Final report

New generation NIRS calibrations to improve diet evaluation and animal growth predictions

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Abstract

The new generation NIRS Calibrations project was initiated to address gaps in the Australian feed testing landscape by (1) broadening the diversity of plant species that are predicted accurately, (2) increasing the number of forage quality traits that can be predicted, and (3) exploring the opportunity to use faecal NIRS to predict diet selection, forage intake and animal performance. These calibrations provide producers, researchers, and consultants with the tools required to maximise the efficiency of red meat production and mitigate risk associated with poor nutrition. The team from CSIRO and NSW DPI identified gaps, leveraged historical samples from across Australia, conducted research, and generated new NIRS calibrations. The overarching goal was to have broad, accurate, fit-for-purpose calibrations that accurately predict key traits for the entire Australian feedbase. Novel calibrations were also developed for minerals, secondary compounds and the Cornell Net Carbohydrate and Protein System.

The accuracy of handheld NIR sensors, as a practical alternative to lab-based units, was evaluated and it was demonstrated that they predict traits with biologically significant errors so are likely to remain a rough screening tool. A series of high-quality faecal calibrations for sheep and sheep and cattle was created covering a large range of important parameters such as dietary digestible organic matter intake*, in vivo* digestibility, dietary fibre, minerals and crude protein. Preliminary evidence for methane predictions from faeces was generated. Utilising the large collection of pasture samples with *in vivo* data, significant improvements were made with NIR calibrations to predict *in vivo* digestibility for sheep and cattle at maintenance and *adlib* feeding levels. The team demonstrated successful transfer of the currently utilised Coates northern cattle faecal calibrations for cattle in Northern Australia onto modern instruments, thus ensuring the technology remains available to pastoralists and consultants.

Executive summary

Background

Forage nutritive value (NV) is the principal determinant of voluntary intake and livestock productivity. Accurate NV estimation is required to predict animal performance, manage diets to achieve production goals, as a selection tool for higher quality forages, and to develop forage quality curves which underpin systems models. Animal house feeding experiments and wet chemistry laboratory analysis are expensive and time consuming. Near Infrared spectroscopy (NIRS) allows the rapid and inexpensive prediction of nutritional traits. The use of NIRS requires the development of statistical relationships between measured traits and light absorbance in the near-infrared region of the electromagnetic spectrum (wavelength range 700 – 2500 nanometres). Some nutritional and production traits are easier to predict by NIRS than others and existing calibrations have generally been developed for a narrow set of species (plants) or for only northern cattle (faecal). There are very few methods to predict intake, diet quality and performance of ruminants in extensive grazing systems. Intake markers are difficult to administer and achieve uniform release rates. Methods based on animal sensors can be very inaccurate when applied to different species, breeds and feedbase systems. Faecal NIRS offers a tool to predict aspects of selection, intake and diet quality from a faecal sample. This methodology has been developed for tropical cattle systems. It is unclear if it can be applied to southern cattle and sheep systems.

Digestibility is a key nutritional measure used to evaluate the quality of feeds and is used to estimate the metabolizable energy (ME) available for sheep and cattle (CSIRO 2007). Feed laboratories regularly use NIR calibrations to predict digestibility at maintenance feeding level, based on *in vitro* digestibility using either rumen fluid (Tilley *et al*. 1963), or pepsin cellulase (PC, Clarke *et al*. 1982) methods, with digestible organic matter in the dry matter (DOMD) used to estimate ME of the feed (CSIRO 2007). NIR calibrations based on primary *in vivo* standards (either faecal or forage) potentially bypasses the error associated with *in vitro* predictions (Kitessa *et al*. 1999). One of the objectives of this project was to determine whether NIR calibrations could be created directly for *in vivo* DOMD in sheep and cattle that covered a range of species at maintenance and *ad lib* feeding levels.

The main target audience was red meat producers, the feed testing industry, consultants, forage breeders and researchers. This work has potential national and international impact. The outputs from this project will be used to deliver more accurate predictions of nutritive value for all species within the Australian feedbase. With refinement, the faecal NIRS work will deliver an ability to predict diet quality, intake, aspects of diet selection and potentially methane emissions for sheep and cattle across Australia.

Objectives

The aim of this project was to bring together two large research and commercial delivery laboratories to combine, test and refine existing plant NIRS calibrations with samples that are taxonomically, spatially and temporally diverse.

Including;

- Expand the calibration database to accurately predict novel forages in the Australian feedbase (including forage brassicas, subtropical species and native shrubs).

- Develop alibrations for nutritional parameters that have not been predicted within the Australian feedbase were also developed (e.g.*in vivo* DOMD, oxalate or fibre digestion fractions).
- Investigate veracity of claims that mineral content (e.g. phosphorus) can be predicted accurately were also investigated.
- Using some samples that were generated in the project, as well as historical samples, expand faecal NIRS calibrations to predict intake, diet selection and performance traits of southern sheep and cattle was explored.
- Test in field hand held NIRS units. For two commercially available portable NIRS units, loss of accuracy associated with in-field scanning of swards was quantified.
- Finally, tropical cattle faecal calibrations were future-proofed by transferring them to modern NIRS machines.

Methodology

Data for more than 70,000 forage and 4656 sheep and cattle faecal samples were utilised during the project. For forage NIRS, the team compared historical databases and identified gaps in plant groups and traits that were not predicted. Samples were classified by both laboratories into 43 groups and 113 sub-groups comprising forages, pastures, hays, straws, silages, concentrates and by-products to aid calibration development. Gaps representing sample type/taxonomy were identified and discussed with industry. Gaps within these sample types/taxonomy were targeted for collection to strengthen the database and ensure it is representative of the Australian feedbase. Additional novel samples were analysed to expand existing calibrations as well as developing new calibrations.

For a number of these plant samples (n=251), fresh material was scanned with two different handheld sensors (ASD FieldSpec Pro and Spectra Vista HR-1024) to test feasibility of trait prediction from fresh material.

For faecal NIRS, new diet/faecal pairs were generated (through an animal house experiment) and historical samples were collated from sheep and cattle experiments where diets with accurate animal metadata was available. These were used to develop preliminary calibrations and to explore the need to have separate calibrations for sheep and cattle.

A total of 358 *in vivo* standards from a range of historical experiments were sourced. These represented various forages and feeds, including tropical grasses and legumes, temperate and tropical silages, native Australian shrub species and some grains. Digestibility estimates were from either sheep and/or cattle and at *ad lib* and/or maintenance feeding levels.

The project focussed on different instruments including the FOSS 6500, FOSS XDS, SpectraStar XT & XTR models, Bruker MPA and MPA II, ASD FieldSpec Pro 3 as well as a Spectra Vista Corporation HR-1024. Calibrations were developed using the standard partial least squares approach. FOSS and SpectraStar are dispersive infrared instruments (or scanning spectrometers) while Bruker is a fourier transform (FT)-NIR instrument. The instruments capture spectra at different intervals and measure reflectance in different ways, so transfer of calibrations between systems can be problematic, compared to transfer of calibrations between machines with the same mode of spectral collection. As anticipated, there were difficulties moving data between FOSS/SpectraStar and Bruker, however this was overcome for transferring between FOSS and SpectraStar, with significant gains made in

conversion of calibrations between FOSS 6500 and XDS instruments. Some promising data was generated to suggest that transfer between FOSS and Bruker instruments is possible, especially if calibrations are further strengthened with additional samples and data. To aid transfer of data between instruments and potentially facilitate cloud-based calibration transfer, a diverse core selection of samples was shared between CSIRO and NSW DPI which were scanned and analysed by both laboratories on all available NIR instruments. For a subset of data, machine learning was tested as an alternative mathematical tool to partial least squares. It was unclear if there was a benefit from the new approach.

There are many different statistics that are used to compare calibration accuracy. Throughout the report, accuracy of the calibrations is expressed in the manner of Williams (2014), using the ratio of standard error of performance to standard deviation (RPD) values. These are calculated on validation statistics (i.e. samples that were not used to generate the calibration tested). RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 provide rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control and acceptable for many predictive purposes, 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

Results/key findings

Broadening forage NIRS calibrations.

During this project, novel NIRS calibrations were developed, broadened and strengthened for the following plant (forage or silage) nutritional traits;

- Digestibility predictions including dry matter digestibility (DMD), organic matter digestibility (OMD) and DOMD using various methodologies (*in vivo*, pepsin cellulase, daisy, Tilley and Terry) (RPD 1.7 to 4.3),
- Organic matter (OM) or ash content (RPD 2.7),
- Ether extract (EE, fat content; RPD 3.4),
- Gross Energy (GE; RPD 1.9),
- Water soluble carbohydrates (WSC; RPD 3.4), ethanol soluble carbohydrates (ESC; RPD 2.0) and starch (RPD 5.3),
- Fibre fractions, including acid detergent lignin (ADL; RPD 1.8), acid detergent fibre (ADF; RPD 4.5), neutral detergent fibre (NDF; RPD 4.0), and Cornell fibre fractions - indigestible neutral detergent fibre (iNDF; after 240 hours; RPD 3.0 to 3.3) and undigested neutral detergent fibre (uNDF; after 30, 120 and 240 hours; RPD 2.8 to 3.9),
- Protein fractions – total nitrogen (N) and crude protein (CP; RPD 7.1), non-protein nitrogen (NPN; RPD2.6), neutral detergent insoluble crude protein (NDICP; RPD 2.2), acid detergent insoluble crude protein (ADICP; RPD 2.0), and rumen digestible protein (RDP; RPD 3.7),
- Anti-nutritional compounds/anions – nitrate (RPD 2.8), oxalate (RPD 2.8), and phosphate (RPD 2.3)
- Methane from fermentation in rumen fluid (RPD 1.8).

In the majority of cases, taxonomically broad calibrations predicted the nutritional traits of samples with greater accuracy than calibrations developed specifically for a plant or taxonomically similar group of similar plants. If more taxonomic, spatial and temporal diversity can be built in without a large reduction in accuracy, these broad NIRS calibrations represent a valuable tool for Australian researchers, feed testing agents and livestock producers, as they encompass nearly all of the species

that appear in monocultures or mixed swards. Errors associated with species identification or mixtures of species are avoided. Many of these calibrations are higher than published values.

Predicting mineral content of plants.

Excellent calibrations were developed for magnesium (RPD 1.2 to 6.2) and calcium (RPD 5.0); however, magnesium was predicted in only one laboratory but not the other. Other minerals that could be predicted included chloride (RPD 2.8), phosphorus (RPD 2.3), sulphur (RPD 2.2), sodium (RPD 2.4) and potassium (RPD 2.1). Commercial NIRS labs offer predictions of calcium, magnesium, phosphorus, sodium, potassium, sulphur, chloride and dietary cation-anion difference (DCAD) which is calculated from these minerals.

In-field sensing.

The highest quality, laboratory grade, hand-held NIRS sensors (ASD FieldSpec and Spectra Vista Corporation HR-1024) were used in this project. The models differed with the Fieldspec ASD using a contact probe with an inbuilt light source, while the HR-1024 relies upon ambient light from the sun. Both instruments employed multiple detectors which scanned the full near-infrared region of the electromagnetic spectrum. They use 3 separate dispersion elements and detectors. The sampling interval varies from 1.4nm from 350-1000nm and 2nm from 1000-2500nm giving resolution of 3nm @700nm, 10nm @1400nm and 12nm @2100nm. Many commercial products capture fewer wavelengths and are reliant on ambient light so are highly unlikely to be more accurate than our machines. There is opportunity to predict some traits using these machines, however, there is a significant statistical and biologically relevant loss of accuracy. The current predictions, based on ~300 samples, do not offer a very useful tool for animal management or plant improvement practices. The predictions included DMD (RPD 2.0), DOMD (RPD 2.0), ADF (RPD 1.1 to 1.6), NDF (RPD 1.9), WSC (RPD 1.6) and CP (RPD 1.7). Loss of accuracy is presumably associated with 'noise' created by ambient light, moisture content and sample heterogeneity. New mathematical approaches could be considered and there is scope to broaden the data set.

Predicting diet selection, intake and diet quality using faeces.

Faecal traits that are predicted from faecal samples.

The stable carbon isotope technique allows a prediction of diet selection (between species with C3 and C4 photosynthetic pathways). In northern Australia, this distinguishes tropical grass (C4) from forbs (C3) and in southern Australia it can determine saltbush (C4) and subtropical grasses (C4) in a temperate diet (C3). Very accurate (excellent)calibrations were developed to predict delta carbon from sheep and cattle faeces. Excellent ruminant faecal nitrogen and faecal organic matter calibrations were generated from a combination of sheep and cattle samples. This could be used to expand the scope of the northern cattle calibrations into southern cattle and sheep systems.

Dietary traits that are predicted using faeces.

Faecal samples were used to predict the quality of the diet the animal had consumed. Diet CP of sheep could be predicted with fair screening potential. There is a very high prospect for NIRS calibrations to predict dietary CP intake for both sheep and cattle. This offers a significant management tool for extensive production systems where diet selection is variable and nitrogen supplementation provides production benefits.

Other aspects of nutritional value of the diet selected by individual sheep and cattle could be predicted from a faecal sample. Diet ADF and NDF content were both predicted with excellent results, for sheep alone or sheep and cattle combined. This is very exciting as ADF is an indicator of a poor-quality diet and often highly correlated to energy values, animal performance and methane emissions. These calibrations are performing better than any others that were identified in published literature in Australia and internationally. Dietary ash content was also predicted with excellent results.

Quality control level calibrations were developed for *in vivo* DOMD in sheep, with an error of prediction of 3.1% units. This is also novel and has significant implications for managing sheep in extensive grazing systems. It could offer a phenotyping tool to identify individual sheep that are able to find and select a higher energy diet when grazing pasture. More work needs to be done to develop the cattle calibration and integrate sheep and cattle.

Unfortunately, historical sheep and cattle faecal samples, with measured methane from respiration chambers, were relatively scarce. Good evidence was generated to suggest that methane could be predicted with faecal NIRS, using cattle grazing tropical forages, although low sample numbers lead to uncertainty. Given the ability of the method to predict diet digestibility and indigestible fibre, combined with these preliminary results, it is probable that methane could be predicted accurately with some additional work. This would be an extremely useful tool for researchers seeking to identify low methane plants and animals and for industry to benchmark Eco credentials. A series of methane chamber experiments with ~12-15 diverse forages is required to generate *in vivo* calibration samples that can be used to benchmark *in vitro* fermentation methods.

Prediction of the dietary concentrations of some minerals from faecal samples was possible. Given the small sample numbers, this was a very good result. Minerals that could be predicted included chloride, sodium, magnesium, calcium, iron, potassium, sulphur and zinc. Other minerals that had potential to be predicted included copper, boron, phosphorus and manganese. These data suggest the high potential of this calibration set as a useful animal management tool, especially if the industry moves towards valuing micro-nutrient profiles of meat. More data are required, however this may just require the measurement of minerals in a selection of the existing faecal/foragesamples that were collected, not new animal research.

Animal performance traits that are predicted using faeces.

Voluntary feed intake is a difficult trait to measure in field grazing trials. Use of intake markers and on-animal sensors is expensive and can have significant errors. 'Fair' screening potential for organic matter intake by sheep (RPD 2.8, R^2 0.87, error of prediction of 1.5 g OM/kg LW.day) was demonstrated in this study. Digestible organic matter intake was predicted to a quality control standard (RPD 3.1, R^2 0.90, error of prediction of 1.03 g OM intake/kg LW.day). This requires more work before commercialisation, especially in developing the cattle component.

Transferring calibrations to new types of NIRS machines.

At the start of the project, the historical CSIRO tropical cattle faecal calibrations (David Coates) were run on two very old (30-40 years) FOSS 6500 machines that were no longer supported by the manufacturer. As part of this project, these calibrations were successfully transferred to a modern SpectraStar XTR and FOSS XDS. This occurred after a tender process to select commercial laboratories to deliver the cattle service. Significant progress has been made in demonstrating the calibrations can be transferred to a Bruker MPA II. These calibrations have been static for some time. More work is required in validating and expanding the calibrations with faecal standards

accompanied by accurate data. This project found that it is possible to generate and commercialise calibrations that cover sheep and cattle (northern and southern) – this will simplify delivery and allow for more opportunities to test predictions with emerging animal feeding/metabolism/methane data.

Benefits to industry

1. Accurate, rapid and inexpensive predictions of a wider range of nutritive characteristics for the vast majority of plant species in the Australian feedbase. This will provide producers, consultants, researchers and plant breeders information that will allow them to make management decisions that will optimise red meat production and animal welfare.
2. NIRS-based tools to predict diet selection, diet quality (energy, fibre, crude protein, some antinutritional compounds and minerals), and digestible organic matter intake. This offers opportunities for producers and researchers to assess performance of grazing animals at the individual or herd/flock level in extensive systems. This will enable more timely supplementation decisions and offers an accurate and inexpensive tool for phenotyping individual animals grazing pasture.
3. Improved profitability and welfare of livestock industries through better management of the feedbase and livestock. This will translate to improved welfare outcomes during times of nutrient shortfalls.
4. Greater awareness of the opportunity to improve profitability by matching the nutritional needs of different classes of livestock and with current and emerging feedbase species.

Future research and recommendations

1. **The broad multi-species NIRS calibrations for many of the forage quality traits for the Australian feedbase (160 plant species) are near-commercial and could be considered for release.** This includes calibrations for novel traits. Some may require a little more work to optimise the product across the Bruker and Foss/SpectraStar methods. At this stage, there will be separate calibrations for the platforms with different predictive potential. This may cause some confusion to industry and requires further consideration during commercialisation.
2. The faecal NIRS calibrations for diet selection, diet quality, intake and possibly methane are showing much more promise than anticipated at the start of this project. It is exciting that evidence has been generated to indicate that sheep and cattle faeces can be used in the same calibration (as sheep are a much easier and less expensive model for generation of new samples for validation and expansion). **These faecal NIRS calibrations could be a game-changer for the red meat industry and should be considered a priority for future investment.** In the latter stages of the project, the team co-designed and gained animal ethics approval for a metabolism crate/methane feeding study to generate faecal samples to fill the critical gaps in the data.
3. **The delivery of information regarding diet selection, diet quality, intake and possibly methane from faecal NIRS calibrations could be linked to animal nutrition models to provide producers with information regarding growth rates, efficiency and the need for strategic**

supplementation. If delivered through a web-based platform, this information could be provided as quickly as samples can be dried, ground and scanned (24-48h).

4. The researchers have considered – in consultation with MLA and industry – the potential commercialisation pathways of plant and faecal calibrations developed during this project. The consensus was that greatest adoption of these improved calibrations would occur if they were provided to all feed testing industry participants, rather than select individual laboratories. Current calibration transfer systems are cumbersome and require labour inputs for updates, and there is a risk that industry will be using many versions of the calibrations. This will require ongoing oversight. **The optimal way forward is for industry to co-invest in a digital platform that links to a centrally maintained data cube that continues to expand as new traits and novel samples are included**. This would be a game-changer for the Australian red meat and research sector and could allow rapid, inexpensive quantification of eco-credentials. A draft model, based on CSIRO delivery into the minerals sector, has been presented.

5. In-field sensing of whole plants remains less accurate than use of dried and ground material, this is presumably associated with moisture masking key wavelengths, heterogeneity of samples and variation in ambient light. **There may be use-cases where the degree of accuracy is less important and there is a role for these hand-held sensors**. For example, sensors with in-build light sources or leaf clips may have a role in plant improvement, assessing plant health or plant nutrient needs. Industry needs to identify acceptable errors for various uses of sensing and this needs to be considered in the prioritisation of future research projects. **New mathematical techniques such as machine learning could be considered**.

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1. Background

1.1 The importance of feeding and nutritional value of forage

The feeding value of forage is the animal production response when grazing under unrestricted (i.e. unlimited biomass) conditions. Feeding value drives production of meat and wool after the animal has met its requirement for growth, maintenance, thermal regulation, disease management and reproduction. It is a function of voluntary feed intake (what an individual chooses to eat, and the quantity eaten) and the nutritive value of the ingested biomass. Nutritive value (NV) refers to the responses in animal production per unit of feed that is eaten and is a function of the digestibility of the nutrients in the feed and the efficiency with which the nutrients are used for animal maintenance or production. The NV of grains and forages impacts on voluntary intake (food can only be consumed as quickly as it can be broken down and pass through the rumen) and live weight gain in ruminant livestock.

NV is therefore a critical factor in predicting the productivity and health of sheep and cattle, and utilisation of the on-farm feedbase. Very few researchers, consultants and farmers have the capacity to monitor the quality of the feedbase due to the technical complexity in measurement methods and cost, and this can lead to less-than-optimal performance and poor management decisions. Equally, few pasture breeding or selection programs measure nutritional traits throughout a plants lifecycle, and this can lead to suboptimal outcomes for industry and higher methane emission intensity. Industry strategies, including MISP 2020 and the MLA Strategic Plan 2020-2025, identify a lift in on farm productivity as a major imperative for research investment. Tools to help producers become more efficient are a major part of this plan. Accurate determination of feed value will contribute to this goal by improving livestock feeding and feedbase utilisation in Australia. Improvements in diet quality and feed utilisation contribute to the MLA goal of a carbon neutral red meat industry. Ability to predict methane emissions from a faecal sample offers a simple and inexpensive tool for farmers to demonstrate best-practice methane reduction.

Anti-nutritional factors also have a direct bearing on animal health and productivity and include compounds such as tannins, nitrates, prussic acid and oxalates. Mineral imbalances can also lead to suboptimal growth or toxicity. These anti-nutritional traits could be exacerbated by future climates, for example, drought conditions can provoke high nitrate and prussic acid levels in sorghum. Early detection of hazardous levels of these components can prevent production losses and eliminate preventable deaths. Rapid detection using NIRS could potentially provide a tool to identify forages high in these components.

Traditional methods of determining nutritional factors influencing intake and digestion are expensive and time consuming. Depending on the number of parameters analysed, and the complexity of testing for these, cost of analysis for each feed can exceed $250 and turn-around time is often more than 2 weeks. This high cost precludes the routine use of traditional testing by farmers and their advisors and limits the number of tests than can be done by forage and ruminant nutrition researchers.

NIRS is a rapid, inexpensive and non-destructive technology that can predict multiple feed quality parameters in a single 10 second scan (Deaville and Flinn 2000). When a sample is analysed, the radiant energy is absorbed selectively according to the specific vibration of the molecules within the sample, which produces absorbances and overtones in the spectrum. Prominent absorptions in NIRS forage spectra include water, aliphatic hydrocarbons, lipids and carbohydrates (Conzen, 2006). NIRS therefore predicts feed quality by relating the absorbance of near infrared light for individual samples, with calibrations developed for each parameter being analysed. Calibrations are constructed by scanning samples with known values for a parameter (previously determined using traditional measurement or wet chemistry methods) and developing mathematical relationships between spectral variation and the measured trait.

The relatively low cost ($30-$70 for multiple traits) and more rapid turnaround time (1-4 days) means that NIR now underpins commercial and research feed analyses in Australia and internationally. The accuracy and reliability of NIRS predictions depends on the integrity and robustness of the calibrations. While some good calibrations have been developed to meet specific needs, there is a lack of broad predictive ability across the Australian feedbase. In 2014 a RIRDC funded project titled “Future proofing fodder quality analysis for Australia”, (Little, 2014) the author reviewed some of the risks and opportunities the industry currently faces. Not all Australian feed test providers have the resources, skills or facilities to develop their own calibrations. Currently there is no incentive for Australian commercial laboratories to develop new calibrations for Australian feeds because the cost of chemistry associated with development is too high relative to the commercial return. Consequently, these laboratories often rely on old or purchased calibrations. In addition, few individuals have the capacity to test the accuracy of NIRS predictions and error statistics are rarely provided with predictions – thus decisions can be based on poor data.

Existing calibrations do not predict for the range of parameters now requested by industry. This has led to a reliance on calibrations from international sources, where they are not developed using the feeds and forages utilised in Australia. Little (2014) estimated that in 2013, 10,000 samples per year (25% of total commercial analyses) were being sent to USA. Little (2014) also reported that more recently a number of Australian laboratories have become affiliated with two of the ‘big 3’ USA forage laboratories (Dairy One and Cumberland Valley Analytical Services). This development, and the use of USA laboratories and their affiliates, is being driven by sectors of the dairy industry. The implication for commercial laboratories in Australia is that without providing these additional analyses business will decline. The implications for feed testing in Australian are loss of capacity in feed testing and the ability to develop calibrations for the Australian meat industry. In summary, while NIRS is a powerful tool, inappropriate use of the technology can lead to suboptimal decisions. Robust calibrations for the Australian feedbase, based on Australian pastures, crops, silages and forage, are required by the red meat industry.

1.2 NIRS is not just for livestock managers, examples where NIRS approaches have been used to improve outcomes from forage improvement or domestication projects

NIRS has the capacity to greatly increase research outputs within budgetary constraints. Three examples of the use of NIRS as a tool in research and breeding projects include;

1. Recent comparison of the nutritional value of different annual and perennial legume species has shown massive differences in energy decline during senescence (a time of year when energy is critical for finishing lambs). While biomass values were similar, differences between common commercial species were dramatic with dry matter digestibility values ranging from 45% to 70%. For mature sheep these differences would result in 50g/day of growth compared to 140 g/day of weight loss (GrazFeed: Freer *et al* 1997; Norman *et al*. 2021; MLA funded ELLE project B.CCH.6540).
2. A benchmarking study with the SARDI lucerne breeding program found biologically significant variation within lucerne germplasm for digestibility at each of the different growth stages. This would lead to a threefold difference in liveweight change for a pregnant ewe (day 100 of gestation) (GrazFeed, Freer *et al* 1997).
3. Over 4000 genotypes of saltbush were screened for nutritional value during the domestication programme. MIDAS economic modelling indicated that improving digestibility by 10% (from 50 to 55%) would be three times more profitable than increasing biomass production by 10% or reducing the cost of establishment by 10% (O'Connell *et al* 2006). In 2014, the cultivar Anameka™ was released, with an organic matter digestibility of 64%. This is predicted to triple the profitability of shrub enterprises on farms.

These examples are based on previous MLA investments, where sheep were the focus. NIRS has already been established as a valuable tool for monitoring cattle productivity and diet selection in extensive northern systems. In this case, faecal samples are used, this is referred to throughout the document as faecal NIRS.

1.3 Developing NIRS calibrations for taxonomically diverse vs narrow groups of species

There are many examples where NIRS calibrations have been developed to predict the nutritional value of a narrow range of forages such as whole cereal plants (Deaville *et al*. 2009; Stubbs *et al*. 2010), lucerne (Halgerson *et al*. 2004; Brogna *et al*. 2009), perennial grasses (Myer *et al*. 2011; Burns *et al*. 2013), forage maize (Hetta *et al*. 2017) and even woody forage shrubs such as tagasaste (Flinn *et al*. 1996) and sagebrush (Olsoy *et al*. 2016). These examples are all characterised by narrow taxonomic diversity with only one or two plant species within the calibration set. Some authors feel that for NIRS predictions of forage quality, species-specific calibrations are more accurate than broad, taxonomically diverse calibrations (Dryden 2003; Landau *et al*. 2006). Accurate, species-specific calibrations are useful for single-species forage improvement programs and assessment of widely sown species such as oaten or lucerne hays. These calibrations are less useful/not feasible for forage testing laboratories and researchers who work with a wide range of species, and/or mixed swards or have samples submitted with uncertain identification.

There have been several studies exploring how much diversity is required to develop robust multi-species NIRS calibrations. Shenk and Westerhau (1993) concluded that if enough samples are utilised, broad multi-forage species calibrations can be nearly as accurate as those for single species. Andueza and colleagues (2011) explored development of calibrations for single forage species and compared them to mixed grass (comprising five species), mixed legume (comprising three species) and a broad, global calibration encompassing all eight species of grasses and legumes. For many traits, prediction accuracy was higher for the most taxonomically diverse data. In southern Chile, a calibration was successfully developed for mixed swards, comprising eight perennial grass and legume species by using nearly 300 spectra/chemistry pairs (Lobos-Ortega *et al*. 2013). In Italy, calibrations have been developed for 13 species that are endemic to native grasslands, including grasses and legumes (Parrini *et al*. 2018). In southern Australia, calibrations were successfully developed for eight woody shrub species (Norman *et al*. 2010). A range of studies are summarised in Appendix table 8.1.1

Extensive grazing systems in southern Australia are based on a diverse range of forage species, dominated by annual and perennial grasses, legumes and forbs. One aim of this project was to investigate the feasibility of developing broad NIRS calibrations to predict the nutritional value of the majority of annual and perennial forage species in the feedbase of southern Australia. The hypothesis that it would be possible to develop a global calibration that provides accurate predictions across a diverse range of forage species for a range of nutritional traits was tested.

1.4 Developing plant NIRS calibrations for novel traits and minerals

Before this project, the teams from CSIRO and NSW DPI developed and refined calibrations on an ad-hoc basis to meet specific project needs or as time permitted. This project aimed to develop novel feed NIRS calibrations to meet current and future feed testing requirements for the Australian red meat industries. At the start of the project in 2019, there were five commercial labs in Australia that were offering NIRS feed/forage testing services, with these laboratories routinely predicting forage DM content, ME, DMD (based on several methods), CP (or total N), NDF, ADF and crude fat, with several laboratories also offering ash, starch and WSC predictions. Additionally, commercial dairy fodder packages provided NIRS prediction of 7 minerals – Ca, Mg, P, Na, K, S & Cl (and DCAD which is calculated form these minerals). These figures were offered as a guide, with recommendations of traditional wet chemistry for accurate mineral profiles. Some labs offered iNDF, NDFD, ADICP, NDICP, lignin, starch, nitrate probability and total fatty acids (Table 1.1 Lists feed and faecal testing parameters that are measured, the standard acronym and general unit of measurement). The hypothesis that it would be possible to use NIRS to accurately predict for a range of additional parameters, including secondary compounds and minerals was tested.

Table 1.1 The majority of feed and faecal testing parameters that are measured, the standard acronym and general unit of measurement (used throughout this report).

Parameter	Common Acronym	Common unit
Digestibility (*in vivo*, rumen fluid methods include *in sacco*, Daisy or Tilley and Terry, the enzyme method is pepsin/cellulase)	DMD	% DM
Organic matter digestibility	OMD	% DM
Digestible organic matter in the dry matter	DOMD	% DM
Gross Energy	GE	MJ/kg DM
Metabolisable energy	[1]ME or M/D	MJ/kg DM
Organic matter (100 - ash)	OM	% DM
Neutral detergent fibre	NDF	% DM
Undigested neutral detergent fibre 30hrs dry matter basis	UNDF30	% DM
Undigested neutral detergent fibre 120hrs dry matter basis	UNDF120	% DM
Indigestible neutral detergent fibre 240hrs dry matter basis	iNDF240	% DM
Undigested neutral detergent fibre 30hrs organic matter basis	UNDFom30	% DM
Undigested neutral detergent fibre 120hrs organic matter basis	UNDFom120	% DM
Indigestible neutral detergent fibre 240hrs organic matter basis	iNDFom240	% DM
Acid detergent fibre	ADF	% DM
Hemicellulose	Hemi	% DM
Lignin		% DM
Water soluble carbohydrates	WSC	% DM
Ethanol soluble carbohydrates	ESC	% DM
Starch		% DM
Total non-structural carbohydrates	TNSC	% DM
Ether extract (crude fat)	FAT	% DM
Nitrogen	N	% DM
Crude protein (total N x 6.25)	CP	% DM
Non-protein nitrogen (as CP)	NPN	% DM
Neutral detergent insoluble crude protein	NDICP	% DM
Acid detergent insoluble crude protein	ADICP	% DM
Rumen digestible protein	RDP	% DM
Buffer insoluble CP		% DM
Volatile fatty acids	VFA	mM
Non grass (%) or C3/C4 ratio of plants	Delta C	
Daily dry matter intake (kg or per unit bodyweight)	DDMI	g or kg
Digestible dry matter intake	DMI	g or kg
Digestible organic matter intake	DOMI	g or kg
Daily live weight change or gain	LWC or LWG	g or kg

[1] M/D (megajoules per day) is measured at the maintenance level of feeding, throughout this document it is assumed that ME is the same as M/D.

