The nutritive value of rangelands plants of Southern and Western Australia

A review of the literature and a scoping study to demonstrate the inconsistencies between the current system of nutritive analysis and animal performance

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Executive summary

Wool-producers in the southern pastoral zone are being hindered in their efforts to improve the nutrition of their flocks by the lack of accurate and meaningful information on the value of rangeland pasture species. Sub-optimal animal nutrition is associated with poor reproductive performance and slow growth rates, which retards gains in genetic merit of flocks.

Current knowledge of the nutritive value of Australian rangeland plants is limited by the analytical methods used to determine parameters such as energy, protein and fibre content of feeds. “Wet chemistry” (*in vitro*) techniques appear to overestimate the energy value of salt-rich species such as members of the *Atriplex* and *Maireana* genera, because the methods cannot make allowances for salts having no energy value. However, newer techniques such as near infrared spectroscopy (NIRS) also produce results that are not consistent with animal performance. The main limitation with NIRS is the requirement for detailed calibrations to be made based on each species under a range of growing conditions being fed to an animal (“*in vivo*”) to enable a detailed study of how the animal digests the feed. While this is not simple to do, without it NIRS calibrations will forever be inaccurate and of limited value to graziers whose systems are based on saltbushes and bluebushes. Even now, it is difficult for producers to design strategic supplementary feeding regimes to improve reproductive performance because they do not have sufficient information to determine which nutrients are required. Even when they send samples of the preferentially grazed plants to a feed testing laboratory, the producer must be aware that the predicted nutritional value of the feed may be quite different to the way animals actually perform on that feed.

Without accurate, reliable and repeatable nutritive information on their key species, wool-growers in the southern pastoral zone of Australia will continue to struggle with low lambing rates, poor weaner growth and impaired genetic gain. This has a negative impact on the whole Australian wool industry.

Background

The nutrition of ruminants grazing in pastoral regions of Australia remains poorly understood. Due to the range of plant species present and the extensive nature of the system, it remains impossible to determine precisely what the grazing animal consumes, in terms of the species eaten and the proportion of the various species in the diet. However most pastoralists are aware of and can identify (although not necessarily accurately by name) those species that are preferentially selected. While there are assorted data on the nutritional value of some of these species, animal performance does not closely reflect the estimated value of these plants. This suggests that the current methods of analysis may not be suited to these plants, which often contain quite high levels of salts, up to 30% of plant dry matter, or that the plant species that are most preferred and therefore form the bulk of the diet, are not being analysed.

Many plants in pastoral areas are halophytes ("*halo*" = salt, "*phyte*" = plant), meaning that they grow best on soils that are too saline for conventional plants. Halophytes tolerate salt by various physiological adaptations, for example by accumulating salts (mainly sodium, potassium, chloride and sulphate) in their leaves at a higher concentration than the soil around them, or by synthesising osmotically-active compounds known as compatible solutes (for example, betaines and oxalates). While the role of betaines in ruminant nutrition is not clear, oxalates react with calcium in the digestive system to make calcium less available while forming stones in the kidneys, which negatively affect renal function. Plants such as *Atriplex* (saltbushes) and *Maireana* (bluebushes) achieve much of their salt tolerance by these methods. While these mechanisms are beneficial to the plant, when grazing animals consume these plants, they consume a diet high in salts.

The current analytical methods available to study the nutritional value of plants include:

- *In vivo*
- *In vitro* (wet chemistry)
- Near infrared spectroscopy (NIRS)

There is evidence that both *in vitro* and NIRS methods appear to produce erroneous results when applied to halophytic plants. Masters (2006) states that sheep production when grazing on halophytes may be complicated by three factors that do not appear to be an issue with conventional plant species:

1. Effect of high dietary ash/salt on digestion and intake (physiology)
2. Indirect methods used to estimate energy content of feeds (methodology)
3. Gap in information leading to nonsensical nutritive value estimates of halophytes (calibration of physiology and methodology)

Ruminants experience nutritional impairment when dietary salt content exceeds about 2% (Masters *et al.* 2001), therefore the salt content of halophytes is a major concern in terms of retarding animal nutrition in these environments. This level of salt is excessive for many rumen microbes, therefore having a direct effect on the ability of the animal to digest feed, in addition to the penalty associated with increased water intake to manage the high salt load. The increase in water consumption due to elevated dietary salt is shown in Figure 1, following a study by Masters *et al.* (2005).

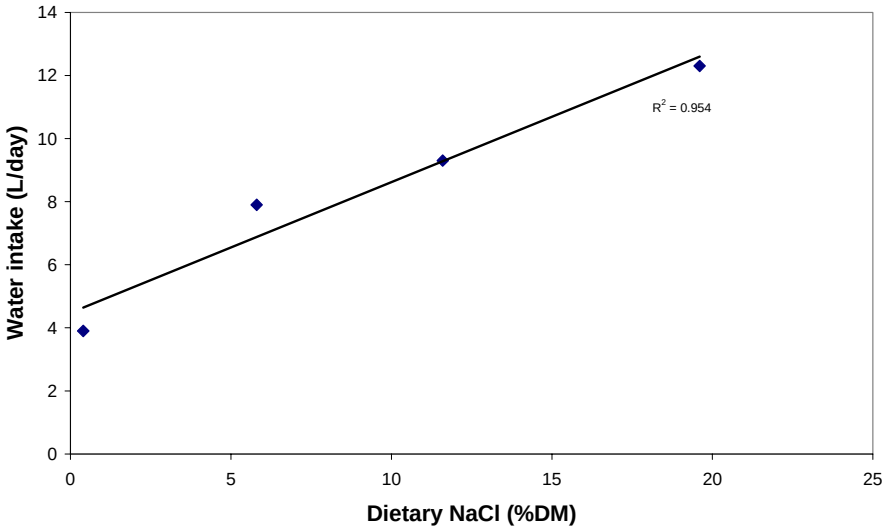

Figure 1 The relationship between salt intake and water consumption (adapted from Masters *et al*. 2005)

This increase in water intake increases the rate of passage through the rumen, allowing less time for rumen microbes to digest organic matter. Hemsley *et al*. (1975) reported a 24% reduction in digestion of organic matter in the rumen when 1% NaCl was included in the diet. Further, increased water intake leads to lower feed intake due to the volumetric constraints of the gastro-intestinal tract. Masters *et al.* (2005) found that, while weaner wethers on a diet low in salt (Na = 0.2%, K = 1.6%) consume 4L of water and 1.4kgDM of feed per day, an increase in Na content to 7.4% leads to water intake of as much as 12L per day, with feed intake falling to 1kgDM per day. This obviously has an immediate and negative impact on animal performance due to lower feed intake, without considering the metabolic penalty of this high level of dietary salt. Additionally, Madsen *et al.* (1997) state that the physical limitation to intake when grazing feed of low digestibility (as many rangeland species are) is a major factor retarding production.

The most accurate method of determining the nutritional value of a feedstuff, although expensive and time-consuming, is to feed it to an animal and observe the animal's performance (for example, weight gain or loss). Such studies are called *in vivo* (Latin: "within the living") studies. Briefly, animals are fed a known mass of feed while housed in metabolism cages for eight days. Each day, the weight of feed remaining and total faecal output are determined. The amount of feed refused is subtracted from the amount fed to determine actual intake. Dry matter digestibility (DMD) is calculated as the percentage difference between the total dry matter eaten and the total dry matter faeces excreted (AFIA 2002). Metabolisable energy (ME) of the feed is then calculated from DMD.

For feed characterisation to remain practical, research has developed statistical relationships to predict *in vivo* outcomes based on chemical analyses and more biologically meaningful assays using rumen microorganisms or commercial cell-free enzymes (Givens & Deaville 1999). These techniques, known as "wet chemistry", involve plant samples undergoing a suite of tests in the laboratory using a range of different methods. Because these studies are performed at the test tube level, they are called *in vitro* (Latin: "within the glass"). Parameters such as energy content, protein, fibre and mineral content can be determined using calculations derived from *in vivo* studies. While wet chemistry provides a reasonable compromise between simplicity and accuracy of prediction, it takes a few days for laboratories

to complete analysis of samples. In commercial laboratories, there are often considerable delays in receiving results.

More recently, near infrared (NIR) spectroscopy (NIRS) has emerged as a system to analyse vegetative samples. NIRS requires small amounts of plant material and is rapid, non-destructive, requires minimal sample preparation and is highly accurate and precise. Compared to traditional methods, it is much cheaper per sample, no chemical reagents are required and no wastes produced (Givens & Deaville 1999; Dynes & Schlink 2002). Once calibrated, the NIR spectrometer appears simple to use and to operate.

The use of NIRS technology is rapidly increasing within the cropping industry for rapid analysis of grain protein and moisture and further calibrations are planned to determine starch concentration. For example, NIRS units are installed on many new headers to allow immediate assessment of crop quality to optimise market access, which has been made possible by progress in miniaturisation of both hardware and software, and improved physical robustness of the instrument. It is therefore likely that ongoing development will allow NIRS systems to be produced that can allow real-time prediction of forage quality for immediate feeding and marketing decisions.

However, the industry is concerned that neither NIRS nor wet chemistry techniques accurately predict the nutritive value of halophytic plants. Much of this is due to the lack of *in vivo* studies using salty or other “unusual” plants, meaning that the equations for calibrations are based on too few, if any, samples, so a relatively high statistical error is likely to exist. Current laboratory NIRS calibrations for roughage species have been developed from a range of cereal hays. Because the NIR signature of each species is unique, it is likely to be misleading to attempt to conduct a NIRS examination of a plant that the system is not calibrated to analyse correctly (Clancy *pers. comm.* 2006).

Wool producers in pastoral Australia cannot be sure of the quality of the plants their sheep consume because of these methodological shortfalls. This report highlights the various issues arising from the apparent inaccuracies in current feed analyses, particularly those relating to rangeland plant species. Producers are aware that some species are preferentially grazed, and with some level of additional support in identifying and naming key species, can provide accurate records of which plant species comprise the bulk of the grazing ruminant’s diet. Accurate testing would allow producers, where possible, to allocate paddocks to specific classes of sheep to optimise productivity. This would also allow them to practice more strategic and appropriate dietary supplementation where required.

