SCREENING OF NEW LINES OF CEREAL GRAINS FOR INCLUSION IN NIRS CALIBRATIONS FOR PREDICTING NUTRITIONAL QUALITY OF FEED INGREDIENTS FOR PIGS

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

By

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Executive Summary

The aim of the project was to improve the accuracy and robustness of NIR calibrations for predicting the energy content of cereal grains for pigs by increasing the number and variety of grains included in the calibrations. Two experiments measuring whole tract digestible energy content (Faecal DE content) were conducted using 70 grains, including 23 'connectivity' grains that had been included in earlier experiments to allow statistical adjustment of measured values across all experiments used to establish the NIR calibrations. The 47 new grains included maize for the first time, several new grain varieties from cereal breeding companies, sprouted grains, large and coloured endosperm grains and grains considered to have scans identified as 'outliers' by the NIR software. Although ileal DE values were originally planned for the project, legal restrictions on ileal cannulation of pigs at the University of Queensland meant that measurements on the digestibility of energy to the end of the small intestine could not be made.

Celite, a source of acid insoluble ash (AIA), had been used as an indigestible marker for calculation of the DE content of the experimental grains in previous Premium Grains for Livestock (PGLP) and Pork CRC experiments. The AIA assay had been conducted for previous CRC projects at the DEEDI laboratory in Brisbane. However, this laboratory was closing and the assays were moved to the Symbio Alliance laboratory in Brisbane. An experiment designed to assess the relative accuracy of AIA measurements between the laboratories using samples from the first run of the first experiment, showed a positive bias and greater variance in values obtained from Symbio compared with the DEEDI laboratory. The analyses were repeated by Symbio following a review of procedures. Nevertheless, the variation between duplicate measurements was still substantial and the estimated variance in the Faecal DE content of the grains used in the two experiments was larger than in previous experiments.

Statistical analysis of the experimental results showed that the diet pelleting process accounted for more than 50% of the non-grain variation in Faecal DE measurements and reinforced the need for partial replication of the pelleting process in pig digestibility experiments.

There were highly significant ($P<0.01$) differences in Faecal DE values between grain types (wheat, barley, triticale, sorghum, maize) and within grain types. Natural paddock germination of a wheat and a barley sample tended to reduce the Faecal DE content of these grains, but it was only significant for wheat when expressed on a dry matter basis. Artificial germination of sorghum for 24 h increased Faecal DE content by approximately 1 MJ/kg as fed, but continuing germination for 48 h reduced the response to approximately 0.5 MJ/kg as fed greater than the non-germinated control. The marked increase in available energy content of sorghum with early germination was most likely related to natural proteases degrading the protein matrix encapsulating starch granules and enhancing the access of amylase to starch within the digestive tract. However, continuing germination appears to increase energy use through shoot and root development and reduce the positive response observed for short germination times.

NIR calibration upgrades were conducted for both whole grain and milled grain scans. Adding grains from the Pork CRC projects to the original set used to develop the NIR calibration in PGLP, has increased the accuracy or precision, as determined by a reduction in SECV, and the robustness or ability to predict values for unknown samples (RPD) of the NIR calibrations predicting the Faecal DE content of cereal grains. However, adding results from the two experiments in this project did not lead to a further increase in precision of predictions and actually reduced the RPD measure of the calibrations. The latest NIR calibration can predict Faecal DE with a standard error of ±0.26 MJ/kg as fed compared with ±0.38 MJ/kg as fed in the PGLP calibration. The latest calibration can predict Faecal

DE content within 0.52 MJ/kg as fed with 95% confidence. The robustness of the calibration as indicated by a final RPD value of 2.65 is regarded by NIR specialists as being good for predicting values for unknown samples. However, a RPD value >3 is considered excellent.

There are two reasons for the negative effects of adding results from the experiments in this project to the calibrations. First, the grains used in these experiments were selected because they were different and/or had different scans from those used in previous experiments. The selection of these grains was deliberate so the ability of the calibrations to predict values for unknown samples could be improved to cover maize and grains with unusual characteristics. Second, the variance in Faecal DE values derived from the experiments was considerably greater than observed in previous experiments. This increase in variance in Faecal DE values was partially due to greater variance in AIA measurements in these experiments compared with earlier experiments.

Comparison of calibrations based on whole grain scans with those based on milled grain scans suggests there is little difference in calibration statistics or the mean values predicted by each calibration for all grains used in PGLP and Pork CRC experiments. However, the predicted values for individual grains varied from -0.53 to + 0.65 MJ/kg as fed, with R^2 for the relationship of only 0.92. Thus for most grains, different values would be predicted from calibrations based on whole grain scans compared with those form the milled grain calibration.

Differences of this magnitude between the calibrations are unacceptable for commercial application. It is recommended strongly that milled grain calibrations are removed from the AusScan suite. Scanning whole grains is faster, removes milling effects/differences and does not involve additional expense of grain milling.

Recommendations

1. More experiments need to be conducted with both 'normal' and unusual grains, particularly maize samples and other grains selected because they are GH or NH outliers to further validate and upgrade the NIR calibrations for Faecal and Ileal DE. Selection of these grains should further improve the RPD value for the calibrations, with a target for a RPD value greater than 3.0.
2. NIR calibrations based on milled grain scans are removed from the AusScan suite of commercially available calibrations because the values predicted for individual grains differs widely depending whether the calibration is developed using whole grain or milled grain scans.

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1. Introduction

Previous research from the Premium Grains for Livestock Program (PGLP) showed that current methods used for trading grains for livestock in Australia (crude protein, test weight (kg/hl) and screenings percentage) have little relationship with either the available energy content (MJ/kg) or energy intake (MJ/d) of cereal grains for pigs (see Black et al. 2009). The Australian pork industry, in collaboration with PGLP funding organisations and RIRDC Chicken Meat Program, has established a more accurate and unique system for measuring the energy value of cereal grains for livestock based on whole grain near-infra red reflectance (NIR) calibrations. The system is being promoted as a new basis for trading grains for livestock in Australia through the Pork CRC AusScan project.

Research in the first CRC projects 1B-101 and 1B-104 (funded entirely by GRDC) has substantially improved the precision with which faecal digestible energy (DE) can be predicted with 95% confidence decreasing from ±0.38 to ±0.27 MJ/kg. Similarly the robustness of the calibration estimated by the Ratio of Precision to Deviation (RPD) has increased from 2.08 (approximately quantitative) to 2.63 (good prediction of unknown samples). The R^2 for the relationship between predicted and measured faecal DE has also improved from 0.84 to 0.89. Although the reliability of the calibrations has improved substantially through research within the Pork CRC, further experiments were needed to enhance the value of the calibrations for the pig industry and their adoption as the basis for trading feed grains in Australia.

Although considerable effort was put into selecting and producing grains for previous upgrades of the calibrations, which included weather damaged (water-stressed and sprouted) grains, it is clear that the addition of more sprouted and water-stressed grains would further improve the robustness of the calibrations. NIR scans of these weather damaged grains are still frequently seen as 'outliers' by the NIR analysis software and their energy values cannot be predicted with confidence. In addition, it has been an intention of the Pork CRC to make the NIR calibrations available to the international market. For this internationalisation to be successful, maize (commonly called corn in overseas markets) must be added to the calibrations. Thus, grains selected for examination in the project have included additional water-stressed and sprouted grains, extended the range of triticale, sorghum and barley samples using near release germ-lines and added nine maize samples collected for variation in genotype and growing conditions.

There is likely to be a significant royalty stream for the Pork CRC if the AusScan calibrations become the main basis for trading feed grains in Australia and overseas. Hence, in the longer-term it is expected that the AusScan business will be self sustaining and can fund the upgrading and maintenance of the NIR calibrations. However, for this to happen, confidence in the calibrations is needed in the short-term and this can only be gained by improving the precision and robustness of the existing calibrations. This project is a move towards this goal.

The value of a grain as a source of energy to an animal depends on the total amount of energy made available for metabolism (available energy intake, MJ/d), which is determined primarily by the energy released during digestion (available energy content, MJ/kg) and the amount of grain consumed (kg/d). Available energy content (MJ/kg) is used traditionally by the animal industries to formulate

diets with predefined energy content. The amount of a grain-based diet eaten by animals depends largely on factors affecting rate of passage of digesta through the whole digestive tract. Animal performance is driven by the total intake of available energy (MJ/d), whereas feed conversion efficiency is determined primarily by available energy content (MJ/kg) with total intake being important mainly when energy intake by the animal is close to maintenance.

The value of energy released during digestion varies substantially depending on whether digestion is a result of animal secreted enzymes or enzymes of microbial origin. In the latter case, dietary constituents are converted into growing microbes, volatile fatty acids and other compounds with the release of methane, ammonia and heat of fermentation. This microbial fermentation process can result in loss from the animal of 10-15 percent of the energy in digested material depending on diet composition, conditions of fermentation and species of microbes present. Hence, for a pig it is important to know both total disappearance of energy across the whole digestive tract (commonly called faecal digestible energy (DE), which accounts for animal enzyme and microbial digestion) and digestion to the end of the ileum (commonly called ileal DE, which accounts primarily for digestion by animal secreted enzymes).

Thus, it is important when determining the energy value of a grain, to measure the available energy content of the grain to the ileum and across the whole digestive tract as well as the total intake of available energy from the grain when it is incorporated into a diet.

A NIR calibration for DE intake index was developed in PGLP. Experiments measuring the intake of diets containing the same grains used in the digestibility trials were conducted in Projects 1B-101 & 1B-104 with the aim of strengthening this calibration. However, the experiments were poorly conducted, variation in measurements was excessive and a difference of approximately 18% in intake and growth rate was needed to obtain significance between grains ($P<0.05$). Subsequently, considerable effort was put into ensuring that the intake experiments could be conducted with higher precision at Wacol, with the experimental variation being reduced and a difference of 5% in FCR being statistically significant at $P<0.05$. However, lack of supply of pigs with uniform weight from the Gatton piggery meant that differences of approximately 8% were required for significance in feed intake and growth rate. The Wacol facilities were no longer available to the Pork CRC at the start of this project and the proposed new facilities at Darbalara, University of Queensland, were never built. Hence, although extremely important for predicting the energy value of grains for pigs, experiments measuring the intake of diets containing the selected grains did not proceed. Although ileal DE was measured in the first run of the experiments at the University of Queensland, there was a legal challenge to the way ileal cannulation was conducted for the experiments. This was not resolved in the short-term and resulted in only Feacal DE measurements being made to provide additional results for NIR calibration upgrades.