1.5 Developing faecal NIRS calibrations to predict intake, nutritional value of the diet and livestock performance

Much of the energy from ingested forage is not retained by ruminants and is voided as faeces (20-65%) and methane (6-10%). These losses represent an economic inefficiency and are associated with negative environmental outcomes. Measuring the digestibility (or indigestibility) of diets selected by grazing animals in extensive systems presents difficulties because the diet selected by individuals differs substantially from that of the pasture on offer. Individuals may achieve the same growth

rates by selecting different diets. Except for uniform monocultures, it is not possible to manually harvest plant material that accurately represents the diet selected by the grazing animal. This led to the development and adoption of sampling procedures using oesophageal fistulate animals so that samples of selectively grazed forage representing the diet of the grazing animals could be collected and analysed in the laboratory. This approach involves variable errors and the need for surgically modified animals. In response to a need for a better methodology, Queensland researcher David Coates started a novel research programme in 1994. The Australian faecal NIRS calibrations were developed by CSIRO and MLA for cattle production systems in the subtropical zone of Queensland (Coates and Dixon 2011; MLA project NAP3.121, 2001–2004).

The highlight of this historical work was development of faecal NIRS calibration equations to estimate the DMD of forage diets that are ingested by cattle grazing in the rangelands of northern Australia. To achieve this, a large and diverse calibration data set of matched diet–faecal pairs was obtained over 10 years using three sampling methods: (1) grazed pasture with diet samples collected from oesophageal fistulated steers and faeces collected from resident cattle; (2) *in vivo* digestibility experiments with penned cattle fed forage hays; and (3) penned cattle fed pasture freshly harvested from the field (Coates and Dixon 2011). Estimated *in vivo* DMD reference values were determined using pepsin–cellulase *in vitro* analysis of diet samples. The final calibration set of 1052 samples represented 264 diets with DMD ranging from 38% to 75%. DMD% was predicted with a standard error of calibration of 1.87% and a coefficient of determination (R^2) of 0.90 (Table 1.2).

Table 1.2. Livestock performance and diet traits and errors of prediction by Coates and colleagues (MLA project NAP3.121, 2001–2004).

Parameter	Unit	Standard error of calibration	R^2 calibration	Standard error of validation	R^2 validation	RPD
Faecal nitrogen (%)	%	0.1	0.96	0.1	0.96	4.8
Dietary CP	%	0.1	0.95	0.2	0.95	4.3
Daily dry matter intake	g/kgLW.day	2.4	0.74	2.5	0.72	1.8
Diet DMD	%	1.9	0.90	1.9	0.89	3.0
Diet OMD	%	1.7	0.89	1.8	0.89	3.0
Average daily liveweight gain	g/day	157	0.88	162	0.87	2.8
Digestible dry matter intake (DDMI)	g/kgLW	0.8	0.95	0.9	0.94	4.2
Non grass (%) or C3/C4 ratio of plants	Delta carbon (absolute value)	0.8	0.93	0.8	0.93	3.8

The Coates team also developed faecal NIRS calibrations for estimation of faecal nitrogen, dietary nitrogen, daily dry matter intake (DDMI), diet OMD, average daily liveweight gain, digestible dry matter intake and non-grass percentage (based on carbon isotope rations that are associated with the C3/C4 photosynthetic ratio of plant species). The prediction statistics of the Coates calibrations are presented in Table 1.2.

At the start of this project, commercial use of these faecal NIRS calibrations was licenced by MLA (on behalf of CSIRO) to a single commercial provider. CSIRO maintained the 'master' NIRS machine (a 40-

year-old FOSS 6500) and provided background technical support. Unlike plant NIRS, where the calibrations can be tested and updated relatively easily with laboratory-derived data, with faecal NIRS is more difficult to test or expand calibrations. For expansion of calibrations (or new ones) for the prediction of intake, digestibility and liveweight change, faecal samples must be generated from large numbers of sheep and/or cattle, ideally in metabolism crates, fed a range of known diets. The oesophageal fistulate method is no longer used. This activity is time consuming, expensive and involves intensive animal studies. The hypothesis that the faecal NIRS technology could be applied to southern cattle and sheep (given availability of sufficient reference samples) was tested. Further, the hypothesis that sheep and cattle diet/faecal pairs would have to be separated by species to optimise development of faecal NIRS calibrations was tested. Finally, the team worked proactively to ensure that any samples generated in the LPP that may contribute to future calibration development were collected, NIRS scanned and stored appropriately.

1.6 Quantifying the loss of accuracy associated with in-field scanning of swards

The convenience and instant feedback available from the use of handheld NIR spectrophotometers is a major incentive driving further technological development and availability of these devices. The prospect of coupling this technology with cloud based NIR calibrations is also advantageous.

Most commercial feed testing calibrations using benchtop instruments in laboratories are based on samples that have been dried and ground to a homogenous sample. Drying is important because moisture in samples can have spectral reflectance peaks that sit over areas that are important for prediction of other traits. Grinding improves accuracy by reducing error associated with the part of the plant that is scanned. The hypothesis that some traits can be predicted with hand-held scanners, with the understanding that error of prediction would likely be larger than for dried and ground samples in a laboratory was tested.

The number of handheld NIRS spectrophotometers available has been rapidly expanding over the last five years. The models differ quite a lot in terms of size, cost and quality. The miniaturisation of electronics and improvements in stability and size of detectors has allowed these instruments to become a viable option for in-field analysis (Evangelista, 2021). Detectors are usually comprised of photo-diode arrays or miniaturised In GaAs detectors used in conjunction with micro-electro-mechanical systems (MEMS) to maintain signal stability. The scientific community is somewhat divided on the quality of predictions and suitability of handheld devices for infield evaluation of forages and silages (Krzysztof 2020).

2. Objectives

The objectives (achieved) of this project were to;

1. Work collaboratively; with project teams across LLP to optimise resources use efficiency and data collection across projects, to test the performance of the existing southern feedbase NIRS calibration equations with plant samples from a range of other sites and species. Develop NIRS calibrations that accurately predict for samples sourced from different environments (locations) across southern Australia.
2. Build new data into the calibration(s) (including temperate and subtropical grasses, legumes and forbs), develop and test a new series of prediction equations. Define the boundaries for appropriate industry use of the equations.
3. Work collaboratively to develop NIRS calibrations based on Australian feeds for protein (e.g. N, ADICP, NDICP, NPN), carbohydrate (WSC, starch, pectin, ADF, NDF, lignin) fractions, digestibility (Pepsin/cellulase and rumen fluid).
4. Assess the likelihood of successfully developing calibrations to predict nitrate, oxalate and saponin.
5. Assess the reliability of NIRS to accurately predict mineral content, as reported in USA style feed reports.
6. Investigate means to take spectra from various machines and merge them in a common database. Generate a set of samples in sealed ring cups for use by industry to monitor quality control and to facilitate the alignment of participating spectrophotometers to a master NIRS machine.
7. Through experimentation the opportunity to move to handheld technologies to scan and predict the chemical composition of wet samples in the field was explored. All samples that were subject to wet chemistry were preserved so future technologies can be tested and calibrated with minimum of cost.
8. In addition to seven primary objectives, the transfer the Coates NIRS calibrations to modern NIRS machines emerged as a priority. This was to mitigate the risk of the calibrations being lost if the aging FOSS 6500 failed (no longer supported by FOSS). It was also to enable labs that do not have FOSS 6500 machines are able to provide a commercial service to industry.

Historical samples from CSIRO, NSWDPI and other laboratories and livestock researchers across Australia were sourced to develop proof of concept for faecal NIRS calibrations for intake, diet selection, production and methane parameters for southern cattle and sheep.

3. Methodology

3.1 How does NIRS work?

NIRS is a rapid, inexpensive and non-destructive technology that can predict multiple feed quality parameters in a single 10 second scan (Deaville and Flinn 2000). Although development of an NIRS laboratory can be expensive, it is relatively inexpensive in the long term as individual sample processing costs are low. There are other advantages to NIRS over conventional laboratory analytical methods; it is non-destructive; requires no reagents; and allows for the determination of multiple traits (Stuth *et al*. 2003). An NIR spectrometer projects a known quantity of NIR light onto a sample and then records the reflectance from that substance. The stretching and bending of primarily CH, NH, OH, CO, and CC bonds as a result of the interaction between this radiation and a biological material yields chemical information about that material (Stuth *et al*. 2003). In addition to the chemical features of a substance, physical attributes such as particle size also affect NIR spectra by creating “scatter”. Scatter is the dispersion of reflected light from the surface of sample particles without penetrating the sample and can have a significant impact in observed variation in NIR spectra (Stuth *et al*. 2003). Therefore, scattered light contains no information concerning the chemistry of the sample but may have implications of the physics related to particle size. This is why particle size and grinding methodology is an important feature of the technology.

NIRS predicts feed quality by relating the absorbance of near infrared light for individual samples, with calibrations developed for each parameter. NIRS relies on analysis of a particular product with both NIRS and traditional wet chemistry, then the pairs of information in the calibration set are used to generate a predictive equation. This is referred to as spectro-chemical prediction models (Shenk and Westerhaus, 1996). This low cost and rapid turnaround time methodology (compared to traditional chemical analysis) is the reason why NIRS underpins commercial feed analyses in Australia and internationally. The accuracy and reliability of NIRS predictions depends on the integrity and robustness of the calibrations, which relies on the strength of the absorption related to the component of interest in the NIR spectrum, as well as the accuracy of the method used to measure the analyte.

Prediction of selection and productivity traits with faecal samples (faecal NIRS) is more complex as it is an indirect method. Diverse calibration data sets of matched diet–faecal pairs are required, and these are costly to obtain. Samples that may be useful include (1) grazed pasture with diet samples collected from oesophageal fistulated steers and faeces collected from resident cattle; (2) *in vivo* digestibility experiments with animals in metabolism crates fed forage hays or freshly harvested forage from the field.

3.2 Methods for developing and testing calibrations

Calibration consists of both physical and electronic steps. The process begins with obtaining a sample set of the desired material, in this case forage and faeces. To optimise the predictive ability, the calibration set should be well distributed, representing the range of expected variation in the constituent of interest (Stuth *et al*. 2003). Considerations include temporal variability, spatial variability and biological variability (eg plant growth stage or parts of plants. Drying and grinding procedures are very important due to the fact that water is a strong absorber of NIR light and

particle size affects the shape of the spectrum (Stuth *et al*. 2003). The conditions under which samples are scanned (NIR spectra obtained) should also be as uniform as possible with respect to ambient temperature and moisture. Temperature affects the shape of the spectra, shifting the expression of absorption peaks.

Statistical procedures begin after careful collection of matched spectra and accurate laboratory reference data. Data pre-treatment steps such as multiplicative scatter corrections (Martens and Stark, 1991) or detrending and standard normal variate transformations (Barnes *et al*., 1989) reduce the effect of particle size (scatter) on the calibration set (Stuth *et al*. 2003). Calculating derivatives of the spectra can assist with baseline shifts and overlapping absorption bands (Hruschka, 1987). The data are then generally subject to multivariate regression procedures. A range of commercial software packages assist with the process. Emerging mathematical methods such as neural networks/machine learning may have a role where traditional techniques have been unsuccessful.

For the FOSS and SpectraStar analyses, the spectrum file data from the NIRS machine was converted to a multifile, and the chemometric software package Ucal (Unity Scientific) was used to generate predictions using partial least squares regression methods. For each calibration, a range of pre-treatment options including standard normal variate detrending, scatter correction, and derivatization with different derivative gap and smoothing was tested. From this, the best performing equations were selected. No wave specification trims were utilised, the entire available spectra from 680 nm to 2500 nm was employed. Critical levels to remove outliers were left at default settings with the T limit equalling 2.5. The GD limit was 3.0 and neighbourhood size was set to 0.20.

For the Bruker Spectral data for all samples was imported into the OPUS software package (Bruker Optik™, OPUS version 7.5, 2014), where the distribution of the spectral population was checked by principal component analysis (PCA) and plotted in Opus using PCA spectra scores and calibrations developed using partial least squares (PLS) regressions within the OPUS software package. Calibrations were optimized within Opus to investigate the different mathematical pre-treatments with the software suggesting the best pre-treatment according to the lower root mean square error of cross validation (RMSECV). Treatments tested included multiple scatter correction (MSC) and standard normal variate (SNV) in combination with first and second derivatives. Once complete, the performance of the calibration models was validated, and root mean squared errors of prediction (RMSEP) were calculated.

Equation validation is conducted to assess the predictive ability of the selected calibration equation. Validation entails prediction of either an independent set of samples, i.e. from a different population than the calibration set, with known reference values, or removing a certain number of samples from the calibration set, and not using them in the calibration process (Stuth *et al*. 2003). Key statistic include;

- **Standard error of prediction (SEP)**. This is used to judge the predictive ability of a calibration equation and presented in the units of measurement. It should be as small as possible.
- **Standard error of cross validation (SECV)**. This is where a pre-determined proportion of samples, is sequentially removed from the calibration set and predicted by an equation developed with the remaining samples.
- **Coefficient of determination for a linear model (R^2 value),**
- **1 minus variance ratio (1-vr).**
- **Ratio of standard error of performance to standard deviation (RPD).**

There are several ways to calculate RPD. When they have been compared during this project, there is a slight difference to the second decimal place. This is not a significant factor when comparing the predictive ability.

1. R^2 values from validation statistics can be used to calculate the RPD using the following equation;

$$\mathbf{RPD = 1 / (1 - R^2)^{0.5}}$$

2. Calculation using the SD and SEP;

$$\mathbf{RPD = SD/SEP}$$

The guide of Williams (2014) was adopted in this project. They suggested RPD values of 0 to 1.9 are very poor and not recommended for forage testing; RPD values of 2.0 to 2.4 are poor and only useful for rough screening; RPD values of 2.5 to 2.9 offer a fair screening potential; RPD values of 3.0 to 3.4 are good (quality control); RPD values of 3.5 to 4.0 are very good (suited to process control) and RPD values greater than 4.1 are deemed excellent. Throughout the report, a 'traffic light' system to colour RPD values from red (very poor and not recommended for forage testing at this stage), through green to blue (excellent) was utilised.

Machine learning

Machine learning and its branch deep learning is a rapidly evolving mathematical method. These techniques must be correctly applied to a problem to produce an acceptable solution. The objective was to take some of the spectra and chemistry and apply an alternative statistical method to the traditional Partial Least Squares. The methodology is incredibly complex and summarised in Richetti *et al* (2023).

3.3 Analysis of samples using desktop and handheld NIRS machines

At CSIRO Floreat, Spectra were collected using a Unity Spectrastar 2500XT and 2600XTR- rotating top window system (Unity Scientific) and some samples were scanned with a FOSS 6500. The software UCAL was used to create models by selecting wavelengths, mathematical pre-treatments PLSR factors, outlier determinations and PLSR regression.

At NSW DPI Wagga, spectra were collected using either the BRUKER™ Multi-Purpose NIR Analyser or the FOSS XDS™. Samples scanned using the Bruker MPA instruments were scanned a total of 32 times each with a resolution of 8 $^{cm-1}$ between 12500 and 3600 $^{cm-1}$. The spectral data pre-treatment used mean centring and a 17 point Savitzky-Golay smoothing function. The final frequency region(s) chosen was based on data obtained using the optimisation tool in the OPUS software. Individual sample scans were averaged to provide a single spectra per sample. Models were created using the Bruker Optik software. OPUS^{TM} software version 7.5 (Bruker Optik, Germany, 2014) was used to create models by selecting wavelengths, mathematical pre-treatment's, PLSR factors, outlier determinations and PLSR regression.

The ASD Fieldspec Pro (Picture 1.1) is a spectroradiometer designed for field environmental remote-sensing using fibre optics, with optical energy collected through precisely cut, sealed and polished fibers. It has a high signal to noise ratio, repeatability, and resistance to vibration/changes in

temperature or humidity and operates across VNIR (350-1000 nm) and SWIR1 and 2 (1000 to 1830nm and 1830 to 2500nm). This device employs three detectors: silicon photodiode array (350-1000 nm) and two separate, TE cooled, InGaAs photodiodes (1000-2500 nm). Integrating a contact probe illuminated with a halogen bulb into the sample collection avoided the confounding interference of sources of natural illumination, atmospheric transmission, the presence of clouds and wind, and viewing geometry.

Figure 1.1 ASD FieldSpec Pro System (pictures obtained from the ASD website).

The Spectra Vista HR-1024 (Picture 1.2) is a high resolution NIRS portable unit that has a conical field of view which allows it to collect spectral data from curved surfaces suited to heterogenous samples like forage samples. This unit has three detectors to process spectral data a Si unit (350 – 1000 nm) as well as two InGaS detectors – InGaAs detector 1 (1000 – 1890 nm) and an extended InGaAs (1890 – 2500 nm).

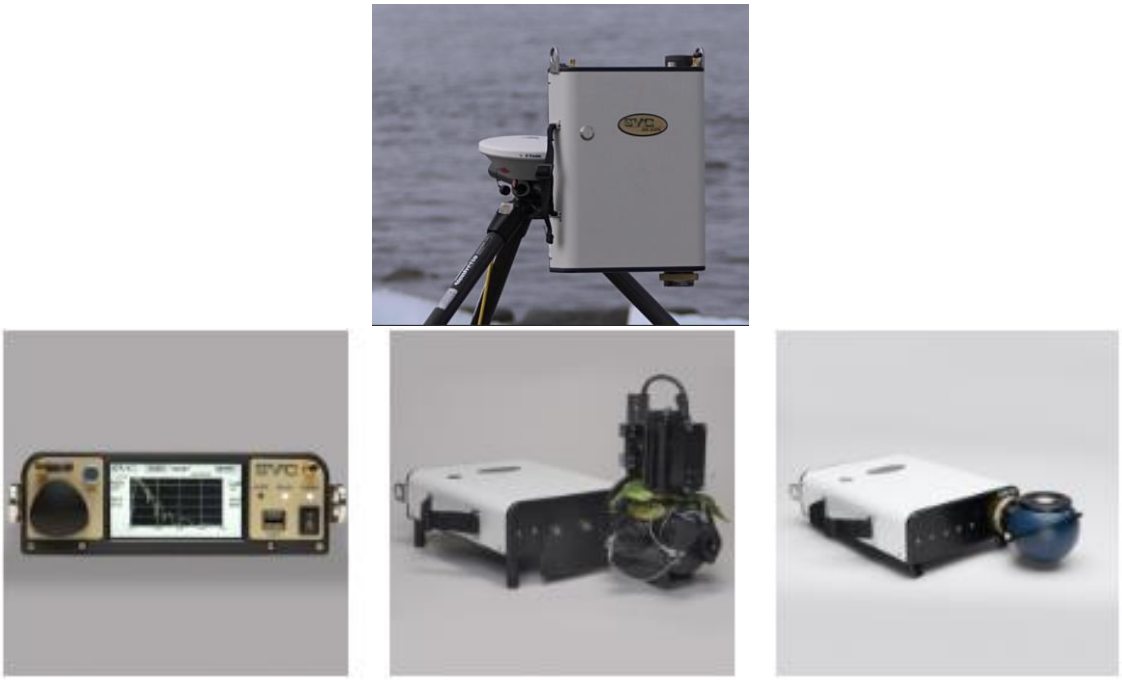

Figure 1.2. The Spectra Vista HR-1024 is a high resolution NIRS portable unit, pictures from the Spectra Vista website.

3.4 Methods for transferring calibrations between machines

Transferring calibrations between machines can be difficult. If two instruments collect spectra in the same way, it is easier to transfer e.g., FOSS to FOSS (wavelengths), FOSS to SpectraStar (a bit more difficult as 1 nm or 2 nm data collection) or Bruker to Bruker (Fourier transferred to wavenumbers). Calibrations can be transferred from one to the other over the range of wavelengths or wavenumbers that overlap, as long as both use wavelengths or wavenumbers. The range of the machines can be important. For example, if one machine is scanning from 700 to 2500, and you are transferring to a machine that scans from 700 to 2000, you can trim the spectra. It is relatively simple if there are not important data in the range that was left out. The calibration needs to be tested on both instruments to assess the success of the transfer. If one instrument collects wave number and the other wavelengths, as in the case of the WWAI and CSIRO labs, transfer is very difficult and requires specialist skills. Until the model for commercialisation is determined, it is simpler to just scan common samples on both machines and develop separate calibrations for both platforms. In this report, calibrations are reported as WWAI (for Bruker FT) or CSIRO (for FOSS or SpectraStar).

3.5 Sample Selection

The project accessed approximately 70,000 stored forage samples and 4656 sheep and cattle faecal samples that were generated between 1968 and 2023 by the project partners, other Livestock Productivity Project (LPP) projects and other research projects. All samples had NIRS scans, and a large number of the samples had been subject to wet chemistry analyses for various parameters or had paired livestock data. The numbers of wet chemistry analyses which have been conducted on all LPP forage samples are included in Appendix Table 8.2.1 and 8.2.2

Of the 70,000 forage samples a significant number were submitted by research projects for which more detail on sample type etc. exists or could be accessed. The categories with the least number of samples were those flagged in the project submission – tropical species, newly introduced species etc. Samples from individual producers generally had less detail but were still valuable for calibration development as they represented the complexity of samples (mixes, stage of growth, care of handling post-collection etc.) that exists with commercial samples.

A database with details such as submitter, location, sample type (e.g., by-product, forage etc.), sample detail (e.g., canola, cottonseed meal) and all analyses that had been conducted by NIRS and chemistry was produced. This included a unique coding system which has been developed to identify sample categories (Appendix Table 8.5). Deficiencies in sample type and associated wet chemistry were rectified by sourcing additional samples and conducting wet chemistry analyses on these and currently held samples.

3.6 Sample processing

The bulk of samples were either placed in a paper bag then oven dried for 48 h at 60^{o} (CSIRO), 24 h at 80^{o} (Wagga) or immediately frozen before eventual freeze drying. Samples were ground to pass through a 1 mm screen using either a Cyclotech (FOSS), Labmill 3100 (Perten) or Cyclone Mill Twister (RETSCH) grinder. A preliminary study was conducted with unground samples that were divided and

subsequently ground in each of the grinders to establish whether the type of grinder created any spectral bias, however no significant spectral bias associated with these grinders was detected. As samples were scanned on both machines, any residual bias would be built into new calibrations.

3.7 *In vivo* Standards

In vivo standards are forages which have been fed to sheep and/or cattle and for which apparent whole tract digestibility has been determined. As such, *in vivo* standards are a critically important reference. The project has acquired 490 *in vivo* samples of different feed types from various sources (Table 3.1), which represent a range of forage types and preservation methods including maize, temperate and tropical silages from NSW DPI Wagga Wagga Agricultural Institute (WWAI) as well as tropical and subtropical forages fed to sheep and cattle from CSIRO's Coates and Minson collections. Temperate hays, grains, forages, native shrubs and mixed diets from the other sources including CSIRO, NSW DPI and DPIRD in WA. The samples collected represented digestibility values measured in both sheep and cattle at *ad lib* and maintenance levels of feeding. A more detailed list of samples is attached (Appendix Table 8.6), and these have been collated into an Excel database.

The samples comprise a set of sub-tropical, and tropical grasses which have had digestibility studies conducted on them. Many also have original intake data, proximate data such as crude protein, ash and fibre analysis, as well as comprehensive mineral data. Accordingly, they would represent a valuable set if they proved suitable for use in the current NIR project.

Table 3.1. Number of samples with known *in vivo* digestibility for sheep and cattle at the *ad libitum* or restricted level of feeding.

Source	Sample type	Sheep		Cattle	
		Intake level	n	Intake level	n
NSWDPI, Wagga Wagga	Temperate & tropical dried forages & silages	*Ad lib*	22	*Ad lib*	29
		Restricted	33	Restricted	32
		Ad lib & Restricted	2	*Ad lib* & Restricted	11
	Grains, concentrates	Restricted	32	*Ad lib*	23
CSIRO, Floreat	Dry forage including hay, annual legumes, native shrubs and pellets.	Restricted	22	-	-
CSIRO, Coates	Various, mostly subtropical forages			*Ad lib* & Restricted	24
CSIRO, Minson	Dried subtropical and tropical forage	*Ad lib*	238	-	-
Vic DPI, Hamilton	Dried temperate forage	*Ad lib* & restricted	10	-	-
DPIRD, Bunbury	Dried temperate forages including annual legumes	Restricted	12	-	-

Samples sourced from Dr Dennis Minson (CSIRO) samples were generated between 1964 and 1970 by CSIRO in Townsville and comprised a total of 238 subtropical samples listed in the AFIA *in vivo* standard records. Of these 154 were in storage in tin drums in the loft of an unairconditioned shed in Northam WA. A subset of approximately 60 of these samples were stored in a dry cool room (4^{o}C)

by David Coates in Townsville for 40 years and subsequently relocated to CSIRO Floreat in 2015 where they have been kept in a dry cool room (4^{o}C). These samples from controlled storage conditions were compared to the samples from uncontrolled storage conditions to investigate degradation associated with storage method. The first part of the study was to determine if the proximate analyses including the *in vitro* pepsin-cellulase digestibility were significantly different between the two sets of samples. Any significant difference could indicate degradation of the uncontrolled storage set due to storage conditions, which would render them unusable in this project. Secondly, to determine if the storage conditions had a measurable effect on the NIR spectral characteristics. This could have implications for the use of the larger set as NIR calibration standards in any subsequent calibrations constructed using these standards. If there was a measurable effect, and if so, is the magnitude significant enough to cause inaccurate predictions of unknown samples. The development of the Minson samples is described in six publications; Minson and Milford (1968), Milford and Minson (1968 a & b), Minson (1971), Minson (1972) and Minson and McLeod (1970).

3.8 Faecal samples from known diets, intakes and diet quality

In this project historical faecal samples with known reference data were sourced from a range of animal house feeding experiments and new samples (n=16) were generated with sheep at WWAI (Table 3.2). Cattle faecal samples were obtained from a Livestock Productivity Partnership grazing experiment at CSIRO Chiswick (n=5). A feeding experiment for perennial wheat +/- lucerne, serradella or clover yielded 24 samples and Lucy Watt's PhD project provided sheep faecal samples from various legume and oat combinations (n=24). The CSIRO shrub improvement work yielded 193 faecal samples with a good range of mineral intakes from individual sheep offered 16 diets with faecal harnesses or metabolism crates at restricted levels of feeding (Norman *et al*. 2010, plus two additional unpublished metabolism crate experiments). The CSIRO carbon isotope experiment gave 161 faecal samples and involved sheep offered 11 diets that were combinations of hay, saltbush and/or Rhodes grass (Norman *et al*. 2009). This collection was valuable for having carbon isotope data (delta C) for all the faecal samples. The Australian Wool Innovation Shrub Nitrate project offered a legacy of 14 sheep faecal samples with metabolism crate and methane chamber data (Li *et al*. 2018). Kevin Bell (Pardoo Station) kindly provided 31 diet faecal pairs for northern WA cattle grazing irrigated forage. Finally, the recent CSIRO Dryland Pasture Legume Systems Project yielded 62 faecal samples of sheep offered a pelleted ration with different pasture seed and pod supplements. An additional 476 sheep and cattle faecal samples have been scanned. Many of these have been analysed in the laboratory for faecal nitrogen and faecal ash, so have been included in the data. Ed Charmley (CSIRO) is sourcing metadata from a range of subtropical cattle digestibility and methane studies that have been conducted at Lansdowne in Townsville. A subset of 84 sheep faecal samples from Floreat have individual intake, liveweight, methane chamber and metabolism crate data associated with them. This includes 51 samples from the UWA/CSIRO/MLA 'ELLE' project (NLMP) and 33 from the CSIRO/JCU/MLA algae project.

It was particularly important that the data associated with the faecal samples had very low errors associated with the reference measurements. Data were discarded where there were doubts regarding the methods used for collection or where the individual faecal samples were associated with mean metadata – rather than an individual animal.

Table 3.2. Key faecal samples collected as part of project contribution to faecal NIR development.

Source	Species	n	Feed type	Feeding method	Collection method	Sample times
WWAI, this project	Sheep	3	Mature annual ryegrass/bladder clover/medic and Japanese millet pastures	Grazed	Group	1
WWAI, this project	Sheep	13	Japanese millet, Lucerne/oaten chaff mix	Pen	Individual	1
Perennial wheat project	Sheep	24	Perennial wheat +/- lucerne, serradella or clover	Grazed	Group	1
Chiswick intake	Cattle	5	Pasture (predominately lovegrass, phalaris and soft brome)	Grazed	Group	5
PhD project (Lucy Watt)	Sheep	32	Legume or oat/ legume hay	Pen	Individual	1
CSIRO shrub improvement	Sheep	60	Hay control and 5 saltbush diets with hay (0.5:0.5).	Met crate, maintenance	Met crate, individual	2
[1]CSIRO shrub improvement	Sheep	133	Shrubs, grass and lucerne control. Saltbush, tagasaste, bluebush, rhagodia, acacia, NyPa grass	Met crate, maintenance	Faecal harness, individual	1
[2]CSIRO carbon isotope	Sheep	161	Combinations of hay and saltbush and Rhodes grass. Lots of C isotope data.	Met crate, maintenance	Faecal harness, individual	1
Kevin Bell	Cattle	31	Northern cattle grazing irrigated forage.	Grazed	Group	7
CSIRO Dryland Pasture Legume Systems Project	Sheep	62	Sheep fed a pelleted ration with different types of pasture seeds in pod.	Met crate, maintenance	Met crate, individual	2
CSIRO Shrub Nitrates project	Sheep	14	Rhagodia preissii, old man saltbush and hay. *In vivo* and methane chamber data.	Met crate, maintenance	Met crate, individual	2
CSIRO/UWA ELLE project	Sheep	51	Serradella and biserrula in various combinations	Met crate and methane chamber	Met crate, individual	2
[3]CSIRO Algae project	Sheep	33	Pelleted ration and algae at various feeding levels.	Met crate and methane chamber	Met crate, individual	2
QAAFI AGO faeces	Cattle	24				
Other	Sheep and cattle	476	Samples that have been scanned but corresponding animal data unavailable. N content and ash in faeces measured for some.	Grazed and pen	Individual	?

Published in [1]Norman *et al* (2010), [2]Norman *et al* (2009), [3]Li *et al* (2018).

3.9 Wet Chemistry Analyses

All wet chemistry analyses were conducted using standard methods routinely used for forage quality analysis in Australia. Method references for the CSIRO Floreat laboratory and NSW DPI's Feed Quality Service at the Wagga Wagga Agriculture Institute (WWAI) are provided in Table 3.3. Methods were often very similar and included quality controls and standards, where differences existed, data from the two labs was compared to ensure no systemic method-related biases. For samples with reference data from other sources (e.g. Minson), where methodology was obscure or no longer best-practice, the biomass was reanalysed with the methods listed in Table 3.3.
For the cross checking of digestibility methods (where WWAI primarily uses rumen fluid and CSIRO uses the enzyme pepsin/cellulase method), 75 samples (silage and hay) ex WWAI were sent to CSIRO for digestibility analysis using the pepsin/cellulase technique in their laboratory to cross validate their values.

Table 3.3. Analysis methods used for determining feed quality parameters.