A survey of pastoralists acknowledged that analytical systems must be more accurate to enable them to make animal nutrition decisions that are more informed, leading to improved sheep and wool production and ultimately, better whole farm economic status.

Key terms used in this report

ADF (acid detergent fibre): the proportion of the plant sample composed of cellulose and lignin. These values relate to the ability of an animal to digest the forage. As ADF increases, digestibility usually decreases. Acid detergent refers to the type of reagents used to complete the analysis.

Ash: the mineral component of a plant, mainly comprised of salts. Ash is defined as material remaining after the sample has been placed in a 500^{o}C furnace for 12 hours. Ash has no energy value. High levels of ash have the effect of diluting the nutrient status of the plant, and present the grazing animal with various nutritional limitations such as the energetic cost of removal and various affects on the function of the rumen and lower gastro-intestinal tract.

DOMD (dry organic matter digestibility): the proportion of the plant dry matter that is organic (ie, non-ash), meaning it can be digested by the grazing ruminant.

DM (dry matter): the non-water component of a plant. It is determined by drying the plant sample in an oven at 135^{o}C for two hours. Most plant nutritional parameters are expressed as a proportion of dry matter.

DMD (dry matter digestibility): correctly it is determined *in vivo*, but is normally estimated from *in vitro* digestibility or chemical composition.

In vitro: refers to the technique of performing a given experiment in a test tube, or, generally, in a controlled environment outside a living organism.

In vivo: refers to an experiment that which takes place inside an organism. In science, i*n vivo* refers to experimentation done in or on the living tissue of a whole, living organism as opposed to a partial or dead one.

ME (metabolisable energy): usually expressed in MJ (megajoules) per kilogram of dry matter (MJ/kgDM), this is the unit of energy that is most relevant to ruminants. As a guide, a 50kg wether needs a diet of 8MJ/kgDM, while a lactating ewe or weaner lamb needs 11MJ/kgDM

NDF (neutral detergent fibre): the proportion of the plant sample composed of cellulose, hemicellulose and lignin. NDF values indicate the amount of the forage the animal can consume. As NDF percentages increase, the amount of dry matter intake generally decreases. The “neutral detergent” part of the name refers to the type of reagents used.

NIRS (near infrared reflectance spectroscopy): a scientific analysis technique that uses the radiation of a sample to determine its structure and composition using, utilising some level of data manipulation using a computer. Near infrared light is found near the red part of the light spectrum but is invisible to human eyes.

Wet chemistry: see *in vitro*.

Wet chemistry analysis

It is logistically too difficult to conduct large-scale animal experiments for every feed sample each time a feed value is needed (Dynes & Schlink 2002), hence, for feed characterisation to remain practical, *in vitro* studies are necessary (Givens & Deaville 1999).

In vitro (wet chemistry) studies are currently the main method used to analyse feed samples, however, they are relatively slow and expensive.

Moir (1961) states that DMD determined *in vitro* is a simple and accurate indication of feeding value (i.e. digestible energy content), given the very close relationship between digestible energy and DMD ($R^2 = 0.98$) as determined by a large range of intakes and feed compositions.

Wet chemistry estimates are based on *in vivo* studies so are not always highly accurate with every sample. Further, there are many plants, especially rangeland species, which have not been included in any *in vivo* studies, meaning that wet chemistry estimates cannot be considered accurate, as these estimates will be based quite possibly on unrelated species. This is especially problematic when dealing with halophytic plants.

Halophytic plants may have ash contents which make up over 20% of the dry matter. When analysed by *in vitro* studies, the energy value of these plants has often been over-estimated due to the high total ash and the assumption that the percentage of ash that is soluble is constant. The ash has no energy value: the more ash in the plant, the lower the nutritive value.

The ash content of plants may be excluded from the digestibility calculation by expressing it as dry organic matter digestibility (DOMD); however, according to Masters *et al* (2001) and as illustrated in Figure 2, this does not account for relative differences in the proportion of ash that is soluble.

Increasing the amount of salt in the diet leads to a profound reduction in metabolisable energy for a given *DMD*. This highlights both the penalty for the grazing animal in consuming plants that are high in salt, and the importance of analysing feed samples for the digestibility of the organic matter (that is, the non-salt component) rather than for total dry matter.

In the southern pastoral region of South Australia, a producer with a monoculture of *A. nummularia* has found that ewes in late pregnancy have difficulty maintaining condition despite the energy content appearing sufficient based on DMD analyses (Jolly, 2004, unpub).

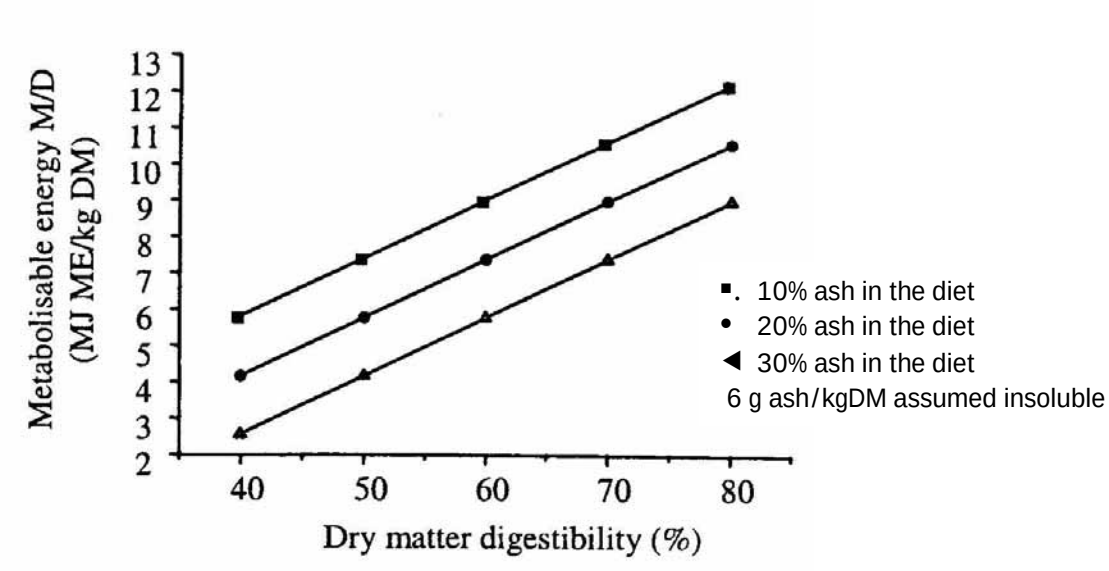

Figure 2 The relationship between dry matter digestibility and metabolisable energy (MJ ME/kgDM) (Masters *et al.* 2001)

It is therefore imperative to determine the soluble and insoluble ash components of forages and correct the *in vitro* digestibility (IVD) to account for these data, which will make the results much more accurate (Don Law, *pers. comm.* 2006). Norman *et al.* (2002) showed little difference between IVD and IVD-corrected-for-soluble-ash in grasses (R^2 = 0.93), but a large difference for halophytes (R^2 = 0.34). This substantial error is associated with the over-estimation of energy value of halophytic plants.

Perennial halophytes, uncorrected for soluble ash content, were found to have an IVD of 70% (ME 11.2) however when IVD was corrected for ash content, the IVD was 50% (ME 8); the practical implications of this reflect the difference between optimising productivity of a lactating ewe or a weaner lamb and weight loss of both of these classes of animal.

Possibly the same argument exists for plants that are high in minerals such as lucerne; this has yet to be investigated.

McDonald *et al.* (2002) proposes that a more accurate estimation of the ME value of halophytic plants may be obtained from the formula *ME (MJ/kgDM) = 0.16DOMD* which corrects for ash.

When forages have low digestibility, low ME and are high in fibre, an animal can still grow, in theory, by increasing intake. However, when IVD falls below about 55%, and NDF exceeds 30% (lactating ewes and weaner lambs) or 60% (dry sheep), physical limitations on the rate of eating, digestion and passage through the gastrointestinal tract mean that intake is restricted, so weight loss in inevitable (Dynes & Schlink 2002).

Further, feed intake is reduced by the high salt level in the plant, both as a direct effect on palatability and the additional water intake reducing appetite (Masters *et al.* 2001). Feed intake may also be depressed by anti-nutritional factors in various species affecting palatability. For example, saltbushes contain varying levels of tannins, nitrates and oxalates that reduce feed intake (Masters *et al.* 2001). Salt itself limits feed intake; 16% NaCl in the diet

reduces feed intake by almost half before sheep have exceeded their salt tolerance. An oxalate content of 3% in the diet (a common level in saltbushes) depresses intake significantly. Feed intake may also be limited due to;

- sparse distribution of pasture over big areas
- forage characteristics such as low palatability
- high resistance to structural degradation by chewing
- resistance to digestion (Weston 1996)

The importance of basing energy estimation on DOMD rather than DMD is not a new concept. Almost 40 years ago, Kellaway (1969) noted that DOMD is far preferable to DMD. In an *in vivo* trial with 20 forage species fed to Merino wethers, Kellaway found a closer correlation between energy digestibility (ED) and DOMD (CV = 1.3%) than ED and DMD (4.4%). DOMD is more robust when soil contamination occurs (Kellaway 1969; Gunderson 2006). While some commercial laboratories have begun including DOMD in their current analysis reports, the Australia Fodder Industry Association (AFIA 2002) has not officially validated DOMD for plant analysis.

Masters (2006) reports the results of applying four different methods to determine the DOMD of a set of three *Atriplex nummularia* samples. These data appear in Table 1 and clearly demonstrate the inaccuracy of wet chemistry and uncalibrated NIRS compared with animal measurements. *In vivo* digestibility as determined in this trial is lower than found by Ben Salem *et al.* (2005) who report Tunisian *A. nummularia* with (*in vivo*) dry matter digestibility of 65% and organic matter digestibility of 70% of organic matter.