2. **Methodology**

Two experiments were conducted at the University of Queensland animal research facilities at Gatton. These experiments were given code names NG001 and NG002, respectively. Faecal DE values were determined for 70 grains across both experiments, with 40 grains in NG001 and 30 grains in NG002. These two experiments were regarded as being part of a much larger experiment that included all the pig digestibility experiments conducted in PGLP and the Pork CRC projects 1B-101 and 1B-104. Consequently, approximately 30% of the grains in each experiment had been used in previous experiments and were included to allow statistical 'connectivity' between experiments. The connectivity grains provide the basis for the statistical modelling of between and within experiment variation and enable a valid comparison of all grains. Hence, statistically adjusted values for measured variables across all experiments included in the NIR calibrations were derived.

2.1 Grains selected for experiments

The new grains were selected for the experiments on the basis of the following criteria:

- To increase the number of wheat, barley, triticale and sorghum samples in the calibration.
- Grains that had been shown by the NIR software to be outliers from the scan profiles incorporated into the current NIR calibrations. This criterion applied particularly to three wheat and two barley samples collected in Western Australia during 2009 and to three sorghum samples provided by a feed mill in eastern Australia.
- Grains from new or soon to be released lines from plant breeding companies such the durum wheat, Zulu and hybrid wheat Crusader, the new barley cultivar ND19119 (Rawson) and the triticale cultivars JRCT74 (Berkshire) and JRCT400 (not released).
- Grains that had characteristics different from those already included in the calibrations such as a naked barley (Finniss) and white sorghum (Liberty).
- Grains that had been weather damaged included the high screenings Bolac cultivar of wheat that had been water stressed and barley, wheat and sorghum samples with different degrees of sprouting.
- Wheat sample 1892 and barley sample 3933 were harvested from the same respective commercial crops that approximately 2 weeks apart; before and after rain and high humidity that caused pre-harvest sprouting. Sorghum sample 7887 was divided into three portions with one portion kept as a reference sample and the other two portions soaked for 24 h and for 48 h respectively, before being oven dried at 40^{o}C, to create moderately and highly sprouted grains.
- Cultivars of maize were collected opportunistically from northern and southern New South Wales. These maize samples consisted of varieties grown for both animal feed and silage or processing into human foods such as corn chips.

The grains used in experiment NG001 are shown in Table 1, while the grains used in experiment NG002 are shown in Table 2.

Table 1. Grain unique identification number and treatment code, grain type, variety and other information for each grain used in experiment NG001.

Sample ID + info code	Grain type	Variety and other information	Harvested
1763_RAW0	wheat	fed in DS004	connectivity
1766_RAW0	wheat	fed in DS004	connectivity
1770_RAW0	wheat	fed in DS004, HR001	connectivity
1882_RAW0	wheat	Unknown - Agracorp, ASW (Sue Low)	2009
1883_RAW0	wheat	Unknown - Curruthers, ASW (Sue Low)	2009
1884_RAW0	wheat	Unknown - Sutherland, ASW (Sue Low)	2009
1886_RAW0	wheat	Jandaroi from Rob Coleman	2009
1887_RAW0	wheat	Zulu - new feed durum (D Ronald - Spring Ridge)	2009
1888_RAW0	wheat	Crusader - Hybrid wheat (D Ronald - Spring Ridge)	2009
3871_RAW0	barley	fed in DS004, HR001	Connectivity
3873_RAW0	barley	fed in DS004, HR001	Connectivity
3874_RAW0	barley	fed in DS004	Connectivity
3875_RAW0	barley	fed in HR001	Connectivity
3897_RAW0	barley	fed in DS004, HR001	Connectivity
3922_RAW0	barley	Grout from PBI - opportunity grain.	2009
3923_RAW0	barley	Fitzroy from PBI - opportunity grain	2009
3924_RAW0	barley	Unknown - Danubin, feed (Sue Low)	2009
3926_RAW0	barley	Unknown - Nicoletti, feed (Sue Low)	2009
3927_RAW0	barley	Fleet from John Sturgess	2009
3928_RAW0	barley	Fleet2 from John Sturgess	2009
3929_RAW0	barley	ND19119 (Rawson) from John Sturgess	2009
3930_RAW0	barley	Shepherd from John Stugess	2009
3931_RAW0	barley	ND19119 (Rawson) from Ken Cameron	2009
3932_RAW0	barley	Finniss (naked barley) from Chris Farlow	2009
6830_RAW0	triticale	fed in DS004, HR001	Connectivity
6831_RAW0	triticale	fed in DS004	Connectivity
6849_RAW0	triticale	Berkshire (JRCT74) Dave Henman - Rivalea	2009
6850_RAW0	triticale	JRCT400 (newest Usyd line)	2009
6859_RAW0	triticale	Bogong (Chris Farlow)	2009
7855_RAW0	sorghum	fed in DS002, DS004	Connectivity
7857_RAW0	sorghum	fed in DS002, DS004, HR001	Connectivity
7869_RAW0	sorghum	fed in HR001	Connectivity
7885_RAW0	sorghum	Liberty (White) sorghum	2009
7886_RAW0	sorghum	Buster from PBI Narrabri	2009
7887_RAW0	sorghum	MR43 normal unsprouted grain (from Hermitage)	2009
7887_SPROUT_MED	sorghum	slightly sprouted (from Hermitage)	2009
7887_SPROUT_HIGH	sorghum	heavily sprouted (from Hermitage)	2009

8082_RAW0	maize	PAC345 processing corn (from Rob Coleman)	2009
8083_RAW0	maize	Unknown - corn from Better Blend	2009
8084_RAW0	maize	31G66 feed and silage corn (from Dave Turner)	2010

Table 2. Grain unique identification number and treatment code, grain type, variety and other information for each grain used in experiment NG002.

Sample ID + info code	Grain type	Variety and other information	Harvested
1763_RAW0	wheat	fed in DS004, NG001	Connectivity
1766_RAW0	wheat	fed in DS004, NG001	Connectivity
1770_RAW0	wheat	fed in DS004, HR001, NG001	Connectivity
1885_RAW0	wheat	Bolac (Hart Bros seeds 17% screenings)	2009
1889_RAW0	wheat	Kidman (biscuit wheat)	2009
1890_RAW0	wheat	Qal2004 (soft wheat)	2009
1892_RAW0	wheat	sound durum from Martin Dunlop (FN 426)	2010
1892_SPROUT	wheat	sprouted durum from Martin Dunlop (FN 142)	2010
1893_SPROUT	wheat	Sunlin - PBI Narrabri (sprouted)	2010
3871_RAW0	barley	fed in DS004, HR001, NG001	Connectivity
3874_RAW0	barley	fed in DS004, HR001, NG001	Connectivity
3933_RAW0	barley	unsprouted Grout feed barley from Bill Wall	2010
3933_SPROUT	barley	Sprouted Grout feed barley from Bill Wall	2010
36830_RAW0	triticale	fed in DS004, HR001, NG001	Connectivity
6860_RAW0	triticale	Canobolas	2009
6861_RAW0	triticale	Tobruk	2009
6866_SPROUT	triticale	JRCT400 - PBI Narrabri (sprouted)	2010
7855_RAW0	sorghum	fed in DS002, DS004, NG001	Connectivity
7861_RAW0	sorghum	fed in DS002, DS004	Connectivity
7889_RAW0	sorghum	Unknown - Weston Animal Nutrition	2010
7890_RAW0	sorghum	Unknown - Weston Animal Nutrition	2010
7891_RAW0	sorghum	Unknown - Weston Animal Nutrition	2010
8082_RAW0	maize	fed in NG001	Connectivity
8084_RAW0	maize	fed in NG001	Connectivity
8085_RAW0	maize	Unknown - Rob Coleman, Quirindi	2010
8086_RAW0	maize	N43N34 (Andrew Pursehouse), Quirindi	2010
8087_RAW0	maize	32P55 (Andrew Pursehouse) , Quirindi	2010
8088_RAW0	maize	Unknown - 2009 season Harris Bros, Moree	2010
8089_RAW0	maize	Unknown - 2010 season grain (Tynams), Hay	2010
8090_RAW0	maize	32P55 (James Keen), Bellata	2011

2.2 Diets and processing procedures

Each grain was milled through a 3.4 mm screen in a hammer-mill before being included in a diet. The diets fed to pigs consisted of 94.5% cereal grain with the remainder being minerals, vitamins and celite (Table 3). Celite was used as an

acid insoluble ash indigestible marker for calculating the proportion of energy consumed that was digested.

Table 3. Composition of the diet offered to pigs when determining the DE content of grains.

Diet Ingredient	%	kg
Grain	94.505	94.505
Dicalcium phosphate	3.00	3.00
Salt	0.275	0.275
Minerals	0.07	0.07
Vitamins	0.05	0.05
Choline chloride	0.10	0.10
Celite	2.00	2.00
Total	**100**	**100**

The diets were cold-press pelleted through a 5mm diameter die at the DEEDI, Alexandra Hills feed processing unit. Cold-press pelleting was used to minimise the effect of heat and/or gelatinisation on the structure of the grain and its digestibility.

Moisture content of a grain and ambient conditions can influence the amount of structural change a grain undergoes during processing. These variables are difficult to control, so the diets were pelleted in accordance with a partially replicated experimental design. Thus, in experiment NG001, 40 grain samples were offered to the pigs, but 50 pellet batches were made. Similarly, in experiment NG002, 30 grains were fed, but 40 pellet batches were made.

The experimental design for pelleting the diets for NG001 is shown in Table 4 to illustrate the degree of replication and the variation in order of pelleting. A similar pelleting design was used in experiment NG002.