Parameter(s)	Method
Digestibility (rumen fluid)	1. Modified Ankom Daisy digestibility (Technology Method 3) 2. Modified Tilley and Terry (1963) A two-stage technique for the *in vitro* digestion of forage crops
Digestibility (pepsin/cellulase)	WWAI - Australian Fodder Industry Association (AFIA) Method 1.7R - *Determination of Digestibility using the Pepsin-Cellulase Method* CSIRO - Modification of Clarke *et al*. (1982) see Norman *et al*. (2021).
Digestibility (*in vivo*)	50 kg Merino wethers fitted with faecal harnesses or in metabolism crates as described by Norman *et al*. (2010) OR 277 kg British breed steers (*Bos taurus*) in metabolism crates as described by Bailes (2020)
Methane (*in vivo*)	Li *et al*. (2018)
Methane (*in vitro*)	Durmic *et al*. (2010)
Ash (organic matter)	WWAI - AFIA Method 1.10R – *Determination of Ash* CSIRO - Faichney and White (1983)
Ether extract (crude fat)	AOAC Official Method 2003.06[1]. *Fat (crude) or Ether Extract in Animal Feed*
Acid detergent fibre	ANKOM^{200} Acid Detergent fibre in Filter Bag Technique VER 19.05.17, AFIA Method 1.9A(a) *Determination of Acid Detergent Fibre - Ankom*
Neutral detergent fibre	AFIA Method 1.8A(a) *Determination of Amylase Neutral Detergent Fibre – Ankom*, ANKOM^{200} Neutral Detergent Fibre in Filter Bag Technique VER 10.21.05
Lignin	ANKOM Technology – 9/99
Water soluble carbohydrates	AFIA 1.11A - Determination of Water-Soluble Carbohydrates – Water Extraction – Alkaline Ferricyanide
Ethanol soluble carbohydrates	Method – 1.11R: Determination of Soluble Carbohydrates - Anthrone
Starch	Enzymatic digestion by AOAC Method 996.11, with analysis of converted dextrins by flow injection analysis see Piltz (2022)
Total non-structural carbohydrates	Modification of AOAC Method 996.11, with analysis of converted dextrins and sugars by flow injection analysis see Piltz (2022)
Nitrogen	NSW - AOAC official method 990.03. (1990) Protein (crude) in animal feed. Combustion method CSIRO - Leco CN628, Sweeney and Rexroad (1987)
Parameter(s)	Method
Non-protein nitrogen	Licitra *et al*. (1996)

Ammonia nitrogen	AFIA Method 1.6R Determination *of the Ammonia-N Content of Silages*
Neutral detergent insoluble crude protein (NDICP)	Licitra *et al*. (1996)
Acid detergent insoluble crude protein (ADICP)	Licitra *et al*. (1996)
Buffer insoluble CP	Licitra *et al*. (1996)
Nitrate	WWAI - Modification of the Rayment and Higginson Method 7C1 (1992) *Determination of Nitrate in 1 M KCl Soil Extracts by Flow Injection Analysis* CSIRO - extracted with slight modifications of Cataldi *et al*. (2003) and analysed by HPLC using suppressed conductivity
Oxalate	Modification of Martz *et al*. (1990) *Determination of Oxalate in Forage ·by Reverse-Phase High Pressure Liquid Chromatography* CSIRO - extracted with slight modifications of Cataldi *et al*. (2003) and analysed by HPLC using suppressed conductivity
Malate	Extracted with slight modifications of Cataldi *et al*. (2003) and analysed by HPLC using suppressed conductivity
Phosphate	Extracted with slight modifications of Cataldi *et al*. (2003) and analysed by HPLC using suppressed conductivity
Sulphate	Extracted with slight modifications of Cataldi *et al*. (2003) and analysed by HPLC using suppressed conductivity
Prussic acid	Cyanide measured in aqueous homogenate of material by evolution under mild acid conditions, capture of CN in alkaline picrate and spectrophotometric quantitation of CN-picrate complex (RLS123).
Minerals	CSIRO – CSBP, ICP-AES method after complete digestion of the plant material with a combination of nitric acid and hydrogen peroxide at high temperature (McQuaker,Brown, & Klucker, 1979).
Tannins	Li, Tanner & Larkin. J Sci Food Agric 1996, **70**, 89-101
Silage ammonia	AFIA Method 1.6R Determination *of the Ammonia-N Content of Silages*
Silage pH	AFIA Method 1.12R Determination of silage pH
Volatile fatty acids	Modification of Packer *et al* method (2011).

4. Results

4.1 Ability of NIR to accurately predict forage quality parameters on the majority of species in the Australian feedbase

As discussed previously, transfer of calibrations between FOSS/SpectraStar and Bruker is complex. For the majority of traits, calibration data has been presented for both types of machines. Comparisons of machine capability are not possible as the different labs had a range of historical samples (spectra with wet chemistry), where the data were available, but the original sample had been discarded. They, therefore could not be scanned by the other laboratory. Where possible, samples were scanned by both machines and variability in plant samples was optimised for both laboratories. Rather than restrict the data inputs all data are utilised, and sample numbers are presented for each calibration. The numbers therefore represent current capability for predictions using FOSS/SpectraStar or Bruker. There is a significant opportunity to pull these data together using alternative mathematical techniques.

Digestibility or energy

Accuracy of prediction varied with individual parameters (Tables 4.1A and 4.1B). There were some very promising results for broad calibrations for the digestibility (or the energy value) of forage. Excellent results were obtained for DOMD (PC) with SpectraStar (RPD 4.3) and Bruker (RPD 4.3). Both labs also obtained very good results for DMD (PC) with a RPD's of 3.8 and 3.7 (Tables 4.1A and 4.1B respectively, Figure 4.1). For DMD (PC), the error of prediction was just 2.7 or 3.1 units (%DM). These could be converted to ME using standard linear equations, so they will predict ME equally well. Most other digestibility predictions had fair or quality control screening potential, including DMD (TT, Bruker), OMD (PC, SpectraStar). Where the Bruker was giving poor or rough screening potential results, it tended to be associated with very low sample numbers (<100), so it may not be a good reflection of the potential. These traits included DMD (daisy), OMD (*in vivo*), OMD (daisy) and DOMD (daisy).

These calibrations offer an excellent prediction tool for the entire southern feedbase. The possibility that calibrations could be optimised for plant improvement programs if samples were restricted into 'like' taxonomic groups or a single species was tested. For DOMD (PC), separating did not improve the predictions with RPD values ranging from 3.0 to 4.0 (Tables 4.2 A and B).

The broad predictions, and even the taxonomically narrow ones generated in this project, were better than the examples found in the literature, with RPD's ranging from 1.3 to 2.5 (De Boever *et al* 1996; Olsoy *et al* 2016; Hetta *et al* 2017) (Appendix Table 8.1). Norman *et al*. (2010) achieved an RPD of 3.5 for Australian native shrubs.

The rumen fluid methods were generally predicted less successfully than the enzymatic methods, both in our project and in the literature. This is likely associated with higher laboratory errors or more complex spectral relationships associated with interactions of diverse rumen flora, as compared to chemically uniform enzymes. When comparing the performance of NIR calibrations based on *in vitro* methods compared to *in vivo* methods it is important to consider that NIR calibrations developed on *in vitro* techniques generally achieve good correlation coefficients (typically between 0.70 – 0.95, Kitessa *et al*. 1999), these *in vitro* techniques are only a prediction of *in vivo* digestibility. Error can also be compounded by the use of *in vivo* standards in the *in vitro* assays that do not match the samples being analysed (Soressa, 1999).

Ideally, *in vivo* digestibility would be predicted as this is a primary measurement and not associated with laboratory based errors. This has largely remained elusive in the literature with and RPD of 1.3 for Deaville *et al* (2009) and RPD of 2.2 for De Boever *et al* (1996) for animals consuming whole cereal crops or grass silages. Our best *in vivo* prediction was on the Bruker for DOMD with an RPD of 2.9 (fair screening potential) and error of prediction of 2.7 % units. A reasonable prediction of OMD (*in vivo*) was not achieved. As discussed earlier, when assessing the quality of these *in vivo* based calibrations it is important to reflect that wet chemistry *in vitro* techniques only give a prediction of *in vivo* digestibility and error can be compounded by the use of *in vivo* standards in the *in vitro* assays that do not match the samples analysed (Soressa, 1999). Given this, an RPD value of 2.9 represents good value as an alternative to predictions based on the *in vitro* methodology.

Table 4.1A. Calibration statistics for feed quality parameter (predicted from plant samples) from Bruker MPA (WWAI) machine.

	Parameter	n	SEC	R^2	SECV	R^2CV	**RPD**
Digestibility	DMD (*in vivo*)	80	2.64	0.89	3.47	0.78	**2.1**
	DMD (daisy)	97	3.9	0.80	4.76	0.67	**1.7**
	DMD (TT)	252	3.05	0.89	3.59	0.85	**2.6**
	DMD (PC)	2292	3	0.93	3.08	0.93	**3.8**
	OMD (*in vivo*)	80	2.86	0.85	3.62	0.72	**1.9**
	OMD (daisy)	97	2.33	0.95	5.36	0.72	**1.9**
	DOMD (*in vivo*)	97	1.69	0.98	3.83	0.88	**2.9**
	DOMD (daisy)	97	1.4	0.98	4.9	0.74	**2.0**
	DOMD (TT)	252	3.0	0.84	3.6	0.77	**2.1**
	DOMD (PC	2292	3.0	0.93	3.1	0.93	**3.8**
Ash	Organic matter	2128	1.35	0.88	1.55	0.83	**2.5**
Fat	Ether extract	70	0.32	0.95	0.403	0.92	**3.4**
Energy	Gross Energy	75	0.895	0.87	1.27	0.71	**1.9**
Carbohydrates	WSC	7055	1.88	0.92	1.94	0.92	**3.4**
	ESC	151	1	0.82	1.14	0.75	**2.0**
	Starch	151	1.92	0.98	2.41	0.96	**5.3**
Fibre	ADF	1961	2.29	0.92	2.39	0.91	**3.4**
	NDF	1961	3.77	0.94	3.91	0.93	**3.8**
	Lignin	525	1.2	0.78	1.37	0.69	**1.8**
INDFD	INDF240	121	2.79	0.94	3.24	0.91	**3.3**
	UNDF120	118	3.01	0.95	3.47	0.93	**3.9**
	UNDF30	120	3.07	0.95	3.59	0.93	**3.8**
	INDFom240	119	3.64	0.92	4.23	0.89	**3.0**
	UNDFom120	121	4.05	0.92	4.82	0.87	**2.8**
	UNDFom30	119	3.7	0.93	4.29	0.90	**3.2**
Protein	Nitrogen	7055	0.18	0.98	0.18	0.98	**7.1**
fractions	Crude protein	7055	1.1	0.98	1.13	0.98	**7.1**
	NPN	224	0.308	0.90	0.368	0.86	**2.6**
	NDICP	226	0.192	0.84	0.22	0.79	**2.2**
	ADICP	190	0.072	0.87	0.095	0.75	**2.0**
	RDP	202	0.413	0.94	0.436	0.92	**3.7**
Anions	Nitrate	168	1970	0.73	2890	0.36	**1.3**
VFA wet	acetic	68	0.92	0.87	1.78	0.42	**1.3**
scanned	propanoic	68	0.354	0.19	0.365	0.11	**1.1**
	iso-butyric	68	0.1	0.05	0.102	0.03	**1.0**
	butyric	68	0.1	0.05	0.102	0.03	**1.0**
	iso-valeric	68	0.064	0.47	0.0726	0.25	**1.2**
	valeric	68	0.136	0.49	0.149	0.32	**1.2**
	hexanoic	68	0.028	0.97	0.136	0.24	**1.1**
	heptanoic	68	0.017	0.09	0.0173	0.01	**1.0**
VFA dry	acetic	68	5.32	0.59	6.29	0.34	**1.2**
scanned	propanoic	51	0.403	0.78	0.818	0.04	**1.0**
	iso-butyric	51	0.18	0.35	0.211	0.06	**1.0**
	butyric	51	1.57	0.78	2.35	0.38	**1.3**
	iso-valeric	51	0.272	0.04	0.281	0.08	**1.0**
	valeric	51	0.224	0.81	0.383	0.34	**1.2**
	hexanoic	51	0.878	0.05	0.92	0.10	**1.0**
	heptanoic	51	0.077	0.03	0.078	0.04	**1.0**
Silage	ammonia	517	2.93	0.63	3.24	0.54	**1.5**
	pH	517	0.508	0.70	0.556	0.63	**1.7**

TT- Tilley and Terry, INDFD-Indigestible neutral detergent fibre dry matter digestibility, INDFO-Indigestible neutral detergent fibre organic matter digestibility, WSC-Water soluble carbohydrates, ESC-Ethanol soluble carbohydrates, NPN-Non-protein nitrogen, RDP-Rumen degraded protein, NDICP-Neutral detergent insoluble crude protein, ADINCP-Acid detergent insoluble crude protein, ADF-Acid detergent fibre, NDF-Neutral detergent fibre. Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 have rough screening potential, 2.5 to 2.9 have fair screening potential, 3.0 to 3.4 are quality control, 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

Table 4.1B. Calibration statistics for feed quality parameter (predicted from plant samples) from SpectraStar (CSIRO) machine

	Parameter	n	SEC	R^2	SECV	R^2CV	RPD
Digestibility	DMD (PC)	536	2.5	0.94	2.7	0.93	**3.7**
	OMD (PC)	329	5.2	0.86	5.2	0.86	**2.6**
	DOMD (PC)	332	4.7	0.97	6.4	0.98	**4.3**
Ash	Organic matter	1294	1.7	0.89	2.5	0.87	**2.7**
Fibre	ADF	1425	1.8	0.96	1.9	0.95	**4.5**
	NDF	1450	3.3	0.95	3.4	0.94	**4.0**
Protein fractions	Nitrogen	537	0.16	0.97	0.18	0.96	**5.3**
	Crude protein	537	1.0	0.97	1.13	0.96	**5.3**
Anions	Nitrate	613	5.6	0.92	7.4	0.88	**2.8**
	Oxalate	999	7.5	0.90	8.9	0.87	**2.8**
	Malate	263	1.4	0.12	2.1	0.07	**1.0**
	Phosphate	947	1.5	0.86	1.7	0.81	**2.3**
	Sulphate	964	1.8	0.41	1.9	0.38	**1.3**
CH4 (batch culture)		133	3.2	0.88	4.8	0.70	**1.8**

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

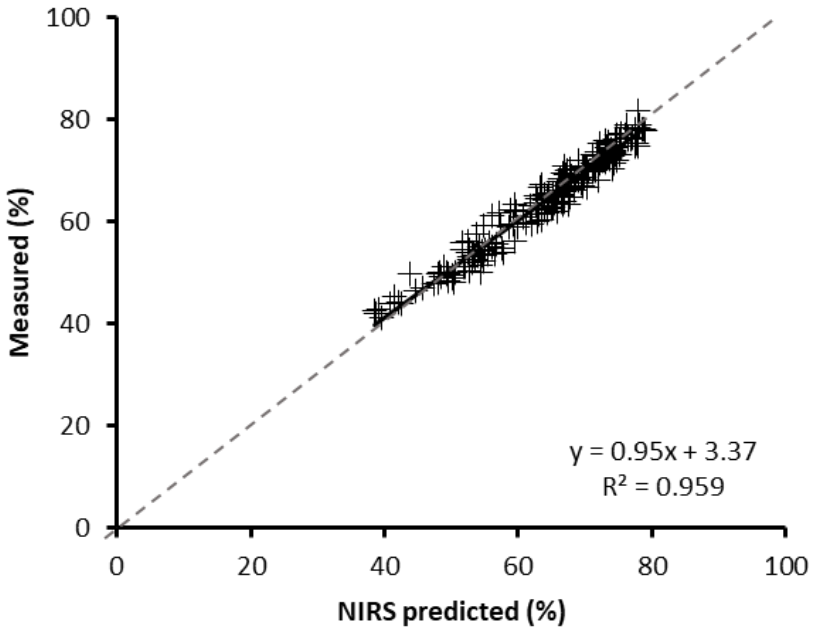

Figure 4.1. Predicted vs measured DMD (pepsin-cellulase)

Table 4.2 A. Calibrations for standard traits, based on predictions developed for specific forage types, developed on the Bruker machine.

Types/Codes	Measure	n	SEC	R^2	SECV	R^2CV	RPD
Cereals	ADF	640	2.0	0.92	2.1	0.91	**3.4**
(Ce FHS)	Ash	564	1.2	0.92	1.3	0.91	**3.3**
	CP	782	1.2	0.98	1.2	0.98	**7.2**
	DMD	575	2.6	0.95	2.8	0.93	**3.8**
	DOMD	579	2.6	0.93	2.7	0.92	**3.6**
	NDF	632	3.3	0.88	3.5	0.87	**2.8**
	WSC	721	2.0	0.96	2.2	0.95	**4.5**
Cereal oats	ADF	313	2.3	0.79	2.5	0.76	**2.0**
(CeO FHS)	Ash	284	1.1	0.87	1.3	0.80	**2.2**
	CP	275	0.8	0.99	0.9	0.98	**7.2**
	DMD	284	2.3	0.91	2.6	0.88	**2.8**
	DOMD	277	1.6	0.93	1.9	0.89	**3.0**
	NDF	302	2.3	0.88	2.4	0.87	**2.8**
	WSC	274	2.3	0.94	2.5	0.93	**3.8**
Lucerne	ADF	141	2.0	0.94	2.5	0.90	**3.2**
(FLLu FHS)	Ash	141	1.2	0.82	1.4	0.77	**2.1**
	CP	253	0.8	0.99	0.9	0.98	**6.8**
	DMD	144	2.6	0.95	3.4	0.90	**3.2**
	DOMD	144	2.6	0.92	2.9	0.90	**3.2**
	NDF	136	3.3	0.91	3.6	0.88	**2.9**
	WSC	263	1.1	0.89	1.2	0.86	**2.7**
Temperate legumes	ADF	211	1.2	0.97	1.4	0.94	**4.2**
(FLT)	Ash	212	0.7	0.93	1.1	0.82	**2.4**
	CP	198	0.6	0.99	0.7	0.98	**8.0**
	DMD	211	2.1	0.93	2.4	0.90	**3.1**
	DOMD	212	1.7	0.93	2.1	0.89	**3.0**
	NDF	214	2.8	0.90	3.2	0.86	**2.7**
	WSC	130	1.4	0.78	1.7	0.69	**1.8**
Tropical legumes	ADF	143	1.2	0.95	1.4	0.92	**3.6**
(FLTr)	Ash	142	0.5	0.94	0.7	0.87	**2.7**
	CP	140	0.8	0.98	1.1	0.96	**5.0**
	DMD	139	2.3	0.94	2.7	0.91	**3.4**
	DOMD	143	1.5	0.96	2.3	0.91	**3.3**
	NDF	142	2.5	0.86	3.0	0.80	**2.2**
	WSC	160	0.8	0.90	0.9	0.84	**2.5**

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent. Codes are listed in the appendix.

Table 4.2 B. Calibrations for standard traits, based on predictions developed for specific forage types, developed on the Bruker machine.

Codes/types	Measure	n	SEC	R^2	SECV	R^2CV	RPD
Native grasses	ADF	86	2.2	0.96	2.5	0.94	**3.9**
(FN)	Ash	95	1.9	0.82	2.6	0.67	**1.7**
	CP	100	0.6	0.99	0.8	0.99	**8.5**
	DMD	93	1.4	0.97	2.1	0.94	**4.1**
	DOMD	95	1.7	0.95	2.2	0.92	**3.5**
	NDF	84	2.5	0.97	3.1	0.96	**4.8**
	WSC	167	1.1	0.91	1.2	0.90	**3.1**
Sorghum	ADF	81	1.1	0.93	1.3	0.90	**3.2**
(FS)	Ash	87	0.6	0.94	0.8	0.88	**2.9**
	CP	102	0.7	0.98	0.8	0.98	**6.3**
	DMD	84	1.1	0.96	1.8	0.89	**3.0**
	DOMD	84	0.7	0.98	1.1	0.94	**4.0**
	NDF	84	1.4	0.93	1.9	0.85	**2.6**
	WSC	112	1.0	0.97	1.3	0.95	**4.3**
Temperate grasses	ADF	95	1.1	0.96	1.4	0.94	**4.0**
(FTG FHS)	Ash	106	1.1	0.94	1.8	0.85	**2.5**
	CP	220	0.7	0.98	0.9	0.97	**5.7**
	DMD	109	3.6	0.91	4.1	0.87	**2.8**
	DOMD	108	3.0	0.91	3.3	0.89	**3.0**
	NDF	92	2.7	0.90	3.3	0.83	**2.2**
	WSC	310	0.7	0.98	0.8	0.97	**6.0**
Tropical grasses	ADF	306	1.1	0.96	1.2	0.96	**4.8**
(FTrG)	Ash	435	0.8	0.83	0.9	0.80	**2.1**
	CP	700	0.8	0.99	0.8	0.99	**8.1**
	DMD	442	2.4	0.91	2.7	0.89	**3.1**
	DOMD	441	2.2	0.90	2.3	0.89	**3.0**
	NDF	303	2.1	0.92	2.3	0.91	**3.3**
	WSC	461	0.7	0.91	0.8	0.89	**3.0**

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent. Codes are listed in the appendix.

Organic matter or ash

Organic matter or ash content is a valuable component in determining NV as inorganic material cannot contribute to dietary energy, a major constraint to production. High ash can also indicate soil contamination in the sample or a low cutting height during harvest. Accuracy of prediction calibrations are presented in Tables 4.1A and 4.1B. Organic matter or ash was predicted with fair screening potential with and RPD of 2.5 to 2.7 and errors of prediction of 1.5 to 2.5 % units. These results compare favourably with other results found within the literature (eg Windham, 1991).

Volatile fatty acids

Volatile fatty acids include acetic, propionic, isobutyric, butyric, isovaleric, valeric, hexanoic and heptanoic acids. VFAs provide an important source of energy to ruminants consuming silage, and the relative proportion gives information regarding the quality of the fermentation process (Kaiser, 2004). VFAs also provide an additional challenge in that they are volatile and samples must be analysed fresh. NSW DPI scanned a diverse series of silages using a 90mm cell on the Bruker MPA,

and then proceeded to analyse the samples for VFA content. Calibration models developed were of poor quality with RPD values ranging from 1.0-1.3. These results were poorer than findings of other workers (Park, 1998; Sinnaeve, 1994).

Silage Ammonia and pH

Silage ammonia and pH are routinely used as measures to indicate the quality of the silage fermentation (Kaiser, 2004). A series of 517 silage samples derived from forages representing tropical and temperate grasses and crops were scanned using a 90mm cell on the Bruker MPA NIRS instrument, and calibrations developed. These calibrations gave R^2 values between 0.6-0.7 and RPD values at the upper end of the poor quality range (1.5-1.7). These results were disappointing compared to some of the other values given in the literature (eg Park, 1998) with an R^2 value of 0.81 for pH and 0.92 for ammonia.

Fibre fractions

Accuracy of prediction calibrations are presented in Tables 4.1A and 4.1B. RPD values for ADF ranged from 3.4 (Bruker) to 4.5 (SpectraStar) with errors of prediction of just 2.4 and 1.9 % units. These are comparable with the ADF from the literature search where six studies had a mean RPD of 3.6 and a range of 2.8 to 5.8 (Appendix Table 8.2. Galili *et al* 2018; Norman *et al* 2010; Henry *et al* 2000; Rothman *et al* 2009; Alomar *et al* 2009; Zicarelli *et al* 2022).

RPD values for NDF ranged from 3.8 (Bruker) to 4.0 (SpectraStar) with errors of prediction of just 3.9 and 3.4 % units. The mean NDF from the 16 reported values in the literature search was 3.6 with a range of 1.8 (poor) to 7.1 (excellent) (Appendix Table 8.1.2. Myer *et al* 2011; Hsu *et al* 2000; Henry *et al* 2000; Norman *et al*. 2010; Galili *et al*. 2018; Hetta *et al* 2017; Hsu *et al* 2000; Stubbs *et al* 2010; Rothman *et al* 2009; Deaville *et al* 2009; Lobos *et al* 2019; Alomar *et al* 2009; Zicarelli *et al* 2022).

When the calibrations were developed on a narrow taxonomic group (Table 4.2 A and B), in 6 of the 10 cases, the ADF prediction was improved, when compared to the global calibration from the Bruker. In contrast, narrowing the taxonomic range generally did not improve NDF prediction in 8 of the 10 cases (Table 4.2 A and B), when compared to the global calibration from the Bruker.

For the majority of the fibre fractions, quality control to very good calibrations were achieved with the Bruker (not tested using the SpectraStar). One of the focus areas of nutritionists using CNCPS oriented testing has been on the degradability of carbohydrate fractions, and the impact this has on intake especially in high performance animal production systems such as dairy.

Indigestible NDF (INDF) represents that portion of the NDF fraction that is not digested after 240hrs of incubation in rumen fluid, whereas undegraded NDF at a time point of 30hrs or 120hrs represents that fraction of NDF which is retained after incubation in rumen fluid for that corresponding exposure time. NDF degradability with rumen fluid at a number of time points and resulting calculated passage rates are integral to calculating these impacts on intake.

Calibrations for iNDF and iNDFom fractions for a diverse set of standards (representing 32 groups and 72 subgroups) produced very good results with RPD values between 2.8-3.9 (Table 4.1A), indicating that these calibrations should be applicable for routine use. These values also compare favourably with other recently published results obtained by other researchers (Refat and Yu, 2022; Zang *et al* 2021). The corresponding graphs (figures 4.2, 4.3, 4.4) for the 3 timepoints, 30hrs, 120hrs and 240hrs respectively are shown below and show very strong prediction relationships.

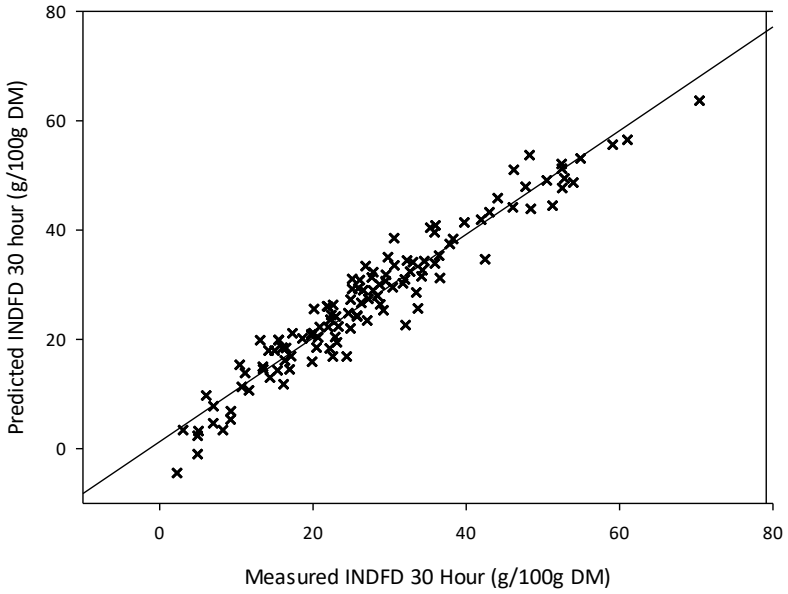

Figure 4.2. Undigested neutral detergent fibre (UNDF-30), 30 hour g/100g DM, y = 0.953x + 1.282, R^2 = 0.953

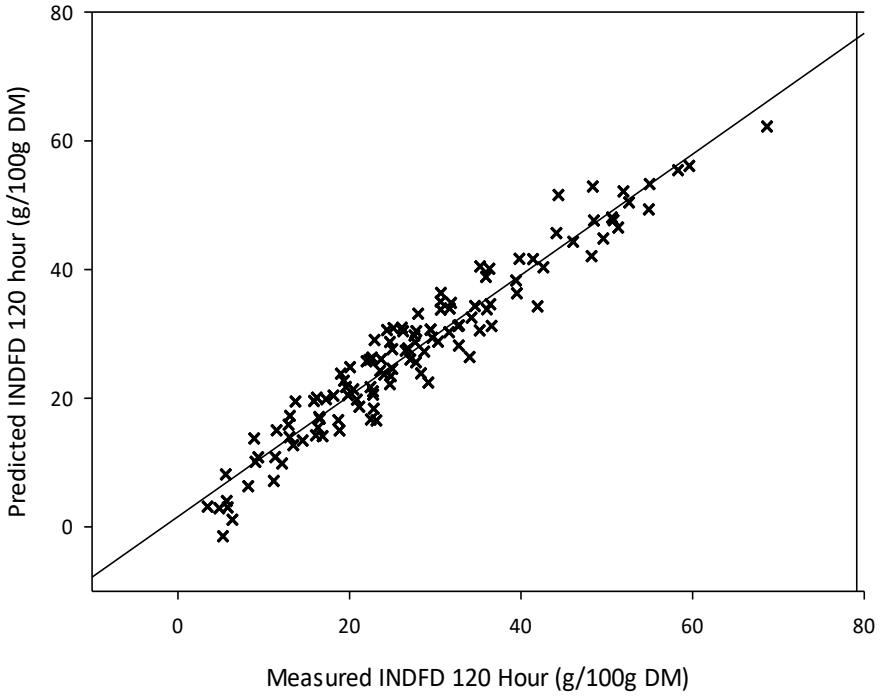

Figure 4.3. Undigested neutral detergent fibre (UNDF-120), 120 hour g/100g DM, y = 0.954x + 1.255
R^2 = 0.954

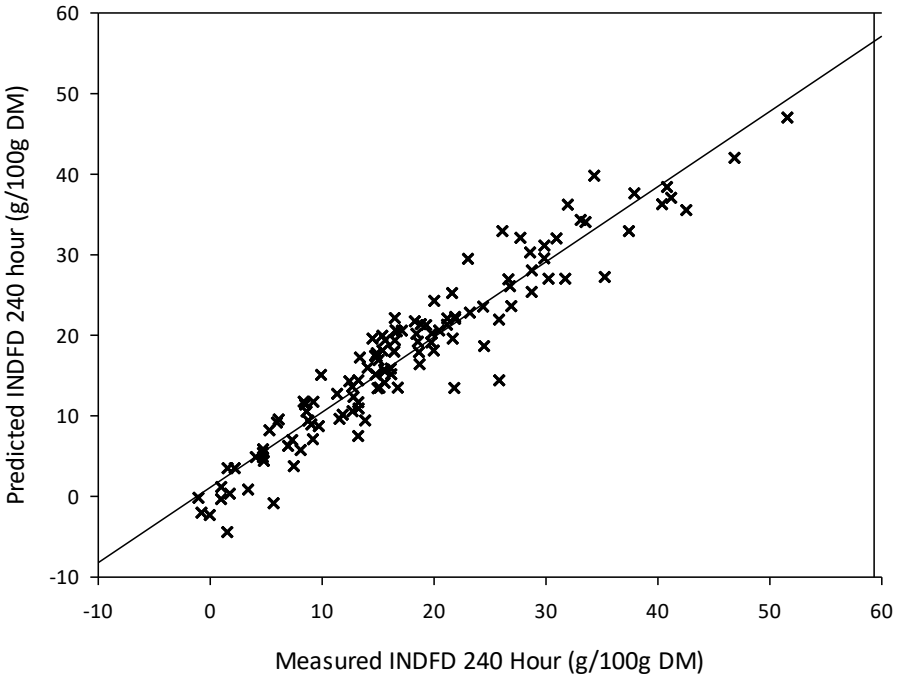

Figure 4.4. Indigestible neutral detergent fibre (INDF), 240 hour g/100g DM, y = 0.937x + 1.104 R^2 = 0.937

Fats and carbohydrates

Accuracy of prediction calibrations are presented in Table 4.1A (Bruker), these traits were not predicted using the SpectraStar. Lipids (crude fat) represent an energy rich fraction of forages, and for most species, are within the range of 1-4%DM. Some forages such as vegetative ryegrass can have lipid contents in the range of 6% or even higher. It is useful to measure the lipid content to determine limits for supplements that can be high in fat – such as canola meal. Crude fat was predicted with a quality control RPD of 3.4, which compares well with published values (Ullmann *et al*. 2017). Gross energy has been shown to be correlated with metabolisable energy and often sought after to use in predictive equations for ME or TDN. Unfortunately, GE was shown to be poorly predicted (RPD 1.9). This may be due to the relatively low range of the analyte (16.5-19.5MJ/kg).

Water soluble carbohydrates represent low molecular weight sugars such as sucrose, fructose and glucose as well as fructans and oligiosaccarides. Ethanol soluble carbohydrates contains only the monosaccharides and disaccharides. Both these fractions are important because they rapidly supply energy to meet the needs of rumen flora and allow production. WSC was predicted with a quality control RPD of 3.4, whereas the ESC calibration gave a disappointing RPD of 2.0. Starch was predicted with an excellent RPD of 5.3.