Table 1 Examples of *in vitro* and *in vivo* estimates of DOMD (adapted from Masters 2006)

	in vivo	**Pepsin-cellulase**	**Pepsin-cellulase (corrected*)**	**NIRS**
Sample 1	56			76
Sample 2	52	77	70	
Sample 3	48	77	71	

*** corrected with non-halophyte calibration**

The range of values in using four different methods is evident. It is also clear that no method as it stands is close to the *in vivo* digestibility of feed, meaning that animal performance will be greatly over-estimated if the producers used any of these *in vitro* methods to make decisions regarding livestock nutrition.

It is worth noting that energy expenditure for grazing and foraging can be quite significant for free-ranging animals. Graham (1964) determined that energy expenditure for grazing is about 0.54kcal/hr/kgLW, or about 0.11MJ/hr for a 50kg sheep, irrespective of sward type. When feed is scarce, sheep may graze for 8-13 hours (Arnold 1960), meaning that the metabolic cost of grazing may need to be considered above “standard” maintenance requirements. It is estimated that muscular work, mainly standing and eating, could account for nearly 40% of the daily energy expenditure of a sheep at maintenance, compared to about 10% for a caged

sheep. Thus, for sheep on hilly pasture or in large paddocks a long way from water, the energy cost of walking could become a major issue, with some studies suggesting grazing animals require 10-100% more dry organic matter (DOM) to maintain body weight than do hand-fed animals.

This has implications for pastoralists who are currently being encouraged to run more ewes and to breed prime lambs for finishing systems. Lactating ewes have a high demand for all nutrients, especially energy, and pastoral ewes are therefore faced with having to expend considerable amounts of energy to forage for feed that may be in short supply and is of low nutritive value.

There appears to be reasonable evidence that the nutritive value of many rangeland species is lower than currently estimated by DMD and DOMD and more accuracy is required to facilitate strategic and cost efficient supplementation.

In vitro digestibility values need to be considered in the context of fibre, soluble mineral content, non-protein nitrogen and tannins (Dynes & Schlink 2002). While DOMD corrects for very high ash content, protein levels provided by plant analysis are often misleading indications of protein available to the animal (Norman *et al.* 2002). The methods used for protein determination may also be overestimating the value of rangeland plants. Currently, *in vitro* analysis of plants reports protein as crude protein (CP), as determined by the Kjedahl technique (Masters *et al.* 2001). This method determines total nitrogen (N) content of the sample, and multiplies the total N content by 6.25 (based on the assumption that the average protein contains 16% N). This assumes that all N in plants is protein; however, many salt tolerant plants contain significant amounts of non-protein-N (NPN) such as betaines (up to 6gN/kgDM, equivalent to 4% CP) and nitrates (3-5gN/kgDM, equivalent to 2-3% CP). The understanding of protein digestibility of halophytes is likely to be compromised by the limited research into the quantitative and qualitative protein needs of rumen microbes (Madsen *et al.* 1997, Masters *et al.* 2001), therefore further research is required in this area. It is generally assumed that rangelands species provide adequate amounts of rumen degradable protein (RDP) which may not be the case.

While rumen microbes can utilise soluble N compounds to produce proteins, this process requires an adequate supply of digestible energy, which may already be limited in rangelands species. Moreover, the high water intake induced by high salt intake also influences true protein digestion in the rumen by increasing the rate of passage of feed through the digestive tract. The degradation of protein in the rumen produces ammonia (which means an amount of N is lost in the urine as urea), and increases the rate of passage of feed which means more protein escapes degradation in the rumen and is absorbed intact from the small intestine.

In this case, while the true protein content of the feed may be lower than *in vitro* analysis predicts, the protein fraction may be undergoing less degradation in the rumen and instead be digested, more efficiently, in the small intestine. Conversely, Weston *et al.* (1970) found that the protein in *A. nummularia* was extensively degraded to ammonia in the rumen, and suggested that the true protein value of this species (and probably other saltbushes) was probably only 60% of the total digestible crude protein as determined by laboratory analysis. This implies that when the crude protein level of a halophytic plant (eg, saltbush) is reported at 20% via the Kjedahl technique, it is more likely to be in the vicinity of 12% CP.

It is not desirable to change *in vitro* methods just for salt-rich plant samples, not least because it is potentially dangerous to declare an arbitrary salt content of plants at which different

analysis is applied. Don Law (*pers. comm*. 2006), chairman of the RIRDC fodder R&D Advisory Committee, states, “wet chemistry methods are untouchable”, suggesting that the methods used and authorised by the AFIA Technical Committee are non-negotiable.

However, he agrees with Masters (2006) and others, that digestibility corrections could make wet chemistry more accurate. AFIA has published a methods manual for feed testing laboratories, but it is unclear as to how many testing laboratories are following these AFIA guidelines precisely. Even within the manual, there are sometimes several different (or slightly different) methods to determine the status of the same nutrients.

The methods described in the AFIA manual have not been validated with high-salt rangeland plant species. The current system should be of concern to pastoral woolgrowers as laboratories are not necessarily using the same methods, or conversion equations from either DMD or DOMD to ME.

Obviously, it is desirable that the plant analysis report clearly states the methods used to determine each nutritional parameter and to provide suggested guidelines for each nutrient using a particular analytical method, however it is unlikely that this information would be meaningful for the majority of woolgrowers or their consultants. It is even more important that producers can rely on the results they receive so that they can use them effectively in their production systems.

NIRS Analysis

Near infrared spectroscopy is a rapid, non-destructive method of analysis requiring minimal sample preparation (Robert *et al.* 1986; Undersander 2006). The NIRS approach requires less operator time, is more independent of operator technique and is less expensive per sample (CAMO 2006).

NIRS uses energy in the wavelength range of 1100-2500mm directed on a sample cell containing the specimen, and the diffuse reflected energy is measured. This near infrared spectrum is literally a reflection of the chemical structure of the sample, especially the hydrogen bonds such as –CH, –OH, –NH and–SH which absorb energy at specific wavelengths (Givens & Deaville 1999; McDonald *et al.* 2002; García & Cozzolino 2006). Thus, a spectrum of reflected energy is recorded, which is then related, by software, by multiple linear regression to their known composition determined by traditional (that is, *in vitro*) methods (Foley *et al.* 1998). Alternatively, data can be processed using a statistical method known as principal component analysis (PCA), which is considered similarly accurate (Robert *et al.* 1986).

As there is no mathematical law to describe the interaction of radiation with a scattering medium containing a heterogenous distribution of absorbing species, NIRS is currently a largely empirical or secondary technique requiring calibration using samples of known composition determined using *in vitro* techniques (CAMO 2006). Thus, currently NIRS can be no better than the *in vitro* or (ideally) *in vivo* standards used to calibrate the instrument. It is therefore imperative that the plant samples are correctly handled to increase the accuracy and reliability of the estimation. Obviously, accuracy would be enhanced further if calibration equations are made directly on *in vivo*-derived data, especially when dealing with halophytic plants where *in vitro* methods are not sufficiently accurate (van der Baan *et al.* 2004).

If we could see infrared light, we would not see colour but instead the forms of ‘protein’, ‘starch’, ‘fat’, and other large molecules. NIR data is normally shown as a graph with peaks of activity at various wavelengths (Figure 3) with each peak referring to a particular nutritional parameter (Norris *et al.* 1976). For example, signature peaks for acid detergent fibre (ADF) occur at about 1500nm and 1660nm, while the peaks for neutral detergent fibre (NDF) are around 2100nm and 2300nm. NIRS has been approved by the Association of Official Analytical Chemists (AOAC) to determine moisture, Kjeldahl nitrogen and ADF for feed and forage analysis, and is used internationally for determining moisture and protein in grain at silos. NIRS determines moisture very well and is more accurate than most wet chemistry analyses, but the machines at many laboratories cannot read samples wetter than 15% moisture with NIRS, so samples must be partially dried before NIR analysis (Undersander 2006).

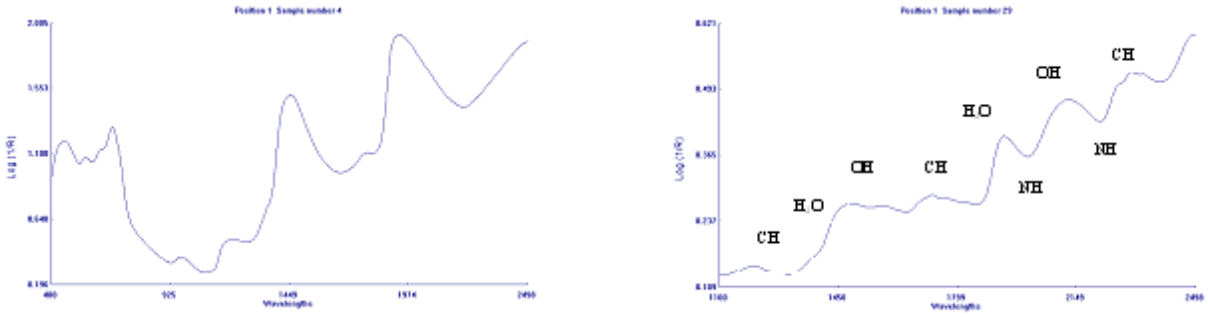

Figure 3 Fresh (left) and dried silage NIRS spectra (Sprague et al. 2003)

The relationship between concentration of a specific component and absorbed energy is further complicated by overlapping of spectral bands from the different constituents present (Givens & Deaville 1999). Therefore, NIRS calibrations are based on the statistical analysis ("chemometrics") of the relationship between mathematically transformed spectra and the frequency of chemical bonds in an organic matrix ("reference values") quantified by classical laboratory *in vitro* procedures (Dynes & Schlink 2002; Landau *et al.* 2006) or *in vivo* studies. For the greatest accuracy, *in vivo* digestibility measurements will always be best, however, if NIRS can be calibrated using *in vivo* data sets, NIRS can be a highly efficient and accurate system for predicting the nutritive value of feed and hence animal performance.

NIRS versus wet chemistry

The literature is conflicting as to the relative accuracy of NIRS *versus* wet chemistry. Like any analysis system, NIRS is dependent on reliable data for calibrations being obtained by metabolism trials with animals.

Prediction of energy value by NIRS is easier for cereal grains than, for example, mixed-species silage, because such feeds are less variable in chemical composition (Sprague *et al.* 2003). The similarity in NIR spectra between four cereal grains is shown in Figure 4.