Table 4. Design for the pelleting process for diets in experiment NG001 showing grain sample ID, day each grain was pelleted, the order of pelleting throughout the day and the grains replicated.

BATCH No.	DAY No.	DAY ORDER	SAMPLE ID + INFO
1	1	1	3871_RAW0
2	1	2	3929_RAW0
3	1	3	3926_RAW0 (1st batch)
4	1	4	8082_RAW0 (1st batch)
5	1	5	8084_RAW0
6	2	1	3874_RAW0
7	2	2	3930_RAW0
8	2	3	3923_RAW0 (1st batch)
9	2	4	6849_RAW0 (1st batch)
10	2	5	6850_RAW0

11	3	1	7886_RAW0 (1st batch)
12	3	2	7885_RAW0 (1st batch)
13	3	3	7857_RAW0
14	3	4	3928_RAW0 (1st batch)
15	3	5	3927_RAW0
16	4	1	3897_RAW0
17	4	2	3928_RAW0 (2nd batch)
18	4	3	3924_RAW0
19	4	4	7885_RAW0 (2nd batch)
20	4	5	7855_RAW0
21	5	1	3922_RAW0
22	5	2	3923_RAW0 (2nd batch)
23	5	3	3875_RAW0
24	5	4	8083_RAW0
25	5	5	8082_RAW0 (2nd batch)
26	6	1	1763_RAW0
27	6	2	1884_RAW0
28	6	3	1888_RAW0 (1st batch)
29	6	4	3932_RAW0
30	6	5	3931_RAW0 (1st batch)
BATCH #	**DAY #**	**DAY ORDER**	**SAMPLE ID + INFO**
31	7	1	3926_RAW0 (2nd batch)
32	7	2	3873_RAW0
33	7	3	3931_RAW0 (2nd batch)
34	7	4	1882_RAW0
35	7	5	1887_RAW0 (1st batch)
36	8	1	7887_SPROUT_HIGH
37	8	2	7869_RAW0
38	8	3	7887_SPROUT_MED
39	8	4	6859_RAW0
40	8	5	6849_RAW0 (2nd batch)
41	9	1	1883_RAW0
42	9	2	1770_RAW0
43	9	3	1888_RAW0 (2nd batch)
44	9	4	6831_RAW0
45	9	5	6830_RAW0
46	10	1	1887_RAW0 (2nd batch)
47	10	2	1886_RAW0
48	10	3	1766_RAW0
49	10	4	7886_RAW0 (2nd batch)
50	10	5	7887_RAW0

2.3 Animal experiments

A similar experimental protocol was adopted for experiments NG001 and NG002. Fifty six pure bred Large White male pigs from the Gatton herd were used within each experiment over four runs. Within each run, there were five feeding and sampling periods where 14 pigs were each fed five different diets, each for a period of seven days. Thus, there was a total of 280 pig x diet measurements.

For each experimental run, 17 male pigs weighing approximately 25 kg were selected from the commercial herd and transferred to the research facility at Gatton. The pigs were housed in individual pens, in an environmentally controlled room with the temperature maintained at 24°C. The pigs were offered water and a commercial 'porker' diet *ad libitum*. For the next 14 days (day 0-14), the pigs were allowed to acclimatise to the environment and handling and were monitored daily for temperament, eating behaviour and signs of distress or disease. At the end of the acclimatisation period, the pigs were weighed and 14 selected for the experimental run based on temperament, an assessment of thrift and general wellbeing, feeding pattern and live weight. The rejected pigs were retained as spare animals in case pigs needed replacing during the experiment.

The pigs were reallocated to pens as shown in Figure 1. Pens 1-14 housed the experimental pigs, pens 16-18 housed the rejected spare pigs, while pens 0, 15 and 19 remained empty. Pigs selected for the experiment were offered the commercial 'porker' diet on day 15 during the reallocation to new pens. The spare pigs remained on the 'porker' diet throughout the whole experiment, unless they were needed to replace one of the selected pigs.

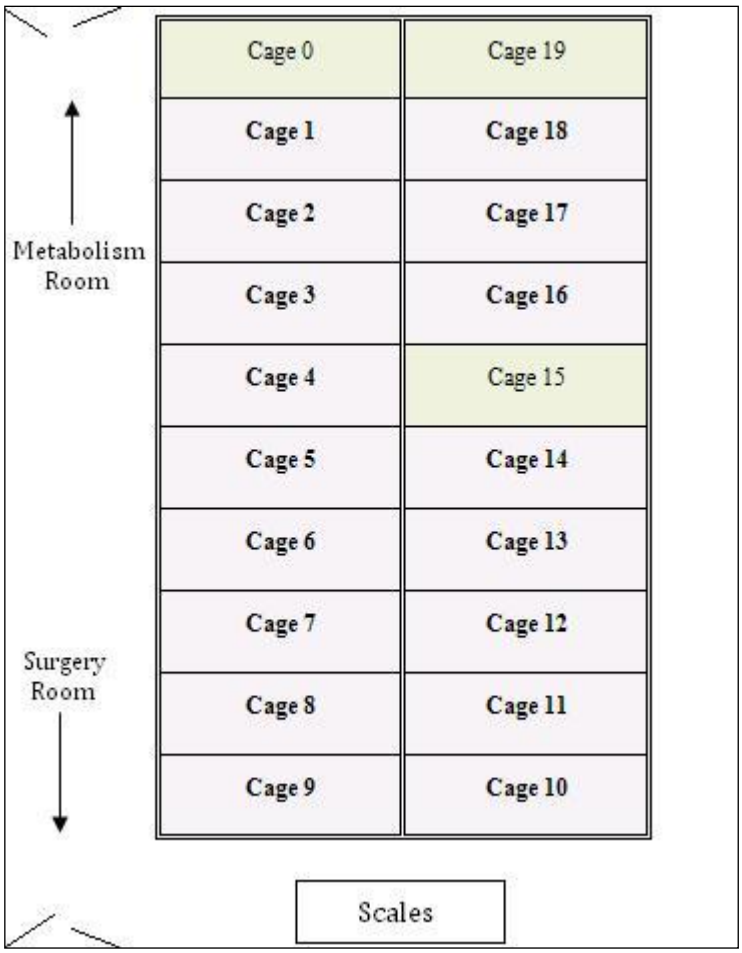

Figure 1. Layout of pens in the holding room, prior to collection of faeces.

Each pig was allocated its five diets on a statistically randomised basis taking account of pig position in the rooms and the order in which the diets were fed. The allocation of diets and experimental design for NG001 is given in Appendix 1. A similar process was used for NG002.

On day 16 of each run, the 14 selected pigs were offered their experimental diets according to the design. The pigs were offered an amount of feed calculated to be 2.5 times energy maintenance [2.5*(0.5MJ DE/kg $LW^{0.75}$)/diet DE]. Where diet DE was assumed to be 14 MJ/kg. The daily ration was divided into two portions and feed at 12 hourly intervals.

On day 21, between 06.30 and 08.00 h the 14 pigs were transferred from the individual pens to individual, elevated metabolism crates and continued to be fed their allocated diet. The layout of the crates in the Metabolism room is shown in Figure 2. Each pig maintained the same crate number that it was allocated for the pens in Figure 1.

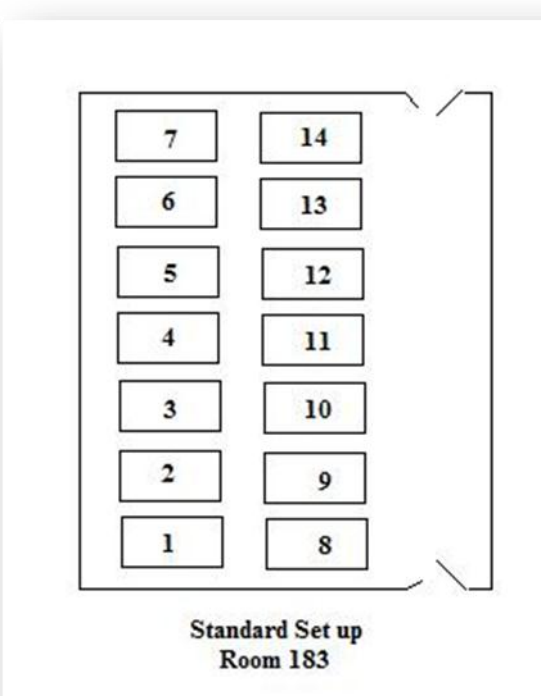

Figure 2. Layout of pens in the metabolism room.

The pigs in metabolism crates were checked every 10 minutes from 08.00 h to 16.00 h on day 21 for faecal excretion. Any faeces collected were pooled and the amount collected by 16.00 h was stored overnight at -4°C. The same procedure was followed from 06.30 to 14.30 h on day 22, with new faecal collections being added to a new bag.

From 15.00 h on day 22, the pigs were moved progressively from the metabolism crates, weighed and placed back to their allocated pens. The recorded weights were used to calculate the amount of the next diet that would be offered to each pig.

Faeces collected for each pig on day 21 were removed from the freezer around 11.00 h on day 22 and kept at ambient temperature. Then, from 16.00 h on day 22, the pooled faecal samples from day 21 and day 22 for each pig were thoroughly mixed and two subsamples taken for future analysis. These subsamples were obtained by the standard sub-sampling method and stored at -20°C until required. One was used for chemical and energy analyses, while the second was maintained in reserve.

All pigs were offered their next diet on day 23 and the procedure from day 16 was repeated. These 7-day cycles of feeding and faecal collection were continued for each pig until 5 diets had been fed and the run completed.

The facilities were then thoroughly cleaned and a new group of 17 pigs selected from the commercial herd and the procedures repeated until all 4 runs were completed.

2.4 Chemical and physical analyses

Each grain sample was analysed for dry matter (DM), nitrogen (N), starch (S), gross energy (GE), phytate, phosphorus (P), acid detergent fibre (ADF) and neutral detergent fibre (NDF). In addition, each grain sample was analysed for 1000 grain weight (g), specific weight (kg/hl) and screenings percentage. The same procedures for these analyses in previous projects assessing the digestibility of grains were used (Final Report Pork CRC project 1B-101). The chemical analyses were conducted by Symbio Alliance, Eight Mile Plains, QLD 4113 (www.symioalliance.com.au), whereas the physical measurements were conducted at the University of Sydney, Narrabri.