When the calibrations were developed on a narrow taxonomic group (Table 4.2A and 4.2B), in just 3 of the 9 cases, the WSC prediction was improved, when compared to the global calibration from the Bruker.

Crude protein, protein fractions and protein degradability

Nitrogen or crude protein remains the easiest trait to predict accurately (Tables 4.1A and 4.1B). Both machines gave excellent results with their different calibration datasets, with an RPD of 7.1 (and an error of prediction of 1.1 % units of CP) for the Bruker and an RPD of 5.3 (and an error of prediction of 1.1 % units of CP) for the SpectraStar. The literature search identified 22 papers where calibrations were developed for total N or CP (Appendix Table 8.1.3). Of these, half achieved excellent RPD values, ranging from 4.1 to 7.1. Our broad calibrations are very sound.

When the calibrations were developed on a narrow taxonomic group (Table 4.2 A and B), in 70% of cases, the RPD for CP could be improved when compared to the global calibration. The best prediction was for native grasses with an RPD of 8.5 and error of prediction of 0.8 % units of CP. Given the accuracy of the broad, global calibrations, you would only narrow the taxonomic range of you were testing N treatments or trying to select for marginally higher CP content.

Protein fractions represent a group of components that classify the protein in terms of decreasing rate of digestibility. They are an important set of data to determine amino acid availability for livestock and to push production levels. Protein fractions were less easy to predict than total N (or CP). Fair screening potential for NPN (RPD 2.6) was achieved. Very good potential for RDP (RPD 3.7) and some promise for NDICP and ADICP (RPD 2.2,2.0 respectively) were also achieved with the Bruker instrument. The range of samples used to develop this set of calibrations again was intentionally diverse with 150 samples representing a range of hays, silages, temperate and tropical pastures. These values reflect a better performance than quoted in the literature by Hoffman (1999) using a range of forages but are not of the same quality of calibrations representing narrower sets of samples such as Nie (2008) who created calibrations based on 230 alfalfa samples.

Anions

Accuracy of prediction calibrations are presented in Tables 4.1A and 4.1B. One lab was able to predict nitrate with fair screening potential (RPD of 2.8), while the other only had a poor prediction potential. While NSW DPI was able to generate a rough screening calibration for canola forage, models with a wider range of samples were unable to predict nitrate with any accuracy. Oxalate was also predicted by the SpectraStar with fair screening potential (RPD of 2.8). There is some promise for the development of a phosphate prediction (RPD 2.3) however little evidence was found for the ability to predict malate or sulphate.

Calibrations with predictive power for nitrate and oxalate in forages is novel, and there is little mention of successful work carried out in the literature by other groups. These new calibrations developed by CSIRO are a new development. Nitrate would be a useful tool used in addition to other nutritional analyses, to flag potentially toxic fodders during drought. For example, a review of nitrate analysis in a range of recovered crop forages, hays and silages during a recent drought in NSW during the summer of 2019, Meyer (2021) showed that up to 30% of recovered canola crops that were either grazed, or preserved as hay or silage had nitrate levels that were potentially toxic, while some excessive levels were recovered in millet and sorghum samples and lower rates detected amongst recovered cereal crops received in the same period. Oxalate is used by plants for osmo-regulation and binds calcium. There are few labs in Australia that measure oxalate so an NIRS calibration would be useful for the ruminant and horse industries.

Methane from Batch culture methods

The accuracy of prediction calibrations is presented in Table 4.1B and Fig 4.5. The preliminary calibration achieved an R^2= 0.88 on a dataset of 133 samples (it dropped on validation), although the RPD was only 1.8, with poor screening potential. This could result from many of the limitations associated with this method of testing such as diet, inoculum collection and processing, substrate and incubation buffers and procedures (Yáñez-Ruiz *et al*. 2016). Batch culture methods also require access to rumen cannulated animals. Ideally, an *in vivo* prediction of methane would be preferred.

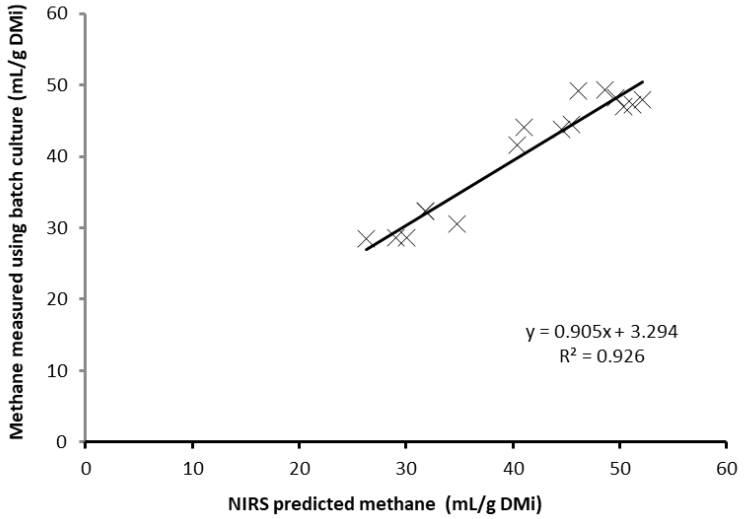

Fig 4.5. Measured and predicted (validation data) methane from batch culture with an RPD of 1.8 (from the ELLE project data, Norman *et al* 2020).

Opportunities to create group-specific calibrations

The project successfully developed broad, robust calibrations for the entire Australian feedbase, in some cases there are opportunities to improve the calibration for particular groups of species for particular traits (Table 4.2A and 4.2B). This could be useful in plant breeding or improvement programmes. Norman *et al* (2020) found that broad calibrations predicted the nutritional traits of annual grasses, annual legumes and forb species with greater accuracy than for perennial grasses or legumes. This could be associated with accuracy of the wet chemistry methods. As a general rule, Norman *et al* (2020) found that separating taxonomically similar species into groups before the development of calibrations, did not lead to more accurate predictions. If more spatial and temporal diversity can be built in without a large reduction in accuracy, these broad NIRS calibrations represent a valuable tool for Australian researchers, feed testing agents and livestock producers, as they encompass nearly all of the species that appear in monocultures or mixed swards. In a cloud-based delivery approach, different statistical methods will negate this issue as the samples will be predicted using 'like' spectra and taxonomic classifications will be redundant.

4.2 Validate NIR for predicting prussic acid and mineral parameters from forage.

A satisfactory calibration for prussic acid was not achieved. The prussic acid calibration that was initially developed gave a good model, unfortunately this translated into poor cross validation statistics. There were only 50 samples in our test group, so more work is required before a definitive recommendation is possible. There is evidence in the literature where groups have been able to successfully predict prussic acid concentration in sorghum forage (Goff, 2011). It is not clear whether the poor calibration statistics are a result of a methodology difference in the wet chemistry method, or some other factor within the preparation or analysis which could explain the poor calibration statistics.

Excellent calibrations were developed for magnesium and calcium (RPD 6.2 and 5.0) using the SpectraStar. The Bruker also predicted calcium very well (RPD 3.1). Interestingly, magnesium models predicted using a diverse sample set (1091) in one laboratory gave lower quality predictions than the other laboratory (RPD 1.7 vs 6.2). This warrants further investigation and is perhaps related to the diversity in the sample set. Other minerals that could be predicted included chloride (fair screening potential) and phosphorus, sulphur, sodium and potassium (rough screening potential).

Commercial NIRS labs offer predictions of calcium, magnesium, phosphorus, sodium, potassium, sulphur and chloride (and DCAD which is calculated form these minerals). Of these minerals, predictions had a rough screening potential for phosphorus, sulphur, sodium and potassium. These results compare favourably with the early literature (Jones 1987, Marten 1989) as well as some more recent studies. Ikoyi (2020) and Halgerson (2004) were able to achieve poor to fair calibration statistics that were of rough screening value for macro-minerals.

The project did not have enough samples with a full suite of micro-mineral analyses to develop calibrations, however, in a later section of this report (Table 4.8) it was found that dietary concentrations of some minerals could be predicted from faecal samples. Minerals (in the diet) that could be predicted with excellent results (from faeces) included chloride, sodium and magnesium (Table 4.8). Calibrations of a quality control standard were achieved for calcium, iron, potassium, sulphur and zinc. Other minerals that had potential to be predicted included copper and boron. Phosphorus and manganese may be possible, but more data are required. This suggests that there is potential to understand mineral nutrition from the animal output rather than the plant input. This has the advantage of accounting for diet selection in extensive systems. It also indicates that the opportunity to predict iron, zinc, copper and boron should be explored further. As the Australian red meat industry adjusts to a future of hotter and drier climates, there will be increasing industry interest in prediction of minerals associated with antioxidant pathways in livestock – predominantly copper, zinc, selenium, manganese and sulphur (Masters 2019). There is evidence in the literature that copper and zinc can be predicted in plant samples (Ouyang *et al*, 2015).

Table 4.3. Calibration statistics for mineral parameters, predicted from plant samples.

	WWAI						CSIRO					
Parameter	**n**	**SEC**	**R^2**	**SECV**	**R^2CV**	**RPD**	**n**	**SEC**	**R^2**	**SECV**	**R^2CV**	**RPD**
Chloride (%DM)[1]	606	0.17	0.90	0.19	0.87	**2.8**						
Chloride (%DM)[2]	310	0.20	0.90	0.24	0.85	**2.6**	568	11.5	0.87	12.30	0.85	**2.8**
Magnesium (%DM)[2]	1091	0.07	0.69	0.08	0.66	**1.7**	197	0.06	0.97	0.09	0.91	**6.2**
Phosphorus (%DM)[1]	1337	0.05	0.83	0.05	0.82	**2.3**						
Phosphorus (%DM)[2]	1041	0.05	0.83	0.05	0.81	**2.3**	201	0.03	0.81	0.03	0.70	**2.3**
Calcium (%DM)[1]	1367	0.16	0.89	0.17	0.88	**2.9**						
Calcium (%DM)[2]	1070	0.15	0.91	0.17	0.90	**3.1**	198	0.05	0.96	0.06	0.92	**5.0**
Potassium (%DM)[1]	1369	0.44	0.79	0.45	0.78	**2.1**						
Potassium (%DM)[2]							190	0.5	0.42	0.60	0.21	**1.3**
Sulphur (%DM)[1]	1360	0.06	0.75	0.06	0.73	**1.9**						
Sulphur (%DM)[2]	1045	0.06	0.78	0.06	0.77	**2.1**	193	0.04	0.79	0.05	0.61	**2.2**
Sodium (%DM)[1]	1281	0.15	0.53	0.16	0.49	**1.4**						
Sodium (%DM)[2]	617	0.12	0.63	0.13	0.57	**1.5**	187	0.69	0.83	0.77	0.76	**2.4**
Prussic acid	50	108	0.87	245	0.18	**1.1**						

Source of analyses; [1] DPI + external provider, [2] DPI only (NSW) or CSBP only (WA). Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

4.3 Test viability of Minson *in vivo* standards

Minson samples subject to uncontrolled storage and controlled storage were analysed for a range of wet chemistry proximate analysis in addition to invitro pepsin cellulase analysis. These analyses were not exhaustive but were chosen to help assess if any likely degradation had occurred in the uncontrolled storage conditions. Sample pairs were analysed in duplicate, within the same batch to eliminate batch effects, in addition to the normal QC standards that were included in the runs. Tests included;

1. Nitrogen by DUMAS (LECO FP-2000) (CP)-(AOAC Crude Protein 2011.11)
2. Neutral Detergent Fibre (NDF) - (+amylase and sodium sulphite, AFIA Method – 1.8A(a))
3. Acid Detergent Fibre (ADF)- (AFIA Method – 1.9A(a))
4. Pepsin cellulase digestibility (DMD, DOMD, ASH, OM) – (AFIA Method – 1.7R)
5. Water Soluble Carbohydrates (WSC)

All wet chemistry parameters studied showed no significant difference between the controlled and uncontrolled storage samples at the $P < 0.01$ significance level. NDF, ADF, CP and WSC also had no significant bias associated with sample source. There was a small bias (0.5%) noted for pepsin cellulase DOMD, with the controlled storage samples showing a slightly greater DOMD value than the uncontrolled storage samples. DOMD results were not significantly different at the $P < 0.01$ significance level. Based on this data, storage has had no significant effect on the nutritional composition as measured by wet chemistry methods.

The samples were then analysed using some generic calibrations, for each of the analytes. The NIR predicted results are arbitrary in a sense, as the object of this exercise was to determine if any spectral changes within the samples due to storage conditions would have an effect on the NIR reflectance spectra, and if so, would this be in a region of the spectra that would be likely to affect the prediction of particular components of interest.

Predicted NDF, CP and WSC showed no significant difference between the controlled storage and uncontrolled storage samples at the $p < 0.01$ significance level. Predicted DOMD gave some interesting results with no significant difference observed for the T&T INVT DOMD, however a significant difference was observed for pepsin-cellulase DOMD with a bias of 1.6% in favour of the controlled storage samples compared to the uncontrolled storage samples. ADF also had a significant bias of 1.1% associated with sample source in favour of the uncontrolled storage sample set.

A review of the 95% Expanded Uncertainty for the wet chemistry method vs the bias results is given in the table below (Table 4.4). Bias values are all less than the corresponding reported wet chemistry 95% Uc. A subsequent follow up experiment creating a PC-DOMD Calibration based on CSIRO data and using the uncontrolled storage data as an independent test set found no significant bias or slope associated with the uncontrolled storage derived NIR predictions. It was concluded that the uncontrolled storage in vivo standards were suitable for use in the calibration sets to be used for NIR development. This nearly tripled the number of in vivo standards that were available and suited to the project, with 178 additional standards being used after uncontrolled storage.

Table 4.4. A review of the 95% Expanded Uncertainty for the wet chemistry method vs the bias for the Minson samples that were stored in controlled and uncontrolled conditions.

Analysis method	Parameter	Unit	95% Uc	NIR BIAS
Crude Protein (forage/silage) by DUMAS	CP	%, g/100g DM	1.6	0.6
Dry Organic Matter Digestibility - Wet chemistry; AFIA Method 1.7R	DOMD	%, g/100g DM	1.8	1.6
Neutral Detergent Fibre (NDF) in Plant Material	NDF	%, g/100g DM	3.0	1.3
Acid Detergent Fibre (ADF) in Plant Material	ADF	%, g/100g DM	2.4	1.1

4.4 Test the ability of NIRS to accurately predict digestibility and animal efficiency parameters from forages

In vivo samples sourced from multiple sources (Table 3.1) have been used to create robust calibrations for digestibility (DMD/OMD/DOMD) at maintenance and *ad lib* feeding levels. Feed intake calibrations were also attempted for daily dry matter intake (DDMI), daily organic matter intake (DOMI), digestible dry matter intake (DMI), digestible organic matter intake (OMI) and these were developed on a g/hd and g/kg liveweight basis.

Calibration models were constructed for sheep only and cattle only as well as combined models for sheep and cattle. All of the models produced for digestibility predictions gave fair to good results (Table 4.5A and 4.5B, Figure 4.6), with lower quality calibration statistics given for the intake models produced. Some of the digestibility models had a relatively low number for the calibration and validation sets – e.g. cattle-adlib DMD-OMD-DOMD (30 samples) which tended to give high calibration R^2 and lower validation R^2 indicating that more samples would likely be required to produce calibrations able to service a wider range of samples.

Calibrations which used combined sheep and cattle data produced robust calibrations for digestibility measures, and also quite good calibrations for intake in terms of dry matter and digestible dry matter on a live weight basis. The combined maintenance DOMD calibration used 97 standards and 14 test spectra, with a range of 53.9-80.4% and used a 2^{nd} derivative data pre-treatment. The combined adlib DOMD calibration used 113 standards and 27 test spectra with a range of 44.0-71.7% and used a 1st derivative data pre-treatment. The Standard Error of Prediction (SEP) for both DOMD-maintenance (3.8%) and DOMD-Adlib (2.0%) were comparable to SEP values obtained for invitro methods, and other calibration statistics such as R^2 were also satisfactory. These calibrations were then tested on a range of forages collected over a year at the NSW DPI Feed Quality Service Laboratory to check the incidence of spectral outliers. These included samples of legume, pasture and cereal hay and silages. Of the 5622 samples scanned, the spectral outlier rate was low (DOMD-Maintenance = 0.1%, DOMD-Adlib 2.4%), indicating the calibrations could be used on a wide range of samples, and have the potential for commercial or research use.

The intake models developed gave calibration statistics that seem to be at the lower end of performance statistics (Table 4.5A and 4.5B, Figure 4.7 and 4.8), but when considered against other available data and methods of intake estimation, especially for pastures, they actually perform quite well. Digestible dry matter intake(%DDMI) models generally outperformed Dry matter intake (%DMI) models for sheep only, cattle only, and in the mixed models. For example, for sheep (n=258) the r^2 for validation for %DDMI was 0.79 vs 0.64 obtained for the %DMI model. For the mixed model (n=312) the r^2 for validation for %DDMI was 0.73 vs 0.57 obtained for the %DMI model, and the RPD is 1.9, giving an SEP of 2 g/kg LWT/day.

Predicting dietary intake is a difficult task for livestock. A review by Gunter (2016) outlining some of the factors that contribute to the lack of accuracy of various empirical models that have been developed concluded that the models generally account for 50 to 70% of the variation, and often have high standard errors of prediction of 5% (of the mean) or greater. Given this context, a mixed model that has an r^2 of 0.73 and an SEP of 2.0 in a set with a range (4.5-22.9) is considered at the upper end of prediction models available. Amongst the variables she lists which can account for deviation from predicted intake are selective feeding, environmental factors such as temperature, sward density, pre and post digestive factors, landscape effects such as topography, social factors such as previous experience on a feed or pasture.

Table 4.5.A. Calibration statistics for prediction of livestock parameters from *in vivo* forage diet samples using the Bruker machine. Predictions included *in vivo* dry matter digestibility (DMD), organic matter digestibility (OMD), dry matter intake (DMI) and digestible dry matter intake (DDMI).

Model	Feeding Level	Component	Parameter	Units	n	SEC	R^2	SECV	R^2CV	**RPD**
Sheep	*ad lib*	Digestibility	DMD	g/100g DM	274	2.4	0.88	2.6	0.85	**2.6**
			DOMD	g/100g DM	274	2.2	0.85	2.4	0.81	**2.3**
			OMD	g/100g DM	274	1.7	0.94	2.3	0.89	**3.0**
		Intake	DMI	kg/hd/day	281	0.1	0.64	0.1	0.58	**1.6**
			DDMI	kg/hd/day	414	0.1	0.78	0.1	0.75	**2.0**
			DMI-LW	g/kg LWT	258	2.7	0.70	2.9	0.64	**1.7**
			DDMI-LW	g/kg LWT	258	1.6	0.78	1.8	0.79	**1.9**
Sheep	Maint.	Digestibility	DMD	g/100g DM	66	2.0	0.94	3.0	0.84	**2.5**
			DOMD	g/100g DM	66	1.7	0.96	2.7	0.87	**2.8**
			OMD	g/100g DM	66	1.0	0.99	2.8	0.88	**2.9**
Cattle	*ad lib*	Digestibility	DMD	g/100g DM	30	1.4	0.92	1.9	0.78	**2.2**
			DOMD	g/100g DM	30	0.6	0.99	2.4	0.73	**1.9**
			OMD	g/100g DM	30	0.7	0.98	2.2	0.72	**1.9**
		Intake	DMI	kg/hd/day	46	0.8	0.81	1.1	0.63	**1.6**
			DDMI	kg/hd/day	46	0.6	0.82	0.8	0.73	**1.9**
			DMI-LW	g/kg LWT	46	1.4	0.91	3.0	0.46	**1.4**
			DDMI-LW	g/kg LWT	46	1.2	0.80	2.1	0.23	**1.1**
Cattle	Maint.	Digestibility	DMD	g/100g DM	57	1.0	0.83	1.8	0.90	**3.2**
			DOMD	g/100g DM	57	0.7	0.99	1.8	0.91	**3.3**
			OMD	g/100g DM	57	1.0	0.99	1.8	0.90	**3.2**
Mixed	*ad lib*	Digestibility	DMD	g/100g DM	286	2.6	0.85	2.7	0.83	**2.4**
			DOMD	g/100g DM	145	1.8	0.94	2.0	0.92	**3.4**
			OMD	g/100g DM	147	1.7	0.95	2.2	0.92	**3.5**
		Intake	DMI-LW	g/kg LWT	288	2.6	0.68	3.0	0.57	**1.5**
			DDMI-LW	g/kg LWT	312	1.8	0.78	2.0	0.73	**1.9**
Mixed	Maint.	Digestibility	DMD	g/100g DM	80	2.6	0.89	3.5	0.78	**2.1**
			DOMD	g/100g DM	111	1.7	0.98	3.8	0.88	**2.9**
			OMD	g/100g DM	80	2.9	0.88	3.6	0.72	**1.9**

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

Table 4.5.B. Calibration statistics for prediction of livestock parameters from forage diet samples using the SpectraStar machine. Predictions included *in vivo* dry matter digestibility (DMD), organic matter digestibility (OMD), dry matter intake (DMI) and digestible dry matter intake (DDMI).

Parameter	Species	Unit	n	Min	Max	SEC	R^2	SECV	R^2CV	**RPD**
Diet N	Sheep	%DM	236	0.6	3.1	0.2	0.91	0.2	0.86	**2.7**
Diet NDF	Sheep	%DM	140	31.9	70.1	1.1	0.98	1.8	0.96	**5.0**
Diet ADF	Sheep	%DM	139	17.5	36.2	0.6	0.99	1.1	0.96	**4.8**
Diet ash	Sheep	% DM	183	3.2	35	1.3	0.98	1.7	0.96	**5.2**
OMD (*in vivo*)	Sheep	%	257	39.9	80.9	3.0	0.86	3.7	0.79	**2.2**
DMD (*in vivo*)	Sheep	%	257	44.6	79.9	2.6	0.82	3.2	0.74	**1.9**
DOMD (*in vivo*)	Sheep	%	191	25.3	72	2.4	0.95	3.3	0.90	**3.1**
DMI	Sheep	g/DM head/day	276	109	1164	149.1	0.52	161.1	0.45	**1.3**
OMI	Sheep	g/DM head/day	69	726	1005	21.7	0.92	24.7	0.90	**3.2**

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

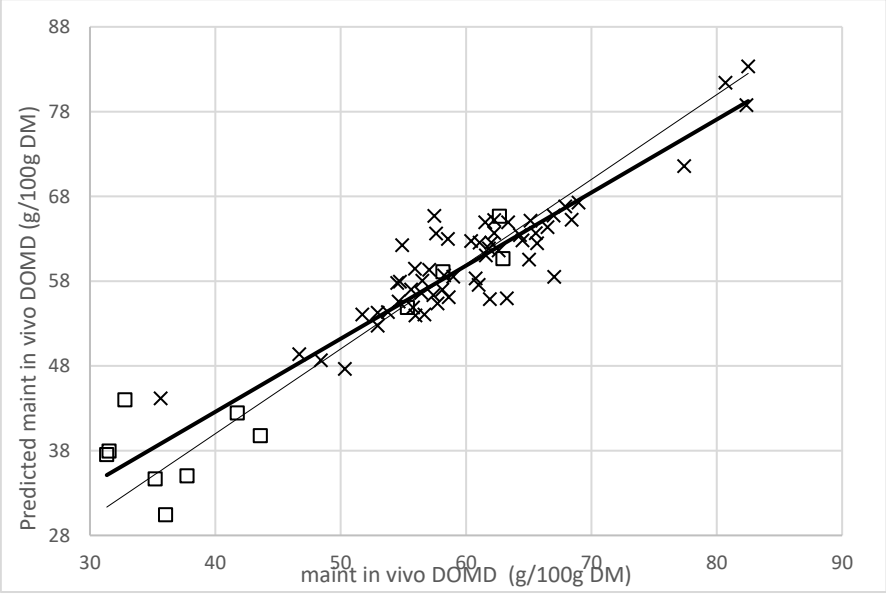

Figure 4.6. Predicted digestible organic matter in the dry matter (DOMD) g/100g DM in sheep, fed at the maintenance level of feeding. The squares represent the CSIRO shrub standards, the crosses are temperate forages. y = 0.86x + 8.089 R^2 = 0.88

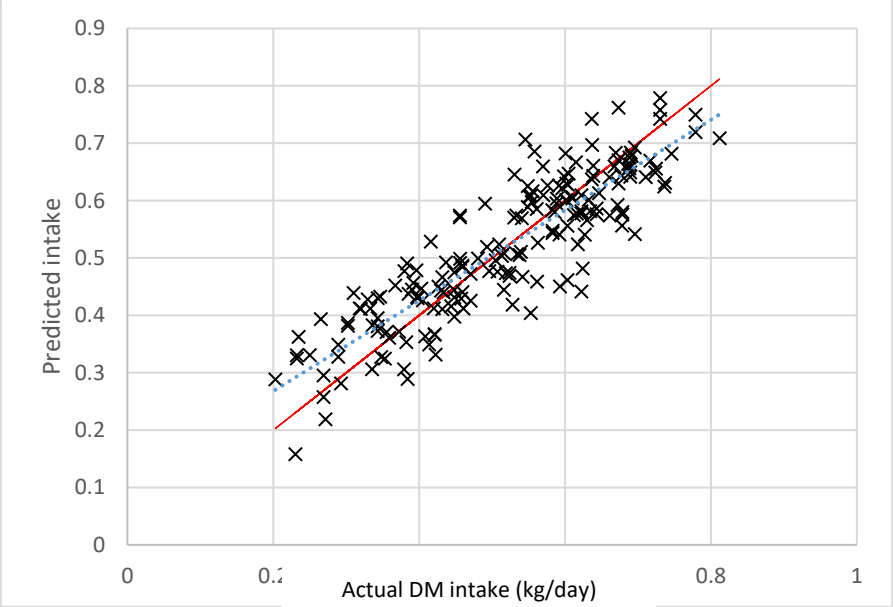

Figure 4.7. Relationship between actual and predicted dry matter intake (DMI) kg/day in sheep (intake predicted from forages using the Minson sample set). y = 0.78x + 0.11 R^2 = 0.79.

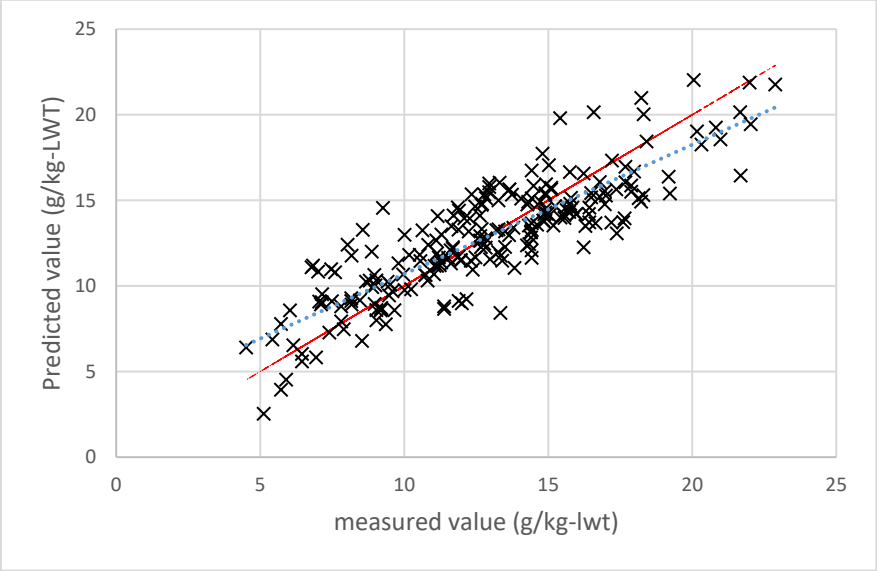

Figure 4.8. Intake prediction combined model for sheep and cattle (g/kg-LWT), y = 0.76x + 3.14, R^2 = 0.73

4.5 Test the ability of NIR to accurately predict digestibility and animal efficiency parameters e.g., intake from faeces

Faeces is the product of eroding and synthesising digestive processes and consists of residues of feed and plant tissue and components of microbial and animal origin. For these reasons faeces should contain information about the amount and characteristics of the diet (Cozzolino *et al*. 2002).

Significant gains were made in the collection and measurement of faecal samples and faecal-diet forage pair samples for use in future calibrations (Table 4.5). In total, spectra from 4656 faecal

samples were utilised. Table 4.6 summarises animal performance and diet quality predictions from sheep faeces, or in several cases, combinations of sheep and cattle faeces.

Table 4.6. Number, mean and range of animal productivity and faecal parameters.

	Trait	Parameter	n	n (paired forage/diet)	mean	min	max
WWAI							
Sheep	Digestibility	DMD	115	17	654	484	799
		DOMD	115	17	598	422	734
		OMD	115	17	670	482	809
	TDMI (kg DM/hd/day)	*ad lib*	190	32	1.7	0.3	6.3
		restricted	-	-	-	-	-
	OMI (kg DM/hd/day)	*ad lib*	115	17	0.9	0.2	1.9
		restricted	-	-	-	-	-
	DMI (g/kg LW)	*ad lib*	75	15	54	3	159
		restricted	-	-	-	-	-
	Diet N		-	-	-	-	-
	Faecal N		85	14	2.7	1.5	3.8
	Faecal delta C		-	-	-	-	-
	Faecal OM		90	19	78	60	88
Cattle	Digestibility	DMD	10	10	543	427	880
		DOMD	10	10	541	430	860
		OMD	-	-	-	-	-
	DMI (kg DM/hd/day)	*ad lib*	5	5	6.9	2.1	9.1
		restricted	-	-	-	-	-
	DMI (g/kg LW)	*ad lib*	-	-	-	-	-
		restricted	-	-	-	-	-
CSIRO							
Sheep	Digestibility	DMD	432	432	61.4	26.6	79.0
		DOMD	527	527	51.9	25.4	76.6
		OMD	629	629	61.3	27.6	80.9
	Diet N		586	-	1.9	0.6	2.9
	Faecal N		582	-	2.07	0.41	4.76
	Faecal delta C		120	-	22	13	29
	Faecal OM		557	-	14	4	35
	Methane		70	-	0.780	0	1.230
	Delta C		120	-	22.1	13.4	29.2
	Diet NDF		515	-	47.2	30.7	72.6
	Diet ADF		515	-	27.6	17.5	36.5
	Diet ASH		557	-	14.2	3.8	35.0
Cattle	Digestibility	DMD	445	-	54.4	43.0	74.9
		DOMD	330	-	50.8	40.2	66.3
	Sheep and cattle DMI	OMD	231	-	56.6	44.5	76.6
	Diet N		427	-	1.10	0.20	4.10
	Faecal N		674	-	1.4	0.7	3.2
	Faecal delta		1592	-	16.6	12.3	26.5
	DDMI herd mean		223	-	8.7	1.9	18.5

*TDMI-Total dry matter intake, TOMI-Total organic matter intake.

4.5.1 Faecal traits that are predicted from faecal samples.

The stable carbon isotope technique has been widely used to infer the dietary ecology of a range of farmed, wild and extinct animal species, including cattle (Jones *et al* 1981). The stable carbon isotope technique is reliant on differences in carbon isotope accumulation in plants with different photosynthetic pathways. Approximately 99% of carbon in nature is the 12C isotope (Ludlow *et al*., 1976). The C3 (Calvin pathway) of photosynthesis discriminates against 13C in favour of 12C considerably more than the C4 (dicarboxylic acid) pathway. This leads to different isotope ratios in the plant biomass and influences isotope ratios throughout the food chain. Deviations in carbon isotope ratios are expressed as δ13C (delta carbon) and measured as deviations from the isotope ratio in a standard carbonate (Lerman and Troughton,1975). Biomass from C3 plants have δ13C values of about −28‰ (range of −20‰ to −35‰) and C4 plants have values of −12‰ (range of −9‰ to −16‰). Norman *et al* (2009) tested the accuracy of the method for predicting diets of sheep grazing various combinations of C3 and C4 plants. For faecal samples, the organic matter content of the diet originating from C4 plants could be predicted with a mean error as low as 2.7%. Fortunately, the faecal samples had been stored were available for this project.

