1976; Fairbrother & Brink, 1990; Berardo, 1997).

The aim of this study was to develop accurate NIRS calibrations for different constituents in lupins and full-fat canola, as well as the total alkaloid concentration in lupins.

Materials and methods

Lupin samples were obtained from different lupin cultivars sampled at different localities in the Western Cape region of South Africa from 1998 to 2000. Approximately 47% narrow leaf lupins (*Lupines angustifolius*), 44% broad leaf lupins (*Lupines albus*) and 9% sweet yellow lupins (*Lupines luteus*) were used in this study. Samples were randomly selected for the development of the different calibrations. Calibrations for ash, dry matter (DM), acid detergent fibre (ADF), neutral detergent fibre (NDF), *in vitro*

organic matter digestibility (IVOMD) and total digestible nutrients (TDN) were only developed for samples collected in 1998. Calibrations for crude protein (CP) and fat were developed for samples obtained from 1998 to 2000. Samples were ground with a Laboratory Mill (Christy & Norris Ltd., Chelmsford, England) using a 1 mm sieve.

Random canola samples were selected from different canola cultivars produced at different localities from 1998 to 2000 in the Western Cape region of South Africa. The calibration for ash was only developed from samples obtained in 1998 and 1999, whereas the rest of the calibrations were developed for samples obtained from 1998 to 2000. Samples were ground with a Knifetec 1095 Sample Mill (Tecator, Box 70, S-263 21 Hoganäs, Sweden) using a 1 mm sieve. Wet chemistry and NIRS analyses were done simultaneously for all the samples. Calibrations were upgraded each year for the relevant constituents in both lupins and full-fat canola. Only the final calibrations were reported.

For the alkaloid calibration twelve *Lupines albus* samples were collected from different locations in the Western Cape region of South Africa. The twelve original samples were mixed in various ratios to create more samples and thereby obtaining larger variation in alkaloid concentrations for the calibration set.

All samples were divided into two sets for each constituent: a larger set for the calibration equations (calibration set) and a smaller set for the validation (validation set) of the calibrations (n values are shown in Tables 1, 2, 4, 5 and 8, respectively). Outliers were removed according to suggestions by the software (Bran+Luebbe SESAME Version 2.00-software, BRAN+LUEBBE GmbH, Norderstedt, Germany). Outliers listed as 'T'- and 'H'-values were taken into consideration. The 'T'-value measures how closely the reference value matches the predicted value. The spectrum is listed and flagged with an asterisk (*) if the 'T'-value is greater than 2.5 times the standard error of calibration. These values can be potential outliers, because they do not fit the calibration equation as well as the other samples. The 'H'-value is a measure of leverage. It puts a numerical value on the influence of a particular spectrum in determining the regression line. It is a measure of multidimensional distance of a spectrum to the regression line. If a spectrum with a large 'H'-value has a small 'T' value, it is likely to be valuable for the calibration. If both the 'H' and the 'T' values are large, it is more likely to be a true outlier. Equations of best fit were chosen for each constituent based on statistical analysis. After removal of the outliers, every fifth sample was selected for the validation sets.

Lupin samples were analysed for ash, DM and fat content, measured as ether extract (EE), by standard methods (AOAC, 1984) (Method numbers 7.009, 7.003 and 7.061, respectively). CP was measured by a FP-428 Nitrogen and Protein Determinator (Leco Corporation, 3000 Lakeview Avenue, St Joseph, MI 49085-2396). NDF and ADF were determined as described by Van Soest (1963) and Van Soest & Wine (1967). IVOMD was determined by the method as described by Tilley & Terry (1963). The IVOMD content of lupin seed was adjusted for its high ether extract content. Average ether extract concentration in cereal grain (20 g/kg) was subtracted from that in lupin seed. The remainder was multiplied by 2.25 and added to the IVOMD value. This was done to adjust for the relatively high oil content of lupin seed, resulting in an increased energy content, but which is not reflected by the *in vitro* technique (Brand *et al.*, 1997). Total

digestible nutrients (TDN) were calculated by the following equation: TDN = [(100 - % ash)/100] x (0.8 IVOMD +15.35) on DM basis (Engels & Van der Merwe, 1967). All values are expressed on a 100% DM basis. Canola samples were only analysed for DM, ash, CP and fat content. The air-dried seed material was extracted from the twelve *Lupines albus* samples and analysed for alkaloids by capillary gas chromatography as described by Priddis (1983). Alkaloid values were determined by the Food and Biological Chemistry Laboratory (Chemistry Centre (WA), 125 Hay Street, East Perth, Western Australia 6004). Analysis of the samples were done simultaneously (wet chemistry and NIRS spectra).

NIRS analyses were done with an InfraAlyzer 500 Near Infrared Reflectance Analyser (IA-500) using Bran+Luebbe SESAME Version 2.00-software (BRAN+LUEBBE GmbH, Norderstedt, Germany). Approximately 6 g of each sample was packed into a sample cup containing a 35-mm-diameter quartz window. Spectra were measured over the wavelength range 1100-2500 nm and recorded as log 1/R at 2 nm intervals. Calibration equations were developed for each constituent following the recommended protocol of Windham *et al.* (1989). Lupin calibrations were developed by means of partial least-square regression (PLSR) on normalised spectra for ash, DM, CP, IVOMD and TDN and on second derivative (segment = 1; gap = 0) spectra for fat, ADF and NDF. Canola calibrations and the alkaloid calibration were developed by means of PLSR on second derivative (segment = 1; gap = 0) spectra.

For determination of the robustness of the alkaloid calibration, the variance in estimated alkaloid content of 8 samples, which were analysed in sextuple, was partitioned in the following mixed liner model:

$$y_{ijkl} = \mu + s_i + rep_j + rot_k + e_{ijkl} \qquad \text{(Equation 1)}$$

With:

y_{ijkl} = A alkaloid analysis on the ijklth sample,

μ = the overall mean,

s_i = the random effect of the ith sample (i = 1, 2, ….. 8),

rep_j = the random effect of the jth replicate (j = 1, 2, …..6),

rot_k = the fixed effect of the kth rotation (k = original position or the original position rotated through 180°),

e_{ijkl} = the random residual variance used as error term to test the other effects for significance.

From this analysis, it was possible to estimate the repeatability of alkaloid analyses, using the NIRS. The following equation were used for this purpose (Turner & Young, 1969):

$$t = \sigma^2_s / (\sigma^2_s + \sigma^2_e) \qquad \text{(Equation 2)}$$

With:

t = repeatability estimate,

σ^2_s = the between sample variance component,

σ^2_e = the within sample or error variance component.

The ASREML program (Gilmour *et al.*, 1999) was used for these analysis. The software allows estimates of variance components for mixed models by restricted maximum likelihood, employing an average information algorithm that concurrently provides estimates of standard errors for parameters (Gilmour *et al.*, 1995).

Results and discussion

The range, mean values, standard deviations (s.d.) and coefficients of variation (c.v.) for the calibration and validation sets of the lupin calibrations are shown in Table 1 and 2, respectively. The variation in the chemical composition of the samples used seem to cover the whole spectrum reported in literature for lupins (Cerning-Beroad & Filiatre, 1976; Guillaume *et al.*, 1987; Brand *et al.*, 1997). The number of samples used for the ash, DM, ADF, NDF, IVOMD and TDN calibrations were less than the minimum number (50) of samples suggested for a narrow-based population (Windham *et al.*, 1989). This was, however, the only samples available and calibrations were attempted to test the accuracy of NIRS for the particular constituents. If the number of samples were to be increased, it would result in more robust, and probably more accurate, calibrations.

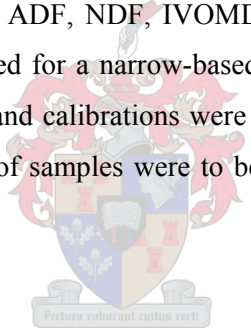

Table 1 Summary of chemical composition (%) of lupins used in the calibration set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	36	3.21	2.60	4.43	0.37	11.53
DM	39	92.65	90.01	96.89	1.53	1.65
CP	122	30.00	21.06	40.57	4.17	13.90
Fat	217	6.93	3.06	11.43	2.18	31.46
ADF	40	18.06	13.50	25.56	2.84	15.73
NDF	40	19.38	12.34	26.69	3.20	16.51
IVOMD	37	81.26	76.74	90.37	3.98	4.90
TDN	38	90.00	84.25	95.57	2.71	3.01

Table 2 Summary of chemical composition (%) of lupins used in the validation set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	13	3.29	2.64	4.26	0.50	15.20
DM	13	92.37	90.40	95.66	1.40	1.52
CP	32	31.36	21.14	40.06	5.03	16.04
Fat	57	6.37	3.70	9.72	2.08	32.65
ADF	12	18.57	14.23	23.76	3.00	16.16
NDF	10	19.81	16.11	24.46	2.82	14.24
IVOMD	12	81.09	77.56	87.81	3.19	3.93
TDN	10	90.25	88.03	92.40	1.57	1.74

Table 3 shows the standard errors of calibration (SEC) and multiple correlation coefficient (r) values for the equations of best fit obtained for each of the constituents measured in the lupins. The r values for the validation sets and standard error of performance (SEP) are also shown in Table 3, as well as the standard error of laboratory (SEL) (Snedecor & Cochran, 1980) and predicted and laboratory mean values.

SEC values ranged from 0.19% for ash to 1.49% for NDF; r values ranged from 0.89 for NDF to 0.99 for fat. In the validation tests the r-values ranged from 0.88 for NDF to 0.99 for fat and the SEP values ranged from 0.16% for ash to 1.57% for NDF. The r values (0.89 and 0.88) for the NDF calibration, compared favourably with values previously noted by other authors for forages, e.g. 0.87 (Marten *et al.*, 1983) and 0.93 (Stimson *et al.*, 1991). The SEP value (1.57%) was better than that reported in the literature, e.g. 2.27% (Shenk *et al.*, 1981), 1.99% (Marten *et al.*, 1983) and 3.1% (Stimson *et al.*, 1991). This calibration could most probably be improved even further by increasing the number of samples in the calibration and validation sets.

IVOMD results indicated an acceptable correlation (r = 0.94), with a rather high SEC (1.37%), which is typical of such biological measures. However, this value was much lower than SEC values reported by Norris *et al.* (1976) (3.5%), Shenk *et al.* (1981) (2.33%) and Bruno-Soares *et al.* (1998) (2.36%) for forages.

Windham *et al.* (1989) stated that if the SEP for validation is less then double the SEL for the primary reference method analysis, the final NIRS equation can be accepted for use, and the SEP for validation can be used as a reliable indication of the accuracy of the final NIRS equation. This rule held true for all the lupin calibrations, except TDN, which leads to the conclusion that the chemical composition determined by means of NIRS is similar to that analysed using conventional laboratory techniques. A possible reason for the inaccurate calibration for TDN can be seen in Figure 2. The sample with the highest concentration (92.4%) was predicted much lower (89.85%) in the validation test. It is conceded that the leverage caused by this particular data point could influence the outcome of the validation. An increase in the number of samples for both the calibration and validation sets would probably solve this problem. Further research is

necessary to conclude to the accuracy of NIRS for prediction of TDN in lupins. The correlation between the NIRS predicted and the laboratory determined values of the validation tests for the various chemical components found in lupins are given in Figures 1 and 2.

Table 3 Statistics of the calibration equations (for lupins) of best fit and validation, including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL).

Chemical Component	Number of PLSR factors	Calibration set		Validation set			Laboratory Mean Values (%)	Predicted Mean Values (%)
		r	SEC (%)	r	SEP (%)	SEL (%)		
Ash	4	0.87	0.19	0.93	0.16	0.14	3.29	3.31
DM	3	0.98	0.34	0.98	0.28	0.39	92.37	92.32
CP	6	0.98	0.78	0.98	1.07	0.89	31.36	31.28
Fat	1	0.99	0.28	0.99	0.33	0.27	6.37	6.37
ADF	4	0.98	0.61	0.96	0.86	0.87	18.57	18.47
NDF	3	0.89	1.49	0.88	1.57	0.89	19.81	20.08
IVOMD	3	0.94	1.37	0.94	1.38	0.92	81.09	80.65
TDN	3	0.91	1.16	0.62	1.23	0.50	90.25	89.89

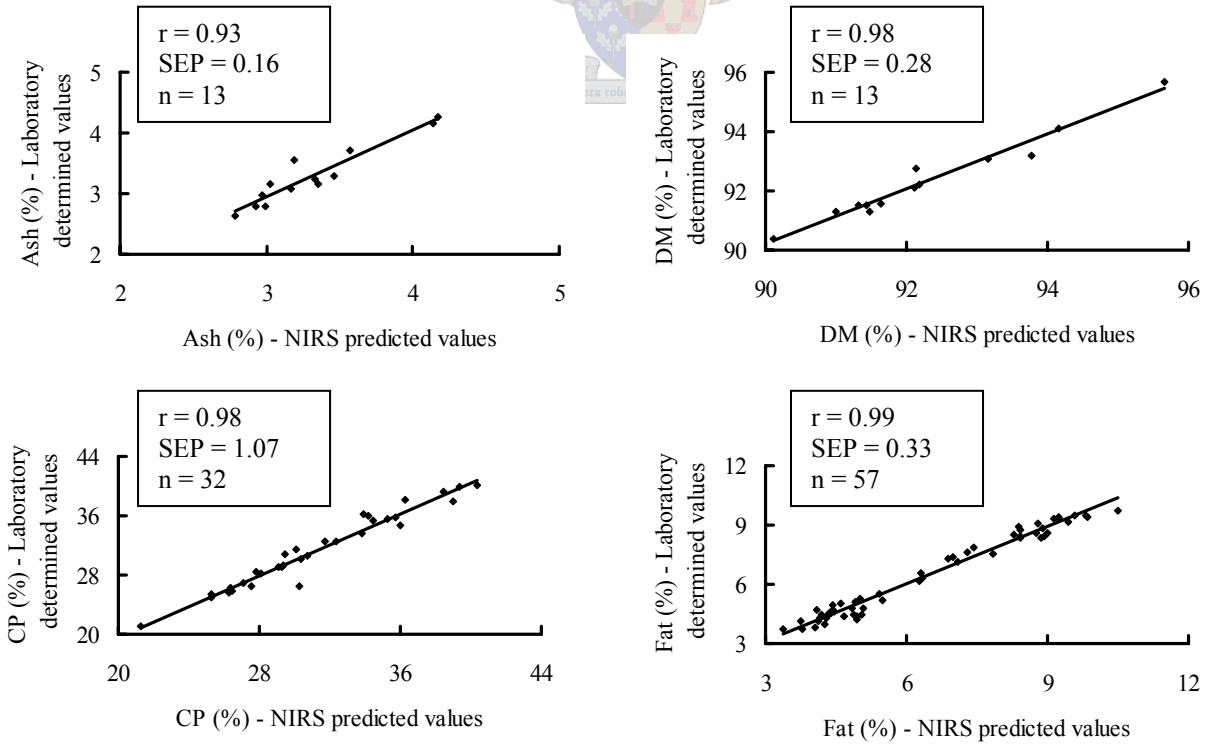

Figure 1 Relationship between laboratory determined and NIRS predicted values for ash, DM, CP and fat for lupins, using between 13 and 57 samples for each validation.