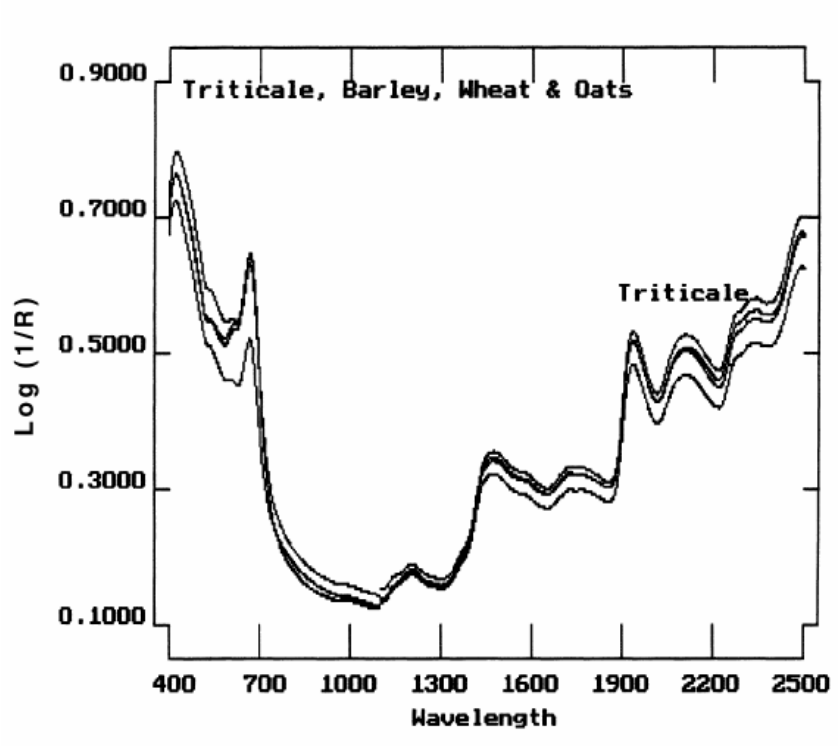

Figure 4 NIRS absorption spectra of green cereal species (adapted from Bruno-Soares *et al*. 1998)

It appears that the NIR spectra are similar across cereal species. Bruno-Soares *et al.* (1998) obtained spectra from 135 samples of green crop oats, barley, triticale, ryegrass and sorghum using the same calibration equations and found high correlations with nutrient parameters determined using wet chemistry. Correlations were 0.90 for ash, and 0.96-0.98 for crude protein, ADF and NDF. This contrasts with the sentiments of García & Cozzolino (2006) who suggested that NIRS be used as a routine procedure to apply in breeding programs only if calibration is done for each species, season and particular conditions, supporting the concerns expressed by Smith *et al.* (1991).

In their study, Bruno-Soares *et al.* (1998) developed the correlations for *in vivo* attributes DMD were 0.86 and DOMD 0.88, but there was poor correlation (0.41) between NIRS and wet chemistry for predicted dry matter intake. They concluded that the DMD and DOMD results are acceptable (this variance is typical of biological measures), but DMI was inadequate. This goes

against the aims of Flinn (1991) to use NIRS to predict feed intake, who cited the work of Barber *et al.* (1990) who claimed that forage intake by sheep is more accurately predicted by NIRS than any combination of "classical" chemical predictors. The use of NIRS in the prediction of dietary quality is developing slowly, and the bottleneck in the process is the establishment of reliable data sets, with adequate variability, for calibration.

García & Cozzolino (2006) reached their conclusion regarding individual calibrations for each species after they studied NIRS spectra obtained from 650 Uruguayan pasture samples and found the technique highly accurate when calibrated using *in vitro* standards. They found high R^2 values (>0.90) for most key nutrition parameters, namely CP (0.98), ADF and DM (0.95), and ash and *in vitro* OMD (0.90). They considered NIRS inadequate for NDF prediction, where the correlation was 0.86. The poor correlation for NDF may reflect the variability in the wet chemistry method as well as the effect of sample characteristics such as high starch or nitrogen content. However, they confirmed, as others have found, that CP is predicted in a wide range of forages with high accuracy. They believed that it is widely accepted that *in vitro* OMD is not a particularly good predictor of *in vivo* OMD, but the pepsin-cellulase method is more accurate than other *in vitro* methods, although very dependent on both the type of forage and laboratory procedures.

Similar success was found by Delgado & Gutierrez (1992) who studied OM digestibility of 187 samples of fresh forage from sown and natural pastures at a range of altitudes throughout Europe. Nutritive values were determined by *in vivo* enzymatic and NIRS techniques, and calibration equations were derived from all methods. Correlations between values of the three methods were high (above 0.90), and so they concluded that NIRS analysis was as accurate in estimating OM digestibility of heterogeneous forage as a standard laboratory technique. They noted that the actual transfer of *in vivo* or *in vitro* data to NIRS calibrations is a software-related issue, so varies between different spectrometers.

Another European group with success using NIRS, De Boever *et al.* (1997), found that for grass silages in Belgium, NIRS provided the best calibration relationship for *in vivo* OMD with a R^2 of 0.79, compared to 0.68, 0.64 and 0.53 for (respectively) cellulase digestibility, rumen fluid-pepsin digestibility and acid detergent lignin determinations (so-called 'wet chemistry' techniques).

These findings clearly demonstrate the ability of NIRS to predict the digestibility *in vivo* of forages and for grasses, at least, the ME content, providing calibrations are highly accurate and the correct region of the spectrum is used. They found two clearly important spectral ranges for digestibility that need to be exploited: 1650-1670 and 2260-2280nm.

More recently, Givens & Deaville (1999) and Landau *et al.* (2006) found NIRS to be more accurate than wet chemistry, but Landau *et al.* (2006) report that some others found it less reliable. These include De Boever *et al.* (1997) who fed sheep on maize silages, and Kjos (1990), who worked with grasses cut for forage, silage and hay, and stated that NIR-predicted values were slightly less correlated with digestibility than the values determined by wet chemistry. NIRS has the potential to predict ruminal degradability of DM of forages, but predictive performance has not been outstanding based on rumen cannulae experiments. Both teams of authors state that NIRS generally provides a more accurate prediction of *in vivo* digestibility than traditional laboratory procedures where calibrations exist.

NIRS can also be used to estimate rumen kinetics of NDF and ADF degradation of by-products, which is critically important during drought when these unusual feedstuffs become a major

component of the diet of sheep and cattle. Berardo *et al.* (1993) studied the use of NIRS in evaluating the nutritive value of a range of ruminant feedstuffs. They fed cannulated buffaloes and cattle Dactylis hay, lucerne hay, sugarbeet pulp, barley grain, wheat straw, vine shoots, olive shoots, olive cake, pure cellulose and dried wood sawdust. NIR spectra were recorded for each of the feeds and used to calibrate their instrument. The calibration gave good results, with the co-efficient of determination for NDF and ADF ranging from 89-98% for the various feeds.

In research involving the Spanish native saltbush *Atriplex halimus*, Andueza *et al.* (2004) studied 318 samples of this plant from the Aragon region. The correlations for predictions of key nutritive value (NV) parameters between NIRS and wet chemistry were somewhat mixed;

- CP = 0.92
- DMD = 0.92
- NDF = 0.92
- Cl = 0.90
- ADF = 0.89
- Ash = 0.81
- Na = 0.63
- K = 0.70
- Ca = 0.04

This group believe that the NIR spectra of these samples gave useful and acceptable calibration models for all attributes except Ca. However, note the difference in "acceptable" correlation values between this work and García & Cozzolino (2006); the latter considered NDF inadequate, as the R^2 was 0.86, whereas the former accepted the correlations of 0.63 for Na and 0.70 for K as acceptable.

This highlights the issue of how different researchers accept a different level of analytical error, which obviously has at least some bearing on how NIRS analysis is accepted as a legitimate technique. On the subject of NDF prediction, Andrés *et al.* (2005), in reporting a study of a range of plants from Spanish meadows, concluded that NDF was not accurately predicted using NIRS, and suggested this may be due to the limitations of the *in vitro* method compared to *in vivo* results.

NIRS calibrations must adequately represent all the variation sources (for example, varieties, year of cultivation, harvest time, drying system), according to Andueza *et al.* (2004). Although in their study of ash content of forages they had successfully predicted NIRS, other authors have not achieved good calibrations of different minerals and the NIR spectra, probably due to their lack of association with hydrated inorganic or organic molecules. The main issue in this regard is that the organic form of plant minerals varies seasonally, as confirmed by Foley *et al.* (1998). For example, phosphorus exists in plants in organic forms such as phymata, phospholipids and nucleic acids, making determining total phosphorus content a complicated matter.

Shenk & Westerhasu (1994) support this claim, and they concluded that NIRS was not suitable for macromineral determination. Nevertheless, Smith *et al.* (1991) found NIRS could accurately screen large numbers of perennial ryegrass from one population for magnesium concentration, but with the proviso that the resulting calibration equations were not to be used on independent samples, without verification by analysing a small number of samples using the reference technique.

Interestingly, cheese-researchers Frankhuizen & van der Veen (1984) found high correlations for fat, moisture and protein content in 60 Gouda, 35 Edam and 50 processed cheese samples, indicating that NIR could be used for accurate estimation of these constituents in cheese, but they found poor correlations for salt-in-DM, processing salts or pH. The greatest accuracy was obtained when the instrument was calibrated separately for each cheese variety.

Because so many pastoral plant species contain high level of salt, this finding is relevant, as it would appear that a lot more cheeses have been analysed with NIRS than saltbushes. In addition, Frankhuizen & van der Veen (1984) believe that NIRS could be made to accurately predict ash content with greater input of calibration equations.

