AusScan NIR Grain Standards Development and User Calibration Upgrade

4B-119

Report prepared for the Co-operative Research Centre for High Integrity Australian Pork

By

Peter Flinn

Kelspec Services Pty Ltd, PO Box 31, Dunkeld, Victoria 3294
E-mail: theflinns@bigpond.com

June 2014

An Australian Government Initiative

Established and supported under the Australian Government's Cooperative Research Centres Program

Executive Summary

The development of near infrared spectroscopy (NIR) calibrations for rapid assessment of quality indicators in feed grain has attracted considerable interest from the Australian feed and livestock industries. The calibrations were derived from animal performance and laboratory data obtained during collaborative research projects supported by R&D Corporations, universities, CSIRO, State instrumentalities and private companies. Since 2008 calibration equations have been available, with periodic updates, to the Australian research and industry sectors under the AusScan licensing system managed by the Pork CRC. Currently the equations are licensed to 17 organisations and are installed on around 43 NIR instruments.

The transfer of NIR calibrations among instruments in a network requires that analysis values obtained for a given sample be the same on all instruments, within acceptable error limits. This presents a challenge, especially where the equations are desired on a range of instrument types with different optical configurations, driven by different software and having different sample presentation methods. The results of a ring test in 2012, in which 10 whole grain samples were tested on 16 NIR instruments, demonstrated that more work was required to improve repeatability of analyses between instruments. Particular focus was given to the 12 FOSS instruments which had previously been part of a standardised network. Some of these had undergone major repairs, the sealed whole grain "standards" in use were difficult to access and had deteriorated over time, and the standardisation process was out of date.

A new and representative set of 18 sealed standards was created, and all were scanned on 16 FOSS instruments around Australia. A new "master" instrument was identified, and a new standardisation file was created for each of the other 15 "host" instruments, allowing them to be spectrally matched to the master. In addition, repeatability files were created, comprising NIR spectra from the same samples which had been tested on different instruments and at different temperatures. All current AusScan calibrations were updated to reflect the new master instrument and to incorporate the repeatability files to aid stability and robustness.

The use of repeatability files must not significantly change calibration accuracy, and this was found to be the case. Repeatability values were found to be generally small in relation to calibration error, which is also essential. Temperature changes were found to have more impact on the repeatability scans than instrument changes, which agrees with other studies.

The outcomes of the project are expected to lead to more stable and robust AusScan calibrations, and result in improved uniformity of analysis across the NIR instruments in the network. This should increase confidence in calibration performance and lead to increased uptake by industry. Due to imminent changes in commercial arrangements for AusScan, it was not possible to test and validate the calibration updates and new standardisation files across the network. It is strongly recommended that, once the new data becomes available to the licensees, a new ring test be conducted as soon as possible in order to properly evaluate the performance of the network.

Table of Contents

1. Introduction

There has been considerable interest in and uptake of a set of near infrared (NIR) calibrations, developed over the past 15 years, to enable rapid assessment of a range of quality indicators in Australian feed grain which are important for the livestock industries.

The calibrations were developed from animal performance and laboratory measurements as part of the long running Premium Grains for Livestock Program, with funding from the grain and livestock R&D Corporations and involving collaborative research and financial support by universities, CSIRO, State instrumentalities and private companies. Subsequent and ongoing funding from the pig and poultry industries has allowed some of the calibrations to be validated and expanded to improve accuracy and precision.

In recent years the relevant calibration equations have been made available in Australia to both the research and industry sectors, under the AusScan licensing system managed by the Pork CRC. Currently the equations are licensed to 17 organisations and are installed on around 43 NIR instruments.

It is important to explain the difference between a calibration data set and a calibration equation. A calibration is developed from two sets of data: the spectra (plots of absorption of near infrared light by the sample against wavelength); and the corresponding reference value(s), eg. protein, fibre, energy, etc. measured by traditional methods. A calibration equation is the mathematical relationship between the spectra and the reference values. It is the equations only which are provided to licensees, used by them to test unknown samples.

When a calibration equation is transferred from one instrument to another, the obvious aim is to ensure that the same analysis values will be obtained for a given sample on both instruments, within acceptable error limits. This can be a challenge even when a network of the same type of NIR instruments is available, all using the same calibration software package, and the same sample presentation method. However, some AusScan licensees have also wanted to use other instrument types, with different optical configurations, running on different software and with various sample presentation methods. Calibration equations were requested for both whole and ground grain samples. These demands result in a whole new level of complexity, with consequences for repeatability between instruments.