Pellet samples were analysed for DM, acid insoluble ash (AIA), GE, phytate and P. One of the faecal subsamples for each pig was freeze dried before analyses were conducted for DM, AIA, GE, phytate and P.

Accuracy of the AIA measurements was critical to the calculation of amount of energy digested because it was used as the diet marker and assumed to be indigestible. The AIA analyses were conducted with a replicated experimental design accounting for day and time/order of analysis and position of the analytical crucibles within the ashing oven. AIA analyses in the previous Pork CRC experiments had been conducted by the Chemistry Laboratory at DEEDI in Brisbane. This laboratory was ceasing operations, so an experiment was designed to compare AIA measurements made at the DEEDI laboratory with those derived from Symbio. The 50 pellet samples and 70 freeze-dried ileal and faecal samples from the first run of NG001 were prepared and ground in the Symbio laboratory and then analysed in designed experiments in both the DEEDI laboratory and the Symbio laboratory. For each analysis, if the value of pellet sample replicates differed by more than 0.3 %w/w, or the ileal and faecal replicates differed by more than 0.8 %w/w, the samples were analysed again in another randomised

experiment. The tolerance limits were calculated with respect to the sensitivity of the respective pellet and animal sample AIA values within the digestible energy equation. The analysis of samples for AIA for all PGLP experiments was conducted by the SARDI laboratory (Roseworthy). A comparison in the accuracy of the AIA measurements made by the SARDI and DEEDI laboratories was conducted previously when the change from SARDI to the DEEDI laboratory occurred in 2006.

2.5 Statistical analyses

For all analyses, linear mixed model technology was used as it can be formulated in such a way that it is analogous to ANOVA. Like ANOVA, the linear mixed model can include terms which reflect both the treatment and blocking structure of an experiment. The former are typically fitted as fixed effects and the later as random effects. In the analysis of NG001 and NG002, the fixed effects contained the effect of grains. Random effects include the effect of pellet batches, pellet days, cage, pig, feeding run, and feeding period. The first two were particularly important sources of non-grain variation in both NG001 and NG002, regularly accounting for more than 50% of non-grain variation. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). All models were fitted using the ASReml-R (Butler et. al. 2007) package for the statistical computing software R (R Core Team 2011).

When experiments were analysed in a meta-analysis to determine statistically corrected values across all experiments, the term "experiment" is fitted as a random effect by itself and as an interaction with all other random terms. This is similar to considering individual experiments as incomplete blocks and enables the recovery of inter-block information. This results in more accurate estimates of grain effects compared to the case of considering experiments as fixed effects.

2.6 Calculations

Faecal DE – All diets included approximately 2% celite (AIA) as an indigestible marker, thus as the diet is digested the concentration of the AIA marker increases. The AIA marker was used to determine digestibility with the following measurements needed to calculate faecal digestibility. All values were initially calculated on an as fed basis and then converted to a dry matter basis.

Digestible energy (DE) values were calculated using the following equations:

Faecal DE of the diet (ar) = (pellet GE (DM) - (faecal GE(DM) x pellet AIA(DM)/faecal AIA(DM))) * (pellet DM %/100))

Ingredient (grain) faecal DE (ar) = (diet faecal DE(ar) - DE other diet components)/% grain in diet

Ingredient (grain) faecal DE (DM) = ingredient (grain) faecal DE (ar)/pellet dry matter

Where: ar = as-received basis, DM = dry matter basis, DE of the other diet components = 0.387 MJ/kg, % grain in diet = 0.94505

2.7 NIR methodology

The NIRS spectra were collected in reflectance mode (log 1/R) from each experimental grain using both whole and laboratory milled samples. Whole grain samples were scanned twice using the sample transport module in a model 6500 scanning monochromator (FOSS, Denmark) and the mean spectra obtained. Milled grain samples were scanned once in small ring cups using the spinning sample module in a model 5000 scanning monochromator (FOSS, Denmark). Both instruments had been previously spectrally matched.

WinISI software, version 3 (FOSS, Denmark) was used to pre-treat the spectral data using "standard normal variate" and "detrend" options. Calibrations for Faecal DE were derived using modified partial least squares regression and a second derivative mathematical treatment. The spectral range utilized for the whole samples was from 700 to 2498 nm, and for the milled samples 1100-2498 nm. Spectral outliers were omitted from the final calibrations. Separate calibrations were established for whole and milled grain scans.

The current NIR Faecal DE calibration available through AusScan (May 2011) was initially used to determine its ability to predict the measured values derived from experiment NG001. These values from NG001 were then included in an updated calibration. This new calibration was used to determine its ability to predict the measured Faecal DE values in experiment NG002. Finally, results from NG002 were included in the latest calculation.

3. **Outcomes**

3.1 Comparison between laboratories in AIA assays

A comparison of AIA analytical results between the DEEDI and Symbio laboratories is given in Table 5 and Figures 3-5. It is clear that the mean values from the Symbio laboratory were consistently higher and more variable than those from the DEEDI laboratory that had been used for analysing AIA in the previous Pork CRC experiments. A comparison with earlier experiments conducted in PGLP where the analyses were conducted at the SARDI Roseworthy laboratory, showed that the mean difference between duplicates was 1.2% for pellets compared with 3.2% for the DEEDI laboratory and 18.8% for Symbio.

Table 6 illustrates the consequence of inaccuracy in duplicate measurements on predicted Faecal DE values (MJ/kg) for a selected number of grain samples using the Symbio analyses. The grains included in Table 6 are those with no difference in duplicates of pellets, no difference in duplicates of faecal samples, approximately mean differences in duplicates for both pellets and faecal analyses,

largest difference between pellet duplicate analyses, and largest difference between faecal duplicate analyses.

A summary of the observations is that differences in pellet AIA analyses have a greater influence on predicted DE than differences in faecal analyses. Currently, the Faecal DE NIR calibration is predicted with an accuracy of ± 0.27 MJ/kg. The analysis of the effect of variation in AIA analyses suggests that a difference of >0.3 %w/w for pellets will result in a difference >0.2 MJ/kg in Faecal DE. Similarly, a difference in duplicates >0.8%w/w in faecal AIA will result in a difference >0.2 MJ/kg DE. Hence, all Symbio analyses that had a difference greater than 0.3%w/w in pellet AIA assays and >0.8%w/w in faecal AIA assays were repeated. This meant that more than 50% of the AIA assays needed to be reanalysed.

A review of the Symbio and DEEDI analytical procedures was undertaken. The Symbio laboratory had not conducted AIA assays previously. The Symbio laboratory used larger samples of 3-10 g compared with the traditional amount of approximately 1 g. The Symbio assay involved more handling steps than the DEEDI method and the crucibles used by Symbio differed from those used in the DEEDI laboratory. The Symbio assay involved only a 4 hr ashing period, whereas DEEDI used a system that ashed samples until constant weight. Finally, Symbio did not correct for a filter paper blank. It was agreed that Symbio would repeat all the pellet and faecal samples, using crucibles from SARDI, 3-5 g of pellets, ash the samples overnight and correct for the filter paper blank.

Following reanalysis of the AIA content of the pellet and faecal samples by Symbio, a comparison was made between the Faecal DE values derived from the Symbio and the DEEDI AIA values for all 70 grains used in run 1, period 1 of experiment NG001 (Figure 6). Reanalysis of pellets and faeces for AIA by Symbio removed the bias between the two laboratories and reduced, but did not remove, all the variation. Nevertheless, closure of the DEEDI laboratory meant that the Symbio AIA results were used for all further calculations of Faecal DE for experiments NG001 and NG002.

The large variation observed in AIA analyses has caused a re-evaluation of the suitability of celite as an internal marker. Consequently, the next experiment in the series to improve the accuracy and robustness of NIR calibrations in project 4B-117 will include celite and titanium dioxide as markers for comparison of repeatability of duplicates and effects on calculated Ileal and Faecal DE values. If titanium oxide proves more repeatable, it may be used as the preferred marker in all future experiments.

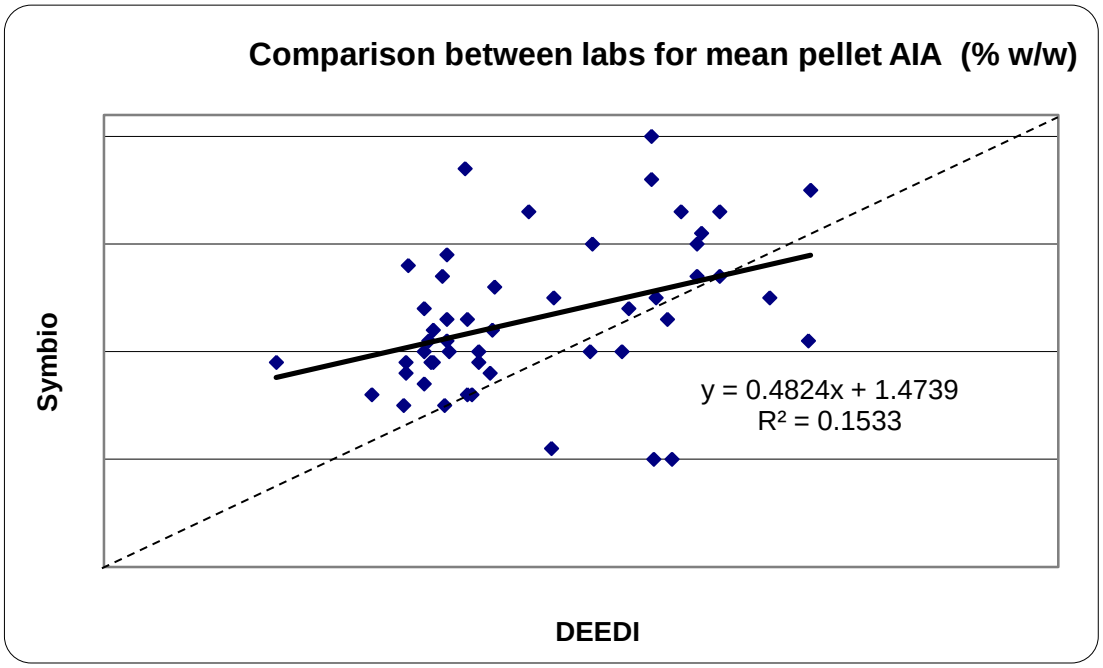

Figure 3. Comparison between the Symbio and DEEDI laboratories in AIA values obtained for the 50 pellet diets used in experiment NG001.