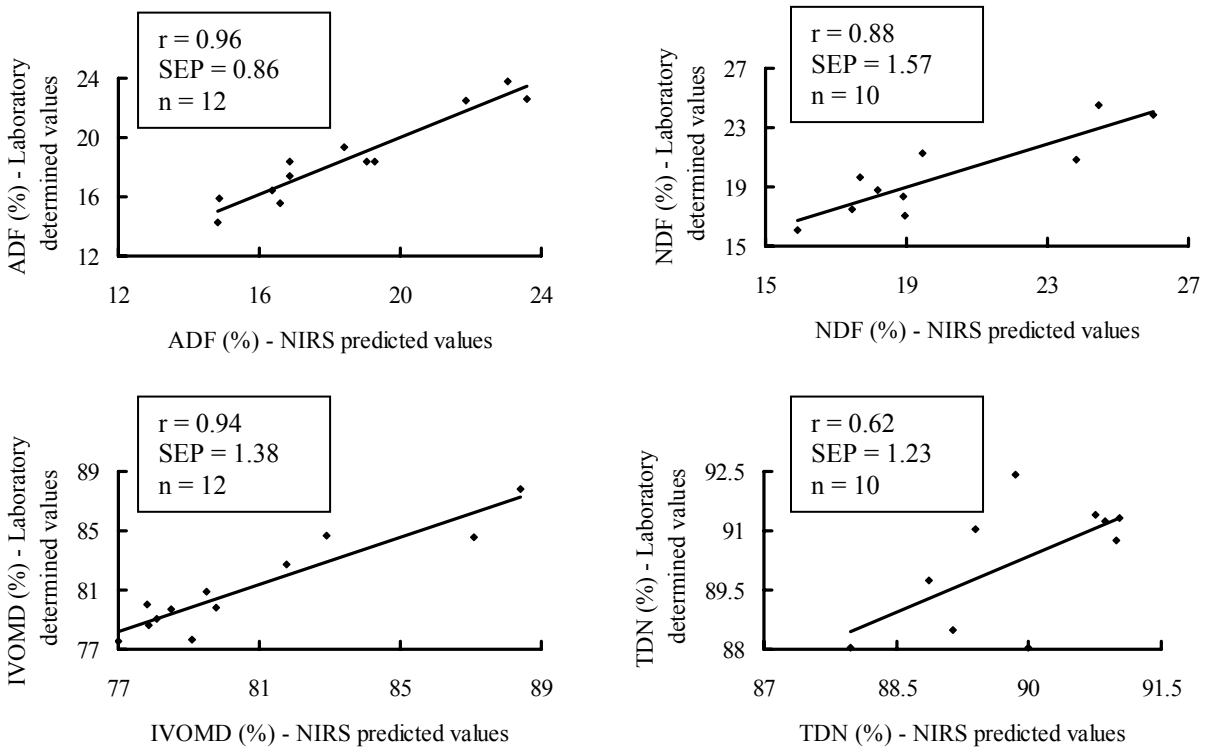

Figure 2 Relationship between laboratory determined and NIRS predicted values for ADF, NDF, IVOMD and TDN for lupins, using between 10 and 12 samples for each validation.

The range, mean values, standard deviations (s.d.) and coefficients of variation (c.v.) for the calibration and validation sets of the full-fat canola calibrations are shown in Table 4 and Table 5, respectively. The variation in the chemical composition of the samples used seems to cover the whole spectrum reported in literature for canola (Shaw *et al.*, 1990; NRC, 1994; Brand & Van der Merwe, 1999).

Table 4 Summary of chemical composition of full-fat canola for the calibration set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component	n	Mean (%)	Min (%)	Max (%)	s.d.(%)	c.v.
Ash	108	3.56	2.83	4.90	0.43	12.08
DM	263	92.97	90.57	95.36	0.96	1.03
CP	212	23.48	14.86	29.50	3.03	12.90
Fat	263	36.89	23.38	48.11	4.85	13.15

Table 5 Summary of chemical composition of full-fat canola for the validation set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component	n	Mean (%)	Min (%)	Max (%)	s.d.(%)	c.v.
Ash	35	3.56	2.97	4.71	0.41	11.52
DM	71	93.01	90.71	95.02	0.95	1.02
CP	61	23.82	16.56	29.50	2.65	11.13
Fat	70	37.05	23.55	48.11	4.68	12.63

Table 6 summarises the statistics for full-fat canola, including SEC and r for the equations of best fit obtained for each of the constituents. The r values for the validation set and SEP are also shown in Table 6 together with the SEL and the means of the laboratory and predicted values. SEC values ranged from 0.22% for DM to 1.25% for fat and r values ranged from 0.81 for ash to 0.99 for CP. In the validation tests the r values range from 0.72 for ash to 0.99 for CP and SEP values ranged from 0.25% for DM to 1.38% for fat. These values were similar to values recorded in the literature (Marten *et al.*, 1984; Smith & Flinn, 1991; Stimson *et al.*, 1991; Berardo, 1997).

Although the nitrogen content of forages varies considerably (14.86 to 29.50%, Table 4) the chemical analysis of this component is very precise which results in successful measurements being performed by NIRS (r = 99; SEP = 0.44%). Several authors reported accurate predictions of this constituent with NIRS (Shenk *et al.*, 1981; r = 0.96; SEP = 0.90%; De Boever *et al.*, 1995; r = 0.98; SEP = 1.1%), which confirms the findings of the present study. Historically the determination of protein in wheat was by far the most important application of NIRS analysis.

The SEP values for the validation of DM, CP and fat in full-fat canola were also less than double the SEL for the primary reference method analysis and the calibrations can therefore be accepted for use (Windham *et al.*, 1989). The correlation between the NIRS predicted and the laboratory determined values of the validation tests for the various chemical components found in full-fat canola are given in Figure 3.

Table 6 Statistics of the calibration equations (for full-fat canola) of best fit and validation including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL).

Chemical Component	Number of PLSR factors	Calibration set r	Calibration set SEC (%)	Validation set r	Validation set SEP (%)	SEL (%)	Laboratory Mean Values (%)	Predicted Mean Values (%)
Ash	3	0.81	0.25	0.72	0.29	0.07	3.56	3.55
DM	6	0.98	0.22	0.97	0.25	0.13	93.01	93.03
CP	4	0.99	0.40	0.99	0.44	0.34	23.82	23.82
Fat	4	0.97	1.25	0.96	1.35	0.72	37.05	37.02

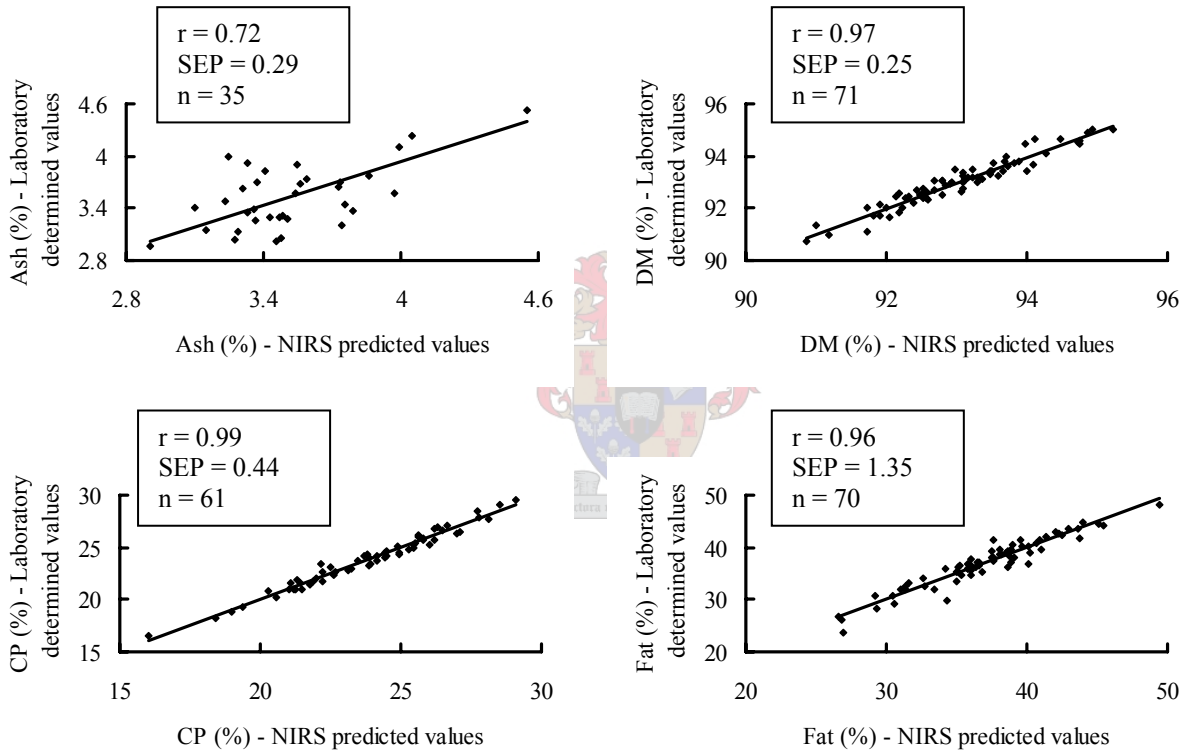

Figure 3 Relationship between laboratory determined and NIRS predicted values for ash, DM, CP, fat for full-fat canola using between 35 and 71 samples for each validation.

The alkaloid concentrations of the original twelve lupin samples are shown in Table 7 and ranged from 220 ppm to 1570 ppm. The samples were mixed in various combinations to yield a larger calibration set with a more even distribution. Mixed samples were then sorted according to increasing alkaloid value and every fifth sample was selected for the validation set.

Table 7 Alkaloid values of the twelve original reference samples.

Sample	1	2	3	4	5	6	7	8	9	10	11	12
Alkaloid (ppm)	1570	220	450	380	260	1080	1240	1140	500	430	280	710

The range, mean values, standard deviations (s.d.) and coefficients of variation (c.v.) for the calibration and validation sets of the alkaloid calibration are shown in Table 8. The variation in the alkaloid concentration reported in Table 8 did not, however, cover the whole range reported in literature for alkaloid concentration (Forbes & Burton, 1954; Ralphs & Williams, 1986; Clark *et al.*, 1987). There was a lack of very low and very high alkaloid concentration.

Table 8 Summary of alkaloid concentration (from lupins) expressed as parts per million (ppm) of the calibration and validation sets showing number of samples (n), means, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Alkaloid values	n	Mean	Min	Max	s.d.	c.v.
Calibration set	221	907.06	220	1570	401.61	44.28
Validation set	45	965.11	280	1560	383.11	39.70

Table 9 shows the SEC and r values for the equations of best fit obtained for the total alkaloid concentration. The r values for the validation sets and standard error of performance (SEP) are also shown in Table 9, as well as the SEL and predicted and laboratory mean values. The r value was high (0.95), which could be an indication that a successful alkaloid calibration is possible (Figure 4). The SEP value, however, was more than double the SEL value, which is an indication that the calibration is not accurate enough for alkaloid concentration prediction purposes. In a study conducted by Clark *et al.* (1987) 246 velvet lupins (*Lupines leucophyllus*) and 273 tall larkspur (*Delphinium occidentale*) with an alkaloid concentration range from 90 - 600 ppm and 260 - 1720 ppm respectively were used for NIRS calibration. The authors reported SEP and r-values of 50 ppm and 0.90 for lupins and 10 ppm and 0.93 for larkspur, respectively. These findings seem to indicate that for a successful alkaloid calibration an increase in the number of samples and larger variation in the calibration set than that used in this investigation is required.

Table 9 Statistics of the calibration equation (for total alkaloid concentration found in lupins) of best fit and validation including the number of PLSR factors standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL).

Chemical Component	Number of PLSR factors	Calibration set		Validation set		SEL (ppm)	Laboratory Mean Values (ppm)	Predicted Mean Values (ppm)
		r	SEC (ppm)	r	SEP (ppm)			
Alkaloids	11	0.99	67.11	0.95	123.06	57.11	965.11	957.09

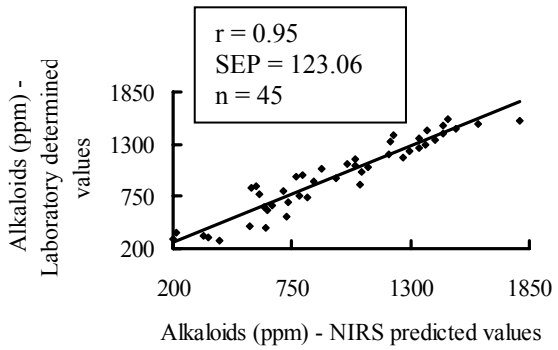

Figure 4 Relationship between laboratory determined and NIRS predicted values for total alkaloid concentrations, using 45 samples.

However, as the SEP value was only 8.84 ppm more than double the SEL value (57.11 ppm), it could be argumented that such an error may be acceptable, if it is taken into account that chemical analysis of alkaloid concentration is very timely and expensive (A$ 80/sample, Food and Biological Chemistry Laboratory, Chemistry Centre (WA), 125 Hay Street, East Perth, Western Australia 6004). Another question was whether this calibration would be suitable to accurately predict samples with a very high or very low alkaloid concentration, due to the lack of such samples in the calibration set. To test the repeatability of the alkaloid calibration, eight samples of known composition were analysed twelve times. The samples were packed into six different sample cups and analysed twice, rotating the sample cup through 180° between repetitions.

Alkaloid analyses were independent of the rotation treatment. Overall means (± SE) were 691 ± 93 ppm for samples read in the original position and 661 ± 93 ppm for samples read in the original position rotated through 180° ($P > 0.05$). Replication number, likewise, had no significant ($P < 0.05$) influence on the realised alkaloid content. The between sample variance component was expressed as a ratio (t ± SE) of the total between and within sample variance as depicted in equation 2. The derived repeatability coefficient amounted to 0.714 ± 0.118. This estimate was not largely influence by the exclusion of the other effects fitted according to equation 1. The within sample variation thus amounted to 28.6 % of the overall variation. When the derived standard error was used, it was evident that the repeatability estimate differed ($P < 0.05$) from unity. Ideally, the between sample variation should have been maximised, while the within sample

variance is minimised, resulting in a t-estimate of near to or equal to 1.

In order to understand the results better solutions for alkaloid concentrations, using chemical analyses, were obtained and plotted against the NIRS analyses values (Figure 5).

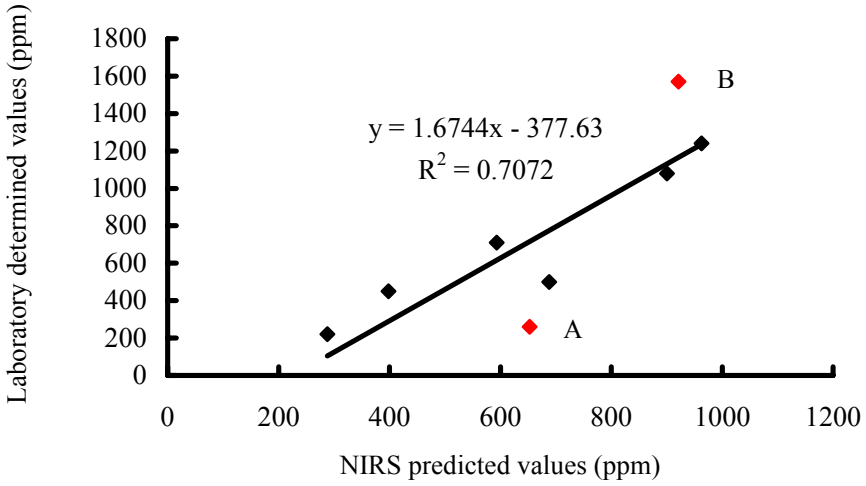

Figure 5 Relationship between laboratory determined and NIRS predicted values for total alkaloid concentration.

From Figure 5 the conclusion can be drawn that the two samples that have the largest negative influence on the regression are the samples marked in red (A = 260 ppm and B = 1570 nm). The mean predicted value for sample A (652.77 ppm) is much higher than the actual value (260 ppm). Whereas the mean predicted value for sample B (921.37 ppm) is much lower than the actual value (1570 ppm). If the minimum (220 ppm) and the maximum (1570 ppm) values used in the calibration set (Table 8) is taken into consideration, the conclusion can be drawn that the accuracy of the calibration failed for prediction of these two samples, due to the variation of the calibration set. This could be due to a lack of low and high alkaloid concentration samples in the calibration set. Another reason for the poor repeatability of the calibration can be the reliability of the reference values used in the calibration set. Due to the high costs of alkaloid analysis, the samples were only chemically analysed once. To get a more robust calibration, it was suggested that more samples (especially samples with very high and very low alkaloid concentration) are included in the calibration set and that existing samples be analysed again to test the repeatability of the chemical analysis.