David Coates used the method to predict the amount of C4 grass and C3 forb in the diets of subtropical cattle. As a general rule, the forbs have higher feeding value and grass dominant diets could be an indicator of a need to consider urea supplementation. In southern systems, temperate grasses and legumes have a C3 photosynthetic pathway and subtropical grasses and shrubs such as saltbush represent the C4 species. The Coates equations used absolute values in the calibrations so faecal samples with values of 14 would indicate a diet dominated by C4 plants, and values of 23 indicated a C3 diet.

Delta carbon from sheep faeces was predicted with excellent results (RPD 5.3, error of prediction 0.97 % units). The sheep calibration has a higher RPD than the original Coates cattle calibration (RPD 3.8, Table 4.6).

An excellent ruminant faecal nitrogen prediction (RPD 4.5) was generated from a combination of 764 cattle faecal samples and 560 sheep samples. Faecal nitrogen can be predicted with an error of just 0.1% units of N. When developed for sheep faecal samples, the calibration only offers fair screening potential (RPD =2.8), the broader multi-animal species calibration being superior. David Coates had a marginally better calibration for just cattle (RPD 4.8).

Faecal organic matter in sheep faeces was predicted with a RPD 3.0. A broad sheep and cattle faecal organic matter calibration gives an error of prediction of just 1.3% units and a very good RPD of 3.7. Again, adding both animal species improved the performance of the calibration. Low organic matter is a good indicator of poor biomass availability (animals consuming more soil) or intake of a high salt diet such as saltbush.

Traits that are measured in faeces make it easier to develop and test calibrations as animal measurements are not required. Dietary traits predicted from faeces are more complex.

4.5.2 Dietary traits that are predicted using faeces.

Faecal samples were used to predict the quality of the diet the animal had consumed using faecal NIRS of diet-faecal pairs. Diet CP of sheep could be predicted with fair screening potential (RPD 2.5, 1.2 % units' error of prediction, Table 4.7). This is not as good as David Coates dietary crude protein for cattle (RPD 4.3, 0.2 % units' error). It's possible that combining the sheep and cattle databases

will lead to a better calibration (as was the case for faecal N). There is a very high prospect for NIRS calibrations to be able to predict dietary CP intake for sheep and cattle.

The quality of the diet could be predicted from a faecal sample. Diet ADF content was predicted with excellent results, with an RPD for sheep alone of 4.0 and for sheep and cattle, 4.8 (Fig 4.7). This is very exciting as ADF is an indicator of a poor-quality diet and often highly correlated to energy values, animal performance and methane. The NDF content of a diet was also predicted using faecal NIRS with excellent results and an RPD of 4.8 for sheep alone and 5.0 for sheep and cattle combined. David Coates was not able to generate good faecal NIRS calibrations for ADF or NDF.

David Coates (MLA Report: NAP3.121 2004) reported that no faecal calibration equations had been developed for determining NDF and ADF dietary concentrations. He identified problems with the accuracy of the chemical analysis procedures. Further, he recommended that where reference values cannot be determined from faecal analysis such as dietary fibre specially conducted experiments for obtaining valid reference values are required. David advised at the time, this would be a necessary adjunct to maintaining useable faecal NIRS technology. He suggested 'to reduce the cost burden of validation experiments, every effort should be made to ensure that every relevant sample and every bit of relevant information from experiments designed for other purposes is acquired for the purpose of maintaining and building up faecal NIRS technology (MLA Report: NAP3.121 2004). While historical samples from as early as 2004 were found and utilised, it is unfortunate that David's advice was not implemented sooner.

Cozzolino *et al*. (2002) developed calibration equations for acid detergent fibre (ADF) and neutral detergent fibre (NDF) using 120 faecal samples scanned on a FOSS 6500. They obtained validation results of 0.74 (SEP: 7.5) for ADF and 0.85 (SEP: 8.5) for NDF. Righi *et al* (2017) reported validations statistics for NDF of 0.62 (SEP: 2.59) and ADF 0.63 (SEP: 1.61) on a calibration developed from faeces collected from lactating dairy cattle. This project achieved much better results, obtaining validation measures for NDF of 0.96 (SEP: 2.30) for sheep and 0.96 (SEP: 1.75) for sheep/cattle calibration, for ADF 0.94 (SEP: 1.50) for sheep and 0.96 (SEP: 1.13) for sheep/cattle calibration.

Dietary ash content could be predicted by faecal NIRS with excellent results (RPD 4.3, Fig 4.10). This was facilitated by the shrub research program which provided samples from a range of high salt diets that had been tested.

Table 4.7 Calibration statistics for SpectraStar (CSIRO) predictions of animal performance and diet quality based on sheep and a combination of sheep and cattle faecal samples.

Parameter	Species	Unit	n	Min	Max	SEC	R^2	SECV	R^2CV	**RPD**
Faecal N	Sheep	%DM	560	0.8	4.1	0.20	0.90	0.27	0.87	**2.8**
	S & cattle	%DM	1324			0.09	0.96	0.10	0.95	**4.5**
Faecal C	Sheep	%DM	534	36.1	48.1	1.40	0.63	1.58	0.57	**1.5**
	S & cattle	%DM	350	35.6	48.1	0.72	0.91	1.06	0.81	**2.3**
Faecal OM	Sheep	%DM	777	73.7	94.9	1.10	0.91	1.20	0.89	**3.0**
	S & cattle	%DM	454	69.5	94.9	1.06	0.95	1.27	0.93	**3.7**
Diet N	Sheep	%DM	570	0.6	2.9	0.20	0.90	0.18	0.87	**2.7**
Diet CP	Sheep	%DM	576	3.8	18.3	1.10	0.87	1.24	0.84	**2.5**
Diet NDF	Sheep	%DM	502	30.7	72.6	1.90	0.97	2.30	0.96	**4.8**
	S & cattle	%DM	140			1.14	0.98	1.75	0.96	**5.0**
Diet ADF	Sheep	%DM	489	17.5	27.7	1.40	0.95	1.50	0.94	**4.0**
	S & cattle	%DM	139			0.63	0.99	1.13	0.96	**4.8**
Diet ash	Sheep	% DM	530	3.8	35.0	1.50	0.97	1.90	0.95	**4.3**
OMD (*in vivo*)	Sheep	%	607	39.9	80.9	2.80	0.84	3.30	0.80	**2.2**
DMD (*in vivo*)	Sheep	%	415	34.4	74.6	2.30	0.88	2.60	0.84	**2.5**
DOMD (*in vivo*)	Sheep	%	506	26.5	72.0	2.50	0.93	3.10	0.90	**3.2**
	S & cattle	%	173	36.2	72.0	2.76	0.76	2.76	0.76	**2.1**
CH4	Cattle	g/kg LW	79	14.0	23.1	1.13	0.76	1.26	0.69	**1.9**
Delta C	Sheep		117	13.4	29.2	0.47	0.99	0.86	0.97	**5.3**
ADG	Sheep	g/day	419	0	280.0	90.60	0.43	95.31	0.42	**1.3**
DMI	Sheep	g/day	649	191.0	1604.0	97.10	0.76	110.00	0.71	**1.9**
DMI	Sheep	gDMkgLWd	434	6.0	25.9	1.59	0.84	1.75	0.80	**2.2**
OMI	Sheep	g/day	516	246.0	1032.0	55.10	0.89	62.40	0.86	**2.7**
OMI	Sheep	gOMkgLWd	434	4.5	23.9	1.27	0.90	1.50	0.87	**2.8**
DDMI	Sheep	g kgLWd	282	2.0	17.6	0.85	0.92	1.00	0.88	**2.9**
DOMI	Sheep	g kgLWd	414	1.8	16.2	0.85	0.92	1.03	0.90	**3.1**
Diet B	Sheep	mg/kg	154	2.8	97.6	6.51	0.89	8.02	0.83	**2.4**
Diet Ca	Sheep	%	156	0.1	1.8	0.08	0.96	0.11	0.91	**3.4**
Diet Cu	Sheep	mg/kg	156	1.7	11.9	0.46	0.94	0.64	0.88	**2.9**
Diet Fe	Sheep	mg/kg	156	65.8	981.7	36.83	0.96	46.96	0.93	**3.7**
Diet Mg	Sheep	%	154	0.1	1.0	0.04	0.98	0.06	0.96	**5.0**
Diet Mn	Sheep	mg/kg	153	22.8	275.5	22.98	0.82	27.77	0.74	**2.0**
Diet P	Sheep	%	156	0.1	0.3	0.02	0.87	0.03	0.77	**2.1**
Diet K	Sheep	%	103	0.4	3.7	0.15	0.97	0.23	0.93	**3.9**
Diet Na	Sheep	%	154	0.0	11.5	0.47	0.98	0.66	0.96	**4.8**
Diet S	Sheep	%	154	0.1	0.8	0.03	0.96	0.04	0.92	**3.6**
Diet Zn	Sheep	mg/kg	154	10.0	150.2	6.84	0.94	9.10	0.90	**3.2**
Diet Cl	Sheep	mg/kg	136	0.3	15.8	0.61	0.98	0.79	0.96	**5.0**

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

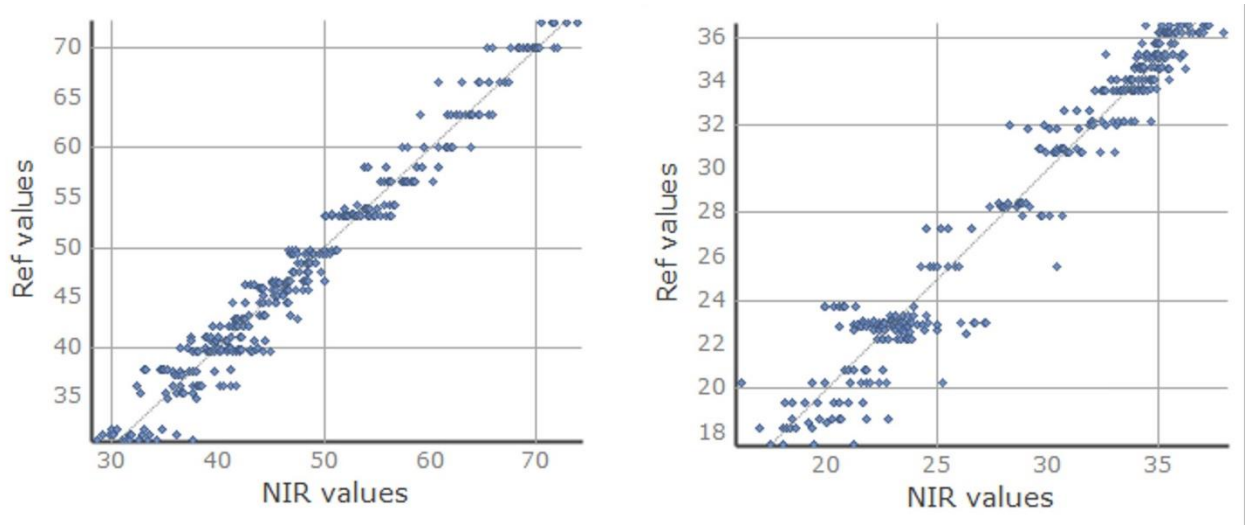

Figure 4.9. Faecal NIR predicted and actual values of neutral detergent fibre in the diet (left) and acid detergent fibre in the diet (right).

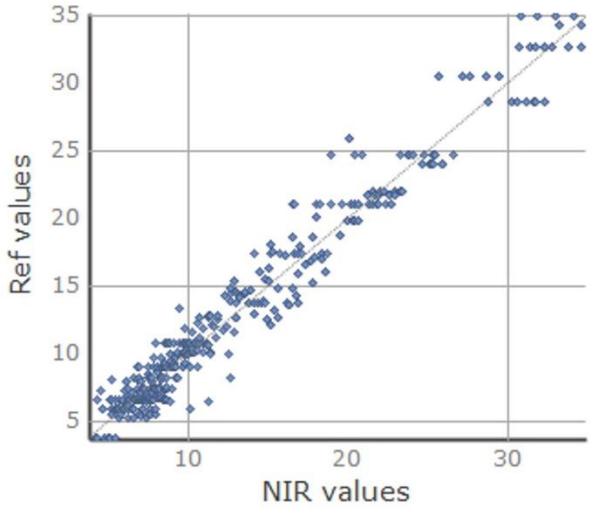

Figure 4.10. Faecal NIR predicted dietary ash.

It is possible to predict the energy content of the ingested diet using faecal NIRS. For *in vivo* DOMD in sheep a quality control level calibration (RPD 3.2) was generated, with an error of prediction of 3.1% (Table 4.7, Fig 4.11). *In vivo* DMD and *in vivo* OMD had lower predictions (RPD 2.2 and 2.5 respectively), however they showed significant promise with further refinement or potentially combining with cattle data. David Coats was able to predict cattle DMD and OMD with RPD values of 3.0.

Unfortunately, sheep and cattle faecal samples, with measured methane from respiration chambers, are relatively scarce. 79 faecal samples and methane data from cattle consuming Leucaena and desmanthus from Ed Charmley's LPP projects were utilised. Evidence was generated that supported

the concept that methane could be predicted with faecal NIRS, although the RPD at this stage is not high (RPD=1.9, Table 4.7, Fig 4.11). More samples are required to test and expand the predictions. Given the ability of the models to predict diet digestibility and indigestible fibre, it is probable that methane could be predicted. This would be an incredibly useful tool for researchers seeking to identify low methane plants and animals and for industry to benchmark Eco credentials.

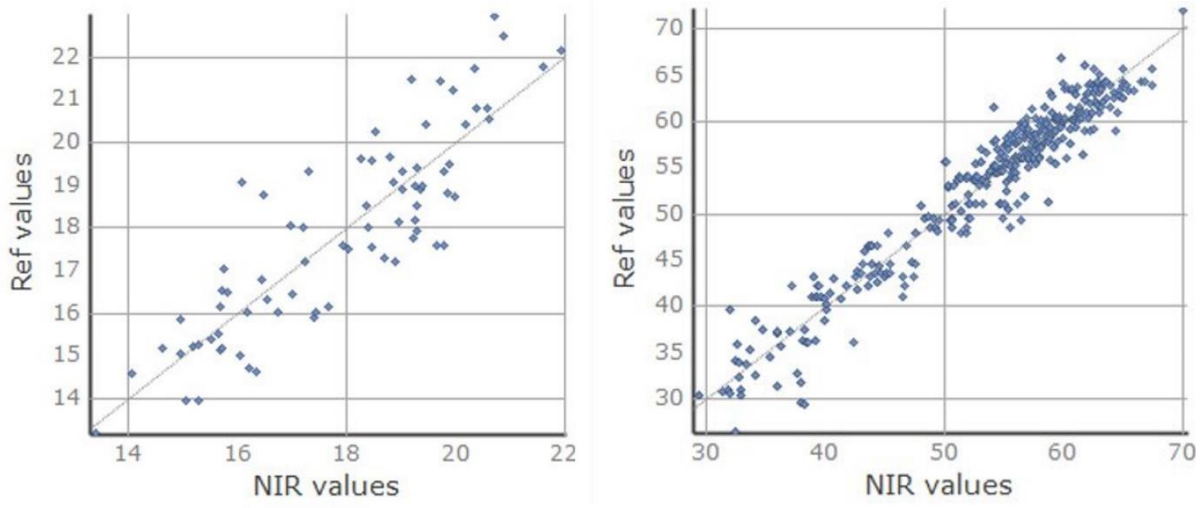

Figure 4.11. Faecal NIR predicted and actual values of cattle methane g/kg liveweight (left) and sheep *in vivo* digestible organic matter in the dry matter (right).

The dietary concentrations of some minerals could be predicted from faecal samples. Given the small sample number, the results were better than anticipated. Minerals that could be predicted with excellent results included chloride, sodium and magnesium (Table 4.9), while calibrations of a quality control standard for calcium, iron, potassium, sulphur and zinc were achieved. Other minerals that had potential to be predicted included copper and boron. Phosphorus and manganese may be possible, but more data are required.

4.5.3 Animal performance traits that are predicted using faeces.

Voluntary feed intake is an extremely difficult trait to measure in field grazing trials. Intake and nutritional value of the forage are both drivers of performance, however, intake is rarely measured. A good calibration for dry matter intake in sheep was not generated but fair screening potential was achieved for organic matter intake (RPD 2.8, error of prediction of 1.5 g OM/kg LW/day). To a large degree the data were biased towards animal house studies at the maintenance level of feeding. More diversity in the data is likely to improve the calibration.

A significant achievement was the generation of a calibration for digestible organic matter intake for sheep (RPD 3.1, Table 4.7, Fig 4.12). Digestible dry matter intake (DDMI) was predicted with an RPD of 2.9 (Table 4.7, Fig 4.12). David Coates generated a calibration equation for digestible dry matter intake (DDMI) of cattle that has an RPD of 4.2. It is likely that the sheep calibrations can be refined and improved. Average daily gain was not predicted with faecal NIRS.

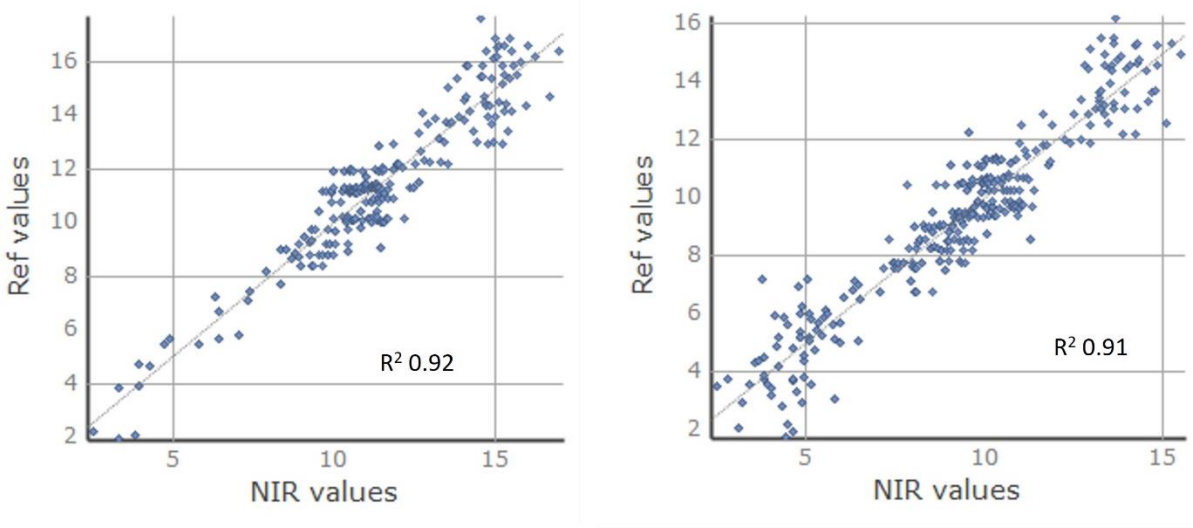

Figure 4.12 Faecal NIR predicted and actual values of sheep digestible dry matter intake (DDMI, left) and digestible organic matter intake (DOMI, right).

4.6 Test potential to transfer northern cattle faecal calibrations to a modern NIRS instrument

One of the key objectives of the project was to evaluate how successfully the Coates faecal calibrations could be transferred to other NIR spectrophotometers to ensure accessibility for use into the future. These calibrations can currently only operate on the FOSS Model 6500 NIR, a model which is now not supported by the manufacturer, and parts are also scarce.

In this project, the calibration set was transferred to four other instruments: Three of these were dispersive instruments which rely upon a diffraction grating for wavelength accuracy and detection. These models included a later model FOSS unit (FOSS XDS), a SpectraStar XT and XTS. The other model tested uses a Michelson interferometer design and Fourier Transformations to collect spectral data.

Wagga XDS spectra were converted to align with spectra collected on the FOSS 6500, so the David Coates Faecal Calibrations could be used to predict sample scans collected on the XDS, SpectraStar to SpectraStar and FOSS to Bruker Instruments.

A Set of David Coates sealed faecal standards were scanned on the FOSS 6500 (MASTER) and Wagga XDS (HOST). The protocol for transferring between Foss Instruments outlined in the WinISI manual Version 1.50 (2000) Infrasoft International, LLC. was used. A single sample standardisation technique was used by the selection of the closest spectra to the average spectrum using 'Make and use scores' to create a new Master file and a new host file which were then used to create a standardization file by calculating the differences between the master and host.

The standardisation file was used to convert data collected on the host instrument and new calibrations generated on that platform. The conversion and recalibration process generated the following calibration statistics (Table 4.8):

Table 4.8. Coates original calibration and Validation XDS against laboratory reference.

Constituent	Original FOSS RSQ	SEC	XDS post standardisation RSQ
Dietary Nitrogen	0.949	0.165	0.912
Dry Matter Digestibility	0.896	1.87	0.896
Organic matter Digestibility	0.892	1.73	0.904
Faecal Nitrogen	0961	0.08	
Faecal Ash	0.903	2.0	
Faecal Delta	0.933	0.766	0.973

Transfer Faecal Calibrations: Unity Spectrastar (CSIRO) to Unity Spectrastar (Gilmac)

In 2018, CSIRO transferred calibrations from the FOSS 6500 to the Unity Spectrastar XTR. This work was undertaken by Paul Brimmer from Unity Scientific. The process requires the wavelengths to be trimmed and spectral ranges aligned. The FOSS 6500 has a range of 400-2500nm at 2nm intervals and the XTR is 680 to 2600nm at 1nm intervals. The David Coates sealed standards were used to collect spectra on both instruments as a matched set for the transfer. Going from the FOSS to Unity required a zero-order approach as the instruments are from different manufacturers. The better matched instruments (master vs host) can use the more complex order. The file generated was used to do an instrument transfer so that the David Coates faecal database spectra file looked like it had been produced by the host instrument i.e., the spectra resembled spectra that could have been generated by the master instrument.

This earlier work meant the process to transferring the Coates calibration to the Unity XTR instrument was very much simplified. The prediction files from the CSIRO SpectraStar XTR were uploaded onto the SpectraStar XTR and the sealed standards were then used to generate predictions which were measured against their laboratory reference values. From that a bias correction was done to attempt to eliminate any residual differences between the instruments (Table 4.9).

Table 4.9. Transfer Faecal Calibrations: FOSS 6500 to Spectrastar XTR

Constituent	Original FOSS - RSQ	SEC	XTR Transfer RSQ	SEC
Dietary N	0.949	0.165	0.898	0.19
DMD	0.896	1.87	0.861	2.11
OMD	0.892	1.73	0.859	1.95
Faecal N	0961	0.08	0.953	0.08
Faecal Ash	0.903	2.0	0.943	1.08
Faecal Delta C	0.933	0.766	0.918	0.78

Transfers from instruments with dispersive optics to interferometer-based instruments present a more difficult challenge due to the differences in the way the machines measure spectra. This is the case when looking at converting calibrations from FOSS based (dispersive) calibrations to Bruker (interferometer). Bruker does have spectra conversion methods that deal with this challenge. To facilitate the conversion the same set of David Coates sealed standards were used to allow conversion of spectra from the FOSS 6500 to the FOSS XDS. Each standard was scanned 5 times each

on the Bruker MPA 2 instrument using the 50mm rotating cup module and scans were then averaged and stored as a single scan.

The following settings were used to scan samples: wavelength range used was 4000-12500 cm-1 (800 – 2500 nm), 64 scans per run, with a resolution of 16 cm-1. Corresponding spectra from the FOSS instrument were then used to run a piecewise direct standardisation to convert all spectra to interferograms, which can then be used to develop calibrations on the Bruker instrument.

Calibration statistics for faecal components developed for the Bruker MPA II are given in table 4.10 below, with original calibration statistics also listed for comparison. Calibration statistics appear to be satisfactory for most of the components with comparable but slightly poorer measures for *in vivo* DMD (Figure 4.13) and OMD (Table 4.10). Faecal N, diet N and delta N also appear to give reasonable calibration statistics but are slightly lower in quality than the original calibrations. Faecal Ash has given lower quality calibration statistics (Figure 4.13) and may require further work.

While the initial Bruker calibrations are encouraging, validation has been limited by available standards and data to check on the Bruker instrument. Testing has been limited by a partial lack of reference data for the sealed standards. Two other sets of samples have been located but again the reference sets are not complete. Laboratory work is being done to enable Faecal N and Faecal ash validations. It would be good to also locate faecal samples from cattle that have intake, dietary nitrogen and *in vivo* digestibility measures.

A set of 30 faecal samples were used as an independent set of check standards that were scanned on the Bruker MPA. Analysis of the results (Table 4.10) indicated that the correlations between the predicted data and the reference data were comparable to the original calibrations developed from the FOSS converted spectra sets, a slope correction was required to correct the data from the Bruker derived scan set. A correlation of the slope corrected predictions and reference data is given in figure 4.14. Bruker recommends that hybrid calibrations should be created with additional reference samples added to the converted FOSS-Bruker scans to ensure the calibrations are robust.

Table 4.10. Transfer Faecal Calibrations: FOSS 6500 to Bruker MPA II

Constituent	Original FOSS - RSQ	SEC	MPA II Transfer RSQ	SEC
Dietary N	0.949	0.165	0.873	0.21
DMD	0.896	1.87	0.835	2.27
OMD	0.892	1.73	0.834	2.10
Faecal N	0961	0.08	0.950	0.09
Faecal Ash	0.903	2.0	0.731	1.24
Faecal Delta C	0.933	0.766	0.851	0.99

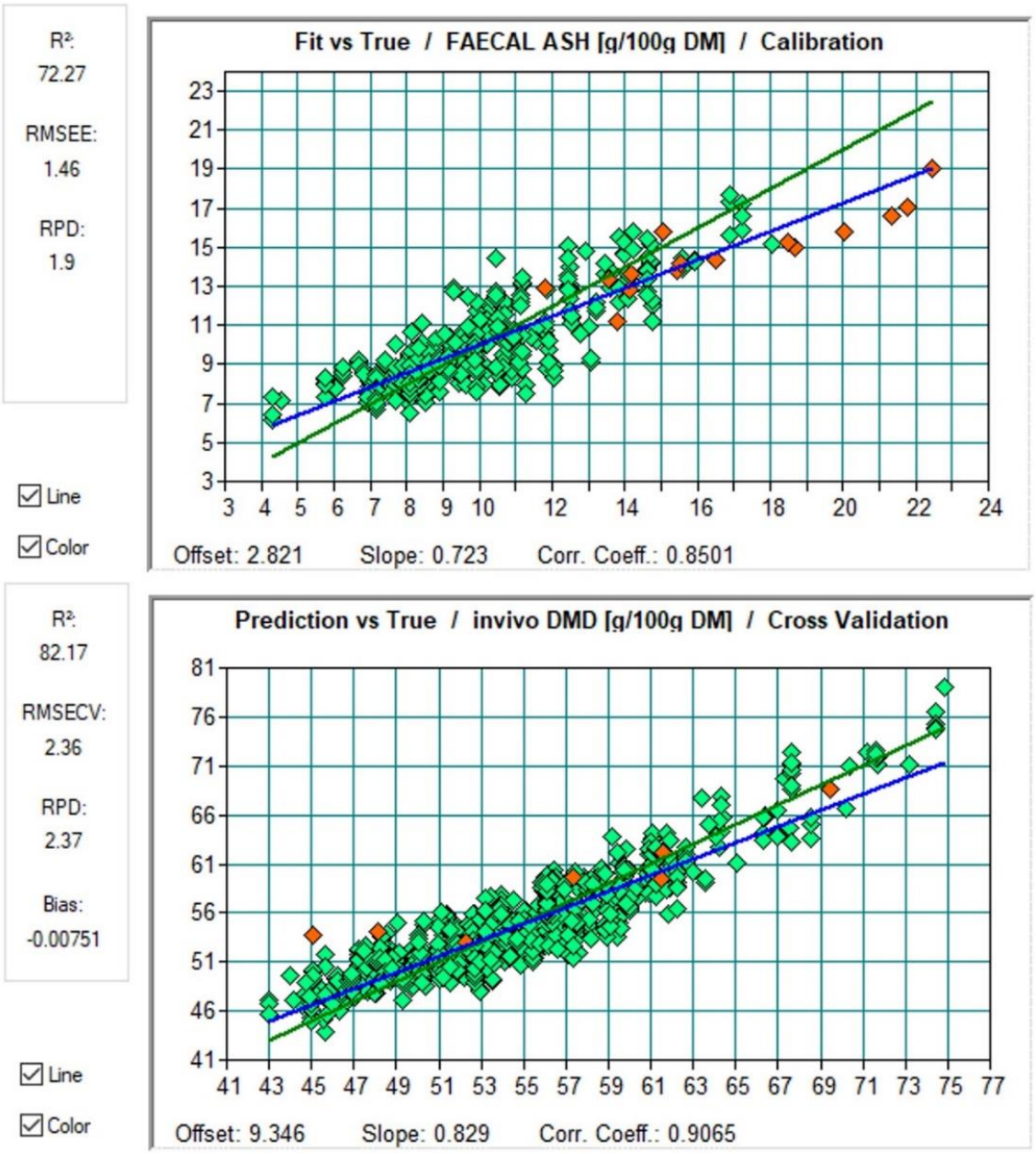

Fig 4.13. Graphs showing Bruker calibrations for Faecal ash and *in vivo* DMD. Orange markers indicate faecal standards used to create new versions of the converted Dixon calibrations. Green markers show spectra acquired originally on FOSS 6500, that have been converted to mimic Bruker spectra.

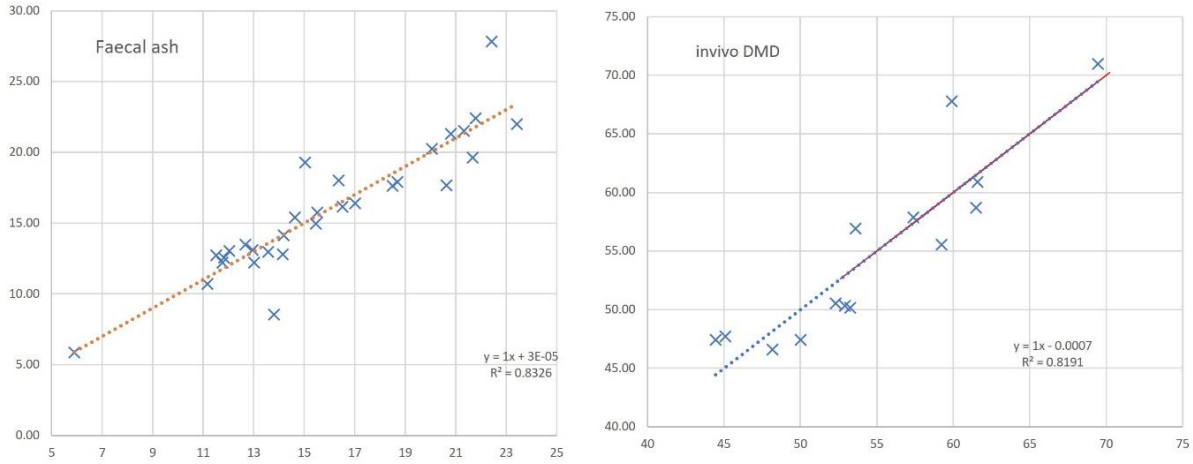

Figure 4.14 Predictions for independent set of faecal samples scanned on the Bruker using the calibrations outlined above (Fig 4.13)

4.7 Generate data to explore use of machine learning to develop predictions for currently difficult to predict traits

Machine learning and deep learning techniques were applied to the problem of predicting nutritional traits. Table 4.11 shows how different deep learning models predict NDF, ADF, DMD, OM and N. These values were obtained using the validation split. The Neural Network with 8 hidden layers and 64 nodes (this means 284,869 parameters in the model) gave the best results (for NDF this was a R^2 of 0.96 and SEM of 2.35), second best was with one hidden layer and 128 nodes. The data suggests that this problem requires more complex models. The model was adjusted, with a much more complex architecture, to predict NDF, ADF, DMD, OM and N at the same time. Machine learning remains a significant opportunity, especially if automated delivery platforms associated with a data cube are developed. In the short term however, it does not appear to offer significant advantages over the traditional partial least squares method. It may be useful to focus future machine learning on key traits that do not appear to be precited using PLS such as phosphorus, wet forage samples or average daily gain from faecal samples.