When the AusScan calibration equations were rolled out, those instruments of the same type (in this case FOSS scanning monochromators with WinISI software) were standardised, or spectrally matched, to a designated "master" instrument, thus enabling the same equation files to be installed on each instrument in the network. Separate standardisations were necessary for scanning whole or ground grains. In other cases, it was necessary to scan all the calibration samples on each instrument, and derive calibration equations specific to each instrument. Clearly this required a lot more work, and introduced additional possible sources of error.

The best way to determine calibration performance across instruments is by means of a carefully organised ring test, in which a small set of new, independent grain samples, rigorously sub-divided into representative subsamples, is sent to each licensee, who then scans the samples on the respective instrument, predicts the quality indicators using the supplied equations, and submits the NIR spectra and analysis values for statistical analysis.

The most recent AusScan ring test was conducted in 2012, involving 16 instruments testing whole grain. Of these, 12 were part of a standardised network of FOSS monochromators, 1 was a Bruker MPA interferometer and 3 were FOSS Infratec transmission instruments with a limited wavelength range of 850-1048 nm. Another 7 instruments were required to test ground grain, and these formed part of a separately standardised network of FOSS monochromators.

One method employed to estimate the agreement between a given instrument and the relevant master instrument (using whole or ground grains) for each quality indicator, was to compare the mean difference between predicted values for the two instruments across all ring test samples with the NIR calibration standard error of cross-validation (SECV)(Spragg, 2012). As an example, for pig faecal digestible energy (FDE), this mean difference significantly exceeded SECV for only one instrument. In the case of broiler apparent metabolisable energy (AME) there were 7 instruments differing significantly from the master instrument.

A more rigorous evaluation was possible for the instruments in the standardised network. In the case of whole grain scanned on the 12 FOSS instruments, instrument performance was satisfactory overall for 13 out of the 19 quality indicators measured (Flinn, 2013, pers comm). However, when examining the specific quality indicators, it was clear that some instruments stood out more than others in terms of excessive differences between the overall mean predicted value across all instruments and the mean for a given instrument. This can indicate that the standardisation for that instrument was out of date, or that it had undergone significant repairs. This was later found to be the case.

A major conclusion from this ring test was that all FOSS instruments used with whole grains needed to be re-standardised. In order to achieve this, it was recommended that AusScan should procure its own set of whole grain sealed "standards", covering the grain types used by industry, rather than relying on access to an older and narrower set owned by GrainGrowers Ltd, which had been borrowed and used for some years but had deteriorated over time. A second conclusion was the need to introduce a "repeatability file" into the calibration process to improve stability and reduce variability among the instruments in the network. This file would consist of the scans of the earlier ring test samples across several instruments, plus repeated scans of grains taken at different temperatures.

This project aimed to fulfill these requirements.

2. Methodology

A set of 20 sealable "quarter cups", designed for scanning whole grain on FOSS model 6500, 5000 and XDS instruments, was purchased from the USA. In all,18 grain samples were selected from the large number stored in freezers at the University of Sydney Plant Breeding Station. The samples were chosen to represent the grain types to be routinely analysed by the instrument network, and to cover an optimum range in optical density at 1680 nm. The chemical composition of these samples was not considered; they were intended as "optical standards" and only for standardisation of NIR instruments. The grain samples chosen comprised 3 wheat, 2 barley, 3 oats, 4 triticale, 5 sorghum and 1 maize. All 18 samples were carefully inserted and sealed in quarter cups.

The 18 "standards" were then scanned on the designated master NIR instrument (a FOSS model XDS located at NSW DPI, Wagga Wagga, NSW). Following that, the standards were shipped around the country and scanned in turn on 15 FOSS instruments, located in New South Wales, Queensland, South Australia and Western Australia. On instruments with a sample transport module, each standard was scanned twice. On XDS instruments without a moving window accessory, each standard was scanned 6 times in different positions.

Standardisation of the 15 host instruments to the master instrument was accomplished using all 18 whole grain standards, following the procedure of Shenk and Westerhaus (1994) incorporated into WinISI software. The spectra of the 18 whole grains on each host instrument were mathematically corrected to match the corresponding spectra from the master instrument, with the correction factors stored in a standardisation (.STD) file specific to that host instrument. This file is then to be used to standardise the spectrum of every sample scanned on the host instrument in future where an AusScan equation is to be applied.