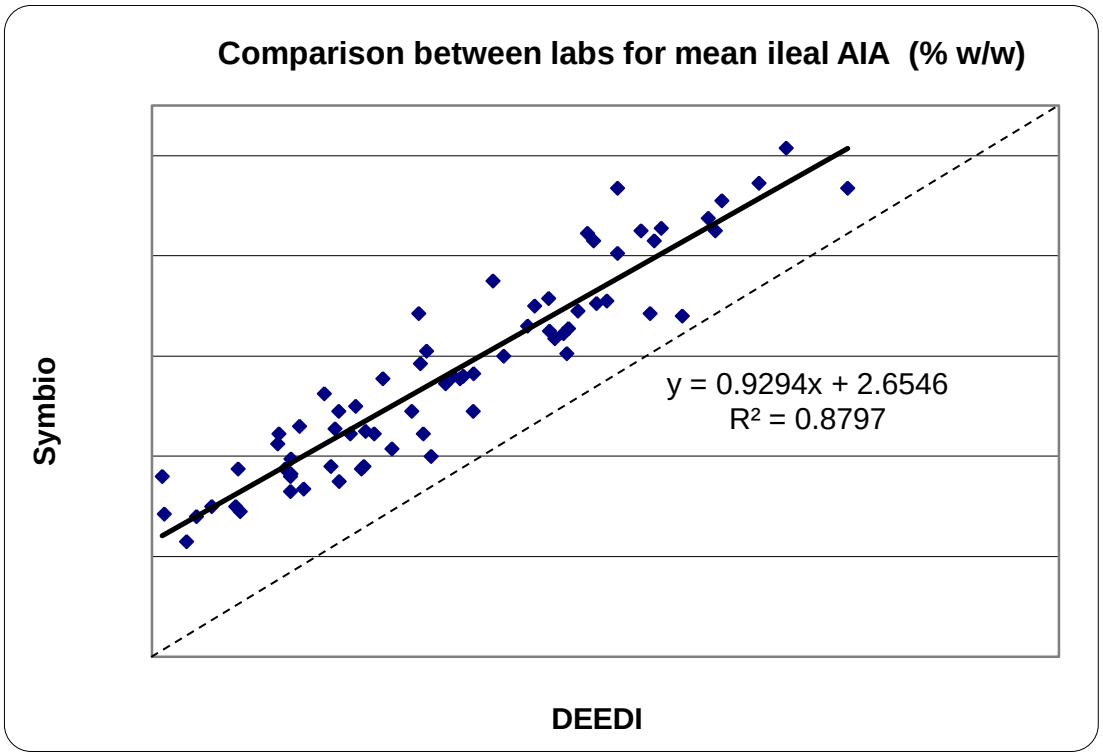

Figure 4. Comparison between the Symbio and DEEDI laboratories in AIA values obtained for the 70 freeze-dried ileal diets from run 1 in experiment NG001.

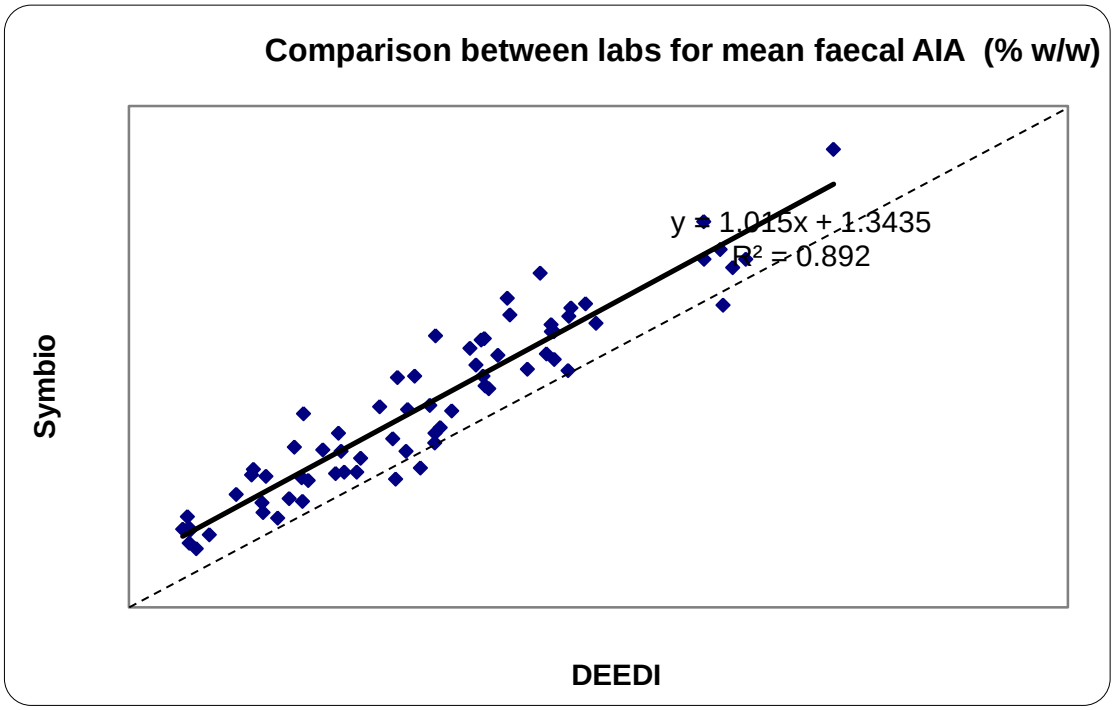

Figure 5. Comparison between the Symbio and DEEDI laboratories in AIA values obtained for the 70 freeze-dried faecal diets from run 1 in experiment NG001.

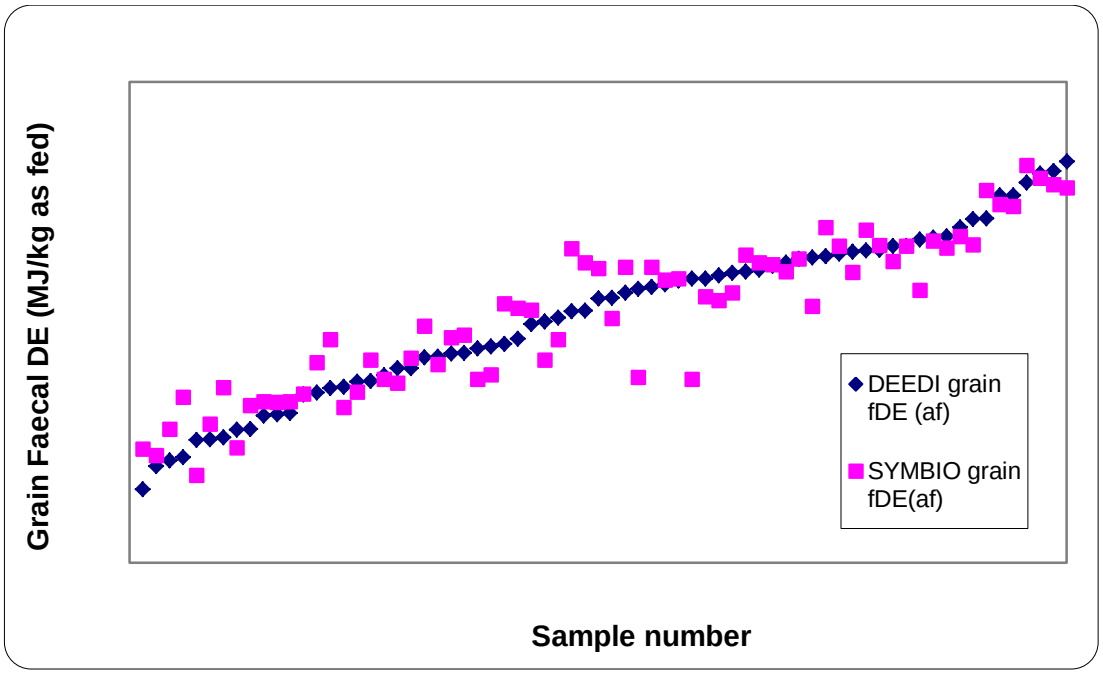

Figure 6. Comparison of Faecal DE values calculated using reanalyed Symbio or DEEDI AIA measured values for pellets and freeze dried faeces for all pigs in run 1, period 1 for experiment NG001.

Table 5. Comparison of AIA analytical results (% w/w) from the DEEDI laboratory and the Symbio laboratory.

Sample type	Number of samples	DEEDI Laboratory				Symbio Laboratory			
		Mean	Mean difference between duplicates	Mean difference in duplicates as % of mean	Range in difference between duplicates	Mean	Mean difference between duplicates	Mean difference in duplicates as % of mean	Range in difference between duplicates
Pellets	50	2.54	0.08	3.24	0.00-0.20	2.70	0.50	18.8	0.00-1.90
Ileal digesta	70	8.63	0.23	2.61	0.00-0.54	10.68	0.97	9.10	0.00-4.70
Faeces	70	15.73	0.27	1.70	0.00-0.63	17.31	0.95	5.47	0.00-1.90

Table 6. Effect in variation in AIA analyses for pellets and feacal samples on predicted faecal DE values (MJ/kg) for selected grains and experimental runs.