Table 4.11. Prediction statistics for the application of machine learning to predict nutritional traits.

	Model 1				Model 2			
	RMSE	R^2	STDE	SEM	RMSE	R^2	STDE	SEM
NDF	17.9	0.98	2.69	1.34	68.7	0.88	8.26	4.13
ADF	21.9	0.95	3.82	1.91	49.1	0.85	6.97	3.48
DMD	193.1	0.74	11.24	5.62	143.1	0.80	11.95	5.98
OM	155.1	0.83	10.50	5.25	166.4	0.79	12.90	6.45
N	1.3	0.79	0.87	0.45	0.8	0.87	0.86	0.45

RMSE = Root Mean Square Error (in the unit of the data, so if n is %, this error is in % if it is in mol/g, this error is also in mol/g), R2 = Coefficient of determination, SEM = standard error of the mean, STDE = Standard deviation of the error (standard deviation of obs. minus sim. value).

4.8 Test the ability of handheld NIR devices to accurately predict digestibility and animal efficiency parameters from fresh forage

Two independent sets of samples and data were acquired and analysed to assess the performance of the two separate handheld instruments (ASD and HR1024). Unfortunately, restricted travel during Covid 19, made a simultaneous comparison impractical. Data comparing the performance of the two handheld units is given in table 4.13.

Evaluation of the ASD Fieldspec unit

A database was developed using the ASD Fieldspec 3 of fresh scans and wet chemistry attributes from numerous fresh pasture species collected in Western Australia such as Sub Clover, Serradella and Trigonella, along with weed species such as Ryegrass, Cape weed and Silver grass. Fresh material was collected and held in the cool room at 4^{o}C until scanning. Prior to scanning, samples were placed in a matt black container and scanned within a day. Ambient light was managed through an independent light source in the wand of the unit. Spectral data was then converted for analysis using UCAL software.

Results for the ASD can be found in Table 4.13. Results indicated that DMD could be predicted with a SECV of 4.5 units (rough screening potential). OM, ADF, NDF, water soluble carbohydrates and total N (or CP) were poorly predicted.

Evaluation of the HR-1024 unit

For the samples analysed by NSW DPI a total of 161 samples and then harvested and taken to the laboratory for analysis in conjunction with project work being carried out by Dr Shawn McGrath, Charles Sturt University.

Paddocks on the Charles Sturt University commercial farm at Wagga Wagga, NSW that were sown in 2020 to barley (*Hordeum vulgare*), Oats (*Avena sativa*) or wheat (*Triticum aestivum*) were identified for sampling. Samples were collected in August-October 2020. Where possible samples from the same site were collected on multiple dates to assess changes in quality through time.

In each paddock, samples were collected from a minimum of three locations, with three samples collected per location on each sampling date. Each paddock/sampling location had GPS coordinates recorded. Samples were collected by cutting all plants at 0.05 m above ground level along a 1 m length. Crop growth stage was recorded. Crop row spacing was recorded to allow an estimate of crop biomass. Samples were collected when surface water was minimal.

Samples were returned to the laboratory and if they could not be processed immediately were stored overnight in a refrigerator (4^{o}C). Samples were chopped into 1-2 cm lengths using a guillotine and then scanned in full sunlight using a Spectra Vista Corporation HR-1024, before drying at 70^{o}C in open aluminium trays. After drying samples were weighed and re-scanned using the HR-1024, following the same procedure as for the fresh samples.

Samples collected from cereal crops across different locations and dates achieved the aim of presenting material for analysis across a range of qualities as determined using wet chemistry. Cereal forage samples ranged from low to very high quality (Table 4.12).

Table 4.12. Summary of wet chemistry results for all cereal crop samples submitted

Component	Units	n	mean	range	std dev
Neutral Detergent Fibre	g/100g DM	161	50.9	36.2-64.2	5.76
Acid Detergent Fibre	g/100g DM	161	27.9	18.7-37.7	4.56
Crude Protein	g/100g DM	161	13.9	3.7-28.3	6.63
Ash	g/100g DM	161	8.9	4.3-15.7	2.46
Organic Matter	g/100g DM	161	91.1	84.3-95.7	2.46
Dry Matter Digestibility	g/100g DM	161	70.8	56.4-87.2	7.75
Digestible Organic Matter Digestibility	g/100g DM	161	66.8	54.6-80.7	6.57
Metabolisable Energy	MJ/kg DM	161	10.6	8.1-13.4	1.33
Water Soluble Carbohydrates	g/100g DM	161	18.7	3.1-52.2	8.79

Calibrations developed using all fresh forage scans on the HR-1024 were generally very poor (RPD 1.1-2.0) and not fit for forage testing (Table 4.13). Developing calibrations using average scans on the HP-1024 improved the statistics slightly (Table 4.13) but are still considered very poor. Calibrations based on the dried ground material, using the high-resolution laboratory instrument (Bruker MPA) were all very good or excellent (Table 4.14) and appear far superior to the calibrations based on the HP-1024 portable unit scans on fresh forage.

This work suggests that calibrations used to determine pepsin digestibility (DMD and DOMD) would be suitable for a rough screening, with NDF approaching just below the 2.0 RPD (at 1.9) threshold for rough screening. Other measures including ADF, CP and WSC would be considered too poor to produce results with any reliability for the HR-1024.

Interestingly, the ASD instrument provided similar performance in terms of r^2 and RPD values obtained. The findings agree well with the findings of other recent reviews of handheld units measuring the quality of fresh forage such as Cherney *et al* (2021), who evaluated four different units and found the following range of correlations CP (0.55 vs 0.48), NDF (0.81 vs 0.74), ADF (0.50 vs 0.61), Ash (0.31 vs 0.48)

This does not offer significant hope for remote prediction of forage quality unless there is a massive mathematical or technological breakthrough. The ability to scan a greater number of samples in less time, thus avoiding changes in the composition due to oven-drying procedures (Alomar *et al*., 2009) needs to be traded against the lower predictive ability for fresh forage (Lobos *et al* 2019). These results also suggest that the water content of fresh forage can often mask NIR signals and generate a limited predictive model (Reeves, 2000; Lobos *et al*. 2019).

Table 4.13. Calibration statistics for the ASD and HR1024 handheld NIRS devices

		ASD WET						HR1024 WET					
	Parameter	n	SEC	R^2	SECV	R^2CV	**RPD**	n	SEC	R^2	SECV	R^2CV	**RPD**
Digestibility	DMD (pepsin-cellulase)	90	2.8	0.91	4.5	0.75	**2.0**	161	3.38	0.81	3.7	0.76	**2.0**
	DOMD (pepsin-cellulase)							161	2.88	0.81	3.1	0.76	**2.0**
	Organic matter	90	1.5	0.71	2.0	0.48	**1.4**	161	1.14	0.79	1.6	0.55	**1.5**
Carbohydrates	Acid detergent fibre	90	4.7	0.77	6.4	0.61	**1.6**	161	2.10	0.80	4.1	0.18	**1.1**
	Neutral detergent fibre	90	5.0	0.82	5.8	0.72	**1.9**	161	2.23	0.93	2.7	0.74	**1.9**
	Water soluble carbohydrates							161	3.43	0.77	4.3	0.62	**1.6**
Protein fractions	Nitrogen	90	0.4	0.92	0.7	0.67	**1.7**	161	0.43	0.85	0.8	0.48	**1.4**

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

Table 4.14. Comparison of calibration statistics using fresh forage scanned on the HR1024 and dried ground sample scanned on the Bruker benchtop MPA

	Treatment	Factors	*n*	RMSECV	R_{cv}^2	RPD	*n*	RMSEP	R_p^2	RPD	SEL
HR-1024											
NDF	First derivative + Vector normalization (SNV)	9	2535	3.4	58.4	**1.6**	659	5.5	30.9	**1.3**	1.2
ADF	First derivative + Vector normalization (SNV)	7	2566	4.1	19.9	**1.1**	664	4.4	6.64	**1.1**	1.0
CP	First derivative + Vector normalization (SNV)	9	2599	4.3	58.5	**1.6**	664	4.5	42.6	**1.4**	0.2
ASH	First derivative	5	2547	1.7	52.2	**1.5**	664	2.0	25.7	**1.2**	0.1
DMD	First derivative + Vector normalization (SNV)	6	2561	4.5	63.3	**1.7**	664	7.0	29.5	**1.2**	1.7
DOMD	First derivative + Vector normalization (SNV)	6	2561	3.9	63.3	**1.7**	664	5.9	29.3	**1.3**	0.9
ME	First derivative + Vector normalization (SNV)	6	2561	0.6	63.3	**1.7**	664	1.0	29.3	**1.3**	0.1
WSC	First derivative + Vector normalization (SNV)	9	2252	4.9	50.1	**1.4**	662	10.3	20.6	**1.2**	0.6
Bruker MPA											
NDF	First derivative + MSC	4	128	1.4	94.2	**4.2**	33	1.8	91.1	**3.6**	1.2
ADF	First derivative + Straight line subtraction	7	125	0.9	96.2	**5.1**	33	1.1	94.0	**4.1**	1.0
CP	First derivative	9	128	0.7	98.9	**9.6**	33	0.8	98.7	**8.6**	0.2
ASH	First derivative + MSC	5	128	0.5	96.2	**5.2**	33	0.7	92.8	**3.7**	0.1
DMD	First derivative + Vector normalization (SNV)	6	125	1.5	96.2	**5.1**	33	2.1	92.8	**3.7**	1.7
DOMD	First derivative + Vector normalization (SNV)	6	125	1.3	96.2	**5.1**	33	1.8	92.6	**3.7**	0.9
ME	First derivative + Vector normalization (SNV)	6	125	0.2	96.2	**5.1**	33	0.3	92.6	**3.7**	0.1
WSC	First derivative + Straight line subtraction	9	128	0.8	99.0	**10.2**	33	3.2	91.3	**3.5**	0.6

RMSECV: Root Mean Squared Error of Cross-Validation, Rcv2: Coefficient of determination in cross-validation, RMSEP: Root Mean Squared Error of Prediction, Rp2: Coefficient of determination in prediction; RPDc=residual prediction deviation in cross validation; RPDp=residual prediction deviation in prediction. Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are rough screening potential, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

5. Conclusion

The new generation NIRS Calibrations project was initiated to address gaps in the Australian feed testing landscape by (1) broadening the diversity of plant species that are predicted accurately, (2) increasing the number of forage quality traits that can be predicted, and (3) explore the opportunity to use faecal NIRS to predict diet selection, intake and animal performance. These calibrations provide producers, researchers, and consultants the tools required to maximise the efficiency of red meat production and mitigate risks associated with poor nutrition. The team from CSIRO and NSW DPI identified gaps, leveraged historical samples from across Australia, conducted research, and generated new NIRS calibrations.

Broad, accurate, fit-for-purpose calibrations that accurately predict key nutritional traits for the entire Australian feedbase have been developed and tested. Novel calibrations were developed for minerals, secondary compounds and the Cornell Net Carbohydrate and Protein System. The accuracy of laboratory-grade handheld NIR sensors, as a practical alternative to lab-based units, was also evaluated. Unfortunately, they were unable to predict the majority of nutritional trats but offered rough screening potential for DMD. The errors of prediction were biologically significant.

A series of high-quality faecal calibrations for sheep and sheep and cattle was created covering a large range of important parameters such as dietary digestible organic matter intake, *in vivo* digestibility, dietary fibre, minerals and crude protein. Preliminary evidence for methane predictions from faeces was generated. Utilising the large collection of pasture samples with *in vivo* data, significant improvements were made with NIR calibrations to predict *in vivo* digestibility for sheep and cattle at maintenance and adlib feeding levels. The team demonstrated successful transfer of the currently utilised Coates northern cattle faecal calibrations to modern instruments.

Broad multi-species NIRS calibrations for existing and novel traits should be considered for commercial release. The faecal NIRS calibrations for diet selection, diet quality, intake and possibly methane are showing significant promise and there is potential to combine sheep and cattle into a single calibration. These could be a game-changer for the red meat industry and should be prioritised for future investment. Current calibration transfer systems are cumbersome, labour intensive and often result in different 'versions' of a calibration across labs. Industry should consider co-investment in a digital platform that links to a centrally maintained data cube that continues to expand as new tests and novel samples are included. This would allow for more groups to maintain NIRS capacity and could be a game-changer for livestock and forage management in the Australian red meat and research sector. Additionally, if methane calibrations are developed, this could allow rapid, inexpensive quantification of eco-credentials.

5.1 Key findings

- During this project, novel NIRS calibrations were developed, tested, broadened and/or strengthened for the following plant (forage or silage) nutritional traits;
  - Digestibility predictions including dry matter digestibility (DMD), organic matter digestibility (OMD) and DOMD using various methodologies (*in vivo*, pepsin cellulase, daisy, Tilley and Terry) (RPD 1.7 to 4.3),
  - Organic matter (OM) or ash content (RPD 2.7),

- Ether extract (EE, fat content; RPD 3.4),
- Gross Energy (GE; RPD 1.9),
- Water soluble carbohydrates (WSC; RPD 3.4), ethanol soluble carbohydrates (ESC; RPD 2.0) and starch (RPD 5.3),
- Fibre fractions, including acid detergent lignin (ADL; RPD 1.8), acid detergent fibre (ADF; RPD 4.5), neutral detergent fibre (NDF; RPD 4.0), and Cornell fibre fractions - indigestible neutral detergent fibre (iNDF; after 30, 120 and 240 hours; RPD 3.3 to 3.8) and undigested neutral detergent fibre (uNDF; after 30, 120 and 240 hours; RPD 2.2 to 3.2),
- Protein fractions – total nitrogen (N) and crude protein (CP; RPD 7.1), non-protein nitrogen (NPN; RPD2.6), neutral detergent insoluble crude protein (NDICP; RPD 2.2), acid detergent insoluble crude protein (ADICP; RPD 2.0), and rumen digestible protein (RDP; RPD 3.7),
- Anti-nutritional compounds/anions – nitrate (RPD 2.8), oxalate (RPD 2.8), and phosphate (RPD 2.3)
- Methane from fermentation in rumen fluid (RPD 1.8).

- **In the majority of cases, taxonomically broad calibrations predicted the nutritional traits of samples with greater accuracy than calibrations developed specifically for a plant or taxonomically similar group of similar plants.** If more taxonomic, spatial and temporal diversity can be built in without a large reduction in accuracy, these broad NIRS calibrations represent a valuable tool for Australian researchers, feed testing agents and livestock producers, as they encompass nearly all of the species that appear in monocultures or mixed swards. Errors associated with species identification or mixtures of species are avoided. Many of these calibrations are higher than published values. Calibrations were expanded to include over 160 plant species from the Australian feedbase. In most cases, taxonomically diverse calibrations predicted the nutritional traits of samples with greater accuracy than calibrations developed specifically for a plant or group of similar plants.
- **Predicting mineral content of plants**. Strong calibrations for magnesium, calcium and chloride were developed. Phosphorus, sulphur, sodium and potassium can be predicted with a lower level of accuracy.
- **In-field sensing is inaccurate.** The highest quality, laboratory grade, hand-held NIRS sensors, including one with an inbuilt light source were utilised. Many commercial products capture fewer wavelengths and are reliant on ambient light so are highly unlikely to be more accurate than our machines. DMD is one of the few traits that could be predicted, however, there was a statisticially significant and biologically relevant loss of accuracy. This is presumably associated with 'noise' created by ambient light, moisture content and sample heterogeneity. Remote sensing of pasture quality using NIRS is unlikely.
- **Faecal traits that are predicted from faecal samples.** The stable carbon isotope technique allows a prediction of key aspects of diet selection, and very strong calibrations to predict delta carbon from sheep and cattle faeces were developed. Excellent ruminant faecal nitrogen and faecal organic matter calibrations were generated from a combination of sheep and cattle samples.
- **Dietary traits that could be predicted using faeces**. These results are novel and internationally significant. There is a very high prospect for NIRS calibrations to predict;
    - dietary CP intake for sheep and cattle.

  - diet ADF and NDF content was predicted with excellent results, for sheep alone or sheep and cattle combined. This is very exciting as ADF is an indicator of a poor-quality diet and often highly correlated to energy values, animal performance and methane.
  - dietary ash content was predicted with excellent results.
  - *in vivo* DOMD in sheep, with an error of prediction of 3.1% units. This has significant implications for managing sheep in extensive grazing systems and perhaps as phenotyping tool for sheep grazing pasture.
  - evidence that methane could be predicted with faecal NIRS, although low sample numbers is a constraint. Given the ability of the method to predict diet digestibility and indigestible fibre, combined with the preliminary results, it is probable that methane could be predicted. This would be an incredibly useful tool for researchers seeking to identify low methane plants and animals and for industry to benchmark ecocredentials.
  - dietary concentrations of some minerals from faecal samples. Minerals that could be predicted included chloride, sodium, magnesium, calcium, iron, potassium, sulphur and zinc. Other minerals that had potential to be predicted included copper, boron, phosphorus and manganese.
- **Animal performance traits that are predicted using faeces**;
  - voluntary feed intake is an extremely difficult trait to measure in field grazing trials. Calibrations were developed that gave fair screening potential for organic matter intake by sheep. More diversity in the data is likely to improve the calibration,
  - digestible organic matter intake for sheep,
  - average daily liveweight gain from faecal samples was not predicted.
- **Transferring calibrations to new types of NIRS machines**. As part of this project, the Coats faecal NIRS calibrations were successfully transferred to a modern SpectraStar XTR and FOSS XDS. Significant progress was made in demonstrating they can be transferred to a Bruker MPA II. More work is required in validating and expanding these calibrations.

5.2 Benefits to industry

- Rapid and inexpensive prediction of nutritional traits enables better decision making at multiple levels across the red meat industry. This project has improved the accuracy and reliability of NIRS predictions for a range of existing and novel traits. The calibrations are broad and cover up to 160 species of plants that are encountered in the Australian feedbase. Traditional methods of determining nutritional factors influencing intake and digestion are expensive and time consuming. For plants or feeds, cost of analysis can exceed $250 with a turn-around time of weeks. This precludes the routine use by farmers and their advisors and limits the number of tests than can be done by forage and ruminant nutrition researchers. NIRS is a rapid, inexpensive and non-destructive technology that can predict multiple feed quality parameters in a single 10 second scan. The relatively low cost and more rapid turnaround time (1-4 days) means that NIR underpins commercial and research feed analyses in Australia and internationally. Existing calibrations do not predict for the range of parameters now requested by industry. This has led to a reliance on calibrations from international sources, where they are not developed using the feeds and forages utilised in Australia.
- Nutritional value is a critical factor in predicting the productivity and health of ruminants, and utilisation of the on-farm feedbase. Very few researchers, consultants and farmers have the capacity to monitor the quality of the feedbase, and this can lead to less-than-optimal

performance and poor management decisions. Equally, few pasture breeding or selection programs measure nutritional traits throughout a plants lifecycle, and this can lead to suboptimal outcomes for industry and higher methane emissions intensity. Industry strategies, including MISP 2020, identify a lift in on farm productivity as a major imperative for research investment. Tools to help producers become more efficient are a major part of this plan. Accurate determination of feed value will contribute to this goal by improving livestock feeding and feedbase utilisation in Australia. Improvements in diet quality and feed utilisation contribute to the MLA goal of a carbon neutral red meat industry.

- A calibration to predict methane emissions for a faecal sample offers a simple and inexpensive tool for farmers to demonstrate best-practice in methane reduction. Anti-nutritional factors also have a direct bearing on animal health and productivity and include compounds such as tannins, nitrates, prussic acid, saponins and oxalates. Mineral imbalances can also lead to suboptimal growth or toxicity. These antinutritional traits could be exacerbated by future climates, for example, drought conditions can provoke high nitrate and prussic acid levels in sorghum. Early detection of risky levels of these components can prevent production losses and eliminate preventable deaths. Rapid detection using NIRS could potentially provide a tool to identify forages high in these components.
- A further benefit is reducing reliance on international NIRS calibrations that are developed for plants and systems that are not representative of the Australian sector. Little (2014) estimated that 25% of total commercial analyses in Australia were being sent to USA. The implication for commercial laboratories in Australia is that without providing these additional analyses then business will decline. The implications for feed testing in Australian are loss of capacity in feed testing and the ability to develop calibrations for the Australian meat industry. While NIRS is a powerful tool, inappropriate use of the technology can lead to suboptimal decisions. This project demonstrated that robust calibrations for the Australian feedbase, based on Australian pastures, crops, silages and forage could be developed and delivered through Australian laboratories.
- There is significant potential for faecal NIRS as a research, breeding and management tool. There are very few ways to measure individual animal diet selection, intake, diet quality, digestion of nutrients and methane emissions. Animal house feeding and metabolism crate studies are the ‘gold standard’ but they are expensive, labour intensive and involve animal experimentation. In genetic comparisons, there is some debate about the value of EBV’s regarding intake and efficiency that are based on animals housed in sheds and offered a uniform diet. In reality, especially in extensive systems with a diverse feedbase, individuals need to make decisions regarding diet composition and these will vary to optimise livestock intake. Many of the tools to estimate intake and diet selection (on-animal sensors, intake markers, pasture depletion scores etc) are expensive and have various degrees of inaccuracy.
- If developed further by filling a few gaps and refining the calibrations, the faecal NIRS tool could have a significant impact on a producer’s ability to optimise diets and growth rates, manage risk associated with poor nutrition and have a way of inexpensively quantifying methane emissions. This could be used in breeding programmes to identify individuals with superior foraging and diet selection ability. It also offers a rapid and inexpensive tool for industry to track changes in carbon efficiency.

6. Future research and recommendations

- Consider the development of a digital NIRS predictive delivery service that is not reliant on lab-to-lab transfers and is able to evolve and expand to meet ongoing industry needs and capitalise on future R&D. This will also allow for future developments in AI and machine learning to be adopted without a need to transfer new calibrations. This system would allow for automatic

collection of data regarding use, royalties and areas where the calibrations are not accurate and perhaps identify areas that may require more wet chemistry to fill gaps.

- There is also an opportunity to run feedback through ruminant nutrition models to provide animal management data at an additional cost (see appendix 8.4).
- Decide on a delivery mechanism for the plant NIRS calibrations that are ready for commercialisation. Outline a plan to finalise others that are promising but require refinement.
- Progress the research into developing faecal NIRS calibrations for sheep and cattle across Australia.
- Conduct an animal house metabolism crate/respiration chamber experiment with sheep to fill critical gaps in the sheep/cattle faecal database. This would encompass 16 diverse feeds, restricted and ad. lib. feeding, determination of intake, nutrient digestion and individual methane emissions. The feeds become forage standards to calibrate fermentability methods (reducing the need for methane chamber experiments) and the faecal samples will fill critical gaps in the faecal/diet reference set.
- The adoption mechanism is already in place as many industry participants utilise feed testing laboratories. The NSWDPI team manage a large commercial testing laboratory so the feed calibrations can be utilised immediately. Future development and adoption activities will be dependent on the form and access mechanism of the product that is commercialised.

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8. Appendix

8.1 Literature search providing examples of calibration development for dry and fresh material, narrow versus broad taxonomy.

Table 8.1.1 Examples of calibrations for digestibility from the literature.

Trait	Fresh/dry	Narrow/broad	Spp	n	SEP	SECV	R^2	**RPD**	Reference
DMD (T&T)	dry	narrow	Paspalum grass (1 spp)	275		3.20	0.56	**1.5**	Myer *et al* 2011
	dry	narrow	Grass silages		3.60		0.64	**1.7**	grass silages
	dry	narrow	Whole cereal crops	145		2.05	0.86	**2.7**	Deaville *et al* 2009
	Fresh	broad	Subtropical pastures	109	2.98	2.41	0.76	**2.0**	Lobos *et al* 2019
DMD or OMD (*in vivo*)	dry	narrow	Whole cereal crops	145		3.05	0.39	**1.3**	Deaville *et al* 2009
	dry	narrow	Grass silages				0.79	**2.2**	De Boever *et al* 1996
DMD or OMD (PC)	dry	narrow	Grass silages		4.70		0.40	**1.3**	grass silages
	dry	narrow	Grass silages				0.64	**1.7**	De Boever *et al* 1996
	dry	narrow	Sagebrush (1 spp)			2.50	0.83	**2.4**	Olsoy *et al* 2016
	dry	narrow	Forage maize (1 spp)				0.84	**2.3**	Hetta *et al* 2017
	dry	broad	Chenopod shrubs (8 spp)	250		2.50	0.92	**3.5**	Norman *et al* 2010

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are poor, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

Table 8.1.2 Examples of calibrations for fibre content from the literature.

Trait	Fresh/dry	Narrow/broad	Spp	n	SEP	SECV	R^2	**RPD**	Reference
ADF	dry	narrow	*Cephalaria joppensis* (1 spp)	73	1.70	1.64	0.91	**3.3**	Galili *et al* 2018
		broad	Chenopod shrubs (8 spp)	250		1.40	0.91	**3.3**	Norman *et al* 2010
		narrow	Oaten hays (1 spp)			1.24	0.93	**3.8**	Henry *et al* 2000
		broad	Gorilla foods (13 spp)	241	2.01		0.97	**5.8**	Rothman *et al* 2009
	Fresh	broad	Grasses, legumes and cereals	107		1.40	0.90	**3.2**	Alomar *et al* 2009
	Fresh (hay)	broad	Mixed temperate hays	48	2.11	1.62	0.87	**2.8**	Zicarelli *et al* 2022
NDF	dry	narrow	Paspalum grass (1 spp)	275		2.60	0.69	**1.8**	Myer *et al* 2011
		narrow	Barley hay (1 spp)		2.46	2.46	0.73	**1.9**	Hsu *et al* 2000
		narrow	Oaten hays (1 spp)			1.82	0.89	**3.0**	Henry *et al* 2000
		broad	Chenopod shrubs (8 spp)	250		2.00	0.90	**3.2**	Norman *et al* 2010
		narrow	Cephalaria joppensis (1 spp)	73	1.70	1.64	0.91	**3.3**	Galili *et al*. 2018
		narrow	Forage maize (1 spp)				0.92	**3.4**	Hetta *et al* 2017
		less narrow	Barley hay and silage (1 spp)		2.34	2.34	0.92	**3.5**	Hsu *et al* 2000
		narrow	Barley silage (1 spp)		1.54	1.54	0.92	**3.5**	Hsu *et al* 2000
		narrow	Cereals only	300	0.94		0.93	**3.8**	Stubbs *et al* 2010
		broad	Gorilla foods (13 spp)	241	1.18		0.95	**4.5**	Rothman *et al* 2009
		narrow	Lucerne (1 spp)		1.46	1.46	0.95	**4.5**	Hsu *et al* 2000
		narrow	Whole cereal crops	145		1.76	0.96	**5.0**	Deaville *et al* 2009
		less narrow	Legumes (4 spp)		2.23	2.23	0.98	**7.1**	Hsu *et al* 2000
	Fresh	broad	Subtropical pastures	113	2.45	2.89	0.78	**2.1**	Lobos *et al* 2019
		broad	Grasses, legumes and cereals	107		3.35	0.80	**2.2**	Alomar *et al* 2009
	Fresh (hay)	broad	Mixed temperate hays	48	2.85	2.20	0.92	**3.5**	Zicarelli *et al* 2022

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are poor, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

Table 8.1.3 Examples of calibrations for crude protein content from the literature.

Fresh/dry	Narrow/broad	Spp	n	SEP	SECV	R^2	**RPD**	Reference
dry	narrow	Cereals only	300	0.56		0.48	**1.4**	Stubbs *et al* 2010
	narrow	Whole cereal crops	145		1.73	0.84	**2.5**	Deaville *et al* 2009
	broad	Chenopod shrubs (8 spp)	250		2.50	0.88	**2.9**	Norman *et al* 2010
	narrow	Paspalum grass (1 spp)	275		1.80	0.89	**3.0**	Myer *et al* 2011
	narrow	One species only	46-228	0.57-1.66		0.90	**3.2**	Andueza *et al* 2011
	narrow	Legume only	140	0.99		0.91	**3.3**	Andueza *et al* 2011
	narrow	*Cephalaria joppensis* (1 spp)	73	0.60	0.95	0.93	**3.8**	Galili *et al*. 2018
	narrow	Grass only	742	1.13		0.93	**3.8**	Andueza *et al* 2011
	narrow	Sagebrush (1 spp)			0.50	0.93	**3.8**	Olsoy *et al* 2016
	broad	Broad forage range (12 spp)	884	1.14		0.94	**4.1**	Andueza *et al* 2011
	narrow	Forage maize (1 spp)				0.94	**3.2**	Hetta *et al* 2017
	narrow	Barley hay and silage (1 spp)			0.52	0.95	**4.5**	Hsu *et al* 2000
	broad	Gorilla foods (13 spp)	241	2.01		0.95	**4.5**	Rothman *et al* 2009
	narrow	Barley hay (1 spp)			0.48	0.96	**5.0**	Hsu *et al* 2000
	narrow	Lucerne (1 spp)				0.96	**5.0**	Nie *et al* 2008
	narrow	Oaten hays (1 spp)			0.32	0.96	**5.0**	Henry *et al* 2000
	narrow	Legumes (4 spp)			1.00	0.97	**5.8**	Hsu *et al* 2000
	narrow	Barley silage (1 spp)			0.41	0.97	**5.8**	Hsu *et al* 2000
	narrow	Lucerne (1 spp)			0.42	0.98	**7.1**	Hsu *et al* 2000
Fresh	broad	Subtropical pastures	456	2.04	2.22	0.84	**2.5**	Lobos *et al* 2019
	broad	Grasses, legumes and cereals	107		1.68	0.93	**3.8**	Alomar *et al* 2009
Fresh (hay)	broad	Mixed temperate hays	48	1.22	0.88	0.95	**4.5**	Zicarelli *et al* 2022

Accuracy is expressed in the manner of Williams (2014) - RPD values of 0 to 1.9 are very poor, 2.0 to 2.4 are poor, 2.5 to 2.9 offer a fair screening potential, 3.0 to 3.4 are quality control; 3.5 to 4.0 are very good, greater than 4.1 are deemed excellent.

8.2 LPP projects that have contributed samples for analyses to LPP NIR laboratories

This project was part of the Livestock Productivity Partnership, and the project team conduced analysis across the projects. All samples (n=7625, Table 8.4) were scanned and predicted using the evolving NIRS calibrations. Any sample that was analysed using wet chemistry has been matched to the spectra and incorporated into the project databases. The samples represented a diverse range of species from subtropical and temperate rainfall zones (Table 8.5). There were also 612 faecal samples from Ed Charmley's cattle projects

Table 8.2.1. Samples that were analysed as part of the Livestock Productivity Partnership, where wet chemistry has been conducted the spectra and chemistry was incorporated into the project databases.

Lab	Project	n
NSW	Perennial wheats (Hayes, Newell)	375
NSW	Grazing brassicas (Hunt, Bell, Watt)	933
WA	Forage brassicas (Bell, Watt and Stutz)	2050
NSW	Temperate perennial legumes (Norton)	287
WA	Temperate perennial legumes and grasses (Culvenor/Stutz)	2290
NSW	Tropical pastures (Boschma, Toole, Newell & Munday)	456
WA	Tropical pastures (Charmley)	622
WA	Faecal samples (cattle), tropical pastures (Charmley)	612

Table 8.2.2. Distribution of Livestock Productivity Partnership samples by species and wet chemistry measurement.