Until 2013, the existing AusScan calibrations had all been derived on the FOSS XDS instrument formerly located at DEPI, Hamilton, Victoria, which was the master instrument in the network. All host instruments had been earlier standardised to that master, based on the old GrainGrowers Ltd standards, over a period of some years. However, the DEPI instrument was no longer available or accessible to AusScan, meaning that a new master instrument was necessary. To accomplish this, all AusScan calibration files were adjusted so that the FOSS XDS instrument at Wagga became the master, using a procedure similar to that of Flinn and Saunders (1995).

The final task was to introduce a repeatability file into each AusScan calibration, with the objective of minimising systematic differences between instruments in the network, and also minimising the effect of sample and instrument temperature, which is known to have a significant impact on NIR spectra (Westerhaus, 1990; Shenk and Westerhaus, 1994). A repeatability file contains spectra of the same samples scanned on different instruments or subjected to different temperature treatments. It does not contain reference values.

Two repeatability files were created, one including the spectra of the 10 ring test grain samples from 2012 scanned on 12 standardised instruments, and the other including the spectra of 20 grain samples scanned 4 times on the master instrument using 4 different temperature treatments. Treatment 1 comprised the sample temperature and the room temperature being both at 20-22^{o}C; treatment 2, sample temperature 28-30^{o}C and room temperature 20-22^{o}C; treatment 3, sample temperature 20-22^{o}C and room temperature 28-30^{o}C; and treatment 4, sample temperature and room temperature both at 28-30^{o}C. Each repeatability file was incorporated separately into each AusScan calibration, then the two repeatability files were combined into one and incorporated into each AusScan calibration.

It was then intended to distribute the updated calibration equations together with the relevant new standardisation file to each licensee whose NIR instrument is part of the FOSS monochromator network, so that another ring test could be conducted, necessary for proper evaluation of network performance. However, this was not done due to the proposed new commercial arrangements for AusScan.

3. Outcomes

The 18 sealed whole grain standards have been scanned on all NIR instruments in the FOSS monochromator network, and will be available for re-standardisation as required in the future. The details of the newly standardised instruments, and the standardisation (STD) filenames, are shown in Table 1.

Table 1 - Standardised FOSS NIR instruments in AusScan network (whole grain)

Instrument type	Organisation	Location	Serial No	STD filename
XDS (Master)	NSW DPI	Wagga NSW	30100647	N/A
6500	NSW DPI	Wagga NSW	76642114	76_WXDW.STD
6500	Westons	Enfield NSW	77672115	77_WXDW.STD
6500	Westons	Bentley WA	B0290845	B0_WXDW.STD
XDS	DAFWA	South Perth WA	30100667	BX_WXDW.STD
XDS	DAFWA	South Perth WA	30100644	NX_WXDW.STD
XDS	DAFWA	South Perth WA	30100600	OX_WXDW.STD
XDS	DAFWA	South Perth WA	30100512	WX_WXDW.STD
XDS	DAFWA	Katanning WA	30100579	KX_WXDW.STD
6500	Milne Feeds	Welshpool WA	58589802	58_WXDW.STD
XDS	Intergrain*	Bibra Lake WA	30101421	X21_WXDW.STD
XDS	Big River Feeds	Murray Bridge SA	30101135	BR_WXDW.STD
6500	DAFF QLD	Toowoomba QLD	47449805	47_WXDW.STD
5000	DAFF QLD	Toowoomba QLD	50709610	50_WXDW.STD

*Intergrain does not hold an AusScan licence at this time, but was keen to scan the standards while they were in WA, in view of becoming a licensee in the future.

The NIR statistics of the updated AusScan calibration equations, with and without the incorporation of repeatability files for the effects of different instruments or different temperatures, are shown in Table 2.

The explanation of the abbreviations in Table 2 is as follows:

NO REP = no repeatability file in calibration
REP_INS = repeatability file included based on different instruments
REP_TEMP = repeatability file included based on different temperatures
REP_INS+TEMP = combined instrument and temperature repeatability file included
N = number of samples
SECV = standard error of cross validation
R^2CV = R^2 in cross validation
REP = repeatability
FDEGAR = Pig faecal digestibile energy of grain, MJ/kg as fed
IDEGAR = Pig ileal digestibile energy of grain, MJ/kg as fed
PIGFDEINTIND = Pig faecal digestible energy intake index, 0-100
AMEGAF = Broiler apparent metabolisable energy (AME) of grain, MJ/kg as fed
AMEGII = Broiler AME intake index, 0-100
AMEGAFENZ = Broiler response in AME to addition of enzymes, MJ/kg as fed