Conclusion

The chemical composition of lupins and full-fat canola predicted by NIRS were highly correlated with values determined by AOAC methods. This indicates that NIRS can be used as a reliable tool in the quality control of both lupins and full-fat canola. This will result in a rapid, less expensive analysis technique, which will benefit the feed industry in formulations of balanced diets. It was also concluded that a NIRS

calibration for alkaloids in lupins could be possible. A lack of samples, however, altered the presentation of a reliable alkaloid calibration in this study.

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Chapter 5

Prediction of the chemical composition of winter grain and maize with near infrared reflectance spectroscopy

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Abstract

Near infrared reflectance spectroscopy (NIRS) calibrations were developed for winter grain samples collected over three years in the Western Cape region of South Africa. Winter grains used in the study include oats, barley, triticale and wheat. Calibrations were also developed for maize samples collected throughout South Africa. Winter grain samples were analysed for ash, dry matter (DM), crude protein content (CP), fat content, acid detergent fibre (ADF), neutral detergent fibre (NDF), *in vitro* organic matter digestibility (IVOMD), lysine and methionine. The digestible energy (DE) content of winter grain samples was determined by the mobile nylon bag technique on pigs. Maize samples were analysed for ash, DM, CP, IVOMD and TDN. Standard errors of prediction (SEP) and multiple correlation coefficient (r) for the different winter grain constituents were respectively; ash (0.22%; 0.87), DM (0.67%; 0.68), CP (0.60%; 0.93); fat (0.19%; 0.99), ADF (0.88%; 0.98), NDF (2.13%; 0.97), IVOMD (2.06%; 0.97), DE (0.76%; 0.91), lysine (0.04%; 0.93) and methionine (0.01%; 0.90). SEP and r values for the maize calibrations were for ash (0.08%; 0.49), DM (0.12%; 0.95), CP (0.12%; 0.95), IVOMD (0.66%; 0.89) and TDN (0.39%; 0.92). These results suggested that NIRS can be developed as a rapid and accurate tool for the prediction of the nutritional value of feedstuffs, which makes it an attractive technique for routine quality control in the industry.

Keywords: winter grains, chemical composition, maize, near infrared reflectance spectroscopy

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Introduction

Different feedstuffs are normally mixed in different concentrations in order to create a final diet that provides the optimum balance of protein, energy and other nutrients, according to the nutrient requirements of different types of animals at different production stages. In practice variability in the nutritional quality of the feedstuffs may result in the formulation of unbalanced diets. Winter grains are important agricultural crops in the Western Cape region of South Africa. Protein content of grains may, however, vary from 8 - 21% (barley), 10 - 22% (wheat), 8 - 21% (triticale) and 8 - 21% (oats) as described by Brandt *et al.* (unpublished data). On average, maize grain consists of 71% starch, 9% protein and 4% oil on a dry weight

basis (Orman & Schumann, 1991). However, genetic background and environmental effects may create significant variation in the chemical composition of the grain (Brandt *et al.*, submitted). Values for maize that range between 6 - 13% for protein, 3 - 7% for oil and 67 - 76% for starch have been reported (Orman & Schumann, 1991; Ward, 1988). The variation in composition and accompanied nutritive value of grain and maize could therefore have a marked effect on animal performance if the diet is formulated on mean table values.

The Feed Industry in many cases still use the measurement of nitrogen as the quality control tool for routine feedstuff evaluation, even though nitrogen does not always correlate well with the amino acid content of feed samples (Van Kempen & Simmins, 1997). Given that near infrared reflectance spectroscopy (NIRS) prediction for amino acids are easier to obtain than a nitrogen measurement using Kjehldahl, Van Kempen & Bodin (1998) suggested NIRS to be the method of choice for rapid prediction of amino acid levels. The development of various NIRS calibrations for wheat (Law & Tkachuk, 1977a, 1977b; Rubenthaler & Bruinsma, 1978; Shenk *et al.*, 1981; Ridgway & Chambers, 1996; Osborne, 2000) and maize (Orman & Schumann, 1991; Hardy *et al.*, 1996; Rippke *et al.*, 1996; Dijkhuizen *et al*., 1998) had the greatest impact in the grain industry, with fewer reports of NIRS measurements for oats (Hymowitz *et al.*, 1974; Krishnan *et al.*, 1994), barley (Lai *et al*., 1984; Edney *et al.*, 1994) and triticale (Marten *et al.*, 1983).

In this study NIRS calibrations have been developed to predict the chemical composition of winter grains and maize as found in South Africa, so as to see whether this methodology is suitable for fast, cost effective routine analysis of certain chemical constituents in these feedstuffs.

Materials and methods

Grain samples were collected over a period of three years from experimental plots at ten different locations in the Western Cape region of South Africa. Seven different grain types and twenty different cultivars were used in this study (Table 1). Samples were pooled for universal winter grain calibrations. The grains used for the lysine and methionine calibrations were wheat (cultivar: *palmiet*), barley (cultivar: *clipper*) and triticale (cultivar: *usgen 19*). Twenty samples of each grain were collected from different locations in the Swartland and Rûens areas of the Western Cape (Brandt *et al.*, 2000). Maize samples were obtained from the South African Grain Laboratory (PO Box 1059, Silverton, 0127, South Africa) and were representative of the grain production of South Africa for the year 2000/2001. Samples were ground with a hammer mill (Scientec, RSA) to pass a 1 mm screen prior to analysis.

All samples were divided into two sets for each constituent: a larger set for the calibration equations (calibration set) and a smaller set for the validation (validation set) of the calibrations (n values are shown in Tables 1, 2, 4, 5 and 8, respectively). Outliers were removed according to suggestions by the software (Bran+Luebbe SESAME Version 2.00-software, BRAN+LUEBBE GmbH, Norderstedt, Germany). Outliers listed as ‘T’- and ‘H’-values were taken into consideration. The ‘T’-value measures how closely the reference value matches the predicted value. The spectrum is listed and flagged with an asterisk (*) if the ‘T’-value is greater than 2.5 times the standard error of calibration. These values can be potential outliers,

because they do not fit the calibration equation as well as the other samples. The 'H'-value is a measure of leverage. It puts a numerical value on the influence of a particular spectrum in determining the regression line. It is a measure of multidimensional distance of a spectrum to the regression line. If a spectrum with a large 'H'-value has a small 'T' value, it is likely to be valuable for the calibration. If both the 'H' and the 'T' values are large, it is more likely to be a true outlier. Equations of best fit were chosen for each constituent based on statistical analysis. After removal of the outliers, every fifth sample was selected for the validation sets.

Table 1 Summary of cultivars collected during 1994, 1995 and 1996 in the winter rainfall region of the Western Cape (Brandt *et al.*, submitted).

GRAIN TYPES	CULTIVAR	GRAIN TYPES	CULTIVAR
2 Row brewers barley	Clipper	Naked oats	Bandicoot
2 Row naked barley	Vloekskoot	Wheat /triticale	Alpha/Kiewiet
6 Row naked barley	Dayan	Wheat (feed-grade)	Alpha
6 Row feed barley	Galleon	Wheat (bread)	Palmiet
6 Row feed barley	Cape-barley	Triticale	Kiewiet
6 Row feed barley	SVG13	Triticale	Rex
6 Row feed barley	Turkish 6-row	Triticale	Usgen 18
Oats	Calgoa	Triticale	Usgen 19
Oats	Heros	Triticale	SCR 13
Oats	Perdeberg		
Oats	Cederberg		

Pectora roborant cultus recti

All samples were analysed for ash, dry matter (DM) and fat content by standard methods (AOAC, 1984) (Method numbers 7.009, 7.003 and 7.061, respectively). The crude protein (CP) of winter grains was measured by a FP-428 Nitrogen and Protein Determinator (Leco Corporation, 3000 Lakeview Avenue, St Joseph, MI 49085-2396) while the CP content of maize samples were analysed by standard methods (AOAC, 1984). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents were determined as described by Van Soest (1963) and Van Soest and Wine (1967). *In vitro* organic matter digestibility (IVOMD) was determined by the method described by Tilley & Terry (1963). Total digestible nutrients (TDN) were calculated by the following equation: TDN = 0.8 IVOMD + 15.35, on DM basis (Engels & Van der Merwe, 1967). Digestible energy (DE) was determined by the mobile nylon bag technique on pigs as described by Brand *et al.* (1989). DE values were corrected for over estimation by the regression equation (y = 1.998 + 0.788x) as described by Brand (2000). All values were expressed on a 100% dry matter (DM) basis. Lysine concentrations were determined with a Beckman System 6300 High Performance Analyser (Beckman Instruments Inc., Palo Alto, California) after acid hydrolysis in 6 N HCl. Methionine concentrations were

determined using performic acid oxidation prior to hydrolysis and were quantified as methionine sulfone (Green *et al.*, 1987). Wet chemistry and NIRS analyses were done simultaneously for all the samples.

NIRS analyses were done with an InfraAlyzer 500 Near Infrared Reflectance Analyser (IA-500) using Bran+Luebbe SESAME Version 2.00-software (BRAN+LUEBBE GmbH, Norderstedt, Germany). Spectra were measured over the wavelength range 1100 – 2500 nm and recorded as log 1/R at 2 nm intervals. Calibration equations were developed for each constituent following the recommended protocol of Windham *et al.* (1989). Calibrations were developed by means of partial least-square regression (PLSR) on second derivative spectra (segment = 1; gap = 0) for all calibrations, except for maize calibrations: ash and TDN, which were developed on normalised spectra.

Results and discussion

The range, mean values, standard deviation (s.d.) and coefficient of variation (c.v.) for the ash, DM, CP, fat, ADF, NDF, IVOMD, DE, lysine and methionine contents of the winter grains used in the calibration and prediction sets for the NIRS, are shown in Tables 2 and 3, respectively. The variation in the chemical composition of the samples used seem to cover the whole spectrum reported in the literature for winter grains (Krishnan *et al.*, 1994; Brand, 2000; Brandt *et al.*, 2000).

Table 2 Summary of chemical composition (%) of winter grains for the calibration set, showing number of samples (n), mean value, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	351	1.66	0.93	3.41	0.46	27.71
DM	313	89.91	88.04	93.5	0.92	1.02
CP	348	13.11	8.43	18.35	1.84	14.04
Fat	138	2.89	1.58	7.48	1.58	54.67
ADF	358	6.63	2.46	16.59	4.25	64.10
NDF	349	21.20	9.79	39.87	8.04	37.92
IVOMD	343	76.25	55.15	84.49	8.45	11.08
DE	356	14.98	10.45	18.45	1.82	12.15
Lysine	49	0.49	0.36	0.78	0.09	18.37
Methionine	55	0.15	0.07	0.21	0.03	20.00

Table 3 Summary of chemical composition (%) of winter grain for the validation set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	85	1.65	0.94	2.76	0.45	27.27
DM	76	89.98	88.26	92.45	0.92	1.02
CP	83	13.05	9.01	16.59	1.62	12.41
Fat	36	3.02	1.71	7.19	1.58	52.32
ADF	89	6.91	2.86	16.4	4.50	65.12
NDF	88	20.92	10.02	38.52	8.62	41.20
IVOMD	70	75.54	55.39	84.27	9.04	11.97
DE	79	14.85	11.21	18.08	1.85	12.46
Lysine	15	0.49	0.37	0.72	0.10	20.41
Methionine	18	0.14	0.08	0.2	0.03	21.43

Table 4 shows the statistics, including standard errors of calibration (SEC), multiple correlation coefficients (r) for the calibrations, standard errors of performance (SEP), standard errors of laboratory (SEL) (Snedecor & Cochran, 1980) and the laboratory and predicted mean values. SEP values for CP (0.60%), ADF (0.88%) and NDF (2.13%) and IVOMD (2.06%) were lower than values previously reported by other researchers (CP = 0.90%, ADF = 1.99% and NDF = 2.27%) (Shenk *et al.*, 1981). Calibration curves for lysine (r = 0.93; SEP = 0.04%) and methionine (r = 0.90; SEP = 0.01%) show higher correlation coefficients compared to values reported by Van Kempen & Bodin (1998) (lysine: r = 0.55; SEP = 0.02%, methionine: r = 0.84; SEP = 0.02%) for wheat grain. If the SEP for the validation is within two multiplications of the SEL for the primary reference method analysis, the final NIRS equation can be accepted for use, and the SEP for validation can be used as a reliable indication of the accuracy of the final NIRS equation (Windham *et al.*, 1989). The calibrations for fat, ADF, IVOMD, lysine and methionine fitted these limitations and could be accepted for rapid predictions of the constituents (fat = 0.19% (SEP) vs. 0.26% (SEL); ADF = 0.88% (SEP) vs. 0.48% (SEL); IVOMD = 2.06% (SEP) vs. 1.08% (SEL); lysine = 0.04% (SEP) vs. 0.03% (SEL); methionine = 0.01% (SEP) vs. 0.01% (SEL)).

The correlation between NIRS predicted values and laboratory determined values for the various chemical components in winter grain are given in Figures 1 and 2.

Table 4 Statistics of the calibration equations (for winter grains) of best fit and prediction, including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of prediction (SEP) and standard error of laboratory (SEL) to predict the chemical composition of grain by NIRS.

Chemical Component	Number of PLSR factors	Calibration set r	Calibration set SEC (%)	Prediction set r	Prediction set SEP (%)	SEL (%)	Laboratory Mean Values (%)	Predicted Mean Values (%)
Ash	9	0.93	0.17	0.87	0.22	0.05	1.65	1.65
DM	5	0.72	0.64	0.68	0.67	0.10	89.98	89.92
CP	7	0.96	0.54	0.93	0.60	0.18	13.05	13.06
Fat	4	0.99	0.18	0.99	0.19	0.26	3.02	3.01
ADF	7	0.98	0.78	0.98	0.88	0.48	6.91	6.70
NDF	8	0.97	1.84	0.97	2.13	0.92	20.92	20.94
IVOMD	7	0.98	1.52	0.97	2.06	1.08	75.54	75.63
DE	11	0.98	0.40	0.91	0.76	0.21	14.85	14.91
Lysine	3	0.92	0.04	0.93	0.04	0.03	0.49	0.50
Methionine	4	0.95	0.01	0.90	0.01	0.01	0.14	0.14

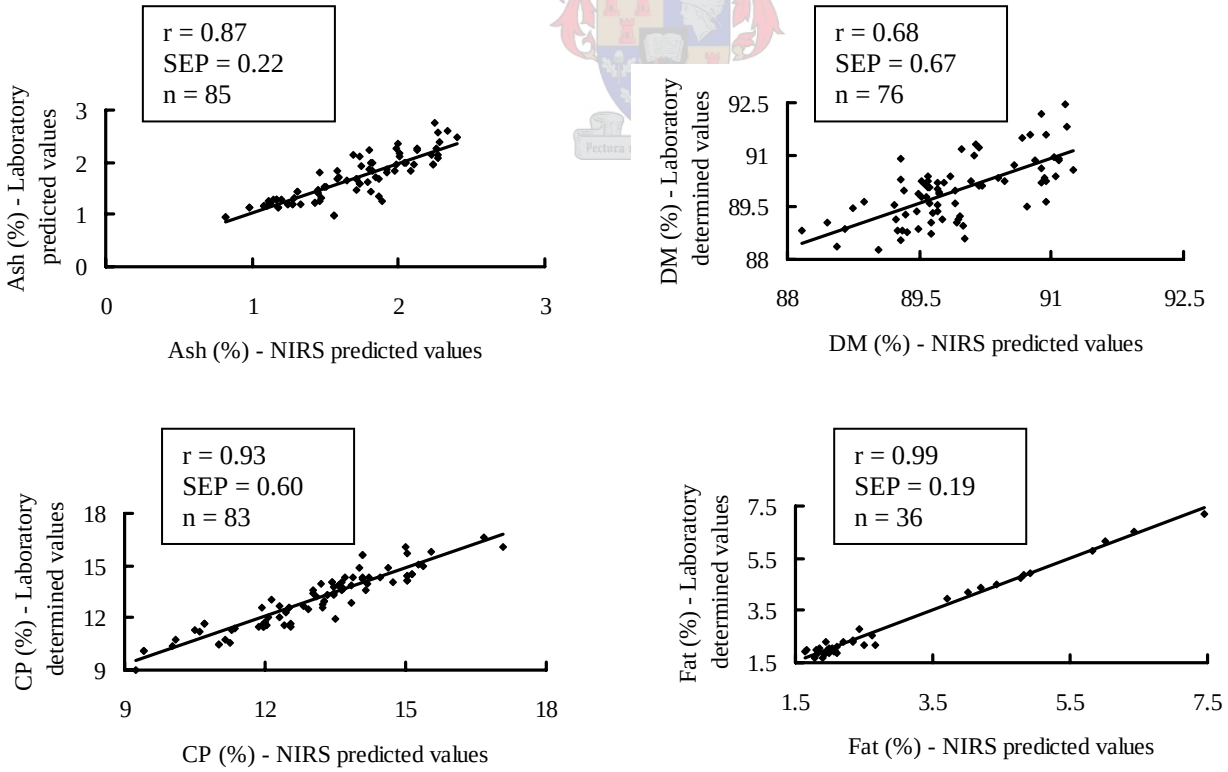

Figure 1 Relationship between laboratory-determined and NIRS predicted values for ash, DM, CP and fat in winter grains, using between 36 and 85 samples for each validation.