The work of Barber *et al.* (1990) highlighted that most traditional relationships for predicting forage digestibility were calibrations based on *in vitro* estimations. Thus, they had never been exposed to the validation regime necessary for true NIR calibrations. Hence, some workers prefer wet chemistry due to its perceived robustness for conventional pastures that have suffered unusual seasonal conditions or unusual samples (Gunderson 2005). Gunderson favours wet chemistry analysis over NIRS, even though cost can be about double; "in an (unusual) year like this, with abnormal growing and harvest conditions that can affect the quality of NIR results, it's well worth the cost", he writes. In addition, plant ash content can vary between years. However, if calibrations with a range of seasonally affected species were obtained from *in vivo* feeding trials it is likely NIR would also be accurate

NIRS can detect some anti-nutritional factors with a high degree of accuracy. Anti-nutritional factors, such as oxalates, phenolics and condensed tannins, are plant compounds that compromise animal performance. They are chemicals that are not directly involved in the process of plant growth (secondary compounds), but act as deterrents to herbivores. Non-ruminants are usually more susceptible to toxicity than ruminants which have the capacity to degrade potential toxins in the rumen.

Although traditional feed testing procedures do not commonly examine feeds for anti-nutritional factors, such as tannins and other phenolics, Foley *et al.* (1998) believe it is only a matter of time before this capability of NIRS is developed and exploited, particularly since many techniques for determining anti-nutritional factors are quite laborious. A recent survey of pastoralists (Franklin-McEvoy 2006, unpub.) provided feedback that indicated support for the development of these calibrations for pastoral species. Further, identifying levels of anti-nutritional factors such as oxalate in plants could have major implications in strategically supplementing sheep in pastoral environments. In the case of oxalate, if pasture analysis revealed high levels in a paddock that was being grazed by lactating ewes, producers could then provide a loose-lick of limestone ($CaCO_3$) to hopefully ensure that ewes ingest sufficient calcium to reduce the incidence of metabolic issues caused by low blood calcium. Previous work by Franklin-McEvoy & Jolly (2006) showed that pastoral sheep in the southern rangelands of South Australia are deficient in calcium, so identifying the calcium and oxalate status of plants would be beneficial to improving ewe nutrition during late pregnancy and lactation.

The use of NIRS for protein prediction appears highly reliable. This is important because the wet chemistry procedure for protein analysis is traditionally done by the Kjeldahl method and requires approximately six hours to perform a test. Other wet chemistry techniques are also slow; for example, moisture evaluation requires between one hour and 72 hours depending on the nature of the sample (Frankhuizen & van der Veen 1984). Because the quality analysis is a laboratory procedure, the sample throughput is constrained. On the other hand, a typical NIRS

instrument requires 30 seconds to load and 30 seconds to measure and process the data, quickly alerting the operator to any process change.

It is apparent from this review that there has been a range of research undertaken to explore the merits of both wet chemistry and NIRS analysis which has given a wide range of results and interpretations. The clear advantages of NIRS analysis include speed, efficiency, the reduced opportunity for operator error, the ability to use NIRS for non-conventional analysis, such as for anti-nutritional factors, together with the potential for NIRS to be miniaturised. It is also clear from the research undertaken that the accuracy of NIRS is dependent on its calibration from high quality data. The absence of the required data appears to have restricted the rigorousness and consistency of NIRS evaluation. It seems many choose to use wet chemistry analysis in the absence of better, proven techniques and equipment.

Faecal NIRS

The key to understanding pastoral nutrition is underpinned by a true understanding of what the animal is actually eating (D. Law 2006, *pers. comm.* 14 June). This can be achieved using faecal NIRS, where faecal samples are analysed using NIRS technology to identify the proportion of indigestible markers of the various plant species consumed (Foley *et al.* 1998; Landau *et al.* 2006).

Faecal NIRS (FNIRS) should not be confused with NIRS used to determine the NV of fresh plant samples; although they both use similar technology, they are in fact measuring different things. FNIRS offers great potential in estimating diet composition based on a plant's unique spectral signature, which when combined with NIRS analysis of the feed, would allow an accurate determination of daily nutrient intake (Coates 2006). Gibbs *et al.* (2006) demonstrated that FNIRS could be developed as a method to predict microbial protein production of grazing ruminants, which would be particularly useful given the potential issues regarding protein digestion of animals grazing halophytic plants. Work by both Coates (2006) and McCosker (2006) use FNIRS to estimate DMD and ME for cattle in Queensland and the Northern Territory (respectively) and have used these data to devise improved supplementary feeding strategies. Using FNIRS to formulate complementary feeding strategies does not take into account the variation in preferential selection, as if FNIRS is done after a period of grazing, completely different species may be selected from that point onwards. It is for this reason that predictive values are of much more importance in formulating feeding strategies, although FNIRS is a useful follow-up measure of diet composition. The main limitation of FNIRS is that although it describes the indigestible proportion of the diet of the grazing animal, it therefore cannot accurately describe the quality and hence predict performance.

Current range of nutritive values of rangeland halophytes

Feed testing laboratories should not attempt to estimate NVs in those plant species for which they lack sufficient calibration points to provide precise values, even though many laboratories do try such predictions. No method can predict NV well unless the relationship between chemical analysis and digestibility has been established beforehand for that species (Madsen *et al.* 1997). As a designer and manufacturer of NIRS analytical equipment for grain growers, Clancy (*pers. comm.* 2006) is adamant that data outputs cannot be considered quantitatively accurate without a large data set of a specific species for calibration purposes. He makes it clear that NIRtech's CropScan range are not to be used to determine protein, moisture, oil, etc. in grains that it is not calibrated for. It is also not advisable to use *in vitro* estimates to compose NIRS calibrations as the result is an estimate based on a calibration from an estimate of a biological system – there are simply too many opportunities for error. While Barber *et al.* (1990) states that various researchers have found that NIRS analysis of grass silage was more accurate for predicting *in vivo* performance than *in vitro* methods, this was only due to thorough calibrations and the use of a relatively complex eight-term regression equation, in addition to continuous monitoring of NIRS instrument performance.

Table 2 Mean levels of key nutritional parameters (Franklin-McEvoy and Jolly 2006)

Species	Common name	CP %	NDF %	DMD %	ME*	ME^
Acacia paporacarpa	Western Myall	13.3	47	26	3.9	2.4
Acacia victoriae	Acacia victoriae	14.1	34	54	8.0	7.1
Atriplex nummularia	Old man saltbush	20.6	27	79	11.8	11.4
Atriplex sp.	Annual saltbush	17.5	30	74	11.0	10.5
Atriplex vesicaria	Bladder saltbush	13.4	30	74	11.1	10.6
Salsola tragus	Buckbush	15.9	32	71	10.7	10.1
Carrichtera annua	Ward's weed	20.6	42	68	10.1	9.5
Centipedia pleiocephala	Soft billybuttons	10.4	46	61	9.2	8.4
Heterodendrum oleifolium	Bullock bush	12.4	43	46	6.9	5.8
Lycium australe	Australian boxthorn	25.4	34	78	11.7	11.3
Maireana appressa	Bluebush	17.9	41	66	9.9	9.2
Maireana astroticma	Bluebush	10.7	44	69	10.4	9.8
Maireana georgii	Sanity bluebush	17.5	40	65	9.8	9.1
Maireana pyramidata	Black bluebush	16.8	38	63	9.5	8.8
Maireana sedifolia	Pearl bluebush	17.5	42	65	9.8	9.1
Maireana triptera	Three-winged bluebush	16.4	41	59	8.8	8.0
Medicago sp.	Medic	19.8	48	57	8.6	7.7
Myoporum platyoarpum	Sugar wood	11.1	28	70	10.6	10.0
Rhagodia sp.	Rhagodia	17.2	31	69	10.4	9.8
Rhagodia spinscens	Thorny saltbush	12.4	49	55	8.2	7.3
Salvia verbenaca	Wild sage	18.0	40	62	9.3	8.6
Sclerolaena ch., dia., eria.	Copperburr	19.3	42	70	10.5	9.9
Sclerolaena obliquicuspis	Limestone copperburr	13.4	53	59	8.8	8.0
Sisymbrium erysimoides	Mustard weed	28.0	27	77	11.6	11.1
Soliva pterosperma	Bindii	13.9	52	56	8.4	7.5
Stipa sp.	Speargrass	7.5	68	53	8.0	7.0
Tetragonia tetragonoides	Spinach	21.0	25	78	11.7	11.2

*ME using formula ME = DMD x 0.15

^ME using formula ME = (DMD x 0.17) - 2

During earlier work by Productive Nutrition Pty Ltd, regular sampling and feed analysis was conducted using a range of plants found in the central northeast of South Australia. These data of preferentially grazed species are shown in Table 2. Of particular interest is the affect of calculating ME using two different methods, both of which are in common use by feed testing companies. For some species, ME is significantly affected by the equation used.

This data set shows both the range of NVs in rangeland species but also the range in estimated ME depending upon which formula is chosen for the calculation. Table 3 shows the range of equations that are currently in use in testing laboratories to convert measured digestibility (DMD, OMD, DOMD) to estimated ME.

A similar trend is shown in Table 3, based on data in a report by Muir (1990). Again, a range of values is present for ME. In this case, six possible calculations for ME generate a range of potential outcomes. This highlights the confusion in the current system for pastoral woolgrowers and their advisors.