AcidT4Ind = Acidosis Index calculated using Acid Time 4 experimental data, 0-100
EstMEcat = Estimated metabolisable energy of grain (cattle), MJ/kg DM
EstMEshp = Estimated metabolisable energy of grain (sheep), MJ/kg DM
AjDMDis = Dry matter digestibility of oats after 48 h *in sacco* in rumen of cattle, %
HullADL = Acid detergent lignin content of the hulls of oats, %
Hull content of oats, % of whole grain
CP_DM = Crude protein content of grain, %DM
CF_DM = Crude fibre content of grain, %DM
ADF_DM = Acid detergent fibre content of grain, %DM
ENG_NDF_DM = Englyst neutral detergent fibre content of grain, %DM
TOT_ST_DM = Total starch content of grain, %DM
TOTINSNSP_DM = Total insoluble non-starch polysaccharide content of grain, %DM
TOTSOLNSP_DM = Total soluble non-starch polysaccharide content of grain, %DM
ARA_XYL_DM = Insoluble arabinoxylans content of grain, %DM
BGLUC_DM = Beta glucans content of grain, %DM
HYDCAP = Hydration capacity - % increase in weight after 16 h in water

It is clear from Table 2 that the use of repeatability files do not significantly change calibration accuracy (SECV). Previous studies have shown that temperature changes have more impact on the repeatability scans than instrument changes (Shenk and Westerhaus, 1994), and this can be seen in Table 2, with the REP values generally lower when a repeatability file based on temperature is used, as compared to a repeatability file based on different instruments. Another positive outcome is that the REP values across the board are generally small in relation to the SECV, even though the repeatability files used here are not based on sealed standards, as is often the case.

Table 2 - Statistics of updated AusScan equations with and without repeatability files (whole grain)

	NO REP			REP_INS				REP_TEMP				REP_INS+TEMP			
	N	SECV	R^2CV	N	SECV	R^2CV	REP	N	SECV	R^2CV	REP	N	SECV	R^2CV	REP
PIG															
FDEGAR	219	0.26	0.87	220	0.26	0.87	0.07	220	0.26	0.87	0.06	218	0.26	0.87	0.07
IDEGAR	212	0.45	0.82	213	0.46	0.80	0.14	215	0.47	0.80	0.07	213	0.45	0.80	0.13
PIGFDEINTIND*	61	11.21	0.52	60	11.04	0.52	5.05	60	11.04	0.52	1.85	59	11.22	0.50	4.24
BROILER															
AMEGAF	295	0.41	0.90	300	0.46	0.88	0.22	294	0.41	0.90	0.11	297	0.44	0.88	0.18
AMEGII	299	4.17	0.82	301	4.47	0.79	1.60	300	4.31	0.81	0.96	296	4.37	0.80	1.32
AMEGAFENZ*	200	0.19	0.70	201	0.19	0.68	0.05	199	0.19	0.72	0.03	200	0.19	0.70	0.04
RUMINANT															
AcidT4Ind*	21	10.56	0.71	21	10.17	0.73	3.30	21	10.70	0.70	2.16	21	10.32	0.72	2.97
EstMEcat	95	0.34	0.78	95	0.33	0.83	0.09	94	0.33	0.80	0.05	94	0.32	0.80	0.08
EstMEshp	103	0.34	0.82	103	0.35	0.80	0.09	103	0.35	0.81	0.10	103	0.36	0.80	0.10
AjDMDis	387	4.84	0.86	392	5.77	0.81	2.91	390	5.31	0.85	2.29	394	5.91	0.80	2.85
HullADL	227	1.40	0.83	228	1.42	0.82	0.34	227	1.40	0.83	0.22	228	1.43	0.82	0.34
Hull	350	2.85	0.84	350	3.29	0.79	2.15	348	3.01	0.82	1.26	349	3.25	0.79	1.89
CHEM															
CP_DM	325	0.68	0.96	322	0.66	0.96	0.37	324	0.66	0.96	0.21	322	0.67	0.96	0.32
CF_DM	175	0.56	0.97	176	0.58	0.97	0.18	178	0.59	0.96	0.18	176	0.58	0.96	0.19
ADF_DM	179	0.91	0.95	176	0.87	0.95	0.35	175	0.85	0.95	0.28	176	0.87	0.95	0.34
ENG_NDF_DM	176	3.20	0.79	176	3.16	0.79	0.82	176	3.18	0.79	0.57	174	3.05	0.81	0.77
TOT_ST_DM	177	3.16	0.93	173	3.04	0.94	1.08	176	3.10	0.93	0.91	170	3.07	0.93	1.10
TOTINSNSP_DM	181	1.88	0.90	180	1.79	0.91	0.61	181	1.87	0.90	0.53	172	1.74	0.91	0.57
TOTSOLNSP_DM	178	0.58	0.85	180	0.59	0.84	0.19	181	0.58	0.84	0.10	180	0.65	0.80	0.18
ARA_XYL_DM	170	1.17	0.89	171	1.24	0.87	0.41	170	1.27	0.87	0.41	170	1.27	0.86	0.38
BGLUC_DM	166	0.56	0.89	165	0.87	0.87	0.31	165	0.63	0.86	0.11	167	0.56	0.89	0.25
HYDCAP	175	7.15	0.86	173	6.58	0.88	2.73	174	6.55	0.89	2.24	172	6.59	0.88	2.58