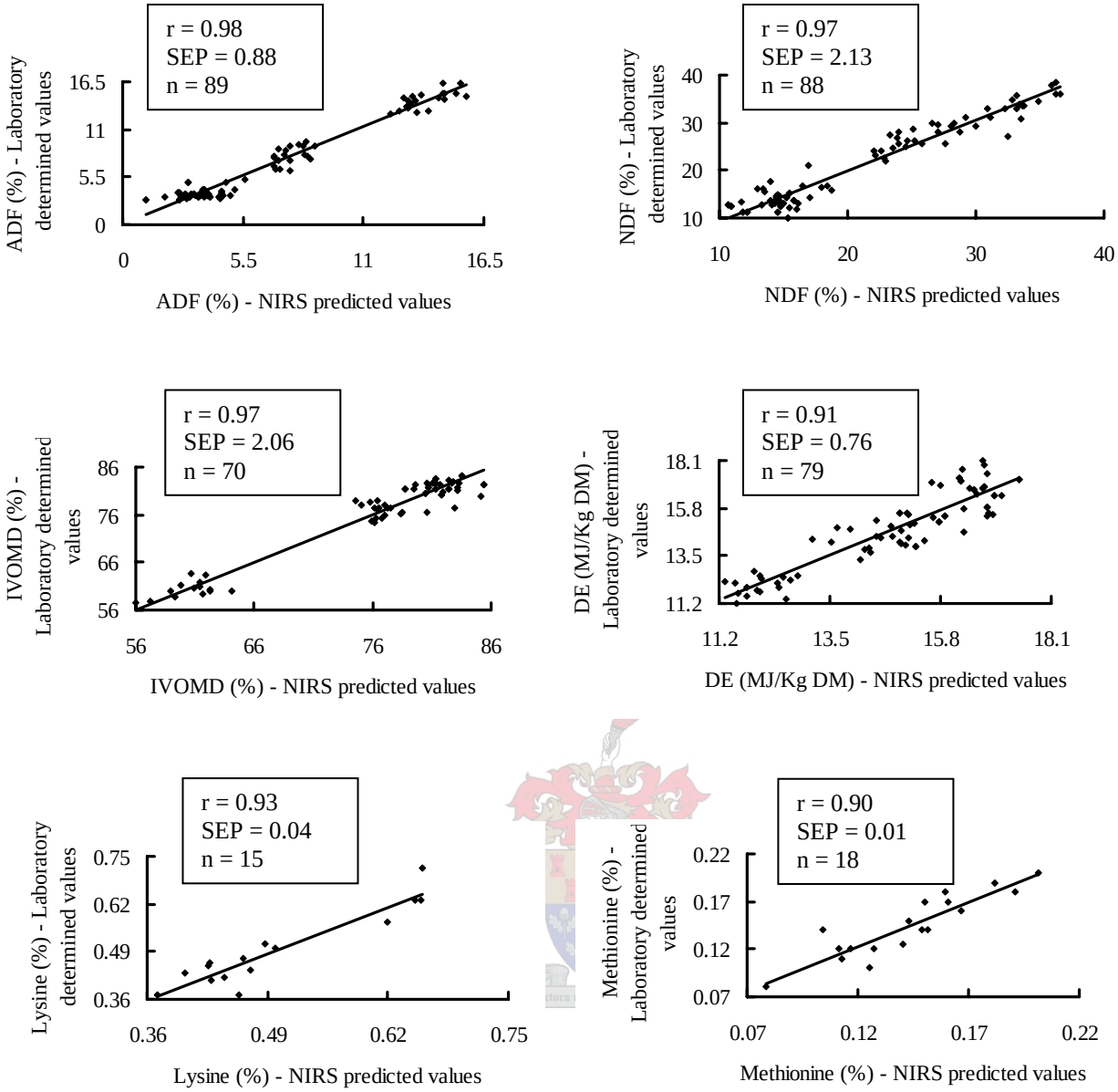

Figure 2 Relationship between laboratory-determined and NIRS predicted values for ADF, NDF, IVOMD, DE, lysine and methionine concentration in winter grain, using 15 and 89 samples, respectively, for each validation

The range, mean values, standard deviation (s.d.) and coefficient of variation (c.v.) for ash, DM, CP, IVOMD and TDN for samples in the calibration and validation sets compiled for maize grain, are shown in Table 5 and 6, respectively. The variation in the chemical composition of the samples used were similar to variation reported in the literature for maize grain (Hymowitz *et al.*, 1974; Orman & Schumann, 1991; Dijkhuizen *et al.*, 1998).

Table 5 Summary of chemical composition (%) of maize for the calibration set showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	154	1.22	0.88	1.47	0.12	9.84
DM	149	87.83	86.68	90.27	0.68	0.77
CP	150	6.89	6.04	8.41	0.44	6.39
IVOMD	148	99.88	90.20	112.36	4.67	4.68
TDN	148	82.63	75.72	90.47	3.22	3.90

Table 6 Summary of chemical composition (%) of maize for the validation set showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	33	1.23	1.07	1.42	0.09	7.32
DM	30	87.69	86.68	88.95	0.62	0.71
CP	28	6.84	6.4	7.74	0.34	4.97
IVOMD	30	100.21	91.94	112.36	4.96	4.95
TDN	30	82.20	77.23	87.17	3.07	3.73

Table 7 shows the statistics, including SEC and r values for the equations of best fit obtained for each of the constituents in maize. The r values for the validation sets and SEP are also shown in Table 7, as well as the SEL and predicted and laboratory mean values. The SEP values for DM (0.12%), CP (0.13%), IVOMD (1.56%) and TDN (1.03%) were less than double the SEL for the primary reference method analysis (Windham *et al.*, 1989), which is 0.11% (DM), 0.06% (CP), 0.90% (IVOMD) and 0.56% (TDN), respectively. This is an indication that the calibrations are acceptable and NIRS could therefore replace standard laboratory methods for routine analysis. The values were also lower than values reported in previous studies for maize (DM = 0.50%, CP = 0.31%; Rippke *et al.*, 1996), (CP = 0.26%; Orman & Schumann, 1991), (DM = 0.64%, CP = 0.38%) (Hardy *et al.*, 1996) and IVOMD for barley (0.96%) (Edney *et al.*, 1994).

The correlation between NIRS predicted values and laboratory determined values for the various chemical components of maize grain are given in Figure 3.

Table 7 Statistics of the calibration equations (for maize) of best fit and validation including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL).

Chemical Component	Number of PLSR factors	Calibration set r	Calibration set SEC (%)	Prediction set r	Prediction set SEP (%)	SEL (%)	Laboratory Mean Values (%)	Predicted Mean Values (%)
Ash	14	0.82	0.07	0.49	0.08	0.02	1.23	1.24
DM	6	0.97	0.17	0.98	0.12	0.11	87.69	87.71
CP	15	0.99	0.05	0.95	0.12	0.06	6.84	6.89
IVOMD	14	0.99	0.50	0.95	1.56	0.90	100.21	100.29
TDN	14	0.99	0.35	0.94	1.03	0.56	82.20	82.42

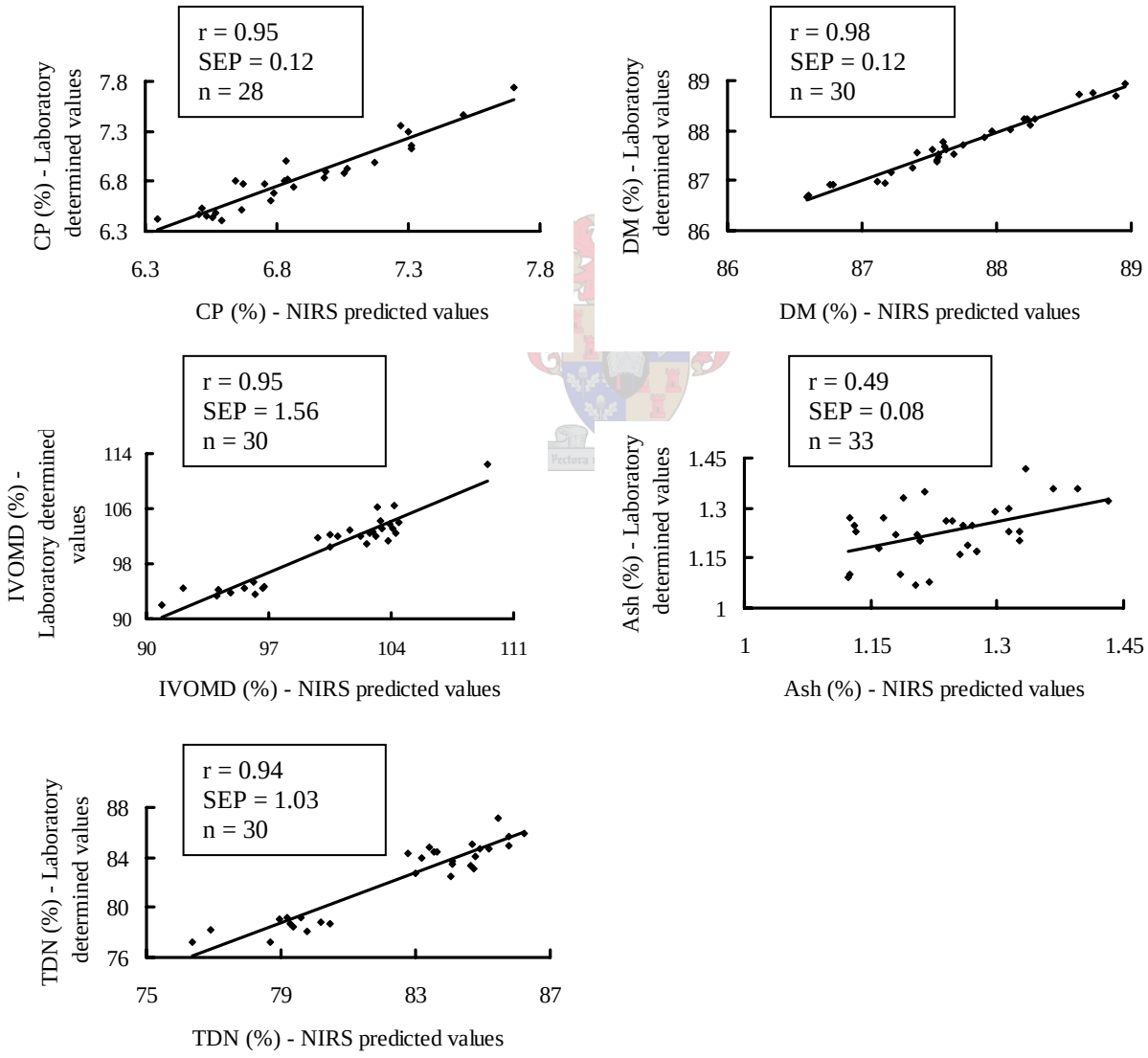

Figure 3 Relationship between laboratory determined and NIRS predicted values for TDN in maize grain, using 30 samples for validation.

Conclusion

High r and low SEC and SEP values showed NIRS to be effective as a rapid screening tool for winter grain and maize samples. A major advantage of NIRS is the ability to predict different chemical constituents simultaneously. NIRS therefore has the ability to predict the feeding values of a large number of samples in a short period of time. This will enable feed companies to monitor the nutrient composition of incoming feedstuffs accurately. Plant breeders will be able to improve the quality of winter grain and maize cultivars by monitoring large numbers of samples obtained from different lines and crosses. It should, however, be noted that each new harvest produces unique characteristics in each feedstuff. Changes in characteristics can, however, be accommodated by regularly adding new crop samples to the calibration and validation sets and in that way updating the NIRS calibrations.

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Pectora roborant cultus recti

Chapter 6

Prediction of the chemical composition of cereal hay, cereal straw, wheat stubble and an alfalfa-grass/hay mixtures with near reflectance infrared spectroscopy

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Abstract

Near infrared reflectance spectroscopy (NIRS) is well established for use in the analysis of forages, grains and silage. However, less information exist about the application of NIRS on cereal hay, cereal straw, grain stubble and alfalfa-grass/hay mixture. In the winter rainfall region of South Africa, cereal straw and stubble dominate the available forage during summer and autumn. Due to certain deficiencies in the available material, animal production from cereal straw and stubble is limited and supplementary feed is necessary to overcome these limitations. Near infrared reflectance spectroscopy calibrations were developed to predict the chemical composition of cereal hay cut at milky dough stage, cereal straws, wheat stubble and alfalfa-grass/hay mixture. The cereal hay types used in this study included barley, oats, wheat and triticale. Samples were collected in the Western Cape region of South Africa and analysed by standard laboratory methods. Standard errors of performance (SEP) and multiple correlation coefficients (r) for NIRS calibrations derived for cereal hays, included ash (r= 0.87; SEP= 0.40%), dry matter (DM) (r = 0.81; SEP = 0.51%), crude protein (CP) (r = 0.87; SEP = 0.71%), acid detergent fibre (ADF) (r = 0.95; SEP = 0.47%), neutral detergent fibre (NDF) (r = 0.93; SEP = 1.44%) and *in vitro* organic matter digestibility (IVOMD) (r = 0.89; SEP = 2.21%). Standard error of performance and r values for cereal straws were respectively, ash (0.33%; 0.99), DM (0.33%; 0.82), CP (0.47%; 0.97), ADF (1.69%; 0.89), NDF (1.16%; 0.95), IVOMD (4.52%; 0.82) and TDN (4.30%; 0.78). Standard error of performance and r values for wheat stubble included ash (1.20%; 0.92), DM (0.70%; 0.59), CP (0.54%; 0.82), ADF (1.41%; 0.95) and NDF (1.19%; 0.97). Calibration statistics for alfalfa-grass/hay mixtures included: ash (r = 0.95; SEP = 1.42%), DM (r = 0.99; SEP = 0.18%), CP (r = 0.98; SEP = 0.77%), ADF (r = 0.99; SEP = 1.69%), NDF (r = 0.99; SEP = 1.13%), fibre (r = 1.00; SEP = 0.72%), IVOMD (r = 0.96; SEP = 2.53%) and TDN (r = 0.96; SEP = 1.30%).

Keywords: alfalfa, cereal hay, cereal straw, stubble, chemical composition, near infrared reflectance spectroscopy,

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Introduction

Conventional analytical methods and animal feeding trials have generally been used to evaluate feed and forage quality. However, the cost and labour requirements to perform these studies are excessive. Near infrared reflectance spectroscopy (NIRS) is a physical technique with the potential of allowing cost effective, rapid and accurate determination of the chemical composition and nutritive value of different feeds (Flinn, 1991).

Near infrared reflectance spectroscopy is well established in the analysis of forages (Brown *et al.*, 1990; Waters & Givens, 1992), cereals and feedstuffs (Givens *et al.*, 1991; Van Kempen & Bodin, 1998) particularly cereal grains (Shenk *et al.*, 1981; Marten *et al.*, 1983), forage grasses (Berardo, 1997; Smith *et al.*, 1998; García-Ciudad *et al.*, 1999) and silage (De Boever *et al.*, 1996; Park *et al.*, 1998; Alomar *et al.*, 1999). However, less information exists about the application of NIRS on cereal hay.