Table 3 NV of a various forage species found in western New South Wales, with estimated ME calculated using various methods (original data from Muir, 1990)

Species*	DM	CP	ADF	OM	DMD (1)	ME*	ME^	ME #	DMD (2)	ME*	ME^	ME #
1	53.8	10.4	38.0	92.7	59.3	8.9	8.1	8.2	56.6	8.5	7.6	7.9
2	57.1	10.3	38.1	93.7	59.2	8.9	8.1	8.3	56.5	8.5	7.6	7.9
3	46.6	11.9	33.4	89.8	62.9	9.4	8.7	8.5	61.1	9.2	8.4	8.2
4	64.5	3.6	49.3	89.7	50.5	7.6	6.6	6.8	44.5	6.7	5.6	6.0
5	71.5	4.4	48.8	87.2	50.9	7.6	6.7	6.7	45.2	6.8	5.7	5.9
6	69.4	4.0	49.2	90.7	50.6	7.6	6.6	6.9	44.8	6.7	5.6	6.1
7	67.2	6.0	43.2	90.1	55.3	8.3	7.4	7.5	50.5	7.6	6.6	6.8
8	46.2	11.1	24.8	76.7	69.6	10.4	9.8	8.0	67.8	10.2	9.5	7.8
9	53.2	7.7	44.8	88.6	54.0	8.1	7.2	7.2	49.9	7.5	6.5	6.6
10	52.6	8.8	33.4	93.8	62.9	9.4	8.7	8.9	59.8	9.0	8.2	8.4
11	63.5	7.7	41.2	87.7	56.8	8.5	7.7	7.5	52.8	7.9	7.0	7.0
12	65.4	5.6	47.2	91.6	52.2	7.8	6.9	7.2	47.1	7.1	6.0	6.5
13	52.0	11.1	48.7	92.0	51.0	7.6	6.7	7.0	48.1	7.2	6.2	6.6
14	37.4	12.7	26.0	80.0	68.7	10.3	9.7	8.2	67.5	10.1	9.5	8.1
15	33.0	15.0	25.6	78.1	68.9	10.3	9.7	8.1	68.8	10.3	9.7	8.1
16	36.2	15.1	26.1	81.4	68.6	10.3	9.7	8.4	68.5	10.3	9.6	8.4
17	30.7	41.1	24.9	89.2	69.5	10.4	9.8	9.3	80.4	12.1	11.7	10.8
18	64.2	6.7	47.5	90.4	51.9	7.8	6.8	7.0	47.3	7.1	6.0	6.4
19	41.4	9.9	33.4	92.8	62.9	9.4	8.7	8.7	60.2	9.0	8.2	8.4
20	38.7	19.1	30.4	82.8	65.2	9.8	9.1	8.1	66.6	10.0	9.3	8.3
21	66.6	6.8	43.4	90.4	55.1	8.3	7.4	7.5	50.6	7.6	6.6	6.9
22	62.9	6.3	48.8	85.5	50.9	7.6	6.7	6.5	46.0	6.9	5.8	5.9

DMD (1) using formula DMD = 88.9 - (0.779 x ADF)

*ME using formula ME = DDM x 0.15
^ME using formula ME = (DDM x 0.17) - 2
ME corrected to OM = (DDM x OM)/100 x 0.15

Table 4 Scientific and common names of species listed in Table 3

Species*	Scientific Name	Common name
1	*Acacia aneura*	mulga
2	*Acacia loderi*	nelia
3	*Acacia victoriae*	prickly wattle
4	*Aristida browniana*	tall kerosene grass
5	*Aristida contorta*	kerosene grass
6	*Aristida jerichoensis*	no 9 wiregrass
7	*Astrebla pectinata*	barley mitchell grass
8	*Atriplex vesicaria*	bladder saltbush
9	*Digitaria brownii*	cotton panic
10	*Dodonaea attenuata*	narrowleaf hopbush
11	*Enteropogon acicularis*	curly windmill grass
12	*Eragrostis eriopoda*	woollybutt
13	*Heterodendrum oleifolium*	rosewood
14	*Maireana astrotricha*	low bluebush
15	*Maireana pyramidata*	black bluebush
16	*Maireana sedifolia*	pearl bluebush
17	*Medicago polymorpha*	burr medic
18	*Monachather paradoxa*	mulga oats
19	*Myoporum platycarpum*	sugarwood
20	*Rhagodia spinescens*	thorny saltbush
21	*Stipa variabillis*	spear grass
22	*Thyridolepis mitchelliana*	mulga mitchell

A further example of analysis is shown in Table 5. In this example energy is expressed as MJ/Kg GE; GE is gross energy expressed on a DM basis. This data, provided by the Department of Agriculture WA, Geraldton, appears to inflate the NV of oily feed such as *Acacia aneura* and is calculated by complete combustion of the plant sample and measuring the energy release. There is no value in reporting energy on a GE basis to woolgrowers as GE is not related to animal performance and further confuses wool growers.

Table 5 Analysis of rangeland shrubs analysed for Dry Matter (DM) and Gross Energy (GE) (adapted from Brennan 2005, pers. comm.)

Sample ID	DM %	GE MJ/Kg
1. *Scaevola spinescens*, Nallan Station, Received 12/9	40	19.8
2. *Ptilotus obovartus*, Munurra Station, Received 12/9	44	14.1
3. *Acacia aneura*, Munurra Station, Received 12/9	63.3	22.4
4. *Rhagodia eremaca*, Munurra Station, Received 12/9	45.1	15.2
5. *Maireana atkinsiana*, Mouroubra Station, Received 20/9	28.4	12.5
6. *Maireana convexa,* Barnong Station, Received 20/9	26.5	15.2
7. *Maireana pyramidata*, Sago Bush, MA, Received 20/9	31	14.8
8. *Maireana thesioides*, Lax Bush, MA Barnong Station, Received 20/9	30.2	14.8
9. *Maireana tomentosa,* Felty Blue Bush, RM, Barnong Station, Received 20/9	41.3	16.8
10. *Enchylaena tomentosa*, Ruby Saltbush, MA Barnong Station, Received 20/9	28.2	16.8
11. *Atriplex bunburyana*, Silver Saltbush, MA Barnong Station, Received 20/9	30.6	15.9
12. *Atriplex nummulara*, Jim Addison, Kalgoorlie, Received 23/9	24.8	13.3
13. *Atriplex vesicaria*, Jim Addison, Kalgoorlie, Received 23/9	23.3	15.3

It is clear that the current NV results for rangeland plant species are unacceptably inaccurate, and that producers should assume that energy and protein levels as indicated by laboratory analysis are somewhat optimistic (D. Masters 2006, *pers. comm.* 4 October). As a result, dietary supplementation regimes based on these results are likely to be inaccurate, which would lead to poor animal responses, which are costly and unproductive. Some of these errors are methodological and some relate to the plethora of possible equations to estimate parameters such as ME. Laboratories that calculate digestibility based on OM, rather than DM, provide more reliable results, but these methods may still be overestimating the value of these plants due to the currently unknown effects of high salt diets on the functioning of the rumen and lower gastro-intestinal tract. *In vivo* determination of DOMD remains the most accurate estimation of ME for rangeland plant species, so should form the basis of NIRS calibrations for halophytes.

Limitations of current laboratory methods

Most laboratories in Australia are encouraged by AFIA, but not enforced, to follow the AFIA laboratory methods manual, meaning that methodology for analysis should be consistent. Meanwhile, in the USA, only 10 of the 136 feed laboratories use the recommended reference methods of ADF and NDF, and 22 use recommended CP methods, as recommended by the (US) National Forage Testing Association (Undersander 2006). Those using the recommended methods had a low variation in results (CP within 0.2% two-thirds of the time), highlighting the importance of using recommended methods and consistent laboratory procedures. The laboratories involved in the checking program but not using recommended methods had errors of 0.8%, 1.4% and 2.3% for CP, ADF and NDF respectively. This implies that CP results would be within 0.8% two thirds of the time, meaning that 34% of the time CP results could be inaccurate by one unit or more, or NDF out by 2.3%.

Nevertheless, there are no set methods for setting-up and working with NIRS machines other than manufacturers' guidelines. Even if a laboratory has accurate wet chemistry, the question is whether the developed calibrations will be used by laboratories (Landau *et al.* 2006; Undersander 2006). When measuring protein digestibility via a range of methods, Madsen *et al.* (1997) found that reproducibility between laboratories was very poor, with standard deviations of up to 16%. Equations cannot accurately predict composition if they contain no samples of the type being analysed, so reputable laboratories recognise the limitations of their equations and use wet chemistry where a sample falls beyond the range of the samples included in the equations.

Current *in vitro* models also fail to account for differences in the population structure of rumen microbes. Moir (1951) obtained rumen liquor samples from sheep grazing annual pastures in a Mediterranean-type climate at monthly intervals over a 19-month period. Her group found marked seasonal fluctuations in the numbers of total organisms, with the population peaking in spring (70-90m/mL) and being lowest in autumn (40-50m/mL) when feed was dry and scarce. Except for minor additions during the period, all morphological types of microbes were constantly present but the balance changed at different times of the year.

The relationship of these various changes, both in number and balance, to the chemical composition of the grazed feed (especially protein content) need further investigation. Similar findings were identified by early work in South Africa, also demonstrating that protein content of the pasture particularly influenced the microbial population. Thus, the sheep's ability to digest certain feeds may change during the course of a year due to fluctuating microbial activity, and this cannot be accounted for with current analytical methods. A more accurate and precise estimate of the true NV of preferentially grazed species would facilitate more accurate complementary feeding of livestock when required. This may be possible with the development of NIRS calibrations directly from animal feeding and metabolism trials.

Feed quality predictions versus actual animal performance

Current *in vitro* analytical techniques were developed for grasses and herbaceous legume plant species, typically clover and lucerne (for example, Tilley & Terry 1963). Meuret *et al.* (1993) claim that NIRS appeared accurate for the prediction of the NV of Mediterranean tree and shrub foliage, but this was only when compared to *in vitro* techniques, and none of the species were halophytes. As illustrated in Table 1, Masters (2006) found a significant difference between *in vitro* and NIRS estimates for oldman saltbush digestibility compared to *in vivo* results. This study was conducted to identify differences in analytical methods of the same plant sample, and it is clear that the estimated energy values of rangeland halophytes from current non-*in vivo* analysis methods are unacceptably flawed. Masters' data shows, for the same plant sample, an NIRS OMD estimation of 76% and an *in vivo* value of 56%.

This is the difference between an animal only maintaining body condition versus one capable of successfully feeding a growing lamb. For example, Konig *et al.* (2006) found that unsupplemented lactating ewes grazing saltbush with DMD of 73% (Tilley & Terry method) lost 120g/day while their lambs gained only 30g/day which is well below current industry standards for Merino lambs, that have an expected growth potential of at least 200g/day.

A producer relying on the digestibility based on current NIRS results (or even the *in vitro* estimates shown) would probably reason that lactating ewes required no supplementary feeding. However, in reality, the ewes grazing halophytes would need a substantial amount of a high-energy supplement, such as barley, to feed successfully a lamb without the ewe losing condition and risking ketosis or milk fever, or affecting her ability to become pregnant next season.