*These equations have limited use, as in each case the ratio of standard deviation (SD) of reference values to SECV is below 2.0, indicating relatively poor calibration performance.

4. Application of Research

The outcomes of this project will lead to the AusScan calibrations becoming more stable and robust, through the re-standardisation process which was possible with the production of new whole grain standards, and the use of a repeatability file (or files). This will result in improved uniformity of analysis across the NIR instruments in the network, and hence improve confidence in the accuracy of the calibrations by licensees in both the research and industry sectors, and hopefully attract further participants to utilise the calibrations.

5. Conclusion

A new set of sealed whole grain "standards", representative of grain types which can be tested by the AusScan calibration equations currently in use, was successfully produced and used to re-standardise all FOSS monochromators being used in the NIR instrument network for whole grain analysis. The calibrations were successfully updated for use on a replacement master instrument, and standardisation files were produced to enable the 15 host instruments to be spectrally matched to the new master. Repeatability files were generated to represent both instrument and temperature differences, and when applied to the calibrations showed that calibration accuracy did not change significantly, and that repeatability values were small compared to calibration error.

6. Limitations/Risks

The main limitation of this study was that, due to the proposed new commercial arrangements for AusScan, it was not possible to distribute the updated equations and standardisation files to the licensees. It was also not possible to undertake the next vital step of organising a new ring test so that the performance of the revamped network could be properly evaluated.

Another limitation is that this study was necessarily confined to the use of FOSS monochromators and whole grain, as an NIR instrument network by definition must use a common software package for establishment and testing of calibrations, and must also use the same sample presentation method.

The third limitation is that the calibrations for PIGFDEINTIND, AMEGAFENZ and AcidT4Ind do not meet normal criteria for accuracy and precision or are based on insufficient samples. In all 3 cases, the ratio of standard deviations of reference values to SECV, known as RPD, is below 2.0. This means that, at best, they can only be used to distinguish high from low values, and should be used with extreme caution. Improvements to the AcidT4Ind calibration may be possible with further investigation of alternative reference values from a different source.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- the new whole grain standards should be carefully stored so that they can be used to standardise additional instruments in future or re-standardise existing instruments as required
- the updated calibration equations and new standardisation files should be made available to the existing licensees if and when appropriate
- it is vital for a new ring test to be organised when possible, so that a set of new grain samples can be tested by all network participants to properly evaluate the performance of the network

8. References

Flinn, P.C. and Saunders, L.D. (1995) pp 129-132 *In* G.D. Batten, P.C. Flinn, L.A. Welsh and A.B. Blakeney (Eds.) "Leaping Ahead with Near Infrared Spectroscopy". Royal Australian Chemical Institute, North Melbourne, Australia.

Shenk, J.S. and Westerhaus, M.O. (1994) pp 406-449 *In* G.C. Fahey, Jr. et al.(Eds.) "Forage Quality, Evaluation and Utilization". American Society of Agronomy, Inc., Madison, WI, USA.

Spragg, J. (2012) "AusScan Ring Test Results Report - December 2012". Pork CRC Ltd, Willaston, SA, Australia.

Westerhaus, M.O. (1990) pp 671-674 *In* R. Biston and N. Bartiaux-Thill (Eds.) "Proc. 3rd Int. Conf. on Near Infrared Spectroscopy". Agricultural Research Centre Publishing, Gembloux, Belgium.