Cereal grain such as wheat, barley, oats and triticale are important agricultural crops in the Western Cape region of South Africa. In this Mediterranean rainfall region these cereal hays are cut at the milky dough stage and conserved as hay. Bruno-Soares *et al.* (1998) conducted a study in Portugal to determine the chemical composition of cereal hay with NIRS and found the technique to be successful.

The nutritional quality of cereal straw is generally very poor, mainly due to its low digestibility and low nitrogen content. Straw alone is seldom able to provide a maintenance diet for sheep. Its main advantage is that it is a readily available and cheap roughage that can be used together with other raw materials to provide a source of roughage to feed sheep during summer and autumn, when better quality feed is unavailable. The quality of cereal straw can vary widely. Soil, fertility, rainfall, time of sowing and time of harvest can all affect straw quality (Aitchison, 1988). The digestibility and energy value of cereal straw can also vary considerably (Givens *et al.*, 1989). Consequently, for the most efficient utilisation of straw in a wide range of ruminant diets, it is necessary to have reliable and cost efficient laboratory methods that can accurately predict its nutritional value.

The digestibility of straw is closely related to the metabolisable energy (ME) content thereof (Givens *et al.*, 1989) and may account for up to 50% of the variability in voluntary dry matter (DM) intake of the animal. The most extensively utilised laboratory method to determine straw quality has been the *in vitro* technique using fluid-pepsin, based essentially on the procedure first described by Tilley & Terry (1963). *In vitro* digestibility techniques to determine straw quality, which utilise cell-free cellulase type enzymes have also been reported (Reid & Ørskov, 1987). However, these evaluation methods are laborious. Studies by Givens *et al.* (1991) demonstrated that the use of NIRS to predict OMD *in vivo* in straw could be more repeatable than the use of rumen fluid or cellulase-based procedures.

The use of NIRS to determine certain chemical components in alfalfa and alfalfa-grass/hay mixtures have also been reported previously (Albrecht *et al.*, 1987; Bertrand *et al.*, 1987; Reeves & Blosser, 1991). Alfalfa is one of the main dryland pasture legumes in the Mediterranean region of South Africa (Van Heerden & Tainton, 1987). Extreme fluctuations in climatic conditions in the areas where Mediterranean pastures are grown result in definite seasonal variation in the quality of the pasture (Brand *et al.*, 1991).

In the winter rainfall region of South Africa the available forage during summer and autumn is dominated by cereal stubble (Brand *et al.*, 1997). About 460 000 ha of wheat stubble are available in the wheat-sheep farming areas (Brand, 1996). Animal production from cereal stubble is, however, limited due to certain deficiencies in the available material. Cereal stubble is characterised by low digestibility and low nitrogen content and supplementary feed is necessary to overcome these limitations, especially where reproduction results in enhanced requirements of ewes grazing cereal stubble (Aitchinson, 1988). In order to provide the necessary supplementation throughout the season, chemical composition of the stubble needs to be monitored on a regular basis.

NIRS analysis is a method involving the absorption of near infrared light (1100-2500 nm) in organic compounds. The method is based on the fact that each of the major chemical components in forages has specific absorption characteristics which are a result of the asymmetric stretching vibrations of hydrogen bonds in the functional groups of molecules (Marten *et al.*, 1985). The method can be used for predicting the content of different components in forage samples, and this is achieved through calibration equations developed for each component of interest. This study was carried out to determine the accuracy for predicting the chemical composition of cereal hay, cereal straw, wheat stubble and alfalfa-grass/hay mixtures, produced in the Mediterranean rainfall region of South Africa, with near infrared reflectance spectroscopy.

Materials and methods

The cereal hay and straw used for the calibrations consisted of barley (Clipper, SVG 13, Gallion and Cape Barley cultivars), oats (Sederberg and Perdeberg cultivars), wheat (Palmiet) and triticale (Usgen 19, Kiewiet, Rex and SCR 13 cultivars), which were grown in the Western Cape region of South Africa. Hay samples were collected during the milky dough stage from experimental plots at 10 different locations in the Swartland and Rûens regions. The population studied included 227 samples: 78 (triticale), 76 (barley), 49 (oats) and 23 (wheat). Straw samples were collected after harvesting the grain from experimental plots at 10 different locations in the Swartland and Rûens regions. The population studied included 208 samples: 79 (triticale), 75 (barley), 39 (oats) and 15 (wheat). Wheat stubble samples were collected from an experiment conducted at the experimental farm, Langgewens, in the Swartland area of the winter rainfall region of South Africa (Brand *et al.*, 2000). Residual grains, ears, straw and green materials from two paddocks were sampled monthly close to the middle of each month by cutting ten replicate quadrates (1.0 x 0.25 m) per paddock. Fifty-six alfalfa-grass hay mixtures were obtained from farmers in the Calvinia region of South Africa.

Samples were dried and ground with a hammer mill (Scientec, RSA) to pass a 1 mm screen prior to analysis. All samples were divided into two sets for each constituent: a larger set for the calibration equations (calibration set) and a smaller set for the validation (validation set) of the calibrations (n values are shown in Tables 1, 2, 4, 5, 7, 8, 10 and 11, respectively). Outliers were removed according to suggestions by the software (Bran+Luebbe SESAME Version 2.00-software, BRAN+LUEBBE GmbH, Norderstedt,

Germany). Outliers listed as 'T'- and 'H'-values were taken into consideration. The 'T'-value measures how closely the reference value matches the predicted value. The spectrum is listed and flagged with an asterisk (*) if the 'T'-value is greater than 2.5 times the standard error of calibration. These values can be potential outliers, because they do not fit the calibration equation as well as the other samples. The 'H'-value is a measure of leverage. It puts a numerical value on the influence of a particular spectrum in determining the regression line. It is a measure of multidimensional distance of a spectrum to the regression line. If a spectrum with a large 'H'-value has a small 'T' value, it is likely to be valuable for the calibration. If both the 'H' and the 'T' values are large, it is more likely to be a true outlier. Equations of best fit were chosen for each constituent based on statistical analysis. After removal of the outliers, every fifth sample was selected for the validation sets.

Samples were analysed for dry matter (DM) and ash content by standard methods (AOAC, 1984) (Method numbers 7.003 and 7.009, respectively). Crude protein (CP) was measured by a FP-428 Nitrogen and Protein Determinator (Leco Corporation, 3000 Lakeview Avenue, St Joseph, MI 49085-2396). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as described by Van Soest (1963) and Van Soest & Wine (1967). *In vitro* organic matter digestibility (IVOMD) was determined by the method as described by Tilley & Terry (1963). Total digestible nutrients (TDN) were calculated by the following equation: TDN = [(100 - % ash)/100] x (0.8 IVOMD +15.35) on DM basis (Engels & Van der Merwe, 1967). Wet chemistry and NIRS analyses were done simultaneously for all the samples, except for wheat stubble samples. These samples were stored in a freezer before analysis with the NIRS. Cross testing were done on the samples to test the accuracy of the reference values before the calibrations were derived.

Near infrared reflectance spectroscopy analyses were done with an InfraAlyzer 500 Near Infrared Reflectance Analyser (IA-500) using Bran+Luebbe SESAME Version 2.00-software (BRAN+LUEBBE GmbH, Norderstedt, Germany). Spectra were measured over the wavelength range 1100 – 2500 nm, recording as log 1/R at 2 nm intervals. Calibration equations were developed for each constituent following the recommended protocol of Windham *et al.* (1989). The log 1/R and second derivative (segment = 1; gap = 0) mathematical treatment were used to analyse the spectra of green crop cereal. Calibrations were developed by means of partial least-square regression (PLSR).

Results and discussion

The range, mean values, standard deviation (s.d.) and coefficient of variation (c.v.) for samples in the calibration and validation sets of the cereal hays calibrations are shown in Table 1 and 2, respectively. The variation in the chemical composition of the samples used seems to cover the whole spectrum reported in literature for cereal hays (Van Wyk *et al.*, 1955; Bruno-Soares *et al.*, 1998).

Table 1 Summary of chemical composition of cereal hay (%) for the calibration set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	173	3.98	2.29	6.29	0.80	20.10
DM	172	90.88	87.85	93.46	0.82	0.90
CP	161	7.83	4.4	12.36	1.63	20.82
ADF	179	28.60	21.46	37.66	3.62	12.66
NDF	181	50.64	42.84	61.85	3.91	7.72
IVOMD	174	66.55	51.32	75.79	4.76	7.15

Table 2 Summary of chemical composition (%) of cereal hays for the validation set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	43	3.91	2.72	6.04	0.75	19.18
DM	42	90.92	88.66	93.25	0.87	0.96
CP	39	7.79	5.43	10.4	1.29	16.56
ADF	42	28.49	22.24	35.82	3.02	10.6
NDF	42	50.92	44.46	60.93	3.91	7.68
IVOMD	39	66.66	55.24	75.17	4.56	6.84

Table 3 shows the statistics for cereal hay, including standard errors of calibration (SEC), multiple correlation coefficients (r) for both the calibration set and the validation set, standard errors of performance (SEP), standard errors of laboratory (SEL) (Snedecor & Cochran, 1980) and the means of the laboratory and predicted values. Results of NIRS calibrations indicated high correlation with all laboratory analysis. Standard error of calibration ranged between 0.20% for ash to 1.66% for IVOMD and r values ranged from 0.90 for DM to 0.97 for ash. SEP values were comparable with values previously reported for the prediction of quality of cereal hay by NIRS (Shenk *et al.*, 1981; Marten *et al.*, 1983; Bruno-Soares *et al.*, 1998). The standard error of performance for CP, ADF and NDF were similar to or lower than those reported for winter grain forages (Marten *et al.*, 1983; Flinn & Murray, 1987; Jones *et al.*, 1987; Brown *et al.*, 1990; Bruno Soares *et al.*, 1998). The SEP value for IVOMD was much higher than reported by Givens *et al.* (1992) for herbage, but lower than values reported by Brown *et al.* (1990) and Bruno-Soares *et al.* (1998) for green crop cereals. SEP values for ash (0.4%), DM (0.51%), CP (0.71%), NDF (1.44%) and IVOMD (2.21%) were, however, more than two multiplications of the SEL for the primary reference method analysis and could therefore not be accepted for prediction (Windham *et al.*, 1989). There are a couple of possible reasons that

could explain the inaccurate calibrations: (i) reference values were not accurately analysed, (ii) samples were milled inconsistently, (iii) sample cups were not constantly packed, (iv) the temperature surrounding the instrument fluctuated too much, and(v) not enough variation included in the calibration. The correlation between the laboratory-determined and NIRS predicted values for the different constituents of cereal hays are given in Figures 1 and 2.

Table 3 Statistics of the calibration equations of best fit and prediction including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL) to predict the chemical composition of cereal hay by NIRS.

Chemical Component	Number of PLSR factors	Calibration set r	Calibration set SEC (%)	Validation set r	Validation set SEP (%)	SEL (%)	Laboratory Mean Values (%)	Predicted Mean Values (%)
Ash	9	0.97	0.20	0.87	0.40	0.11	3.91	3.98
DM	6	0.90	0.37	0.81	0.51	0.13	90.92	90.89
CP	6	0.95	0.54	0.87	0.71	0.21	7.79	7.81
ADF	5	0.95	1.13	0.95	0.93	0.47	28.49	28.44
NDF	6	0.94	1.34	0.93	1.44	0.60	50.92	50.56
IVOMD	6	0.94	1.66	0.89	2.21	0.73	66.66	67.12

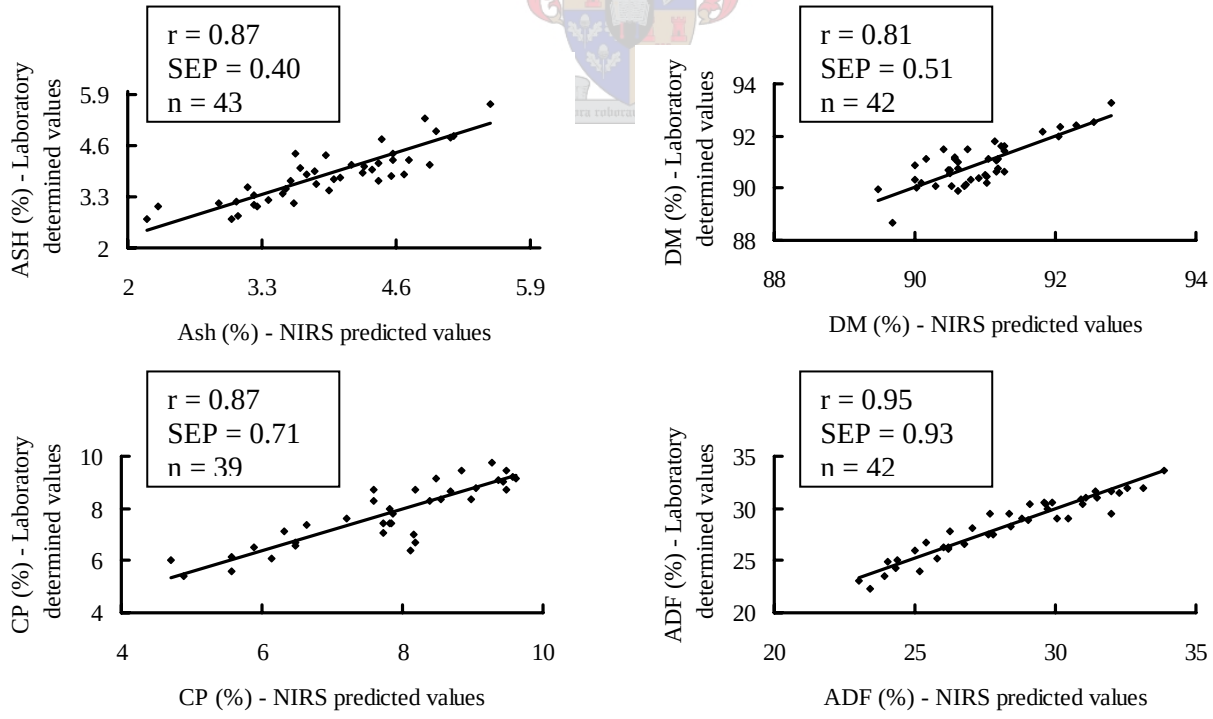

Figure 1 Relationship between laboratory determined and NIRS predicted values for ash, DM, CP and ADF for cereal hay, using between 39 and 43 samples for each validation.

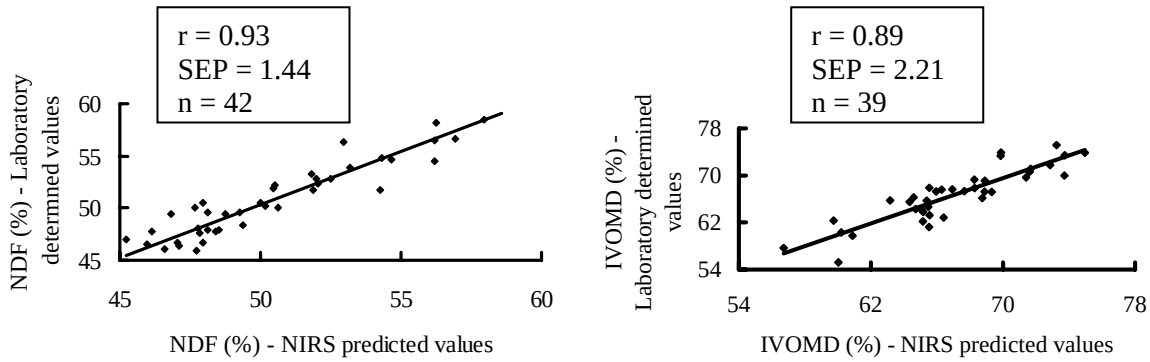

Figure 2 Relationship between laboratory determined and NIRS predicted values for NDF and IVOMD for cereal hay, using between 39 and 42 samples for each validation.