Further, lambs would need to be weaned early, or supplemented on the ewe, and fed a more energy-rich diet to promote growth and development. A significant increase in weight gain has been observed (Franklin-McEvoy 2002; Ben Salem *et al.* 2005) when supplementing wethers and lambs grazing saltbush. Ben Salem *et al.* (2005) showed strong weight gains when supplementing Barbarine lambs (starting weight 18kg) grazing on saltbush with barley. Despite a reported *in vivo* OMD of *A. nummularia* being 70%, lambs lost 35g/day, while those supplemented with 400g of barley per day had *in vivo* OMD of 77% (DMD 72%) but gained 66g/day. The authors highlighted that the addition of an energy supplement (barley) increased utilisation of the nitrogen in the *Atriplex,* with this resulting in the improved growth rates. Further, it is likely that the estimated *in vivo* OMD of the *Atriplex* is somewhat generous, for the reasons already outlined in this report.

Above all, this study highlights the importance of supplementing the diet of lambs with additional energy when consuming *Atriplex*, although the required quantity remains difficult to determine. As a result, producers may be feeding too little or too much, leading respectively to poor growth or poor economics. Supplementary feeding needs to be provided at quite exact rates for optimal animal performance without excessive cost.

Moore and Dolling (1961) found sheep actively selected feed components higher in protein, and rejected lower CP components of the ration, which increased the protein in their diets. They found the ability to select differed significantly between individual sheep. This occurs in grazing situations as well (Masters *et al.* 2001). Therefore, it is not possible to accurately predict animal performance from any given plant due to individual grazing habits, before we consider differences in metabolism and genetic make-up of animals. However, having accurate methods of NV determination gives producers a more complete view on the likely production

potential of their livestock, as many are familiar with and monitor the presence of preferentially selected species.

On-farm work in the southern rangelands of South Australia within a monoculture of *Atriplex nummularia* revealed ewes in late pregnancy were unable to maintain body condition without supplementation with cereal grain. This was despite the nutritive analysis of the saltbush showing that the plant was providing the ewe with energy well in excess of requirements for that stage of pregnancy (Jolly 2003, unpub).

Many of the rangeland species remaining after long periods of dry seasonal conditions appear to have an ME estimate of approximately 7MJ/kgDM. Although level of energy is sufficient to maintain body condition on wool-producing Merino wethers, it is insufficient to support optimal production levels of pregnant and lactating livestock, or growth rates of young animals.

Supplementation and the accuracy of nutritive value estimations

Inaccurate estimation of the nutritive value of sample risks incorrect feeding strategies and/or ewe and lamb losses from inadequate nutrition. If producers have gone to the expense (both time and financial) to test fodder, they deserve dependable results that can be utilised to make decisions such as which paddocks to graze with which class of livestock and whether dietary supplementation is required.

Obviously, estimated NV's being falsely high will pose problems for animal nutrition, as producers may provide or withhold extra feeding with misplaced confidence in the test. Alternatively, producers may blame poor livestock genetics for sub-optimal animal performance rather than inaccurate estimates of plant NV, and may purchase expensive genetics only to find that the potential gain cannot be exploited within the current feeding system.

Table 6 illustrates the potential difference in estimated energy values derived from the feed when NV are determined by either DMD or DOMD, and hence the potential cost to the pastoral woolgrower in terms of lambing ewes. Although realistically, monocultures of *Atriplex nummalaria* are rarely grazed in the rangelands as livestock generally have the opportunity to balance their diet by selection, after prolonged periods of drought, halophyte or salt accumulating species tend to dominate the landscape and hence the diet.

If in this case, woolgrowers were supplementing with 1 kg of barley at a cost of $0.30 cents per head per day (November, 2006), the energy value of the diet would equal ewe requirements and she would produce milk and hence lamb and wool growth to her genetic potential, whilst maintaining body condition.

However, if the "true" energy value of the feed on offer, in this case *Atriplex nummalaria*, was actually 7.7 ME, then 1 kg of barley would improve the energy value of the diet but although still costing $0.30 per head per day, would fail to result in production equalling potential. When multiplied by several thousand sheep, the loss of production, as well as associated and additional costs incurred by inaccurate supplementation strategies is substantial.

Table 6 The effect of ME values on the cost- effectiveness of supplementation strategies (Jolly 2006, unpub.)

Requirements		*DMI*	*MJ ME*	*Digestibility*	*Crude protein*	*NDF*	*Cost per head per day*
Lactating ewe		4% of LW	11		15.0%	30%	
Atriplex nummalaria	Method 1 (DMD)	3.98% of LW	10.2	67.7%	19.5%	31%	
Supplementation required	Barley	1 kg	11		16.9%	25%	$0.30
Atriplex nummalaria	Method 2 (DOMD)	2.7% of LW	7.7	52.7%	19.5%	31%	
Equal supplementation	Barley	1kg	9.32		17.0%	25%	$0.30

The greatest misuse of any analytical system is to start with an unrepresentative sample, and there is significant variation amongst batches of grain, by-products, hay or silage. It is imperative that producers are taught the most accurate methods of taking plant or grain samples to reduce sampling errors. Variation in a silo of corn silage showed 31%DM and 44% NDF at the top of the silo, and 33%DM and 37%NDF at the bottom (Undersander 2006).

To increase accuracy of sampling, multiple samples are required. A largely unrealised opportunity with NIRS is to analyse multiple samples due to the lower per sample cost, thereby reducing sampling errors (Undersander 2006). If accurate and repeatable NIRS calibrations were available, producers could receive greater precision of NV data on their samples, as they could afford to have more than one sample of each species tested to produce a more realistic data set, to overcome the issue of variation within a batch or silo of feed.

Potential value of calibrated NIR analysis

The production of calibration equations for NIRS from *in vivo* studies will be of major benefit to pastoral woolgrowers. This advance in understanding the nutrition of rangeland sheep cannot be underestimated in terms of allowing producers to optimise stock numbers, improve animal growth rates and produce more wool of better consistency and quality.

In vivo studies involve feeding a range of preferentially grazed pastoral species to sheep within specialised facilities at research institutions.

Sourcing sufficient rangeland plants is not likely to be a limiting factor, especially since the work of Graham (1964) found only small differences in feed value of fresh and dried material for *in vivo* studies, with DOM 75% *versus* 73%, and digestible energy 73% *versus* 70%. The development of calibrations on priority species has been already largely determined from a survey of producers. South Australia has good sheep housing facilities at both Middleback Station (*via* Whyalla), and The University of Adelaide's Roseworthy Campus, and the owner of Middleback Station has indicated willingness to facilitate species collection and transfer to Roseworthy. Such work would be valuable to both the University community and to the reputation and recognition of Australian Wool Innovation as a supporter of livestock research.
Undersander (2006) states; "it is vital that there is a concerted effort to develop equations that are truly national and cover as many different environments as possible". Although referring to the situation in Arizona and the USA, this comment also applies to the situation in Australia. NIRS, when properly set-up and calibrated, could play a key role in developing more accurate fibre analysis techniques. NIRS would allow an improvement over wet chemistry because greater replication of calibration standards could reduce the run-to-run error and increase predictability of shorter fermentation times over wet chemistry (Norris *et al.* 1976). In addition, NIRS may also be able to predict voluntary feed intake of feeds by animals, which is not currently possible with present methods (Flinn 1991), which Madsen *et al.* (1997) highlights as being a crucial component of predicting the feeding value of a species.

Calibrations, based on either *in vivo* trials or highly accurate *in vitro* studies are essential before any parameters on any plant species can be analysed (Clancy *pers. comm.* 2006). However, calibrations are instrument-specific - equations can be transferred between identical models but it is very difficult to transfer from one type to another. As a result, it is essential for any such studies to be supported by the key manufacturers of relevant NIR analytical instruments. It is essential for the long-term implications of this work that it be conducted as carefully and accurately as possible, and in such a way that instrument makers can incorporate the calibration equations into the software of their machines. Phil Clancy, owner of NIRtech Instruments and manufacturer of the CropScan series, has already stated his support and interest in this work, which will ensure an Australian company has a part of this potential project.

Current portable NIR analysers (such as the Crop Scan 2000H "On Header Analyser') cost about $14,000, are provided with full factory support and with regular calibration updates available electronically. A more robust 2000G model costs $19,000. Clancy (2006) states that competitive models are up to twice as expensive as the CropScan and are not Australian-made. Foley *et al.* (1998) believe that increasingly portable NIR machines will lead to an increase in utilisation of the technology. This means that high quality calibrations must be obtained as NIRS cannot turn poor analyses into useful ones. Ninety-three percent of producers surveyed demonstrated support for NIR analysis of preferentially grazed species as soon as such research becomes available, and believe they could use a portable system when checking livestock during watering runs.

Focus Groups (Consultation process)

To gather pastoralists' input into this project, a survey was designed and distributed to woolgrower groups in northern South Australia, and the pastoral areas of Western Australia (Appendix 1). The Focus Groups in South Australia were provided by BestPrac and were run by Productive Nutrition Pty Ltd, whereas the Western Australian pastoralists were consulted on an individual basis via the Department of Agriculture and Rosemary Bartle, Rural Business Solutions Pty Ltd.

These Focus Groups showed that there was strong and growing interest in supplementary feeding of pastoral sheep in South Australia, with nine out of eleven participants currently undertaking some level of supplementary feeding. None of the three Western Australian producers indicated that they provided supplementary feed to their stock.

The Focus Group study showed that only one producer attempted to provide supplementary or complementary feeding regimes based on what they knew about the feed quality of their pasture. Instead, most producers hand-fed their animals based on "gut-feel" or on "what worked last year". Although this may sound rather casual, it is the most sensible approach due to the lack of current understanding of rangeland plant species. As a result, both producers and their advisors know that pastoral sheep and cattle are not being fed a balanced diet but current, reliable knowledge of plant quality is so poorly developed that this is the best they can do.

The questionnaire was intended to establish the level of understanding about the nutritive value of rangeland species in relation to animal requirements. Pastoralists were advised it was not important whether or not they gave a correct or incorrect answer, but that the questionnaire was designed to determine their general knowledge about this topic and its importance to their business. It was also designed to give them the opportunity to comment on the potential value of this project and future work in this area to improve their management and subsequent profitability. Although quite a few of the South Australians had completed Sheep Nutrition training, many of the South Australian and Western Australian pastoralists clearly require an improved understanding of animal nutrition before they will be able to make use of improved feed analyses.