Sample	Pellet AIA (%w/w)		Faecal AIA (%w/w)		Predicted Faecal DE (MJ/kg)								
	Range	% mean	Range	% mean	LF-MP[1]	HF-MP	MF-LP	MF-HP	LF-LP	HF-LP	LF-HP	HF-HP	Range
Sorghum 7887	2.5-2.5	0	21.1-22.8	7.3	15.67	15.80	15.74	15.74	15.67	15.80	15.67	15.80	0.13
Barley 3926	2.9-3.1	6.6	13.2-13.2	0	13.99	13.99	14.14	13.91	14.14	14.14	13.91	13.91	0.23
Wheat 1884	2.2-2.7	20.4	19.1-20.4	6.6	15.52	15.64	15.77	15.39	15.72	15.83	15.32	15.46	0.51
Barley 3928	1.1-2.9	90.0	13.5-14.6	7.8	15.22	15.39	16.27	14.34	16.22	16.32	14.21	14.46	2.11
Sorghum 7885	2.7-3.1	13.8	23.5-29.4	22.3	15.59	15.96	15.91	15.68	15.72	16.07	15.46	15.86	0.61

[1]L= Lowest value, M=Mean value, H=Highest value, F=faeces, P=Pellet

3.2 Factors influencing Faecal DE values

Factors contributing to non-grain variation for the variables of Diet Faecal DE and Grain Faecal DE (both as fed and on a dry matter basis) for experiments NG001and NG002 are shown in Tables 7 and 8. The statistically corrected mean values with standard errors for each grain type are shown in Tables 9 and 10, and for each grain sample are shown in Tables 11 and 12, respectively for experiments NG001 and NG002.

3.2.1 Non-grain variation

Processing of the diets contributed most of the non-grain variation for the variable Faecal DE in both experiments. In experiment NG001, processing batch contributed to almost 50% of the non-grain variation, whereas in experiment NG002, it contributed approximately 65% of the non-grain variation. This high amount of non-grain variation in DE being contributed by cold pelleting suggests there is some change in grains during this process, either through physical breakdown of grain structure or mild gelatinisation. In the absence of strict process control during cold-pellet processing, the throughput rate, amount of water added, conditioning time or speed of the pellet press can be changed intermittently to obtain a durable pellet. Clearly, these undefined actions affect reproducibility of processing and pellet quality, confirming the need for partial replication of the processing step within experiments.

Pig number and experimental period both contributed to non-grain variation in DE. It is known that pigs have inherent differences in rate of digesta passage and enzyme concentrations that would contribute to this observed variation. In addition, pig weight changes with experimental period because each pig was offered five diets during each run. Digestibility of diets can tend to increase as pigs become more mature. The position of the metabolism crates also had an impact on diet digestibility in experiment NG001, but not in experiment NG002. The most important observation from Tables 7 and 8 is that proper experimental design reduced non-grain unaccounted variation (residual or random error) from 100% to between 30% (NG001) and 20% (NG002).

Table 7. Factors contributing to non-grain variation for Faecal DE values in experiment NG001.

Factor	Contribution to total error (%)		
	Diet Faecal DE (as fed) MJ/kg	Grain Faecal DE (as fed) MJ/kg	Grain Faecal DE (DM basis) MJ/kg
Processing batch	47.4	47.0	25.6
Processing day	0.0	0.0	0.0
Crate No.	5.1	5.3	7.6
Pig No.	12.5	12.5	16.8
Experiment run	0.0	0.0	0.00
Experiment period	4.5	4.4	7.0
Unaccounted	30.5	30.8	43.0
Total	*100*	*100*	*100*

Table 8. Factors contributing to non-grain variation for Faecal DE values in experiment NG002.

Factor	Contribution to total error (%)		
	Diet Faecal DE (as fed) MJ/kg	Grain Faecal DE (as fed) MJ/kg	Grain Faecal DE (DM basis) MJ/kg
Processing batch	38.6	38.7	20.5
Processing day	26.7	26.6	33.3
Crate No.	0.0	0.0	0.0
Pig No.	6.2	6.3	8.2
Experiment run	0.0	0.0	0.0
Experiment period	7.9	7.9	10.4
Unaccounted	20.6	20.5	27.6
Total	*100*	*100*	*100*

3.2.2 Effect of grain

Statistical analyses showed that there were highly significant differences ($P<0.001$) for all measures of Faecal DE between grain types and between grains within each grain type. Approximate significant differences between each grain can be assessed in Tables 9 and 10, where least significant differences (LSD's) at the 1% and 5% level are provided. If it is of interest to perform a large number of pair-wise comparisons, a method for controlling the family wise error rate (FWER, probability of declaring a difference between two grains when in fact there is none when conducting one or more hypothesis tests) should be used, such as a Bonferroni correction, Tukey-Kramer adjustment, or False Discovery Rates. The later method is preferred.

Faecal DE values were lower for barley than the other grains. A general trend for increasing Faecal DE content across grain types was barley <triticale <wheat <sorghum <maize, although the differences between sorghum and maize were inconsistent across experiments. A thorough comparison of all grains in Tables 11 and 12 shows wide variation within and between grain types.

3.2.3 Effect of experiment

Statistical analysis shows that the standard errors associated with the statistically 'corrected' values for grains was approximately 50% more in experiment NG002 than NG001. The mean values for each grain are also lower in experiment NG002 than NG001. The reasons for this large difference in values and standard errors are not clear, except there was an indication of mould in several of the diet batches in the last run for experiment NG002.

These apparently large differences in values for individual grains across experiments NG001 compared with NG002 are largely removed when the effect of individual experiments is taken into account during the meta-analysis which compares results across all experiments conducted within the PGLP and Pork CRC. For example, 14 grains used in experiments prior to experiments NG001 and NG002 were used as connectivity grains within these experiments. The individual values obtained for these connectivity grains in experiments NG001 and NG002 varied widely from the meta-analysis corrected values established prior to these experiments. However, the mean difference in meta-analysis corrected grain Faecal DE values was only 0.04 MJ/kg as fed prior to and after experiments NG001 and NG002 were added to the analyses. The range in difference between the values for these grains prior to and after inclusion of the NG001 and NG002 results was -0.03 to 0.12. Similarly, mean difference in grain Faecal DE in the meta-analysis corrected values for all grains before and after inclusion of NG001 and NG002 results was only 0.03MJ/kg as fed.

Table 9. Statistically corrected mean values and standard errors (se) for Diet Faecal DE and Grain Faecal DE in experiment NG001.

Grain type	Diet Faecal DE (as fed) MJ/kg		Grain Faecal DE (as fed) MJ/kg		Grain Faecal DE (DM basis)MJ/kg	
	Mean	Se	Mean	se	Mean	se
Wheat	13.54	0.062	13.91	0.066	15.95	0.056
Barley	12.65	0.050	12.97	0.053	14.73	0.047
Triticale	13.32	0.081	13.68	0.086	15.64	0.070
Sorghum	13.73	0.065	14.12	0.069	16.22	0.058
Maize	13.92	0.102	14.31	0.107	16.22	0.088
avsed		*0.100*		*0.105*		*0.083*
Approx	*5%*	*0.210*		*0.221*		*0.174*
LSD	*1%*	*0.270*		*0.227*		*0.224*

Table 10. Statistically corrected mean values and standard errors (se) for Diet Faecal DE and Grain Faecal DE in experiment NG002.

Grain type	Diet Faecal DE (as fed) MJ/kg		Grain Faecal DE (as fed) MJ/kg		Grain Faecal DE (DM basis)MJ/kg	
	Mean	se	Mean	se	Mean	se
Wheat	12.69	0.106	13.02	0.112	15.03	0.109
Barley	11.37	0.160	11.62	0.169	13.47	0.159
Triticale	12.46	0.160	12.77	0.170	14.79	0.161
Sorghum	13.02	0.133	13.37	0.141	15.53	0.133
Maize	12.72	0.106	13.05	0.113	15.24	0.110
avsed		*0.173*		*0.183*		*0.164*
Approx	*5%*	*0.363*		*0.384*		*0.344*
LSD	*1%*	*0.467*		*0.495*		*0.443*

Table 11. Statistically corrected individual grain values with standard errors (se) for Diet Faecal DE and Grain Faecal DE in experiment NG001.

Grain type & ID	Diet Faecal DE (as fed) MJ/kg		Grain Faecal DE (as fed) MJ/kg		Grain Faecal DE (DM basis)MJ/kg	
	Value	se	Value	se	Value	se
Wheat						
1763	13.31	0.185	13.68	0.194	15.69	0.154
1766	13.26	0.188	13.62	0.197	15.63	0.159
1770	13.99	0.185	14.39	0.194	16.49	0.153
1882	13.46	0.185	13.83	0.194	15.83	0.153
1883	13.32	0.185	13.69	0.194	15.63	0.154
1884	13.58	0.183	13.96	0.192	16.05	0.150
1886	13.88	0.185	14.28	0.194	16.42	0.154
1887	13.55	0.138	13.93	0.145	15.92	0.120
1888	13.48	0.136	13.86	0.143	15.93	0.117
Barley						
3871	12.80	0.188	13.13	0.197	14.88	0.159
3873	12.72	0.185	13.06	0.194	14.71	0.153
3874	12.67	0.183	13.00	0.192	14.77	0.150
3875	12.64	0.185	12.97	0.194	14.76	0.153
3897	12.16	0185	12.46	0.194	14.21	0.153
3922	12.78	0.188	13.12	0.197	14.97	0.159
3923	12.82	0.138	13.15	0.145	15.01	0.120
3924	12.20	0.188	12.51	0.197	14.21	0.159
3926	12.78	0.137	13.11	0.144	14.89	0.120
3927	12.15	0.185	12.45	0.194	14.12	0.153
3928	12.17	0.141	12.47	0.148	14.28	0.126
3929	12.59	0.183	12.91	0.192	14.65	0.150
3930	12.80	0.183	13.14	0.192	14.95	0.150
3931	12.96	0.137	13.30	0.144	15.08	0.120
3932	13.46	0.185	13.83	0.194	15.48	0.153

Triticale						
6830	13.60	0.185	13.98	0.194	15.95	0.153
6831	12.77	0.185	13.10	0.194	15.04	0.153
6849	12.87	0.137	13.21	0.194	15.26	0.120
6850	13.85	0.185	14.24	0.194	16.10	0.154
6859	13.49	0.185	13.86	0.194	15.84	0.153
Sorghum						
7855	13.86	0.188	14.26	0.197	16.35	0.159
7857	13.58	0.188	13.96	0.197	15.98	0.159
7869	13.75	0.185	14.14	0.194	16.16	0.153
7885	13.83	0.138	14.22	0.144	16.40	0.120
7886	13.89	0.137	14.29	0.144	16.25	0.120
7887-Raw	13.17	0.183	13.52	0.192	15.79	0.150
7887-Sprt-Med	14.12	0.183	14.53	0.192	16.43	0.150
7887-Sprt-Hgh	13.63	0.183	14.01	0.192	16.37	0.150
Maize						
8082	14.11	0.137	14.52	0.144	16.53	0.120
8083	14.35	0.185	14.77	0.194	16.42	0.154
8084	13.28	0.193	13.65	0.202	15.70	0.167
avsed		*0.237*		*0.248*		*0.193*
Approx LSD	5%	*0.498*		*0.521*		*0.405*
	1%	*0.640*		*0.669*		*0.521*

Table 12. Statistically corrected individual grain values with standard errors (se) for Diet Faecal DE and Grain Faecal DE in experiment NG002.