The range, mean values, standard deviation (s.d.) and coefficient of variation (c.v.) for samples in the calibration and validation sets of cereal straw are shown in Table 4 and 5, respectively. The variation in the chemical composition of the samples used seems to cover the whole spectrum reported in literature for cereal straw (Kernan *et al.*, 1979; O'Donovan, 1983; Nicholson, 1984; Givens *et al.*, 1989).

Table 4 Summary of chemical composition of cereal straw for the calibration set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component	n	Mean (%)	Min (%)	Max (%)	s.d.(%)	c.v.
Ash	160	4.13	2.00	15.14	1.52	36.8
DM	159	92.72	90.36	94.67	0.82	0.88
CP	163	3.90	1.66	12.53	1.92	49.23
ADF	149	46.61	27.82	55.31	4.61	9.89
NDF	161	74.50	52.03	82.06	4.49	6.03
IVOMD	157	31.26	8.62	58.05	8.92	28.53
TDN	162	37.07	19.84	56.00	7.02	18.94

Table 5 Summary of chemical composition of cereal straws for the validation set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component	n	Mean (%)	Min (%)	Max (%)	s.d.(%)	c.v.
Ash	39	4.20	2.20	14.46	1.97	46.90
DM	32	92.70	91.68	93.58	0.57	0.62
CP	39	4.09	1.68	11.00	1.92	46.94
ADF	33	46.51	38.54	51.58	3.17	6.82
NDF	41	74.44	62.77	81.56	3.66	4.92
IVOMD	38	31.75	11.21	46.28	7.90	24.88
TDN	41	37.06	21.74	48.90	6.77	18.27

Table 6 shows the statistics for cereal straws, including SEC, r for both the calibration set and the validation set, SEP and SEL. Results of NIRS calibrations indicates high correlations with all laboratory results, except for TDN (r = 0.89). The SEP values for IVOMD (4.52%) and TDN (4.30%) were very high. Givens *et al.* (1991) also reported high values for IVOMD in straw (3.24%). This results is, however, typical of such biological measures (Norris *et al.*, 1976; Shenk *et al.*, 1979). Hence also the high SEP value for TDN, for TDN is a derivative from IVOMD. Calibrations for DM, ADF, NDF, IVOMD and TDN were also inaccurate (SEP values were more than two multiplications of the SEL for the primary reference method analysis, Windham *et al.*, 1989) and the possible reasons would be the same as were discussed for the cereal hay calibrations. The correlation between the laboratory-determined and NIRS predicted values for the different constituents of cereal straws are given in Figure 3.

Table 6 Statistics of the calibration equations of best fit and prediction including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL) to predict the chemical composition of cereal straws by NIRS.

Chemical Component	Number of PLSR factors	Calibration set r	Calibration set SEC (%)	Validation set r	Validation set SEP (%)	SEL (%)	Laboratory Mean Values (%)	Predicted Mean Values (%)
Ash	10	0.99	0.19	0.99	0.33	0.32	4.20	4.25
DM	8	0.91	0.34	0.82	0.33	0.10	92.70	92.67
CP	7	0.98	0.35	0.97	0.47	0.31	4.09	4.12
ADF	4	0.96	1.29	0.89	1.69	0.55	46.51	46.76
NDF	7	0.98	0.96	0.95	1.16	0.57	74.44	74.20
IVOMD	6	0.94	3.22	0.82	4.52	1.27	31.75	32.08
TDN	5	0.89	3.27	0.78	4.30	1.06	37.06	37.66

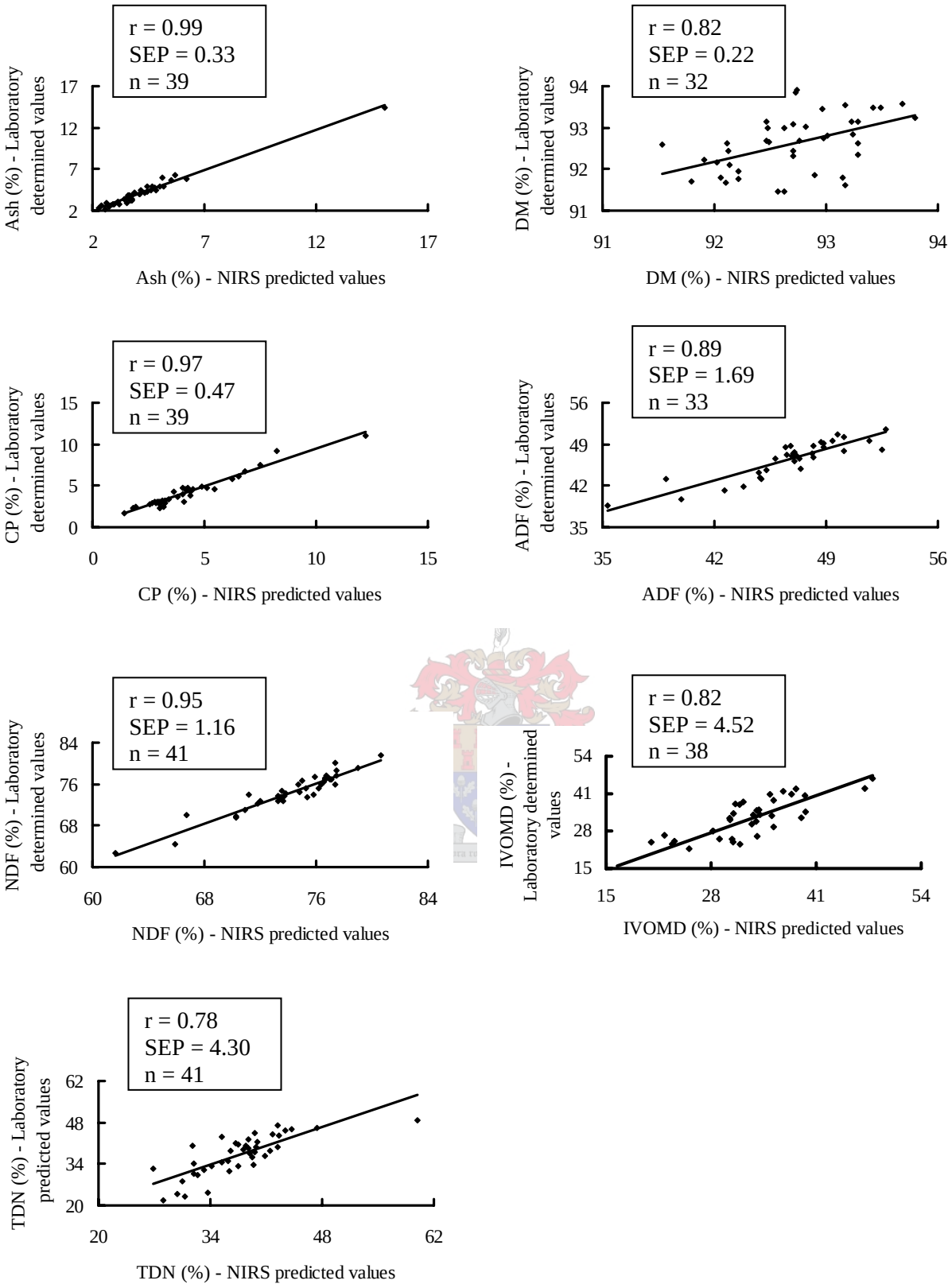

Figure 3 Relationship between laboratory determined and NIRS predicted values for ash, DM, CP, ADF, NDF and IVOMD for cereal straw, using between 33 and 41 samples for each validation.

The range, mean values, standard deviation (s.d.) and coefficient of variation (c.v.) for samples in the calibration and validation sets of wheat stubble are shown in Table 7 and 8 respectively. The variation in the chemical composition of the samples used seems to cover the spectrum reported in literature for wheat stubble (Aitchison, 1988; Brand, 1996; Brand *et al.*, 2000).

Table 7 Summary of chemical composition (%) of wheat stubble for the calibration set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	86	4.55	1.44	16.77	2.49	54.73
DM	88	95.34	93.63	97.45	0.89	0.93
CP	83	2.97	1.52	6.48	1.16	39.06
ADF	84	56.19	37.73	63.26	4.75	8.45
NDF	86	75.82	47.24	82.07	5.03	6.63

Table 8 Summary of chemical composition (%) of wheat stubble for the validation set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	19	4.59	1.72	14.99	2.96	64.49
DM	21	95.37	93.98	97.18	0.85	0.89
CP	20	2.81	1.67	4.92	0.94	33.45
ADF	22	56.56	47.63	62.13	3.99	7.05
NDF	20	75.91	60.99	81.07	4.87	6.42

Table 9 shows the statistics for wheat stubble, including SEC, r for both the calibration set and the validation set, SEP, SEL and the means of the laboratory and predicted values. The SEP values for ash (1.20%), ADF (1.41%) and NDF (1.17%) were within two multiplications of the SEL for the primary reference method analysis (Table 9). The final NIRS equations could, therefore, be accepted for use and the SEP for validation could be used as a reliable indication of the accuracy of the final NIRS equations (Windham *et al.*, 1989). The reason for the inaccurate DM calibration could possibly be due to the time delay between laboratory analysis and collection of the spectral data. CP is usually a highly predictable constituent (Shenk *et al.*, 1981). A possible reason for the inaccurate CP calibration could be that there was an uneven distribution of the constituent in the presented samples. The correlation between the laboratory-determined and NIRS predicted values for the different constituents of wheat stubble and alfalfa-grass hay mixtures are given in Figure 4.

Table 9 Statistics of the calibration equations of best fit and prediction including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL) to predict the chemical composition of wheat stubble by NIRS.

Chemical Component	Number of PLSR factors	Calibration set r	Calibration set SEC (%)	Validation set r	Validation set SEP (%)	SEL (%)	Laboratory Mean Values (%)	Predicted Mean Values (%)
Ash	4	0.96	0.72	0.92	1.20	0.68	4.59	4.36
DM	4	0.92	0.36	0.59	0.70	0.18	95.39	95.32
CP	4	0.96	0.33	0.82	0.54	0.21	2.81	2.85
ADF	5	0.99	0.78	0.95	1.41	0.85	56.56	56.14
NDF	6	0.99	0.70	0.97	1.19	1.06	75.91	75.67

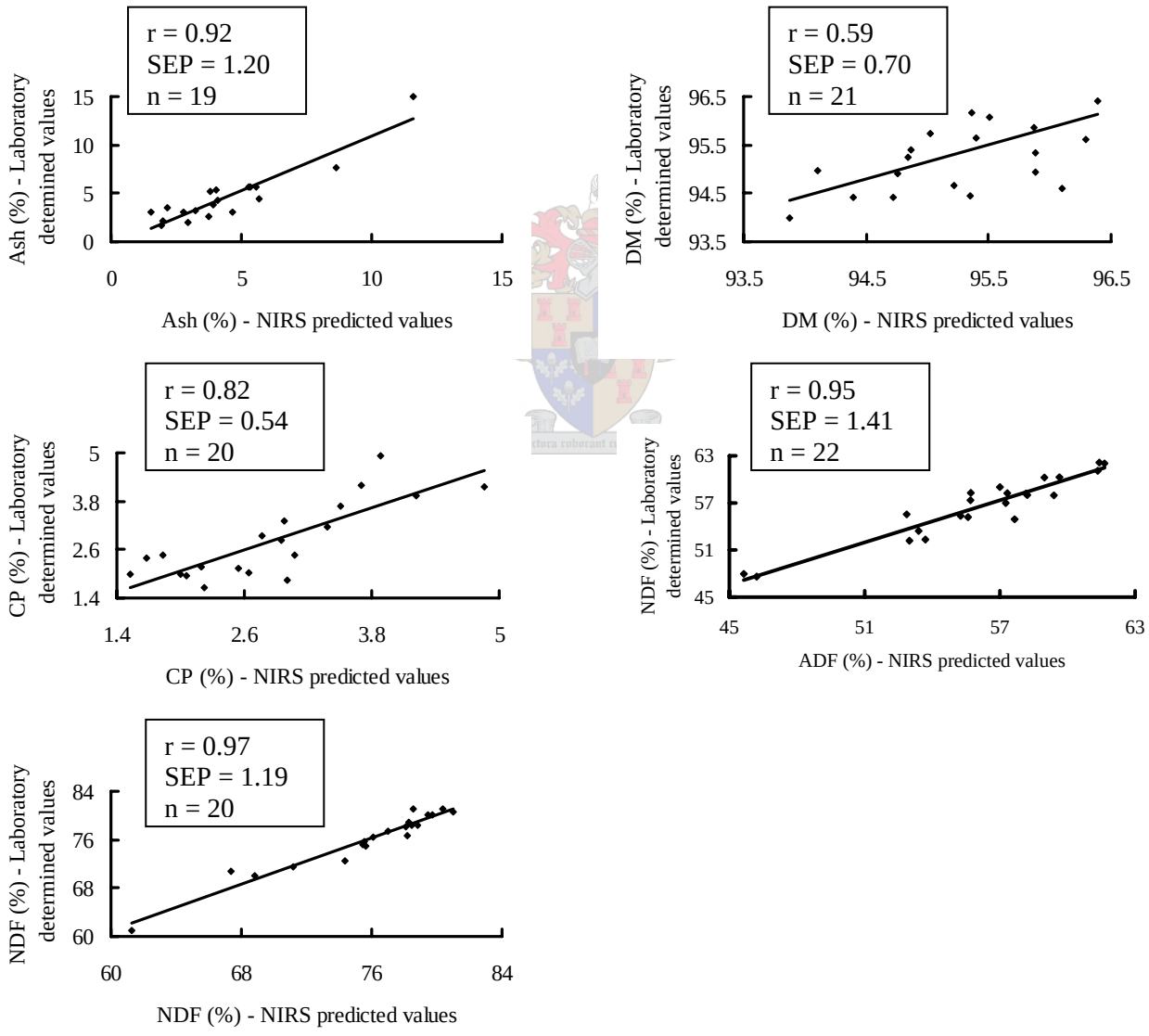

Figure 4 Relationship between laboratory determined and NIRS predicted values for ash, DM, CP, ADF and NDF for wheat stubble, using between 19 and 22 samples for each validation.

The range, mean values, standard deviation (s.d.) and coefficient of variation (c.v.) for samples in the calibration and validation sets of alfalfa-grass hay mixtures are shown in Table 10 and 11 respectively. The variation in the chemical composition of the samples used seems to cover the spectrum reported in literature for alfalfa and grass samples (Van Heerden & Tainton, 1987; Park *et al.*, 1998; Smith *et al.*, 1998).

Table 10 Summary of chemical composition (%) of alfalfa-grass hay mixtures for the calibration set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	80	8.85	5.19	12.27	1.48	16.72
DM	87	89.64	86.82	93.12	1.32	1.47
CP	86	16.50	6.41	21.66	3.68	22.30
ADF	82	32.85	20.72	56.06	7.59	23.11
NDF	87	39.11	25.73	64.85	8.80	22.50
Fibre	83	26.95	17.34	47.03	7.03	26.09
IVOMD	78	73.34	49.72	83.87	8.00	10.91
TDN	75	59.91	46.90	66.32	4.02	6.71

Table 11 Summary of chemical composition (%) of alfalfa-grass hay mixtures for the validation set, showing number of samples (n), mean, range of values, standard deviation (s.d.) and coefficient of variation (c.v.).

Chemical component (%)	n	Mean	Min	Max	s.d.	c.v.
Ash	16	8.87	5.19	11.05	1.65	18.60
DM	17	89.49	87.43	90.88	1.01	1.13
CP	17	16.15	6.41	21.36	4.01	24.83
ADF	18	34.32	20.72	56.06	8.77	25.55
NDF	17	41.00	29.60	54.83	7.57	18.46
Fibre	17	28.88	18.68	47.03	7.68	26.59
IVOMD	15	72.93	49.72	80.80	9.22	12.64
TDN	15	59.63	46.90	64.11	4.86	8.15

Table 12 shows the chemometric statistics for alfalfa-grass hay mixtures, including SEC, r for both the calibration set and the validation set, SEP, SEL and the means of the laboratory and predicted values. The SEP values were less than double the SEL for the primary reference method analysis (Windham *et al.*, 1989), which is an indication that the NIRS is suitable for predicting the chemical composition of alfalfa-grass/hay mixtures. The SEP for DM, CP, ADF and NDF (0.18%, 0.77%, 1.69%, 1.13%) were also smaller than

values previously reported by Bertrand *et al.* (1987) (DM = 2.22%; CP = 0.77%), Reeves & Blosser (1991) (DM = 1.99%; CP = 0.94%; ADF = 2.27%) and Smith & Flinn (1991) (CP = 0.85%; NDF = 2.17%) for alfalfa and Park *et al.* (1998) for grass silage (DM = 23.3%; NDF = 34.4%). The correlation between the laboratory-determined and NIRS predicted values for the different constituents of alfalfa-grass hay mixtures are given in Figure 5.