The relative lack of accurate information on the nutritional value of these plants, makes providing the pastoralist with accurate feeding strategies difficult, as there is insufficient data to know what animals are actually lacking in their diet. It appears that current techniques overestimate ME by around 2MJ, when compared to actual animal performance. For example, a saltbush that tests as 11MJ ME/kgDM is possibly only around 8MJ – a 28% deficit. This is the difference between sufficient energy for an animal at maintenance compared to a lactating ewe. That is, the difference between a ewe successfully feeding a lamb or both starving.

Given the low weaning percentages recorded in pastoral Australia, it is likely that some of this is due to inadequate ewe and lamb nutrition. With lambs worth around $50 (April 2006), a 10% increase in lambing percentage means an extra $5,000 of saleable lamb per 1,000 ewes. For those producers investing in improved genetics on an annual basis, the number of replacement ewe lambs coming into the system will also increase. Obviously, increased numbers of replacement lambs allow greater culling opportunities too, which will more rapidly improve the genetic merit of the flock.

Accurate feed analysis – a tool for decision making

It is evident that further education of producers is needed to understand fully the nutritional requirements of their livestock, particularly in Western Australia. Most producers (90%) had at least a basic understanding of the functions of dietary energy, protein and calcium, but only about 45% could name the functions of fibre or explain the acronym NDF. DMD and DOMD were known to 80%, with some idea of what each term referred. When Focus Group participants were asked wether they knew the nutritional requirements of wool-producing sheep, 30% did know, and a further 20% could quote with good accuracy some target nutritional guidelines. Interesting answers to the question included "Merino sheep cannot be run sustainably as their energy requirements are too high" and "green pastures provides all". Seventy percent showed at least some understanding of nutrition fundamentals (for example, could name some required minerals). Most (80%) participants knew where they could find out such information, with indicated sources being the internet, PIRSA (or AgWA) and Productive Nutrition workshops.

Most producers (80%) had at least some knowledge of their property's key plant species, with the most important species considered as bindii, saltbush, spear grass and bluebush. This was mainly due to their abundance, as their nutritional quality was only considered average-to-good. Clover and ward's weed were also considered important due to their perceived quality. Bluebush and to a greater extent saltbush were considered valuable due to their resilience to environmental insult. Producers stated that they determined the nutritional value of the various species by observing stock preference ("palatability") and their subsequent performance on areas dominated by those species. Importantly for this report was the finding that 40% of respondents had actually had key species tested for nutritive value. However, several producers stated that they did not know which species *should* be more important, and as one producer stated, "the knowledge of pasture species is poor at the moment". Another comment was that grasses and herbs are very important for production, and bushes are the "back-up".

Our study found that balancing animal production with environmental sustainability was a key issue. Only one participant did not answer this question. Thirty percent of graziers used supplementary feeding to reduce the impact on the pasture base, with a similar number expressing the importance of long-term management being more important than short-term economic gain. As one WA producer stated, "have a bit of income and stay afloat rather than taking risks and causing damage to country and create animal welfare issues". Conservative stocking and careful environmental system management were the key issues mentioned. Only one producer believed that you could not balance animal production with environmental care, unless "you had the $ for infrastructure for better control".

On the subject of management of livestock during drought, 80% of those studied destock during a prolonged dry period, and 70% include supplementary feeding in their drought management strategies. Unfortunately, they are currently unable to calculate requirements with any accuracy due to the unreliability of current plant analytical methods. Other strategies include increased observation of stock condition, early weaning and rotational grazing. Importantly, most producers focus on the environmental impact of drought, with 40% actively monitoring for overgrazing, especially perennials. One SA producer plans to increase watering points to reduce grazing intensity, and one WA producer uses licks (ingredients not stated) to "increase grass utilisation", while another did not mate in dry years. Seventy percent of those surveyed use destocking as a management tool to reduce overexploitation of the pasture resource, centred around monitoring of key species.

The Focus Group surveys found 80% of SA participants (but no WA participants) undertook supplementary feeding, with 40% routinely feeding when sheep and lambs are losing condition on paddock feed. Being able to accurately test pasture would allow a proactive rather the current reactive approach to animal nutrition. A similar number fed lupins to rams before mating, and 30% fed ewes before lambing – one producer stated that such a strategy increased lambing percentage by 7%. Hay was the most commonly fed supplement (50%), followed by pellets (including 'sheep nuts'), barley and lupins (all 40%), oats and straw. One producer stated their supplementary feeding regime comprised hay, urea, minerals, molasses and water.

Currently price is the main factor when determining which feeds to use, although several producers stated that nutritive value was an important consideration. Acidosis was noted as a major concern when feeding cereal grains. There was no consensus on how much supplementary feeding was required, with only one participant seeking advice from a consultant, and others following advice from publications, neighbours, PIRSA and a few from observing stock performance and adjusting feeding rates accordingly. Again, not being able to feed an exact amount and type of supplement has led to suboptimal animal performance, but the extent of this is unknown.

The negative effect of frost on plant growth was also highlighted in the Focus Groups, with some areas in the Lake Eyre Basin experiencing four months of frosty weather. Not only does frost slow or even stop plant growth, but Milford (1960) found that frost was "an important reason for the decline in nutritional value of grasses" in pastoral systems. If ewes were lambing at this time, supplementary feeding strategies would potentially alleviate losses of ewes and lambs.

The response from the Focus Groups clearly demonstrates the interest from the pastoralists in better understanding sheep nutrition. It is also evident that all livestock producers need a better understanding of animal nutrition before new technologies can be fully appreciated. These surveys highlighted that educating woolgrowers about sheep nutrition should be a key role for Australian Wool Innovation.

Pastoralists' surveys – looking to the future

The Focus Group survey indicated that around 90% of pastoralists would send feed samples for NV analysis if they could be assured the result would be accurate, applicable and inexpensive, although only one-third of the Western Australians supported this. Interestingly, many respondents believed that a portable, on-farm NIRS machine would be ideal so they had real-time results to make immediate decisions regarding paddock allocation to a specific class of livestock, and the type and amount of supplement.

Over half said that they would use this information to identify the most nutritious areas to graze lambing ewes, while a similar number would frequently test feed quality to make decisions where to shift all stock to suit their production needs.

Greater than 50% of those surveyed did not know what NIRS referred to, although two participants had a good understanding of faecal NIRS. All but one SA group member answered positively to the question, "If rapid, accurate, inexpensive technology meant that quality evaluation of rangeland species was readily available, would you use it?" However, only one Western Australian believed this was important. Interestingly, one (SA) participant stated that they would prefer their own NIRS machine for real-time analysis of their plants to assist in decision-making. Subsequent discussion with other SA group members showed strong interest in such a device, modelled on the machines used for grain analysis on-farm and at grain silos (for example, the CropScan 2000 series) (NIRtech, 2006). NIRtech recognise that NIRS instruments that are capable of accurately predicting the nutritive value of rangeland species is an important potential market.

Pastoralists indicated that this project area was a good investment of their levies; they believed that they had a real-world application for the technology that would result in better animal and environmental management, ideally leading to better financial returns. If such technology was available, over half of the participants said that they could graze lambing ewes in better paddocks, alter stocking rates to manipulate grazing pressure, and to supplement with more precision. One producer would consider analysing their key species on a regular basis (fortnightly) to determine a complete nutrition strategy. Another stated that it would be useful if plant analysis could also provide information on the oxalates and tannin content of pasture species, and that if accurate NIRS calibrations were to be developed then oxalate and tannin should be included due to their effect on animal production.

Once thorough calibrations are established for rangeland plant species, "NIRS offers ecologists enormous analytical power" (Foley *et al.* 1998). NIRS can improve forage analysis through rapid, inexpensive fodder analysis allowing multiple samples to be run, improving analytical accuracy (Undersander 2006).

Conclusion

It is clear from this review of the literature that current methods of analysing plant samples fail to recognise and account for the subtleties and peculiarities of rangeland pastures. This was highlighted by Milford (1960) as being a key limitation to animal production in subtropical and other pastoral environments. The high salt content of many pastoral species affects both the analytical techniques and the grazing animal itself to an extent that is not fully clear. Thus, it is necessary to conduct scientifically valid metabolic trials using these species, in such a way that the data can be accessible to manufacturers of NIRS analytical equipment to enable them to calibrate their instruments.

With ever-improving and ever more affordable technology, it is indeed possible for accurate and reliable plant analysis to be made available to pastoralists and wool producers. Utilising this, woolgrowers can make strategic decisions for the nutritional wellbeing of their animals which maintain or improve the condition of the pasture base. Such technology exists in the grain industry to assist producers make harvesting and marketing decisions. Woolgrowers, particularly in pastoral Australia, could also make great use of such innovative technology and they have demonstrated a strong interest in so doing.

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Appendix 1: Focus Group Questionnaire

The Nutritive Value of Rangeland Plants

Productive Nutrition Pty Ltd - an AWI funded project

FOCUS GROUP QUESTIONNAIRE

Why do livestock need …

Protein

Energy

Calcium

Fibre

Why are the following factors important…

NDF

Digestibility – organic dry matter (DOMD)

Digestibility – dry matter (DMD)

Do you know the nutritional requirements of wool producing sheep?

If the answer to the above question was no, do you know where to find this information?

During a prolonged dry, how do you manage

a) livestock

b) plants

How do you balance animal production issues with environmental concerns?

Which species do you consider the most important?

Species name ***(Please list 1 or more)***	***Is this related to …***			
	quality?	abundance?	preferential grazing or selection?	resilience to environmental insults (drought / flood)?
1				
2				
3				
4				

Comment:

If quality is important, how do you know this plant(s) has superior feed value?

What do you understand by the term NIR(S)?

If rapid, accurate, inexpensive technology meant that quality evaluation of rangelands species was readily available, would you send samples for analysis to assist in your decision making?

If you answered 'yes" to the previous question, how would you use this information?

Do you do any supplementary feeding?

If 'yes':

When do you feed?

What do you feed?

What determines your choice of supplementary feed?

How do you decide how much to feed?

Any other comments:

Name and contact details ***(optional)***:

Thank you

San Jolly

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