Grain type & ID	Diet Faecal DE (as fed) MJ/kg		Grain Faecal DE (as fed) MJ/kg		Grain Faecal DE (DM basis)MJ/kg	
	Value	se	Value	se	Value	se
Wheat						
1763	12.91	0.274	13.25	0.290	15.22	0.251
1766	12.28	0.273	12.58	0.289	14.56	0.249
1770	13.09	0.268	13.44	0.283	15.42	0.240
1885	12.65	0.200	12.98	0.212	15.06	0.188
1889	12.55	0.202	12.87	0.214	14.96	0.191
1890	12.97	0.208	1332	0.220	15.32	0.202
1892 - raw	12.71	0.273	13.04	0.290	14.97	0.250
1892 - sprout	12.30	0.267	12.61	0.282	14.64	0.238
1893 - sprout	12.78	0.274	13.12	0.290	15.11	0.250
Barley						
3871	11.89	0.273	12.18	0.289	14.08	0.249
3874	11.36	0.273	11.61	0.290	13.57	0.248
3933 - raw	11.20	0.203	11.44	0.283	13.21	0.239
3933 - sprout	11.00	0.203	11.23	0.215	13.02	0.194
Triticale						
6830	12.92	0.274	13.26	0.290	15.22	0.251
6860	12.20	0.203	12.50	0.215	14.51	0.194

6861	12.82	0.267	13.16	0.283	15.16	0.240
6866	11.89	0.274	12.17	0.290	14.27	0.251
Sorghum						
7855	12.93	0.267	13.28	0.283	15.35	0.239
7861	12.49	0.268	12.80	0.283	15.02	0.240
7889	13.11	0.201	13.46	0.212	15.74	0.190
7890	13.64	0.269	14.02	0.285	15.96	0.244
7891	12.93	0.201	13.27	0.213	15.57	0.190
Maize						
8082	12.74	0.269	13.07	0.284	15.32	0.241
8084	12.45	0.205	12.76	0.217	14.91	0.196
8085	13.07	0.215	13.42	0.227	15.86	0.209
8086	12.11	0.268	12.40	0.283	14.54	0.240
8087	13.04	0.273	13.39	0.289	15.43	0.250
8088	12.79	0.201	13.12	0.212	15.26	0.189
8089	12.79	0.199	13.13	0.211	15.42	0.187
8090	12.74	0.202	13.07	0.214	15.16	0.192
avsed		*0.201*		*0.213*		*0.123*
Approx LSD	5%	*0.422*		*0.447*		*0.258*
	1%	*0.543*		*0.575*		*0.332*

3.2.4 Effect of grain germination

The experiments provided an opportunity to investigate the effect of sprouting of wheat, barley and sorghum on Faecal DE in pigs. Wheat and barley samples were harvested from commercial farms in northern NSW in 2010. One batch was harvested before rain and a second batch harvested after sufficient rain and humidity to allow sprouting. The sprouted grain dried naturally before being harvested. The Falling Numbers value for the wheat sample fell from 426 to 142 after natural sprouting. In addition, a sample of MR43 sorghum grown at the Hermitage Research Station, Warwick, Queensland, was divided into 3 portions, with one portion used as a control, one portion soaked in water for 24 h and the other soaked for 48 h, before being oven dried at 40^{o}C, to create moderately and highly sprouted grains.

The effects of germination on Faecal DE values are shown in Table 13. Natural sprouting of wheat on farm reduced diet and grain Faecal DE, but the change was only significant ($P<0.05$) when grain Faecal DE was expressed on a dry matter basis. Natural sprouting of barley on farm also tended to reduce Faecal DE, but the difference between the control and sprouted grain was not significant. Previous research within PGLP and earlier Pork CRC projects have shown similar results with little effect of natural sprouted wheat or barley samples on DE in pigs. A negative effect would be expected with excessive sprouting because of loss on energy once starch is used to provide energy for root or shoot growth.

On the contrary, artificial sprouting of sorghum significantly ($P<0.01$) increased the Faecal DE content. There was a large effect of germination for 24 h of around

1 MJ/kg, but the Faecal DE content fell significantly as the germination period was extended from 24 to 48 h. The large increase in Faecal DE seen with 24 h germination is presumably related to breakdown of the protein matrix by grain proteases and increased access of pig derived amylase to the starch granules. The decline in Faecal DE with progressive time of germination suggests that a significant amount of energy was being used by the grain in the germination process. These results may have consequences for natural sprouting of sorghum, with the effect on available energy content for pigs being related to the time un-harvested grain remains moist prior to hot, dry weather returning to dry the grain in the paddock. The results suggest that there would be a significant improvement in available energy content of sorghum for germination periods up to 48 h. However, the positive effect is likely to disappear and turn negative as germination period extends beyond this time.

Previous experiments in the NIR series tend to confirm that natural germination enhances the DE content of sorghum. In PGLP, Faecal DE content of naturally sprouted sorghum samples were approximately 0.4 MJ/kg (as fed) greater than the mean values for un-sprouted sorghum.

Table 13. Effect of sprouting on the Faecal DE content of specific grain samples.

Grain Type	Grain ID	Diet Faecal DE (as fed) MJ/kg	Grain Faecal DE (as fed) MJ/kg	Grain Faecal DE (DM basis)MJ/kg
Wheat	1892-Raw0	12.71	13.04	14.97
	1892-Sprout	2.30	12.61	14.64
Significance		*NS*	*NS*	*P<0.05*
Barley	3933-Raw0	11.20	11.44	13.21
	3933-Sprout	11.00	11.23	13.02
Significance		*NS*	*NS*	*NS*
Sorghum	7887-Raw0	13.17	13.52	15.79
	7887-Spr-24	14.12	14.53	16.43
	7887-Spr-48	13.63	14.01	16.37
Significance Raw0-24		*P<0.01*	*P<0.01*	*P<0.01*
Significance Raw0-48		*NS*	*NS*	*P<0.01*
Significance 24-48		*P<0.05*	*P<0.05*	*NS*

3.3 Validation and upgrade of NIR calibrations

Although most licensees for the calibrations that have been released to the animal and grain industries through the Pork CRC AusScan project typically scan whole grains with NIR because of its speed and convenience, some feed testing laboratories only work with milled samples. Consequently, results are presented for calibrations based on whole and milled grain scans. However, more details are given for calibrations based on whole grain scans.

3.3.1 Accuracy of Faecal DE prediction for new samples

Validation of NIR calibrations refers to the ability of an existing calibration to predict accurately measured values from a new set of grains not previously used to establish the calibration. The outcomes from the validation process are described below.

The statistics for linear regression equations fitted to NIR predicted and experimentally measured values for Faecal DE for grains in all six Pork CRC experiments (DS002+DS005, DS004, HR001, NG001 and NG002) are given for whole grain scans in Table 14. The details of experiments DS002, DS004, DS005, and HR001 are available in earlier Pork CRC reports. Results from experiments DS002 and DS005 are combined because DS005 contained a small number of pearl millet samples and connectivity grains from DS002. In each comparison, the latest NIR calibration at the time was used to determine the accuracy with which the values for unknown samples in the next experiment could be predicted. The results from the new experiment were then included in the development of a new NIR calibration and the results for that experiment again predicted.

Table 14. Linear regression coefficients for equations fitted to NIR predicted values for Faecal DE (MJ/kg as fed) using whole grain scans and measured values for grains used in experiments all Pork CRC experiments.

Calibration used for prediction	R^2	Slope	Intercept
DS002+DS005[1]			
PGLP calibration (1)	0.79	0.87	1.77
PGLP + DS002+DS005 calibration (2)	0.87	1.03	-0.46
DS004			
PGLP + DS002+DS005 calibration (2)	0.87	0.95	0.89
PGLP + DS002+DS005 + DS004 calibration (3)	0.91	1.00	-0.00
HR001			
Calibration (3)	0.83	1.01	-0.18
Calibration (3) + HR001 calibration (4)	0.85	1.05	-0.65
NG001			
Calibration (4)	0.78	0.81	2.37
Calibration (4) + HR001 calibration (5)	0.78	0.91	1.22
NG002			
Calibration (5)	0.83	0.94	0.30
Calibration (5) + HR001 calibration (6)	0.80	0.93	0.91

[1]The DS and HR experiments were conducted in Pork CRC projects 1B-101 and 1B-104

Table 14 shows there have been small improvements in the ability to predict the Faecal DE values for new grains as more grains have been added to the calibrations. This lack of major improvement in predicting values for unknown samples is not surprising because an effort has been made in each experiment within the Pork CRC to introduce into the calibrations new grains with different characteristics. For example, experiment NG001 included several new wheat, barley and sorghum cultivars, NIR scan predicted 'outliers' for wheat and barley, sorghum samples with coloured endosperms, highly germinated sorghum and

maize samples for the first time. The WINISI software identified these samples as 'Neighbourhood' outliers (NH) and the unusual sorghum and maize samples as 'Global' outliers (GH) as well. GH values measure the distance of the sample scan from the centre of the population or of the mean spectrum. NH measures the distance of the sample scan from its neighbours rather than from the mean scan. These H-statistic measures provide information about the similarity of the sample scan to others in the population. Conventionally, NIR scans are regarded as 'outliers' from the calibration population when GH exceeds 4.0 and/or NH exceeds 1.0. The maize samples in NG002 were also identified as GH and NH outliers when the NIR calibration included the grains from experiment NG001. This again is not surprising because there were only three maize samples in the calibration developed after completion of experiment NG001.