Table 12 Statistics of the calibration equations of best fit and prediction including the number of PLSR factors used for each equation, standard error of calibration (SEC), standard error of performance (SEP) and standard error of laboratory (SEL) to predict the chemical composition of alfalfa-grass hay mixtures by NIRS.

Chemical Component	Number of PLSR factors	Calibration set		Validation set		SEL (%)	Laboratory Mean Values (%)	Predicted Mean Values (%)
		r	SEC (%)	r	SEP (%)			
Ash	6	0.96	0.41	0.98	0.37	0.41	8.87	8.89
DM	5	0.99	0.23	0.99	0.18	0.25	89.49	89.55
CP	6	0.98	0.82	0.98	0.77	0.97	16.15	16.31
ADF	6	0.97	1.83	0.99	1.69	2.07	34.32	33.42
NDF	6	0.97	2.24	0.99	1.13	1.83	41.00	40.63
Fibre	8	0.99	1.24	1.00	0.72	1.86	28.88	28.59
IVOMD	7	0.95	2.74	0.96	2.50	2.38	72.93	73.24
TDN	7	0.91	1.71	0.96	1.30	1.25	59.63	59.45

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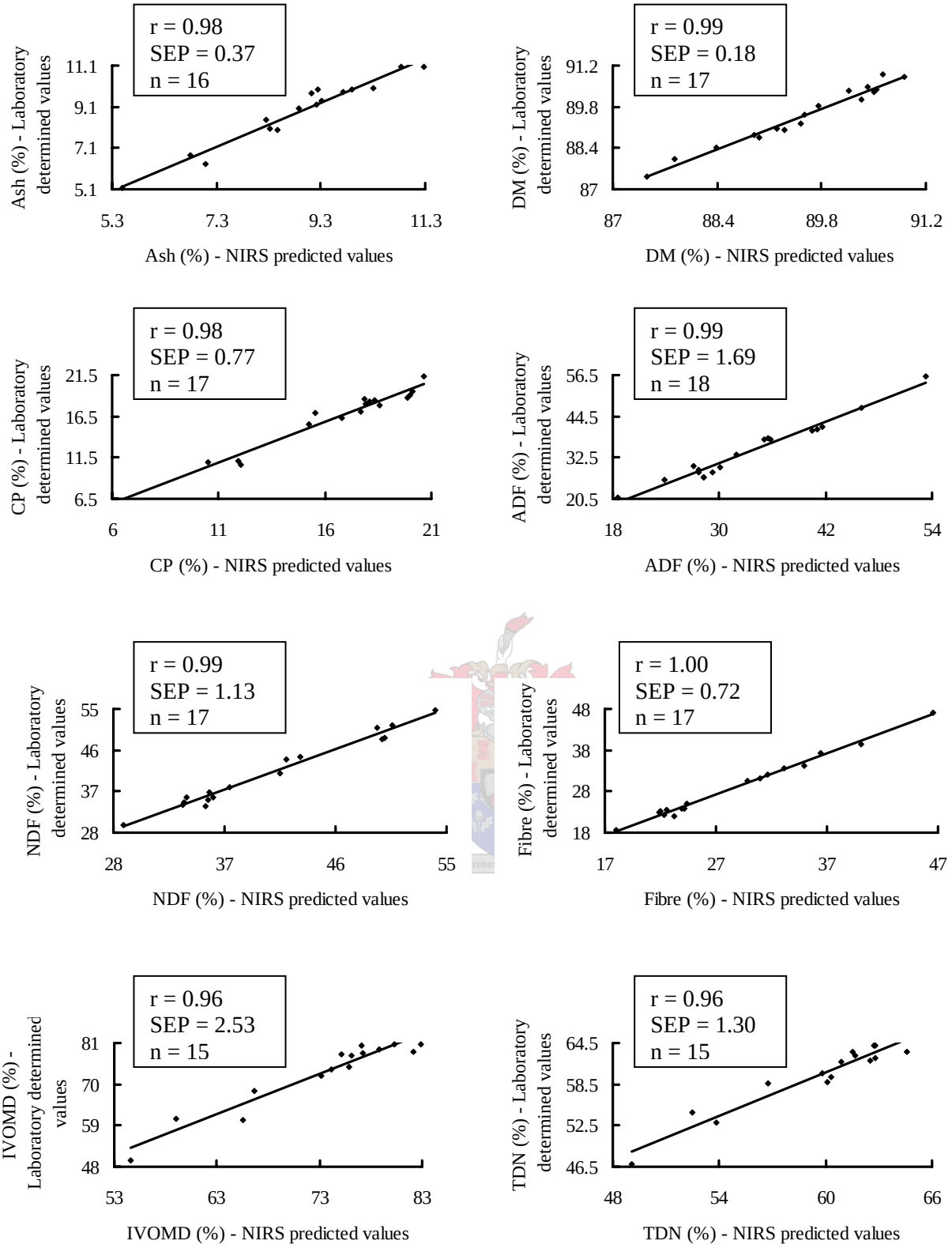

Figure 6 Relationship between laboratory determined and NIRS predicted values for ash, DM, CP, ADF, NDF, fibre, IVOMD and TDN for alfalfa-grass/hay mixtures, using between 15 and 18 samples.

Conclusion

NIRS calibrations developed for wheat stubble and alfalfa-grass hay mixtures in this study resulted in predictions with similar or slightly higher accuracy than calibrations reported in the literature. This could be due to the fact that the samples were from closed population groups and little variation therefore occurred between the samples. The calibrations would therefore lack robustness and could possibly not be suitable for quality control purposes. The calibrations developed for cereal hay and straw were not suitable for prediction purposes. Possible reasons were discussed to explain these inaccurate calibrations. Whilst calibrating, all possible measures should be taken to ensure that external influences (like milling, packing of the sample cup, temperature, etc) on the samples, reference values and spectra be minimised as far as possible, thus resulting in more accurate calibrations.

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Chapter 7

Variation in the chemical composition of different types of winter grain produced in the Western Cape area of South Africa

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Abstract

Five grain types (2-row barley, 6-row barley, oats, wheat and triticale) were produced at two localities (Swartland and Rûens) in the Western Cape area of South Africa. A randomised square experimental design with four replicates per cultivar was used. The grain samples were analysed for ash, dry matter (DM), crude protein (CP) and fat content. The chemical composition of the different grain types was compared with each other by analysis of variance with grain type and area of production as main factors. Interaction between constituent and locality was found with regard to ash and CP content, therefore the means for these constituents of the different grain types are reported separately for each locality. Crude protein content did not differ greatly in the Swartland area, the values ranged from 12.08% (2-row barley) to 13.8% (wheat). In the Rûens area larger difference was found in the CP content. Values ranged from 9.21% (6-row barley) to 11.68% (wheat). There was no difference in fat content between localities, but there was a significant difference between the fat content of oats (5.54%) and that of other grain types (triticale, 1.77%, 6-row barley, 1.91%, wheat, 2% and 2-row barley, 2.04%).

Keywords: Chemical composition, barley, oats, triticale, wheat

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Introduction

Maize is the energy source traditionally used in animal feedstuffs (Brand *et al.*, 1995; Brand & Van der Merwe, 1996). Due to high transport costs from the north, which leads to high costs of maize in the Western Cape, the use of other feeding grains need to be examined as an alternative source for animal feedstuffs in this region (Burger *et al.*, 1996). Cereals are concentrates rich in carbohydrates and are therefore used as energy sources. Their low protein level and deficiencies in some minerals and vitamins can be relatively easily overcome by protein, mineral and vitamin supplements (Todorov, 1988). It was found, however, that the crude protein of feeding grain tends to be higher than that of maize, which can lead to an advantage when used in balanced diets for animal feeds (Heydenrych *et al.*, 2000). Most feeding grains have been tested in pig and poultry diets with success. Feeding grains include barley (Kritzinger & Olckers, 1985), naked oats (Brand & Van der Merwe, 1996), feed grade wheat (Kemm *et al.*, 1986), triticale (Brand *et*

al., 1995) and oats (Todorov, 1988). Brandt *et al.* (submitted) reported that the variation in the chemical composition between individual samples of cereal grains were higher than values documented in the literature. The variation in composition of grain could therefore have a significant effect on animal performance if the diet is formulated on mean table values. The aim of this study was to provide information regarding the variation in the chemical composition of different types of cereal grains grown under similar climate and soil conditions in the Western Cape region of South Africa.

Materials and Methods

Samples were collected at two experimental farms, Langgewens (33°7'S 18°36'E) (Swartland) and Tygerhoek (34°7'S 19°56'E) (Rûens), of the Department of Agriculture, Western Cape, South Africa. Five different grain types and nine different cultivars were compared in this study. The grain types that were used included 2-row barley (Clipper, B 94/5, B 94/7), 6-row barley (SVG 13), oats (Kompasberg, Sederberg), wheat (SST 57, Kariega) and triticale (Kiewiet). A randomised square design with four replicates per cultivar was used.

Samples were analysed for dry matter (DM), ash and ether extract (EE) by standard methods (AOAC, 1984) (Method numbers 7.003, 7.009 and 7.061, respectively). Crude protein (CP) was measured by a FP-428 Nitrogen and Protein Determinator (Leco Corporation, 3000 Lakeview Avenue, St Joseph, MI 49085-2396). All values are expressed on a 100% DM basis.

Statistical analyses were done with Statgraphics 5.0 (Statistical Graphics Inc., 1991) as described by Snedecor & Cochran (1980). Differences between the different types of grain and locality were determined by a multi-factor analysis of variance using a protected F-value.

Pectora roborant cultus recti

Results and Discussion

A comparison between the chemical composition of different cultivars within the different grain types was conducted (Table 1). Significant differences were found between the fat content of wheat (Kompasberg 6.12%; Sederberg 4.96%) and oats (Kariega 1.93%; SST 57 2.07%) cultivars. A significant difference also occurred in the CP content of 2-row barley cultivars, Clipper (10.81%) and B94/5 (12.10%).

A second statistical analysis was conducted to compare the chemical composition of the different grain types collected at the two selected localities. The interactions ($P < 0.05$) between grain type and locality for CP and ash content are depicted in Table 2. Crude protein between grain types did not differ in the Swartland area, with the mean values ranging from 12.08% (2-row barley) to 13.8% (wheat). In the Rûens area significant differences were found in the CP content of different grain types, with values ranging from 9.21% (6-row barley) to 11.68% (wheat). Values reported in the literature for CP content were 11% (barley), 11.4% (oats), 14% (triticale) and 14.1% (wheat) (NRC, 1994). In the absence of a significant interaction ($P > 0.05$) between grain type and locality for fat and dry matter content, the main effects are presented in Table 3. There were no differences in fat content between localities, but there was a significant difference ($P <$

0.05) between the fat content of oats (5.54%) and that of other grain types (triticale, 1.77%; 6-row barley, 1.91%; wheat, 2% and 2-row barley, 2.04%). These values correlate with values obtained by Van der Merwe (1977) (oats 5%; barley, 2.1%; wheat, 2%) for winter grain in South Africa. The NRC (1994) values for fat content are: oats (4.2%), barley (1.8%), wheat (2.5%) and triticale (1.5%). The differences ($P < 0.05$) noted in the dry matter composition of the different types of grain between localities could be due to sampling in different temperature and handling conditions, which resulted in the grains from the Swartland area having a higher DM composition than the grains from the Rûens area.

Table 1 Mean values of the chemical composition of different cultivars between grain types (100% DM basis).

	Oats		Wheat		2-Row Barley		
	Kompasberg	Sederberg	Kariega	SST 57	Clipper	B94/7	B94/5
Ash	2.40[a]	2.83[b]	1.38	1.40	2.35	2.51	2.40
DM	91.74	91.76	90.10	89.76	90.42	90.27	90.48
CP	11.51	11.84	12.63	12.85	10.81[a]	11.06[ab]	12.10[b]
Fat	6.12[a]	4.96[b]	1.93[a]	2.07[b]	2.01	2.04	2.07

[a, b] Row means within grain types with common superscripts do not differ ($P < 0.05$)

Table 2 The interaction between grain type for ash and crude protein for different types of winter grain produced in the Western Cape area of South Africa (100% DM basis).

		Ash		CP	
Grain Type	n	Swartland	Rûens	Swartland	Rûens
2-row barley	24	2.32[bc]	2.52[b]	12.08[a]	10.56[b]
6-row barley	8	2.31[bc]	2.55[b]	12.27[a]	9.21[a]
Oats	16	2.59[c]	2.65[b]	12.09[a]	11.27[c]
Wheat	16	1.52[a]	1.25[a]	13.80[b]	11.68[c]
Triticale	8	2.23[b]	1.59[a]	13.14[ab]	10.31[b]
Mean		2.19	2.11	12.68	10.61

[a, b, c, d,] Column means with common superscripts do not differ ($P < 0.05$)

Table 2 A comparison of the dry matter and fat composition between different types of winter grain produced in the Western Cape area of South Africa (100% DM basis).

		DM	Fat
Main effect	n	LS Mean	LS Mean
Grain Type			
2-row barley	24	90.39[c]	2.04[a]
6-row barley	8	90.54[c]	1.91[a]
Oats	16	91.75[d]	5.54[b]
Wheat	16	89.93[b]	2.00[a]
Triticale	8	89.33[a]	1.77[a]

[a, b, c, d,] Column means with common superscripts do not differ (P<0.05)

Conclusion

The chemical composition values of different types of grain obtained in this study differed from the values documented and normally used for feed formulation. Variation occurred between the chemical composition of different grain types as well as between localities. The study accentuated the need for the analysis of different batches of grain produced under different conditions for use in animal feed. Care should therefore be taken when maize is replaced by small grains as energy source in animal feed. Composition of small grains may possibly be affected by factors such as species, cultivar, climate, rainfall, soil fertility and fertilizer application (Brandt *et al.*, submitted).

Pectora roborant cultus recti

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Chapter 8

Differences in the chemical composition and digestibility of cereal straw and hay produced in a Mediterranean rainfall area of South Africa

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Abstract

Straw and hay samples were collected from 10 different localities in the Swartland and Rûens areas of South Africa. A randomised square experimental design with four replicates per sample was used. Hay samples were collected during the milky dough stage of the grain, while straw was sampled after harvesting the grain. Samples were analysed for dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and *in vitro* organic matter digestibility (IVOMD), while total digestible nutrient values (TDN) were calculated. There was no significant difference between the CP of the different types of hay. Barley hay had the highest IVOMD value (75.32%), followed by lower values for wheat (74.49%), triticale (72.04%) and oats (69.77%). Wheat hay had the highest TDN value (67.07%), with oats having the lowest value (62.75%). Oats was higher in NDF (60.28%) compared to the other types of hay. Significant differences ($P < 0.05$) existed in the ADF values of barley (29.79%), oats (34.24%) and triticale (32.30%). Oat straw had a much higher IVOMD value (39.20%) compared to values obtained for the other grains. Barley had the lowest IVOMD value (29.32%). There was no significant difference ($P > 0.05$) between the crude protein content of the different types of straw. The TDN value of oats (44.48%) was higher than values obtained for the other straw types. There was no significant difference between the ADF value of the different straw types, but barley had the highest NDF value (81.77%), which differed from the value obtained for oats (78.12%) and triticale (79.54%). There was a significant difference in the ash, CP, IVOMD, TDN and ADF contents of straw produced in the Swartland and Rûens areas, respectively. Ash, CP, IVOMD and ADF values of cereal straw samples were higher when produced in the Rûens. Neutral detergent fibre values were higher in cereal straw samples collected in the Rûens, except for barley, which had a higher NDF value when samples were collected in the Swartland. The TDN value of cereal straw was higher for the samples collected in the Rûens compared to that collected in the Swartland, although wheat and triticale showed higher TDN values when sampled in the Swartland. Values obtained in this study provide a database for accurate values of hay and straw types produced in the Western Cape area of South Africa.