Forage Type	Digestibility	ADF and NDF	Crude protein	WSC
Temperate Grass	538	462	624	624
Temperate Legumes	1408	1521	1633	701
Temperate Grass Legume Mix	40	171	91	745
Temperate Grass Cereal Mix	0	9	5	7
Cereal	879	1113	4131	1409
Cereal Legume Mix	36	23	79	16
Chicory	103	103	165	2
Plantain	29	26	28	5
Brassica	331	359	1429	178
Tropical Grasses	1210	1035	1252	783
Tropical Legumes	213	233	49	274
Tropical Grass Legume Mix	0	4	1	2
Millet	50	33	36	52
Sorghums	88	95	109	202
Maize	21	23	129	84
Native	478	493	532	230
Unspecified	988	1248	5774	1173
Total	**6412**	**6951**	**16067**	**6487**

8.3 Results of the patent search for freedom to operate.

Results of the patent search for freedom to operate.

CSIRO conducted a patent search relating to technology which uses Near Infrared Reflectance Spectroscopy (NIRS) to determine parameters for animal management models. We included:

- NIRS analysis of animal faecal samples and / or NIRS analysis of feed or grain;
- determination of animal management (or performance) model factors or parameters from the NIRS analysis; and
- system calibration testing and refining, and the broadening and development of additional calibrations (for new animals and quality and animal performance parameters).

The aim of the searching was to form a basis for an Australian Freedom to Operate (FTO) opinion by identifying any potentially relevant patents published in Australia which may prohibit the use / licencing of our existing technology. Two sources were searched; GOOGLE Patents and Orbit FAMPAT Database.

Forty seven potentially relevant patent families (which are published in Australia or could potentially still come into Australia via the PCT National Phase) were discovered. These are listed in the attachment. Of these, we would consider only 6 to have some relevance to our project. They are highlighted in the attachment and listed below with an abstract. At this stage, we do not see a lot of overlap with our faecal NIRS goals.

- **EP3361248 A1**. Method for the determination of processing influences on the nutritional value of feedstuff raw materials (EVONIK DEGUSSA).
  The present invention relates to a method for the determination of processing influences on the quality of feedstuff raw materials and/or feedstuffs, in which the processing conditions indicator of the of feedstuff raw materials and/or feedstuffs is determined and the specific digestibility coefficient of an amino acid of a feedstuff raw material and/or feedstuff in an animal species is determined. The present invention also relates to a process for the optimization of feedstuffs considering the determined processing influences and the thus obtained and/or obtainable feedstuffs
- **FR2737781 A1**. Feed digestibility measurement in ruminants (RHONE POULENC)
  Method for determining *in vivo* the nutritional value of animal feed during the transit of the feed in the digestive system of ruminants, comprising analysing said feed, more particularly ensilage, by near infrared spectrophotometry.
- **WO200813941 A2**. System for real-time characterization of ruminant feed components (NUTRI INNOVATIONS)
  A computer-based system for characterizing in real time the nutritional components of one of more ingredients for a ruminant feed ration, including dry matter, NDF, NDFd, lignified NDF ratio, percent starch, IVSD, and particle size for a forage material; and IVSD and particle size for a grain material. The system utilizes proprietary NIRS equations based upon prior samplings of a variety of crop species like dual-purpose com silage, leafy corn silage, brown midrib ("BMR") corn silage, grass (silage/dry), alfalfa (silage/dry), BMR forage sorghum, normal dent starch grain, floury endosperm starch grain, and vitreous endosperm grain, and applies those equations to current samplings of a corresponding crop to predict in real time the characteristics of such forage or grain material.. The real-time characterization system may also utilize the predicted data to calculate a "ration fermentability index" value that takes into account the total NDFd and IVSD characteristics (including RAS and RBS) of the forage and starch ingredients to be used in a feed ration to ensure that the ration will not contribute too much or too little digestibility to the cow.

- **WO2004113506 A2**. A method for the development of ruminant feed formulations (FORAGE GENETICS INTERNATIONAL)
  A method that accounts for environmental factors by measuring the starch and fiber degradation characteristics of a variety of genetically different crop plants and grain from crop plants in real time to determine how the crop plants should be conserved, processed and blended into a feed formulation that results in optimum productivity of the ruminant animal. A method further including determining starch digestibility characteristics of a set of crop plant samples comprising grain of said crop plant; developing a prediction equation based on said starch digestibility characteristics, obtaining a grain sample from a crop plant, determining in real time starch digestibility characteristics by LAIRS of said sample by inputting data from said LAIRS into said equation, storing and/or milling said grain on an identity preserved basis, and determining the amount of said crop plant to incorporate into a feed formulation based on the starch digestibility characteristics.
- **WO2005111560 A1.** A method and a system for the assessment of samples (CHEMOMETEC)
  The present invention offers an alternative strategy for the correlation of interference information to chemical and/or physical properties of a sample. This strategy can be implemented in a method and a system, which offer substantial technical and commercial advantages over state of the art techniques based on interference spectroscopy. The invention further provides a method for standardizing an interferometer, as well as a method and a system using the standardized interferometer.
- **CA2839029 A1.** Systems and methods for estimating feed efficiency and carbon footprint for meat producing animal (ALLTECH)
  Systems and methods for estimating meat producing animal feed conversion efficiency and carbon footprint, such as to allow adjustments to be made in the animals feed to improve meat production, reduce waste, and/or reduce the carbon footprint. In embodiments of the present application, a system is provided that integrates a digestion model of an animal feed with weight gain efficiency and carbon footprint. Such systems and methods are useful to analyze and compare different animal feed compositions that differ from one another in one or more components and/or to analyze the effect of the addition of a feed supplement on weight gain efficiency and/or carbon footprint. In embodiments, the systems and methods described herein provide a feed parameter-carbon footprint compromise.

8.4 Opportunity for a digital delivery system.

Transfer of calibrations to other machines is laborious and it not simple to expand the calibrations so they become static and increasingly obsolete. We believe the best solution is a digital platform that could provide near real time predictions from spectra uploaded from a range of machines. CSIRO Mineral Resources have an NIRS delivery prototype that was being used by external mining clients (see schematic below). The platform allows for spectra to be uploaded, quality assessment of data, spectral matching to the 'mother' machine, charging a credit card and automated feedback to the client. If the forage/faecal NIRS capability could be accessed by industry in a similar way – it would have significant impact. There is also an opportunity to run feedback through ruminant nutrition models to provide animal management data at an additional cost.

The digital platform has a need for an industry-facing digital capability and a separate laboratory quality control and expansion capability. If additional plant or livestock advice was to be offered, it would have a modelling node. While useful, we may not need to address differences in data streams associated with how labs capture spectra if collections are scanned with both machines (as is the case currently). There is a significant opportunity to predict methane from livestock in extensive systems as well as the key productivity traits. Historical samples have been exhausted and there are

a few gaps. This work will require an animal house experiment to generate faecal/diet pairs with associated methane chamber data. MLA have indicated a willingness to invest, and we have the capability to do this in association with other industry funded research.

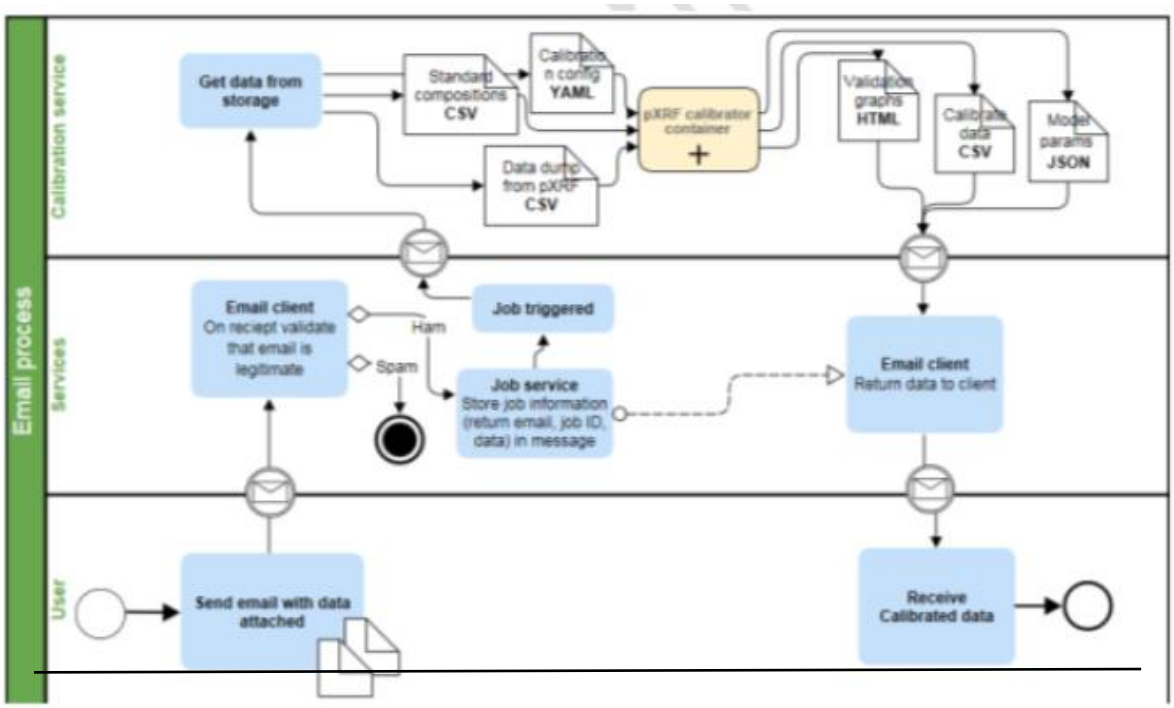

Figure 8.1 Model of how the CSIRO Minerals team deliver NIRS to the mining industry.

Table 8.5 Coding system used for labelling samples used in the project

Group	Subgroup	Type
B	B	Byproducts unspecified or unclassified
B	BWCS	Whole Cotton seed
B	BP	Byproducts, apple, olive, pomice,almond hulls, citrus pulp
BDG	BDG	Byproducts, distellers grain
BGM	BGM	Byproducts, grape marc
BM	BM	meal unspecified
BM	BMCS	Cotton seed meal
BM	BMCa	Canola meal
CH	CH	Chenopod, unspecified
CH	CHSB	Chenopod saltbush
F	F	Forage, unspec
FBr	FBrTG	Forage brassica, temporate grass mix
FBr	FBrC	Forage Canola
FBr	FBr	Fresh forage, forage brassicas
FCe	FCe	Forage Cereal
FCe	FCeW	Forage Cereal wheat
FCe	FCeB	Forage Cereal barley
FCe	FCeO	Forage Cereal oats
FCe	FCeT	Forage Cereal triticale
FCe	FCeR	Forage Cereal rye
FL	FLTr	Fresh forage, tropical legumes
FL	FLLu	Fresh Forage, Lucerne
FL	FLT	Fresh forage temperate legumes
FM	FMTL	Fresh forage, mixed temperategrass-legume pasture
FM	FMTrL	Fresh forage, mixed tropical grass-legume pasture
Fmi	Fmi	Fresh forage, forage pennisetum and other millets
FN	FN	Fresh forage, Native
FS	FS	Fresh forage, forage sorghums
FT	FTC	Fresh Forage, temperate, chickory
FT	FTG	Fresh Forage, temperate grass
FTr	FTrG	Fresh forage, tropical grasses
G	GMz	Grain, maize
G	GC	Grain, canola
G	GB	Grain, brewers /distillers
GB	GCe	Grain, cereal unspec
GCe	GCeW	Grain, cereal wheat
GCe	GCeO	Grain, cereal oats
GCe	GCeB	Grain, cereal barley
GCe	GCeT	Grain, cereal triticale
GCe	GL	Grain, legume, (faba beans, chick peas, lentils, lupins)
GL	GM	Grain,Meal
GM	GTr	Grain, tropical
GTr	H	Hay, unspec
H	HBrC	Hay Canola
HBr	HCe	Hay Cereal, unspec
HCe	HCeW	Hay Cereal wheat
HCe	HCeB	Hay Cereal barley
HCe	HCeO	Hay Cereal oats
HCe	HCeT	Hay Cereal triticale
HCe	HCeR	Hay Cereal rye
HCe	HL	Silage, legume unspec

HL	HLLu	Hay, Lucerne
HL	HLV	Hay, legume vetch
HL	HMTL	Hay, mixed temperategrass-legume pasture
HM	HCeL	Hay, cereal-legume mix
HM	HMTrL	Hay, mixed tropical grass-legume pasture
HM	Hmi	Hay, forage pennisetum and other millets
Hmi	HN	Hay, Native
HN	HTC	Hay, temperate, chickory
HT	HTG	Hay, temperate grass
HT	HTrK	Hay tropical, kikuyu
HTr	HLTr	Hay, tropical legumes
HTr	HTrS	Hay tropical, forage sorghums
HTr	HTrG	Hay, tropical grasses
HTr	HTrRh	Hay tropical, rhodes grass
HTr	TMR	Total mixed rations
MR	PMR	Partial mixed ration
MR	P	Pellets
P	S	Silage, unspec
S	SMz	Silage, maize
S	SBrC	Silage Canola
SBr	SCe	Silage Cereal, unspec
SCe	SCeW	Silage Cereal wheat
SCe	SCeB	Silage Cereal barley
SCe	SCeO	Silage Cereal oats
SCe	SCeT	Silage Cereal triticale
SCe	SCeR	Silage Cereal rye
SCe	SL	Silage, legume unspec
SL	SLCl	Silage, legume clover
SL	SLLu	Silage, legume lucerne
SL	SLV	Silage, legume vetch
SL	SMTL	Silage, mixed temperategrass-legume pasture
SM	SMTC	Silage, mixed temperategrass-cereal pasture
SM	SCeL	Silage, cereal-legume mix
SM	SMTrL	Silage, mixed tropical grass-legume pasture
SM	Smi	Silage, forage pennisetum and other millets
Smi	StS	Straw, sorghum stubble
St	StC	Straw, cereal
St	StL	Straw, legume
St	STG	Silage, temperate grass
ST	STrK	Silage tropical, kikuyu
STr	SLTr	Silage, tropical legumes
STr	STrS	Silage tropical, sorghums
STr	STrG	Silage, tropical grasses

8.6. *In vivo* feeding samples that were collected and used in this project.

Source	Original ID NO	Feed description	Animal	Feeding	*In vivo* DMD g/kg ODM	*In vivo* OMD g/kg ODM	*In vivo* DOMD g/kg ODM
Ag Vic, Hamilton	FQS042	BAL R2 Balansa Clover hay	sheep	*ad lib*	662	668	594
	FQS042	BAL R2 Balansa Clover hay	sheep	M	671	681	605
	FQS044	LUC R1 Lucerne hay	sheep	M	619	626	583
	FQS044	LUC R1 Lucerne hay	sheep	*ad lib*	626	633	590
	FQS049	LUC R2 Lucerne hay	sheep	M	587	608	566
	FQS049	LUC R2 Lucerne hay	sheep	*ad lib*	604	621	578
	FQS043	MED R1 Medic hay	sheep	*ad lib*	686	686	617
	FQS043	MED R1 Medic hay	sheep	M	687	694	624
	FQS047	PAG R1 Pasture good hay	sheep	*ad lib*	685	695	617
	FQS047	PAG R1 Pasture good hay	sheep	M	686	702	623
	FQS045	PAS R2 Pasture hay	sheep	*ad lib*	605	620	570
	FQS045	PAS R2 Pasture hay	sheep	M	610	627	576
	FQS048	PER R1 Persian clover hay	sheep	M	677	686	617
	FQS048	PER R1 Persian clover hay	sheep	*ad lib*	678	682	614
	FQS046	VET R1 Vetch hay	sheep	*ad lib*	531	553	507
	FQS046	VET R1 Vetch hay	sheep	M	558	576	528
	FQS050	VET R2 Vetch hay	sheep	*ad lib*	658	673	608
	FQS050	VET R2 Vetch hay	sheep	M	667	687	621
	FQS051	WHT R2 Frosted wheat hay	sheep	M	557	605	529
CSIRO, David Coates	CSIRO-3021	Blue couch	sheep		535		
	CSIRO-2227	Buffel 1	sheep		540		
	CSIRO-3019	Cavalcade	sheep		577		
	CSIRO-3250/3251	Clitoria diet	sheep		534		
	CSIRO-3022/3020/3024	FCR/Verano (2parts/1part)	sheep		594		
	CSIRO-3460	Humidicola	sheep		493		
	CSIRO-3346	LDN Buffel	sheep		420		
	CSIRO-3345	LDN Pertusa	sheep		472		
	CSIRO-3249	LDN Verano	sheep		501		
	CSIRO-3249/3252	LDN Verano/Buffel (60:40)	sheep		518		
	CSIRO-3347	Lucerne	sheep		683		
	CSIRO-3455/3456/3457	Lucerne/Oats/Pigeon	sheep		710		
	CSIRO-3454	Millet	sheep		600		
	CSIRO-3453	Mitchell	sheep		512		
	CSIRO-2727	native pasture	sheep		457		
	CSIRO-3343/3344	Oaten hay/FCR (75:25)	sheep		727		
	CSIRO-3254/3246	Peanut hay/FCR (70:30)	sheep		544		
	CSIRO-3253	Purple pigeon	sheep		565		
	CSIRO-3458/3459	Seca/Buffel	sheep		489		
	CSIRO-2228	Uro 1	sheep		547		
	CSIRO-2728	Uro 3	sheep		455		
	CSIRO-2729	Uro 4	sheep		511		
	CSIRO-3020/3024	Verano - Harts	sheep		565		
	CSIRO-3342	Wheat	sheep		685		
	CSIRO-3342	Wheat	sheep		688		

CSIRO, Hayley Norman	CSIRO-Diet 7	100% C4 grass (rhodes)	sheep	M	547	569	**508**
	CSIRO-Diet A	Acacia saligna	sheep	M	357	356	**328**
	CSIRO-Diet B	Bluebush	sheep	M	528	423	**313**
	CSIRO-Diet C	Creeping saltbush	sheep	M	479	484	**387**
	CSIRO-Diet #1	legume hay - 100%	sheep	M	663	676	**631**
	CSIRO-Diet K	Lucerne	sheep	M	616	641	**582**
	CSIRO-Diet Nypa	Nypa grass	sheep	M	491	492	**464**
	CSIRO-Diet oaten hay	Oaten hay	sheep	M	661	666	**626**
	CSIRO-Diet MIX	Oldman saltbush	sheep	M	635	543	**378**
	CSIRO-Diet CAR	Oldman saltbush (car)	sheep	M	616	549	**418**
	CSIRO-Diet F	Oldman saltbush (EGG)	sheep	M	592	480	**315**
	CSIRO-Diet LEF	Oldman saltbush (Lefroy, spath)	sheep	M	592	506	**360**
	CSIRO-Diet D	Oldman saltbush (spath)	sheep	M	609	476	**309**
	CSIRO-Diet YAR	Oldman saltbush (spath)	sheep	M	621	523	**352**
	CSIRO-Diet H	Oldman saltbush (Yealering)	sheep	M	674	640	**500**
	CSIRO-Diet pea / lupins	Pea hay and lupin grain	sheep	M	562	589	**553**
	CSIRO-Diet Pellet prefeed	Pellet prefeed	sheep	M	656	676	**640**
	CSIRO-Diet I	River saltbush	sheep	M	597	558	**436**
	CSIRO-Diet J	Tagasaste	sheep	M	646	654	**630**
DPIRD, Bunbury	CSIRO-DB6	1-barley 2-hay	sheep	M	710		
	CSIRO-DB12	3-grain 7-hay	sheep	M	710		
	CSIRO-DB13	4-grain 6-hay	sheep	M	730		
	CSIRO-DB3	avg clover grass weed hay	sheep	M	570		
	CSIRO-DB38	clover rye	sheep	M	780		
	CSIRO-DB15	hay 2-grain	sheep	M	820		
	CSIRO-DB11	lupin	sheep	M	900		
	CSIRO-DB21	meadow silage	sheep	M	670		
	CSIRO-DB43	oats	sheep	M	530		
	CSIRO-DB1	poor cereal straw	sheep	M	490		
	CSIRO-DB4	poor clover grass weed hay	sheep	M	530		
	CSIRO-DB5	rain damage kikuyu	sheep	M	500		
Minson	MIN205	buffel grass	sheep	*ad lib*	510		
	MIN192	buffel grass	sheep	*ad lib*	560		
	MIN195	buffel grass	sheep	*ad lib*	610		
	MIN377	buffel grass	sheep	*ad lib*	620		
	MIN389	buffel grass	sheep	*ad lib*	620		
	MIN391	buffel grass	sheep	*ad lib*	620		
	MIN392	buffel grass	sheep	*ad lib*	620		
	MIN416	buffel grass	sheep	*ad lib*	620		
	MIN362	buffel grass	sheep	*ad lib*	640		
	MIN373	buffel grass	sheep	*ad lib*	640		
	MIN375	buffel grass	sheep	*ad lib*	640		
	MIN378	buffel grass	sheep	*ad lib*	640		
	MIN374	buffel grass	sheep	*ad lib*	650		
	MIN376	buffel grass	sheep	*ad lib*	650		
	MIN372	buffel grass	sheep	*ad lib*	660		
	MIN617	commercil Green Panicum	sheep	*ad lib*	480	500	**439**
	MIN595	commercil Green Panicum	sheep	*ad lib*	600		
	MIN422	cowpea	sheep	*ad lib*	590		
	MIN406	cowpea	sheep	*ad lib*	640		
	MIN423	dolichos	sheep	*ad lib*	580		
	MIN407	dolichos	sheep	*ad lib*	590		
	MIN797	kikuyu	sheep	*ad lib*	450		

MIN646	kikuyu	sheep	*ad lib*	570	580	**519**
MIN461	lucerne	sheep	*ad lib*	700		
MIN618	nth Qld Guinea grass	sheep	*ad lib*	510	540	**467**
MIN543	nth Qld Guinea grass	sheep	*ad lib*	590	630	**522**
MIN529	oats	sheep	*ad lib*	680		
MIN505	oats	sheep	*ad lib*	690		
MIN525	oats	sheep	*ad lib*	690		
MIN528	oats	sheep	*ad lib*	690		
MIN515	oats	sheep	*ad lib*	700		
MIN535	oats	sheep	*ad lib*	700		
MIN536	oats	sheep	*ad lib*	700		
MIN538	oats	sheep	*ad lib*	700		
MIN513	oats	sheep	*ad lib*	710		
MIN531	oats	sheep	*ad lib*	710		
MIN501	oats	sheep	*ad lib*	720		
MIN517	oats	sheep	*ad lib*	720		
MIN519	oats	sheep	*ad lib*	720		
MIN524	oats	sheep	*ad lib*	720		
MIN533	oats	sheep	*ad lib*	720		
MIN504	oats	sheep	*ad lib*	730		
MIN511	oats	sheep	*ad lib*	730		
MIN514	oats	sheep	*ad lib*	730		
MIN526	oats	sheep	*ad lib*	730		
MIN532	oats	sheep	*ad lib*	730		
MIN509	oats	sheep	*ad lib*	740		
MIN516	oats	sheep	*ad lib*	740		
MIN521	oats	sheep	*ad lib*	740		
MIN530	oats	sheep	*ad lib*	740		
MIN510	oats	sheep	*ad lib*	750		
MIN512	oats	sheep	*ad lib*	750		
MIN523	oats	sheep	*ad lib*	750		
MIN502	oats	sheep	*ad lib*	760		
MIN522	oats	sheep	*ad lib*	760		
MIN506	oats	sheep	*ad lib*	770		
MIN518	oats	sheep	*ad lib*	770		
MIN520	oats	sheep	*ad lib*	770		
MIN716	pangola grass	sheep	*ad lib*	580		
MIN770	pangola grass	sheep	*ad lib*	620		
MIN351	pelleted buffel grass	sheep	*ad lib*	360		
MIN352	pelleted buffel grass	sheep	*ad lib*	390		
MIN349	pelleted buffel grass	sheep	*ad lib*	450		
MIN346	rhodes grass	sheep	*ad lib*	490	520	**451**
MIN345	rhodes grass	sheep	*ad lib*	510	540	**464**
MIN337	rhodes grass	sheep	*ad lib*	550	570	**486**
MIN338	rhodes grass	sheep	*ad lib*	560	590	**519**
MIN310	rhodes grass	sheep	*ad lib*	570	600	**526**
MIN311	rhodes grass	sheep	*ad lib*	570	590	**519**
MIN312	rhodes grass	sheep	*ad lib*	570	590	**518**
MIN307	rhodes grass	sheep	*ad lib*	580	600	**534**
MIN332	rhodes grass	sheep	*ad lib*	580	600	**520**
MIN327	rhodes grass	sheep	*ad lib*	590	630	**544**
MIN329	rhodes grass	sheep	*ad lib*	590	640	**552**
MIN331	rhodes grass	sheep	*ad lib*	590	610	**526**
MIN318	rhodes grass	sheep	*ad lib*	600	630	**546**
MIN330	rhodes grass	sheep	*ad lib*	600	630	**527**
MIN316	rhodes grass	sheep	*ad lib*	610	640	**569**
MIN325	rhodes grass	sheep	*ad lib*	610	650	**561**
MIN326	rhodes grass	sheep	*ad lib*	610	640	**561**

MIN333	rhodes grass	sheep	*ad lib*	610	650	**553**
MIN334	rhodes grass	sheep	*ad lib*	610	640	**558**
MIN309	rhodes grass	sheep	*ad lib*	620	630	**559**
MIN314	rhodes grass	sheep	*ad lib*	620	640	**557**
MIN328	rhodes grass	sheep	*ad lib*	620	660	**571**
MIN697	rhodes grass	sheep	*ad lib*	620		
MIN315	rhodes grass	sheep	*ad lib*	630	660	**570**
MIN304	rhodes grass	sheep	*ad lib*	640	670	**578**
MIN313	rhodes grass	sheep	*ad lib*	650	680	**583**
MIN323	rhodes grass	sheep	*ad lib*	650	690	**601**
MIN324	rhodes grass	sheep	*ad lib*	650	680	**577**
MIN317	rhodes grass	sheep	*ad lib*	660	670	**574**
MIN321	rhodes grass	sheep	*ad lib*	660	690	**591**
MIN322	rhodes grass	sheep	*ad lib*	660	690	**593**
MIN462	rompha grass	sheep	*ad lib*	680		
MIN436	rompha grass	sheep	*ad lib*	740		
MIN463	ryegrass	sheep	*ad lib*	740		
MIN397	setaria	sheep	*ad lib*	610		
MIN366	setaria	sheep	*ad lib*	630		
MIN353	setaria	sheep	*ad lib*	640		
MIN357	setaria	sheep	*ad lib*	640		
MIN365	setaria	sheep	*ad lib*	640		
MIN369	setaria	sheep	*ad lib*	640		
MIN396	setaria	sheep	*ad lib*	640		
MIN356	setaria	sheep	*ad lib*	650		
MIN367	setaria	sheep	*ad lib*	650		
MIN382	setaria	sheep	*ad lib*	650		
MIN383	setaria	sheep	*ad lib*	650		
MIN355	setaria	sheep	*ad lib*	660		
MIN277	Tropical Grass	sheep	*ad lib*	360		
MIN348	Tropical Grass	sheep	*ad lib*	380		
MIN348	Tropical Grass	sheep	*ad lib*	380		
MIN488	Tropical Grass	sheep	*ad lib*	400		
MIN219	Tropical Grass	sheep	*ad lib*	410		
MIN404	Tropical Grass	sheep	*ad lib*	450		
MIN400	Tropical Grass	sheep	*ad lib*	460		
MIN566	Tropical Grass	sheep	*ad lib*	460	460	**419**
MIN402	Tropical Grass	sheep	*ad lib*	470		
MIN472	Tropical Grass	sheep	*ad lib*	470		
MIN489	Tropical Grass	sheep	*ad lib*	470		
MIN494	Tropical Grass	sheep	*ad lib*	470		
MIN211	Tropical Grass	sheep	*ad lib*	480		
MIN399	Tropical Grass	sheep	*ad lib*	480		
MIN429	Tropical Grass	sheep	*ad lib*	480		
MIN403	Tropical Grass	sheep	*ad lib*	490		
MIN567	Tropical Grass	sheep	*ad lib*	490	500	**450**
MIN619	Tropical Grass	sheep	*ad lib*	490	510	**459**
MIN276	Tropical Grass	sheep	*ad lib*	510		
MIN445	Tropical Grass	sheep	*ad lib*	510		
MIN783	Tropical Grass	sheep	*ad lib*	520		
MIN802	Tropical Grass	sheep	*ad lib*	520		
MIN448	Tropical Grass	sheep	*ad lib*	530		
MIN616	Tropical Grass	sheep	*ad lib*	530	560	**480**
MIN419	Tropical Grass	sheep	*ad lib*	540		
MIN458	Tropical Grass	sheep	*ad lib*	540		
MIN800	Tropical Grass	sheep	*ad lib*	540		
MIN552	Tropical Grass	sheep	*ad lib*	550	550	**492**
MIN804	Tropical Grass	sheep	*ad lib*	550		

	MIN196	Tropical Grass	sheep	*ad lib*	560		
	MIN435	Tropical Grass	sheep	*ad lib*	560		
	MIN491	Tropical Grass	sheep	*ad lib*	570		
	MIN418	Tropical Grass	sheep	*ad lib*	580		
	MIN467	Tropical Grass	sheep	*ad lib*	580		
	MIN191	Tropical Grass	sheep	*ad lib*	590		
	MIN411	Tropical Grass	sheep	*ad lib*	590		
	MIN412	Tropical Grass	sheep	*ad lib*	590		
	MIN417	Tropical Grass	sheep	*ad lib*	590		
	MIN420	Tropical Grass	sheep	*ad lib*	590		
	MIN544	Tropical Grass	sheep	*ad lib*	590	620	**533**
	MIN764	Tropical Grass	sheep	*ad lib*	600		
	MIN433	Tropical Grass	sheep	*ad lib*	610		
	MIN490	Tropical Grass	sheep	*ad lib*	610		
	MIN545	Tropical Grass	sheep	*ad lib*	610	630	**550**
	MIN547	Tropical Grass	sheep	*ad lib*	610	640	**542**
	MIN580	Tropical Grass	sheep	*ad lib*	610	620	**548**
	MIN762	Tropical Grass	sheep	*ad lib*	610		
	MIN540	Tropical Grass	sheep	*ad lib*	620	640	**553**
	MIN469	Tropical Grass	sheep	*ad lib*	630		
	MIN473	Tropical Grass	sheep	*ad lib*	630		
	MIN572	Tropical Grass	sheep	*ad lib*	630		
	MIN550	Tropical Grass	sheep	*ad lib*	640	660	**575**
	MIN503	Tropical Grass	sheep	*ad lib*	720		
Minson, WWAI	MIN206	buffel grass	sheep	*ad lib*	480		
	MIN358	buffel grass	sheep	*ad lib*	560		
	MIN388	buffel grass	sheep	*ad lib*	620		
	MIN390	buffel grass	sheep	*ad lib*	620		
	MIN361	buffel grass	sheep	*ad lib*	630		
	MIN387	buffel grass	sheep	*ad lib*	640		
	MIN360	buffel grass	sheep	*ad lib*	650		
	MIN363	buffel grass	sheep	*ad lib*	670		
	MIN569	commercil Green Panicum	sheep	*ad lib*	480	480	**424**
	MIN605	commercil Green Panicum	sheep	*ad lib*	490	500	**435**
	MIN589	commercil Green Panicum	sheep	*ad lib*	520	530	**461**
	MIN563	commercil Green Panicum	sheep	*ad lib*	600	620	**538**
	MIN611	commercil Green Panicum	sheep	*ad lib*	610		
	MIN574	commercil Green Panicum	sheep	*ad lib*	620	640	**554**
	MIN415	cowpea	sheep	*ad lib*	570		
	MIN409	cowpea	sheep	*ad lib*	600		
	MIN455	dolichos	sheep	*ad lib*	510		
	MIN408	dolichos	sheep	*ad lib*	550		
	MIN624	green Panicum	sheep	*ad lib*	640	660	**558**
	MIN654	kikuyu	sheep	*ad lib*	470	480	**440**
	MIN690	kikuyu	sheep	*ad lib*	470	480	**435**
	MIN684	kikuyu	sheep	*ad lib*	480	490	**440**
	MIN785	kikuyu	sheep	*ad lib*	480		
	MIN637	kikuyu	sheep	*ad lib*	490	520	**467**
	MIN784	kikuyu	sheep	*ad lib*	500		
	MIN798	kikuyu	sheep	*ad lib*	500		
	MIN723	kikuyu	sheep	*ad lib*	570		
	MIN767	kikuyu	sheep	*ad lib*	570		
	MIN725	kikuyu	sheep	*ad lib*	580		
	MIN713	kikuyu	sheep	*ad lib*	590		
	MIN661	kikuyu	sheep	*ad lib*	600	610	**542**
	MIN666	kikuyu	sheep	*ad lib*	600	600	**524**
	MIN699	kikuyu	sheep	*ad lib*	600		