Keywords: barley, chemical composition, hay, oats, straw, triticale, wheat

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Introduction

Most grain types, cultivated with the purpose of the production of grain for human and livestock consumption, yield considerable amounts of crop by-products. These are normally consumed by ruminant animals. Such by-products usually contain high amounts of fibrous substances (Kosilla, 1984). Weight-for-weight, almost as much straw is produced as grain, the quantity involved is therefore enormous and will continue to be so in the future (FAO, as cited by Sundstøl & Owen, 1984). In the Western Cape region of South Africa, 374 000 hectare (ha) wheat was planted in 2001/02, with a crop estimation of 785 400 ton. Seventy thousand ha of barley was also planted, with a crop estimation of 112 000 ton (Crop Estimates Committee, 2001).

The demand for crops that can be used directly for human consumption, as well as feed for monogastric animals or cereals for industrial purposes increased in recent years (Kristensen, 1984). The cost of high-quality roughage has also risen, thus increasing the need to look for alternative feedstuffs, especially for ruminant animals. Although straw is used for other purposes (e.g. heating and bedding), straw is still relatively cheap and is an essential alternative feed source for cattle and other ruminant animals. There seems to be a demand for low priced low-to-medium-quality feedstuffs in the diets of cattle and other ruminants at certain production stages (Kristensen, 1984). The lactating dairy cow, for example, is probably the most efficient domestic ruminant to utilise low quality roughage like cereal hay and straw (Reid *et al*., 1980). Competition with humans and monogastric animals for high quality feedstuffs may necessitate feeding of more non-competitive feedstuffs, like grain residues, to ruminants. The chemical composition of different types of hay and straw produced at different localities may, however, differ due to different climatic and soil conditions (Sundstøl & Owen, 1984). Ley cropping can also influence soil conditions (Reid *et al.*, 1980)

For many decades research workers in various regions of the world have conducted experiments to determine the contribution which poor quality roughages can make towards reducing the cost and increasing the efficiency of ruminant livestock production. Few publications exists in which the nutritional values of straw types are compared (O'Donovan, 1983) and hardly any exists referring to the Western Cape Region of South Africa. This chapter provides information regarding the differences in the chemical composition and digestibility of straw and hay of the winter grains wheat, barley, oats and triticale produced in the Western Cape area of South Africa.

Material and methods

Samples were collected from experimental plots at 10 different localities in the Swartland and Rûens regions of South Africa. Samples were produced on 10.5 m^2 experimental plots at Moorreesburg (33º7'S 18º36'E), Ceres (33º25'S 19º15'E), Hopefield (33º5'S 18º20'E), Velddrif (32º45'S 18º10'E), Eendekuil (32º40'S 18º52'E), Riviersonderend (34º7'S 19º56'E), Bredasdorp (34º30'S 20º3'E), Swellendam (34º3'S 20º27'E), Riversdale (34º7'S 21º15'E) and Caledon (34º13'S 19º27'E). Four different grain types and eleven

different cultivars were compared in this study. The grain types that were used included barley (Clipper, SVG 13, Gallion, Cape Barley), oats (Sederberg, Perdeberg), wheat (Palmiet) and triticale (Usgen 19, Kiewiet, Rex, SCR 13). A randomised square experimental design with four replicates per sample was used. Hay samples were collected during the milky dough stage of the grain, while straws were sampled after harvesting the grain.

Samples were analysed for dry matter (DM) and ash by standard methods (AOAC, 1984) (Method numbers 7.003 and 7.009, respectively). Crude protein (CP) was measured by a FP-428 Nitrogen and Protein Determinator (Leco Corporation, 3000 Lakeview Avenue, St Joseph, MI 49085-2396). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as described by Van Soest (1963) and Van Soest and Wine (1967). *In vitro* organic matter digestibility (IVOMD) was determined by the method as described by Tilley & Terry (1963). Total digestible nutrients (TDN) were calculated by the following equation: TDN = 0.8 IVOMD +15.35 on DM basis (Engels & Van der Merwe, 1967). A statistical analysis showed no difference between the different cultivars within grain types, therefore the samples were pooled for further statistical comparison. Statistical analyses were done with Statgraphics 5.0 (Statistical Graphics Inc., 1991), as described by Snedecor & Cochran (1980). Differences in the composition and digestibility of the different types of hay and straw were determined by analysis of variance using a protected F-value.

Results and discussion

Means, ranges and standard error of the chemical composition and digestibility of the different types of hay are given in Table 1. There were no significant difference ($P > 0.05$) between the CP of the different types of hay. Barley had the highest value for IVOMD followed by wheat, triticale and oats. All differed from each other, except barley and wheat. Wheat had the highest TDN value, which differed from the value obtained for oats and triticale, while oats had the lowest value. Significant differences ($P < 0.05$) existed in the ADF values of barley, oats and triticale. Oats was higher in NDF content compared to the other types of hay.

The composition and digestibility of straw samples are presented in Table 2. It is generally accepted that oat straw is more digestible than barley straw, and barley straw is more digestible than wheat straw (Nicholson, 1984). Results from this study were in accordance with the literature (Sundstøl & Owen, 1984; Bredon *et al*., 1987), with oats having a much higher IVOMD value than the other types of straw. Barley straw had the lowest IVOMD value. The IVOMD values for wheat and oat straw was, however, much less than that reported by Bredon *et al*. (1987) (41.22%) and Van Wyk *et al.* (1955) (64.8%).

Oat straw in cattle diets is the popular choice for most producers (Kernan *et al.*, 1979). The fact that oats do not have any awns, compared to wheat and barley, contribute to its popularity. Although barley straw is usually ranked second to oat straw, many cultivars of barley will give a higher quality straw compared to certain oat cultivars (Horton & Stacey, 1979). Barley straw, particularly the two-row cultivars, tends to be higher in CP than the other cereal straws (Nicholson, 1984). In this study, no significant

difference between the CP of the different types of straw was found. Barley had the highest protein value in absolute terms.

Table 1 Minimum, maximum and means of the chemical composition and digestibility of different types of cereal hay (100% DM basis).

Type of hay		Chemical composition and digestibility						
		DM	ASH	CP	IVOMD	TDN	ADF	NDF
Barley	mean	90.75^{ab}	4.67^{b}	8.60	75.32^{c}	66.78^{c}	29.79^{a}	54.58^{a}
(n = 95)	min	87.36	2.52	3.19	57.01	52.66	24.08	47.46
	max	93.46	6.73	18.29	88.86	75.02	45.72	65.65
	s.e.	0.10	0.10	0.23	0.60	0.44	0.43	0.41
Oats	mean	90.52^{a}	4.74^{b}	8.67	69.77^{a}	62.75^{a}	34.24^{c}	60.28^{b}
(n = 49)	min	85.30	2.94	3.95	56.79	53.25	27.10	50.91
	max	92.30	15.07	13.09	81.49	71.89	41.70	68.18
	s.e.	0.16	0.25	0.27	0.89	0.64	0.48	0.54
Wheat	mean	91.26^{c}	3.84^{a}	9.23	74.49^{c}	67.07^{c}	30.85^{ab}	54.40^{a}
(n = 23)	min	90.06	2.93	5.58	68.35	61.94	24.78	48.58
	max	93.25	4.82	15.48	81.45	73.01	36.29	57.98
	s.e.	0.16	0.12	0.52	0.74	0.56	0.53	0.55
Triticale	mean	90.88^{bc}	4.19^{a}	8.73	72.04^{b}	64.88^{b}	32.30^{b}	54.66^{a}
(n = 99)	min	86.65	2.94	4.50	50.84	50.05	23.34	49.70
	max	93.18	5.82	13.48	82.08	71.44	40.14	63.76
	s.e.	0.09	0.64	0.18	0.44	0.30	0.32	0.30

$^{a,\ b,\ c}$ Column means with different superscripts differ significantly ($P < 0.05$)

The TDN value for oat straw was significantly higher than that of the other straw types, although it was much lower than results obtained by Macgregor (1989) (50%) and Van Wyk *et al.* (1955) (64.2%). Macgregor (1989) also found higher TDN values for wheat (44%) and barley (49%).

There were no significant differences ($P > 0.05$) between the ADF values of the different types of straw and values obtained were similar to that obtained by Macgregor (1989) for wheat (54%), barley (59%) and oats (47%). Barley straw had the highest NDF value that differed significantly from the value obtained for oat and triticale straw. These NDF values were similar to that reported by Macgregor (1989) (wheat 85%, barley 80% and oats 70%).

Table 2 Minimum, maximum and means of the chemical composition and digestibility of different types of cereal straw (100% DM basis).

Type of straw		Chemical composition and digestibility						
		DM	ASH	CP	IVOMD	TDN	ADF	NDF
Barley	Mean	92.65[b]	4.29[a]	4.29	29.32[a]	37.15[a]	54.29	81.77[c]
(n = 95)	Min	89.30	2.40	1.98	9.39	21.61	30.37	57.91
	Max	95.41	11.31	13.64	59.88	60.90	86.83	88.14
	s.e.	0.10	0.13	0.21	1.04	0.80	1.01	0.55
Oats	Mean	92.16[a]	4.76[ab]	4.15	39.20[c]	44.48[c]	50.81	78.12[a]
(n = 44)	Min	88.87	2.16	1.79	22.68	30.67	30.26	56.59
	Max	93.86	15.73	11.96	63.32	61.09	83.27	84.93
	s.e.	0.13	0.31	0.35	1.21	0.92	1.63	0.86
Wheat	Mean	93.02[bc]	4.00[a]	4.05	30.92[ab]	38.47[ab]	52.32	80.85[bc]
(n = 23)	Min	91.80	2.23	2.10	14.69	26.33	45.01	70.70
	Max	94.01	5.43	8.12	45.96	49.62	87.05	86.53
	s.e.	0.13	0.17	0.33	1.86	1.42	1.70	0.76
Triticale	mean	92.94[c]	5.01[b]	4.13	33.30[b]	39.90[b]	52.84	79.54[ab]
(n = 91)	min	90.25	2.37	1.95	9.78	22.38	25.18	60.55
	max	95.11	18.83	10.31	53.82	55.41	84.07	86.50
	s.e.	0.10	0.28	0.17	0.75	0.60	1.07	0.40

[a, b, c] Column means with different superscripts differed significantly ($P < 0.05$).

There were differences ($P < 0.05$) in the ash, CP, IVOMD, TDN and ADF content of straw produced in the Swartland and Rûens areas (Table 3). Ash, CP, IVOMD and ADF values were higher when produced in the Rûens. There was no significant interaction ($P > 0.05$) in the chemical composition between straw and locality. However, CP and ADF values were significantly ($P < 0.05$) higher for samples gathered in the Rûens compared to samples gathered in the Swartland. Interactions occurred between straw type and locality for NDF, but there was no difference between the NDF values between the different localities. The NDF values for cereal straw were generally higher in the Rûens, except for barley, which had a higher NDF value in the Swartland. TDN values revealed a significant interaction between the straw type and locality and also showed a significant higher value for the Rûens than for the Swartland. Wheat and triticale, however, had higher TDN values when produced in the Swartland. CP was higher in the Rûens area, most probably because lay cropping with legumes is used in this area, and legumes increase the uptake of soil nitrogen.

Table 3 Means of the chemical composition and digestibility of different types of cereal straw collected at different localities in the Western Cape area of South Africa.

Chemical Composition (%)	Locality	Type of straw			
		Barley	Oats	Wheat	Triticale
DM	Swartland	92.67	92.26	92.62	92.85
	Rûens	92.68	92.07	93.39	93.02
ASH	Swartland	3.92[a]	3.77[a]	3.52[a]	3.31[a]
	Rûens	4.60[b]	5.59[b]	4.44[b]	6.53[b]
CP	Swartland	3.09[a]	2.78[a]	3.02[a]	2.99[a]
	Rûens	5.50[b]	5.29[b]	4.00[b]	5.15[b]
IVOMD	Swartland	25.08[a]	36.74[a]	30.82	32.52
	Rûens	33.56[b]	41.26[b]	31.00	34.00
TDN	Swartland	34.04[a]	43.05[a]	38.58	40.00
	Rûens	40.28[b]	45.68[b]	38.38	39.81
ADF	Swartland	53.76	48.43[a]	49.90[a]	50.43[a]
	Rûens	54.77	52.79[b]	54.54[b]	54.00[b]
NDF	Swartland	83.12[a]	77.21[a]	79.39[a]	78.82[a]
	Rûens	80.42[b]	78.87[b]	82.20[b]	80.19[b]

[a, b,] Column means with different superscripts differed significantly ($P < 0.05$)

Conclusion

The differences in chemical composition and digestibility between the different types of cereal straw sampled in this study were generally lower than the values documented in the literature. The study revealed no large differences in the chemical composition of the different types of straw and hay collected under local conditions. Very little work has been done on hay produced during milky dough stage and future studies on this subject may be valuable to the feed industry. Straw produced in the Rûens area generally showed a higher nutritive value when compared to straw produced in the Swartland area. These differences should be taken in account when hay and straw from the different areas are used in formulating a balanced diet. The study also revealed large variation in the composition of samples of the same type of straw and hay. This accentuates the importance of routine analysis of hay and cereal straw before use in diet formulations.

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Chapter 9

GENERAL CONCLUSION AND FUTURE PERSPECTIVE

Near infrared reflectance spectroscopy (NIRS) has been described as the most exciting technique to hit the agricultural and feed industries since the introduction of the Kjeldahl test (Flinn, 1991). NIRS can rapidly test the quality of agricultural products on a scale not previously imagined, with huge benefits to industry and research (Nelson, 2001). The studies in this thesis only proved the success of NIRS on two types of meat and certain feedstuffs. The subject, however, is immense. The extensive adoption of the success of NIRS technology will, henceforth, have even more valuable and comprehensive consequences. For example, the trade in animal feeds is subject to national and international legislation to safeguard human food, animals and environment and to ensure a competitive market. The techniques at hand, therefore, need to be sufficient, cheap and easy to use in official laboratories, for research purposes and also in the industry at large (Givens & Deaville, 1999). NIRS has the potential to fulfill these requirements.

The need for improved methods to evaluate the quality of meat is growing every day. Manufacturers of meat products are forced to make formulations based on chemical target values for the actual meat cuts. These target values have appeared from spot checks, which were often time consuming and expensive (Givens & Deaville, 1999). NIRS proved to be a faster and cheaper, thus a more effective, way of analysing large numbers of meat samples. NIRS has been evaluated as a tool to predict the chemical composition of freeze-dried ostrich meat and mutton, respectively. Although success has been achieved for both applications, freeze-drying of the meat is not recommended, as it leads to a slower and more expensive analysis.

The use of NIRS for forages is of extreme importance, considering that human food production from ruminants is completely dependent on the efficient utilisation of forages. NIRS has made rapid evaluation of forages possible, consequently allowing rapid strategic and economic decisions to be made concerning supplementation for forage improvement (Givens & Deaville, 1999).

Near infrared reflectance spectroscopy proved to be successful in predicting the chemical composition of most of the locally produced feedstuffs, although certain implications have to be taken into consideration. When calibrating a NIRS instrument, influences like, for example, room temperature, time delay between wet chemistry and NIRS analysis, inaccurate reference values and lack of variation in the calibration set, could lead to inaccurate calibrations. It is therefore imperative that these influences should be limited as much as possible to result in accurate calibrations for quality control purposes. Calibrations have also been attempted for more complex parameters, such as alkaloids, digestibility and amino acids with varying degrees of success. Indications are that accurate calibrations for these constituents should be possible, if enough samples are included in the calibration set to cover the variation found for the constituents.

Information was provided regarding the differences in the chemical composition and digestibility of cereal grains and winter grain hay and straw. Not only has a database been provided for accurate values of these feedstuffs, but also the need for continued analysis of forages under different conditions for use in animal feed has been accentuated.

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