MIN625	kikuyu	sheep	*ad lib*	620	620	**543**
MIN706	kikuyu	sheep	*ad lib*	630		
MIN707	kikuyu	sheep	*ad lib*	640		
MIN570	nth Qld Green Panicum	sheep	*ad lib*	480	490	**433**
MIN590	nth Qld Green Panicum	sheep	*ad lib*	540	560	**486**
MIN575	nth Qld Green Panicum	sheep	*ad lib*	590	610	**519**
MIN596	nth Qld Guinea grass	sheep	*ad lib*	530		
MIN584	nth Qld Guinea Grass	sheep	*ad lib*	590	610	**521**
MIN564	nth Qld Guinea grass	sheep	*ad lib*	620	640	**554**
MIN612	nth Qld Guinea grass	sheep	*ad lib*	620		
MIN795	pangola grass	sheep	*ad lib*	450		
MIN655	pangola grass	sheep	*ad lib*	490	490	**446**
MIN788	pangola grass	sheep	*ad lib*	490		
MIN796	pangola grass	sheep	*ad lib*	490		
MIN789	pangola grass	sheep	*ad lib*	510		
MIN691	pangola grass	sheep	*ad lib*	560	580	**516**
MIN715	pangola grass	sheep	*ad lib*	560		
MIN685	pangola grass	sheep	*ad lib*	570	580	**520**
MIN731	pangola grass	sheep	*ad lib*	570		
MIN732	pangola grass	sheep	*ad lib*	600		
MIN647	pangola grass	sheep	*ad lib*	610	620	**533**
MIN667	pangola grass	sheep	*ad lib*	610	620	**543**
MIN632	pangola grass	sheep	*ad lib*	630	640	**562**
MIN701	pangola grass	sheep	*ad lib*	630		
MIN709	pangola grass	sheep	*ad lib*	640		
MIN769	pangola grass	sheep	*ad lib*	640		
MIN708	pangola grass	sheep	*ad lib*	650		
MIN626	pangola grass	sheep	*ad lib*	670	690	**604**
MIN344	rhodes grass	sheep	*ad lib*	490	520	**450**
MIN347	rhodes grass	sheep	*ad lib*	490	530	**451**
MIN343	rhodes grass	sheep	*ad lib*	510	540	**463**
MIN335	rhodes grass	sheep	*ad lib*	520	550	**480**
MIN336	rhodes grass	sheep	*ad lib*	520	540	**467**
MIN682	rhodes grass	sheep	*ad lib*	520	540	**465**
MIN475	rhodes grass	sheep	*ad lib*	530		
MIN339	rhodes grass	sheep	*ad lib*	540	580	**505**
MIN477	rhodes grass	sheep	*ad lib*	560		
MIN674	rhodes grass	sheep	*ad lib*	580	600	**514**
MIN320	rhodes grass	sheep	*ad lib*	590	640	**554**
MIN704	rhodes grass	sheep	*ad lib*	590		
MIN643	rhodes grass	sheep	*ad lib*	600	620	**534**
MIN711	rhodes grass	sheep	*ad lib*	600		
MIN727	rhodes grass	sheep	*ad lib*	600		
MIN664	rhodes grass	sheep	*ad lib*	620	640	**550**
MIN698	rhodes grass	sheep	*ad lib*	630		
MIN305	rhodes grass	sheep	*ad lib*	640	670	**573**
MIN705	rhodes grass	sheep	*ad lib*	640		
MIN302	rhodes grass	sheep	*ad lib*	650	680	**599**
MIN319	rhodes grass	sheep	*ad lib*	650	680	**590**
MIN301	rhodes grass	sheep	*ad lib*	670	690	**601**
MIN465	rompha grass	sheep	*ad lib*	610		
MIN424	rompha grass	sheep	*ad lib*	660		
MIN460	rompha grass	sheep	*ad lib*	660		
MIN459	rompha grass	sheep	*ad lib*	720		
MIN394	setaria	sheep	*ad lib*	590		
MIN395	setaria	sheep	*ad lib*	610		
MIN368	setaria	sheep	*ad lib*	620		
MIN381	setaria	sheep	*ad lib*	630		

MIN385	setaria	sheep	*ad lib*	640		
MIN354	setaria	sheep	*ad lib*	660		
MIN384	setaria	sheep	*ad lib*	670		
MIN450	Tropical Grass	sheep	*ad lib*	390		
MIN452	Tropical Grass	sheep	*ad lib*	410		
MIN571	Tropical Grass	sheep	*ad lib*	430	440	**401**
MIN651	Tropical Grass	sheep	*ad lib*	430	440	**396**
MIN453	Tropical Grass	sheep	*ad lib*	440		
MIN443	Tropical Grass	sheep	*ad lib*	450		
MIN479	Tropical Grass	sheep	*ad lib*	460		
MIN493	Tropical Grass	sheep	*ad lib*	470		
MIN689	Tropical Grass	sheep	*ad lib*	470	490	**424**
MIN801	Tropical Grass	sheep	*ad lib*	470		
MIN438	Tropical Grass	sheep	*ad lib*	480		
MIN439	Tropical Grass	sheep	*ad lib*	480		
MIN451	Tropical Grass	sheep	*ad lib*	480		
MIN454	Tropical Grass	sheep	*ad lib*	480		
MIN457	Tropical Grass	sheep	*ad lib*	480		
MIN653	Tropical Grass	sheep	*ad lib*	480	490	**434**
MIN425	Tropical Grass	sheep	*ad lib*	490		
MIN441	Tropical Grass	sheep	*ad lib*	490		
MIN497	Tropical Grass	sheep	*ad lib*	490		
MIN597	Tropical Grass	sheep	*ad lib*	490		
MIN683	Tropical Grass	sheep	*ad lib*	490	520	**451**
MIN803	Tropical Grass	sheep	*ad lib*	490		
MIN442	Tropical Grass	sheep	*ad lib*	500		
MIN634	Tropical Grass	sheep	*ad lib*	500	510	**459**
MIN636	Tropical Grass	sheep	*ad lib*	500	520	**466**
MIN688	Tropical Grass	sheep	*ad lib*	510	530	**456**
MIN799	Tropical Grass	sheep	*ad lib*	510		
MIN427	Tropical Grass	sheep	*ad lib*	520		
MIN614	Tropical Grass	sheep	*ad lib*	520	530	**483**
MIN615	Tropical Grass	sheep	*ad lib*	520	540	**480**
MIN780	Tropical Grass	sheep	*ad lib*	520		
MIN414	Tropical Grass	sheep	*ad lib*	530		
MIN601	Tropical Grass	sheep	*ad lib*	530	540	**478**
MIN602	Tropical Grass	sheep	*ad lib*	530	550	**491**
MIN726	Tropical Grass	sheep	*ad lib*	530		
MIN486	Tropical Grass	sheep	*ad lib*	540		
MIN496	Tropical Grass	sheep	*ad lib*	540		
MIN591	Tropical Grass	sheep	*ad lib*	540	550	**493**
MIN604	Tropical Grass	sheep	*ad lib*	540	560	**480**
MIN675	Tropical Grass	sheep	*ad lib*	540	560	**487**
MIN786	Tropical Grass	sheep	*ad lib*	540		
MIN635	Tropical Grass	sheep	*ad lib*	550	580	**506**
MIN413	Tropical Grass	sheep	*ad lib*	560		
MIN430	Tropical Grass	sheep	*ad lib*	560		
MIN431	Tropical Grass	sheep	*ad lib*	560		
MIN444	Tropical Grass	sheep	*ad lib*	560		
MIN447	Tropical Grass	sheep	*ad lib*	560		
MIN588	Tropical Grass	sheep	*ad lib*	560	570	**489**
MIN633	Tropical Grass	sheep	*ad lib*	560	560	**488**
MIN687	Tropical Grass	sheep	*ad lib*	560	560	**498**
MIN763	Tropical Grass	sheep	*ad lib*	560		
MIN421	Tropical Grass	sheep	*ad lib*	570		
MIN565	Tropical Grass	sheep	*ad lib*	570	590	**523**
MIN576	Tropical Grass	sheep	*ad lib*	570	590	**521**
MIN592	Tropical Grass	sheep	*ad lib*	570		

	MIN593	Tropical Grass	sheep	*ad lib*	570		
	MIN622	Tropical Grass	sheep	*ad lib*	570	600	**504**
	MIN665	Tropical Grass	sheep	*ad lib*	570	590	**493**
	MIN471	Tropical Grass	sheep	*ad lib*	580		
	MIN487	Tropical Grass	sheep	*ad lib*	580		
	MIN594	Tropical Grass	sheep	*ad lib*	580		
	MIN610	Tropical Grass	sheep	*ad lib*	580		
	MIN628	Tropical Grass	sheep	*ad lib*	580	610	**544**
	MIN642	Tropical Grass	sheep	*ad lib*	580		
	MIN787	Tropical Grass	sheep	*ad lib*	580		
	MIN645	Tropical Grass	sheep	*ad lib*	590	610	**531**
	MIN681	Tropical Grass	sheep	*ad lib*	590	590	**507**
	MIN761	Tropical Grass	sheep	*ad lib*	590		
	MIN410	Tropical Grass	sheep	*ad lib*	600		
	MIN631	Tropical Grass	sheep	*ad lib*	600	610	**547**
	MIN581	Tropical Grass	sheep	*ad lib*	610	630	**553**
	MIN582	Tropical Grass	sheep	*ad lib*	610	640	**541**
	MIN623	Tropical Grass	sheep	*ad lib*	610	630	**529**
	MIN659	Tropical Grass	sheep	*ad lib*	610	630	**544**
	MIN673	Tropical Grass	sheep	*ad lib*	610	620	**528**
	MIN434	Tropical Grass	sheep	*ad lib*	620		
	MIN609	Tropical Grass	sheep	*ad lib*	620		
	MIN630	Tropical Grass	sheep	*ad lib*	620	640	**552**
	MIN765	Tropical Grass	sheep	*ad lib*	620		
	MIN432	Tropical Grass	sheep	*ad lib*	630		
	MIN629	Tropical Grass	sheep	*ad lib*	630	660	**569**
	MIN660	Tropical Grass	sheep	*ad lib*	630	640	**559**
	MIN663	Tropical Grass	sheep	*ad lib*	630	650	**530**
	MIN546	Tropical Grass	sheep	*ad lib*	640	660	**579**
	MIN627	Tropical Grass	sheep	*ad lib*	640	660	**555**
	MIN641	Tropical Grass	sheep	*ad lib*	640	630	**529**
	MIN468	Tropical Grass	sheep	*ad lib*	670		
	MIN657	Tropical Grass	sheep	*ad lib*	670	670	**567**
Minson, WWAI & CSIRO	MIN359	buffel grass	sheep	*ad lib*	580		
	MIN554	commercil Green Panicum	sheep	*ad lib*	470	500	**441**
	MIN583	commercil Green Panicum	sheep	*ad lib*	600	610	**526**
	MIN542	commercil Green Panicum	sheep	*ad lib*	620	650	**543**
	MIN728	kikuyu	sheep	*ad lib*	530		
	MIN714	kikuyu	sheep	*ad lib*	540		
	MIN676	kikuyu	sheep	*ad lib*	570	570	**501**
	MIN730	kikuyu	sheep	*ad lib*	590		
	MIN700	kikuyu	sheep	*ad lib*	630		
	MIN555	nth Qld Guinea grass	sheep	*ad lib*	500	530	**463**
	MIN606	nth Qld Guinea grass	sheep	*ad lib*	510	540	**473**
	MIN549	nth Qld Guinea grass	sheep	*ad lib*	610	640	**543**
	MIN638	pangola grass	sheep	*ad lib*	570	580	**526**
	MIN677	pangola grass	sheep	*ad lib*	600	620	**538**
	MIN662	pangola grass	sheep	*ad lib*	650	670	**600**
	MIN702	pangola grass	sheep	*ad lib*	670		
	MIN712	rhodes grass	sheep	*ad lib*	590		
	MIN721	rhodes grass	sheep	*ad lib*	610		
	MIN306	rhodes grass	sheep	*ad lib*	650	670	**576**
	MIN722	rhodes grass	sheep	*ad lib*	650		
	MIN303	rhodes grass	sheep	*ad lib*	680	690	**595**
	MIN437	rompha grass	sheep	*ad lib*	660		
	MIN464	rompha grass	sheep	*ad lib*	670		

	MIN449	rompha grass	sheep	*ad lib*	690		
	MIN393	setaria	sheep	*ad lib*	620		
	MIN364	setaria	sheep	*ad lib*	630		
	MIN380	setaria	sheep	*ad lib*	660		
	MIN223	Tropical Grass	sheep	*ad lib*	420		
	MIN652	Tropical Grass	sheep	*ad lib*	480	500	**444**
	MIN551	Tropical Grass	sheep	*ad lib*	490	500	**453**
	MIN782	Tropical Grass	sheep	*ad lib*	490		
	MIN556	Tropical Grass	sheep	*ad lib*	500	520	**471**
	MIN568	Tropical Grass	sheep	*ad lib*	500	510	**449**
	MIN607	Tropical Grass	sheep	*ad lib*	500	510	**460**
	MIN456	Tropical Grass	sheep	*ad lib*	510		
	MIN650	Tropical Grass	sheep	*ad lib*	510		
	MIN492	Tropical Grass	sheep	*ad lib*	520		
	MIN553	Tropical Grass	sheep	*ad lib*	530	550	**477**
	MIN587	Tropical Grass	sheep	*ad lib*	550	560	**488**
	MIN608	Tropical Grass	sheep	*ad lib*	550		
	MIN586	Tropical Grass	sheep	*ad lib*	570	570	**508**
	MIN562	Tropical Grass	sheep	*ad lib*	600	620	**532**
	MIN613	Tropical Grass	sheep	*ad lib*	600		
	MIN561	Tropical Grass	sheep	*ad lib*	610	620	**554**
	MIN585	Tropical Grass	sheep	*ad lib*	610	620	**549**
	MIN541	Tropical Grass	sheep	*ad lib*	620	660	**558**
	MIN539	Tropical Grass	sheep	*ad lib*	630	640	**561**
	MIN548	Tropical Grass	sheep	*ad lib*	640	660	**565**
	MIN766	Tropical Grass	sheep	*ad lib*	650		
	MIN470	Tropical Grass	sheep	*ad lib*	660		
	MIN621	Tropical Grass	sheep	*ad lib*	670	670	**513**
NSWDPI, WWAI	FQS034	1. Unfrosted wheat	sheep	M	820	839	825
	FQS110	15 grains - Abacus (H) Triticale	sheep	M	858	866	831
	FQS110	15 grains - Abacus (H) Triticale	cattle	*ad lib*	869	864	845
	FQS109	15 grains - Abacus (M) Triticale	cattle	*ad lib*	854	850	822
	FQS109	15 grains - Abacus (M) Triticale	sheep	M	870	871	838
	FQS101	15 grains - Apollo wheat	cattle	*ad lib*	843	847	826
	FQS101	15 grains - Apollo wheat	sheep	M	871	876	842
	FQS099	15 grains - Brennan wheat	cattle	*ad lib*	853	848	825
	FQS099	15 grains - Brennan wheat	sheep	M	886	890	859
	FQS100	15 grains - Dollabird wheat	cattle	*ad lib*	872	866	845
	FQS100	15 grains - Dollabird wheat	sheep	M	886	899	865
	FQS106	15 grains - Franklin barley	cattle	*ad lib*	811	810	789
	FQS106	15 grains - Franklin barley	sheep	M	840	845	814
	FQS105	15 grains - Gairdner barley	cattle	*ad lib*	775	776	754
	FQS105	15 grains - Gairdner barley	sheep	M	857	866	831
	FQS095	15 grains - QAL 2000 wheat	cattle	*ad lib*	814	817	793
	FQS095	15 grains - QAL 2000 wheat	sheep	M	915	915	880
	FQS104	15 grains - Skiff barley	cattle	*ad lib*	799	797	776
	FQS104	15 grains - Skiff barley	sheep	M	877	877	844
	FQS107	15 grains - Sloop barley	cattle	*ad lib*	808	814	791
	FQS107	15 grains - Sloop barley	sheep	M	842	852	817
	FQS108	15 grains - Sultan barley	cattle	*ad lib*	822	826	792
	FQS108	15 grains - Sultan barley	sheep	M	852	859	818
	FQS102	15 grains - Sunlin wheat	cattle	*ad lib*	842	836	809
	FQS102	15 grains - Sunlin wheat	sheep	M	878	889	852
	FQS096	15 grains - Tennant wheat	cattle	*ad lib*	822	827	821

FQS096	15 grains - Tennant wheat	sheep	M	900	902	867
FQS103	15 grains - Torrens barley	cattle	*ad lib*	888	878	856
FQS103	15 grains - Torrens barley	sheep	M	929	931	898
FQS097	15 grains - Waxy Janz wheat	cattle	*ad lib*	849	847	826
FQS097	15 grains - Waxy Janz wheat	sheep	M	897	898	863
FQS035	2. Ungraded lightly frosted (LF) wheat	sheep	M	798	818	804
INVIVO 114	2005 conc exp luc chaff	sheep	M	643	652	579
INVIVO 112	2005 conc exp oat straw	sheep	M	504	518	479
INVIVO 111	2005 conc exp pea straw	sheep	M	483	506	442
INVIVO 113	2005 conc exp wheat straw	sheep	M	514	533	488
FQS573	2005 Conc expt 1. Lupins grain	sheep	M	903	923	873
FQS570	2005 Conc expt 10. pellets - OLEO	sheep	M	746	764	714
FQS574	2005 Conc expt 2. peas grain	sheep	M	925	944	894
FQS577	2005 Conc expt 3. maize grain	sheep	M	931	946	907
FQS578	2005 Conc expt 4. sorghum grain	sheep	M	898	913	869
FQS576	2005 Conc expt 5. oats grain-echidna	sheep	M	707	719	688
FQS575	2005 Conc expt 6. oats grain-cooba	sheep	M	796	946	782
FQS569	2005 Conc expt 7. pellets - feedlot	sheep	M	775	819	734
FQS571	2005 Conc expt 8. pellets - lamb	sheep	M	814	861	769
FQS572	2005 Conc expt 9. pellets - sheep	sheep	M	722	781	681
FQS036	3. LF - normal component	sheep	M	819	838	824
FQS037	4. LF - frosted + screenings component	sheep	M	791	811	796
FQS038	5. Ungraded severely frosted (SF) wheat	sheep	M	779	800	785
FQS039	6. SF - normal component	sheep	M	800	820	807
FQS040	7. SF - frosted + screenings component wheat	sheep	M	768	791	774
FQS590	8 grains cooba high	cattle	*ad lib*	839	843	802
FQS588	8 grains cooba low	cattle	*ad lib*	783	798	751
FQS583	8 grains echidna	cattle	*ad lib*	641	643	640
FQS586	8 grains eurabbie	cattle	*ad lib*	754	771	733
FQS587	8 grains MA5237	cattle	*ad lib*	848	856	818
FQS584	8 grains mortlock	cattle	*ad lib*	699	713	678
FQS585	8 grains quoll	cattle	*ad lib*	801	810	770
FQS589	8 grains yiddah	cattle	*ad lib*	819	831	792
FQS041	8. Chaff only	sheep	M	624	651	593
INVIVO 115	ACIAR 2006 bunker 1 Barley 1	cattle	*ad lib*	712	743	648
INVIVO 116	ACIAR 2006 bunker 2 Oats 1	cattle	*ad lib*	646	666	595
INVIVO 117	ACIAR 2006 bunker 3 Barley 2	cattle	*ad lib*	690	705	632
INVIVO 118	ACIAR 2006 bunker 4 Oats 2	cattle	*ad lib*	648	665	608
INVIVO 119	ACIAR 2006 bunker 5 Barley 3	cattle	*ad lib*	639	652	602
INVIVO 120	ACIAR 2006 bunker 6 Oats 3	cattle	*ad lib*	650	668	619
FQS053	C/89 Lucerne chaff	sheep	*ad lib*	550	545	499
FQS053	C/89 Lucerne chaff	sheep	M	576	593	545
FQS053	C/89 Lucerne chaff	cattle	M	625	636	584
FQS052	C/89 Oaten chaff	sheep	*ad lib*	466	497	462

FQS052	C/89 Oaten chaff	sheep	M	476	503	467
FQS052	C/89 Oaten chaff	cattle	M	556	583	541
FQS052	C/89 Oaten chaff	cattle	*ad lib*	564	594	553
FQS057	C85-1 Lucerne cube	cattle	*ad lib*	669	669	591
FQS032	Lucerne silage (8 grains Oat expt)	cattle	*ad lib*	611	619	554
FQS032	Lucerne silage (8 grains Oat expt)	cattle	M	627	627	560
FQS069	M1/90 Maize silage	cattle	*ad lib*	704	728	673
FQS070	M1/90 Sub clover silage	cattle	*ad lib*	712	732	643
FQS071	M1/91 Sorghum silage	cattle	*ad lib*	625	644	585
FQS072	M1/92 Lucerne silage	cattle	*ad lib*	556	591	529
FQS054	MS 86-4 (S4/86)- Mini corn silage	sheep	*ad lib*	620	660	610
FQS023	NFCP ARG (Annual Ryegrass) silage	cattle	M	594	612	549
FQS023	NFCP ARG (Annual Ryegrass) silage	sheep	M	599	622	564
FQS005	NFCP ARG/oat early cut silage	sheep	M	723	761	670
FQS005	NFCP ARG/oat early cut silage	cattle	M	781	816	766
FQS006	NFCP ARG/oat late cut silage	sheep	M	626	647	586
FQS006	NFCP ARG/oat late cut silage	cattle	M	649	656	576
FQS014	NFCP Barley /ARG silage	sheep	M	597	623	565
FQS014	NFCP Barley /ARG silage	cattle	M	615	637	581
FQS001	NFCP Ex 1 Sub clover silage	sheep	M	631	656	576
FQS001	NFCP Ex 1 Sub clover silage	cattle	M	672	695	616
FQS011	NFCP Forage sorghum silage	cattle	M	640	669	611
FQS011	NFCP Forage sorghum silage	sheep	M	654	689	631
FQS012	NFCP Grain sorghum silage	cattle	M	567	593	541
FQS012	NFCP Grain sorghum silage	sheep	M	572	608	559
FQS018	NFCP Italian ryegrass early cut silage	cattle	M	666	699	615
FQS018	NFCP Italian ryegrass early cut silage	sheep	M	686	727	645
FQS019	NFCP Italian ryegrass late cut silage	cattle	M	581	610	547
FQS019	NFCP Italian ryegrass late cut silage	sheep	M	596	635	574
FQS031	NFCP Lucerne irrigated 2nd cut silage (15 grains expt)	sheep	M	668	692	597
FQS031	NFCP Lucerne irrigated 2nd cut silage (15 grains expt)	cattle	*ad lib*	680	689	596
FQS003	NFCP Maize early cut silage	sheep	M	690	715	665
FQS003	NFCP Maize early cut silage	cattle	M	693	710	652
FQS004	NFCP Maize late cut silage	cattle	M	647	668	622
FQS004	NFCP Maize late cut silage	sheep	M	650	687	636
FQS021	NFCP Maize silage	cattle	M	604	632	575
FQS021	NFCP Maize silage	sheep	M	636	680	629
FQS030	NFCP Maize silage	cattle	M	660	695	643
FQS024	NFCP Mixed annual grasses/Cocksfoot silage	cattle	M	622	651	590
FQS024	NFCP Mixed annual grasses/Cocksfoot silage	sheep	M	626	669	610
FQS015	NFCP Oat/ pea silage	sheep	M	565	603	546
FQS015	NFCP Oat/ pea silage	cattle	M	580	614	558
FQS002	NFCP Oaten silage	sheep	M	591	612	556
FQS002	NFCP Oaten silage	cattle	M	617	632	564
FQS029	NFCP Pearl Millet silage	cattle	M	612	650	573

FQS026	NFCP Sorghum x sudan grass silage (BMR)	cattle	M	612	646	579
FQS028	NFCP Sorghum x sudan grass silage (Sweet jumbo)	cattle	M	590	621	548
FQS007	NFCP Sub clover/ARG/lucerne early cut silage	sheep	M	668	704	620
FQS007	NFCP Sub clover/ARG/lucerne early cut silage	cattle	M	685	717	650
FQS008	NFCP Sub clover/ARG/lucerne late cut silage	sheep	M	618	634	571
FQS008	NFCP Sub clover/ARG/lucerne late cut silage	cattle	M	645	661	583
FQS017	NFCP Sub clover/ARG/lucerne silage	cattle	M	680	708	634
FQS017	NFCP Sub clover/ARG/lucerne silage	sheep	M	689	719	628
FQS022	NFCP Sub clover/silver grass/lucerne silage	cattle	M	706	735	665
FQS022	NFCP Sub clover/silver grass/lucerne silage	sheep	M	720	747	657
FQS027	NFCP Sudan grass silage	cattle	M	562	594	535
FQS010	NFCP Sweet sorghum delayed sealing silage	sheep	M	634	662	607
FQS010	NFCP Sweet sorghum delayed sealing silage	cattle	M	669	695	633
FQS009	NFCP Sweet sorghum silage	cattle	M	655	680	614
FQS009	NFCP Sweet sorghum silage	sheep	M	659	686	632
FQS013	NFCP Wheat /ARG silage	cattle	M	550	562	517
FQS013	NFCP Wheat /ARG silage	sheep	M	565	584	539
FQS025	NFCP Wheat with annual grass weeds late cut silage	cattle	M	556	599	538
FQS016	NFCP Wheat/Vetch/ARG silage	cattle	M	516	529	484
FQS016	NFCP Wheat/Vetch/ARG silage	sheep	M	530	551	503
FQS020	NFCP White clover/ARG silage	cattle	M	651	680	604
FQS020	NFCP White clover/ARG silage	sheep	M	676	707	618
FQS033	Oat hull pellets and CSM (final diet 80% pellets:20% CSM)	sheep	M	363	374	356
FQS074	P96 Sub clover silage	sheep	*ad lib*	690	708	637
FQS073	P97 Maize silage + CSM	cattle	*ad lib*	665	685	635
FQS055	S1/86 Jumbo	sheep	*ad lib*	615	658	562
FQS060	S1/87 SC Golden early maize silage	sheep	*ad lib*	614	652	583
FQS060	S1/87 SC Golden early maize silage	cattle	*ad lib*	675	717	641
FQS061	S1/87 SC Honey sweet maize silage	sheep	*ad lib*	621	660	609
FQS061	S1/87 SC Honey sweet maize silage	cattle	*ad lib*	672	714	659
FQS062	S1/87 SC SR 103 maize silage	sheep	*ad lib*	621	660	621
FQS062	S1/87 SC SR 103 maize silage	cattle	*ad lib*	674	716	673
FQS067	S1/87 VxT General Early maize silage	sheep	*ad lib*	649	693	630

FQS067	S1/87 VxT General Early maize silage	cattle	*ad lib*	672	724	659
FQS068	S1/87 VxT General Late maize silage	cattle	*ad lib*	661	699	637
FQS068	S1/87 VxT General Late maize silage	sheep	*ad lib*	665	700	638
FQS065	S1/87 VxT SR 103 Early maize silage	sheep	*ad lib*	681	720	645
FQS065	S1/87 VxT SR 103 Early maize silage	cattle	*ad lib*	695	730	654
FQS066	S1/87 VxT SR 103 Late maize silage	sheep	*ad lib*	649	687	635
FQS066	S1/87 VxT SR 103 Late maize silage	cattle	*ad lib*	650	694	641
FQS063	S1/87 VxT XL 94 Early maize silage	sheep	*ad lib*	644	706	645
FQS063	S1/87 VxT XL 94 Early maize silage	cattle	*ad lib*	669	711	650
FQS064	S1/87 VxT XL 94 Late maize silage	sheep	*ad lib*	649	687	637
FQS064	S1/87 VxT XL 94 Late maize silage	cattle	*ad lib*	673	709	658
FQS075	S1/88 General maize silage	sheep	*ad lib*	652	685	643
FQS075	S1/88 General maize silage	cattle	*ad lib*	695	727	682
FQS076	S1/88 GH 5011 maize silage	sheep	*ad lib*	670	703	663
FQS076	S1/88 GH 5011 maize silage	cattle	*ad lib*	735	763	719
FQS077	S1/88 P3906 maize silage	cattle	*ad lib*	714	745	699
FQS077	S1/88 P3906 maize silage	sheep	*ad lib*	719	748	702
FQS078	S1/88 PX 75 maize silage	sheep	*ad lib*	671	699	661
FQS078	S1/88 PX 75 maize silage	cattle	*ad lib*	725	748	708
FQS079	S1/88 SR 103 maize silage	sheep	*ad lib*	695	725	685
FQS079	S1/88 SR 103 maize silage	cattle	*ad lib*	731	758	717
FQS080	S1/88 XL 72 maize silage	sheep	*ad lib*	700	729	692
FQS080	S1/88 XL 72 maize silage	cattle	*ad lib*	727	752	714
FQS081	S1/89 SR 73 maize silage	cattle	*ad lib*	692	725	**676**
FQS083	S1/89 Supersweet sorghum silage	cattle	*ad lib*	593	636	586
FQS082	S1/89 XL 82 maize silage	cattle	*ad lib*	697	734	**691**
FQS094	S1/90 P3183 Maize silage	cattle	*ad lib*	679	691	657
FQS094	S1/90 P3183 Maize silage	cattle	M	690		
FQS090	S1/90 SR 73 Maize silage	cattle	M	680		
FQS090	S1/90 SR 73 Maize silage	cattle	*ad lib*	710	693	656
FQS091	S1/90 Xl 77a Maize silage	cattle	*ad lib*	667	688	653
FQS091	S1/90 Xl 77a Maize silage	cattle	M	711		
FQS093	S1/90 XL 82 high population Maize silage	cattle	*ad lib*	680	697	660
FQS093	S1/90 XL 82 high population Maize silage	cattle	M	692		
FQS092	S1/90 XL 82 Maize silage	cattle	*ad lib*	680	703	669
FQS092	S1/90 XL 82 Maize silage	cattle	M	696		
FQS084	S1/91 Silage 1 Normal Maize	cattle	*ad lib*	692	719	657
FQS084	S1/91 Silage 1 Normal Maize	cattle	M	716	739	670
FQS085	S1/91 Silage 2 BM3 Maize	cattle	*ad lib*	669	693	634
FQS085	S1/91 Silage 2 BM3 Maize	cattle	M	723	751	679
FQS086	S1/91 Silage 3 CO26 Maize	cattle	*ad lib*	656	678	607
FQS086	S1/91 Silage 3 CO26 Maize	cattle	M	693	719	647
FQS087	S1/91 Silage 4 GH5009 Maize	cattle	*ad lib*	700	720	669
FQS087	S1/91 Silage 4 GH5009 Maize	cattle	M	720	744	684

FQS088	S1/91 Silage 5 GH5019WX Maize	cattle	*ad lib*	690	715	662
FQS088	S1/91 Silage 5 GH5019WX Maize	cattle	M	725	753	690
FQS089	S1/91 Silage 6 DK689 Maize	cattle	*ad lib*	647	673	616
FQS089	S1/91 Silage 6 DK689 Maize	cattle	M	687	720	656
FQS056	S3/87/33A Kikuyu - wilted	sheep	*ad lib*	619	659	594
FQS058	S5/86/22 Clover/ lucerne silage	sheep	*ad lib*	561	586	514
FQS059	S5/86/23 Clover/ grass silage	sheep	*ad lib*	679	712	657