The relationships between the NIR predicted Faecal DE values using the previous NIR calibration and measured values for experiments NG001 and NG002 are shown in Figures 7A and 8A, respectively. Figures 7B and 8B also show the relationships between NIR predicted values and measured values when results from the experiments were added to the calibrations.

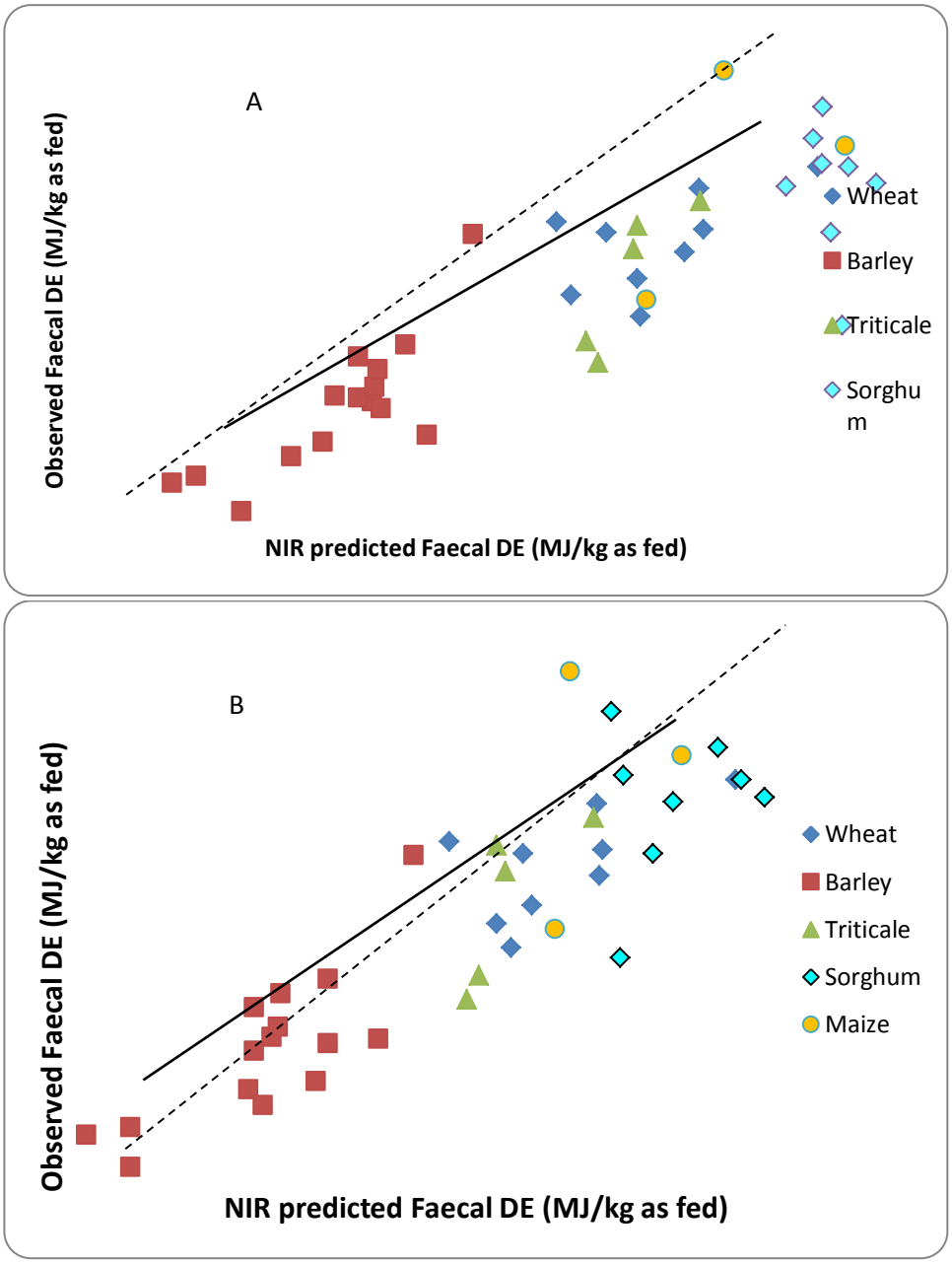

Figure 7. Relationship between NIR predicted values using the previous NIR calibration (A) or calibration including the new grains (B) and measured Faecal DE (MJ/kg as fed) for experiment NG001.

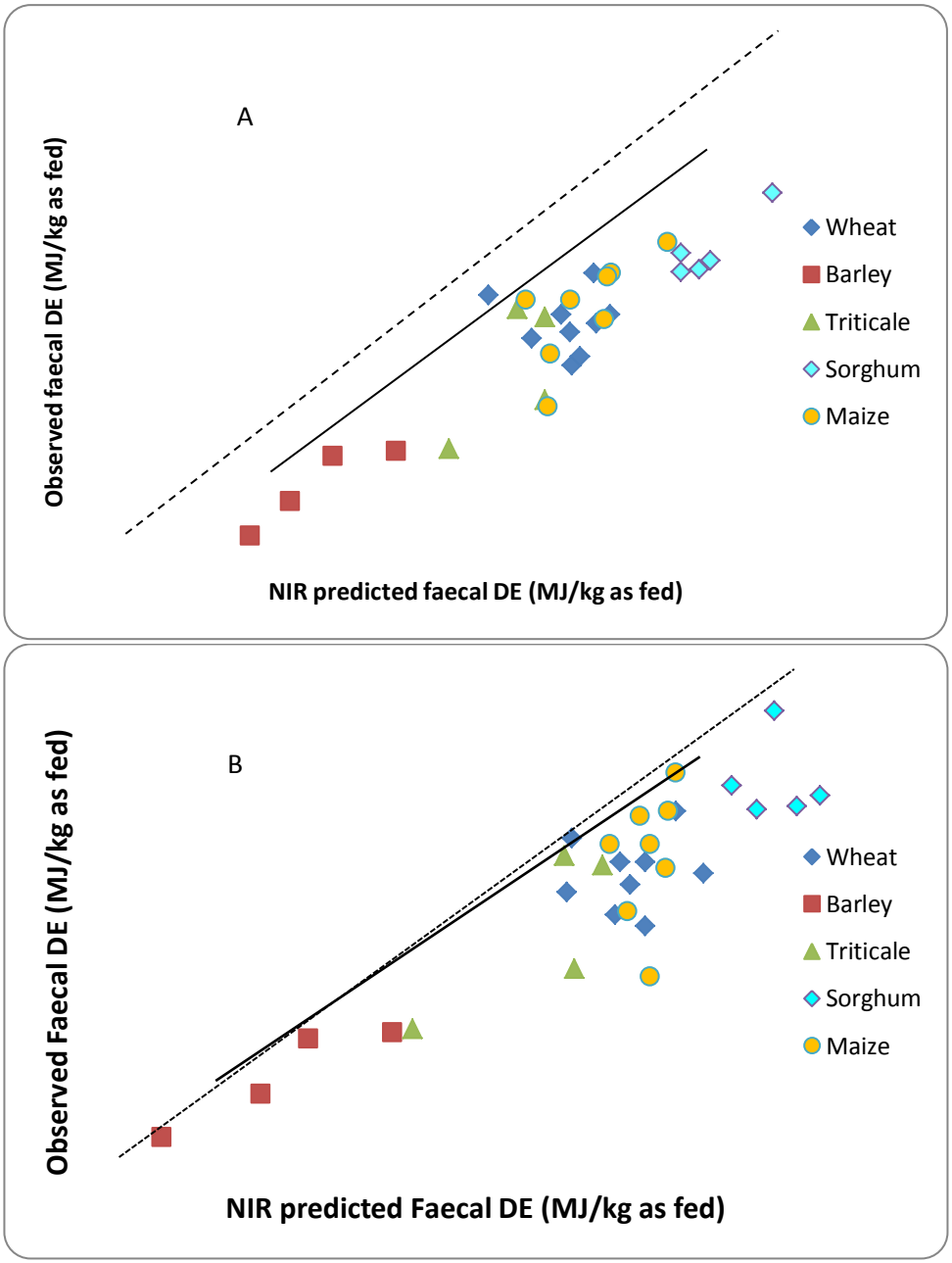

Figure 8. Relationship between NIR predicted values using the previous NIR calibration (A) or calibration including the new grains (B) and measured Faecal DE (MJ/kg as fed) for experiment NG002.

The results presented in Figures 7 and 8 suggest that experiments NG001 and NG002 could not be regarded as true validations of the calibrations. The presence of GH and NH outliers for maize suggests that many more maize samples need to be added to the calculations for these predictions to become accurate. In addition, the presence in these two experiments of a large number of new cultivars and unusual grains recognised as NH and GH outliers, means that a 'true' validation of the latest calibration can be made only when more of these grain types are added to future calibrations.

3.3.2 Upgrade of NIR calibrations

Improvements in the NIR calibrations are assessed in relation to the criteria shown in Table 15. The three most important criteria for judging the suitability of a calibration are:

- RSQ: indicates the reliability with which values are predicted from the calibration in relation to the measured values
- SECV: indicates the accuracy expected for a predicted value (± the true value with a probability of 95%)
- RPD: indicated the robustness and reliability of the calibration for predicting values for unknown samples

Table 15. Criteria used to assess the value of NIR calibrations[1].

Term	Meaning
N	Number of observations used in final calibration – excluding outliers
Mean	Mean of experimental observations
SD	Standard Deviation of experimental observations
RSQ	R^2 values – fraction of the variance accounted for by the NIR calibration when all accepted observations are included in the relationship
SEC	Standard error of the calibration – when all accepted observations are included in the relationship
1-VR	1-Variance Ratio – Fraction of variance accounted for in NIR prediction when some observations are used for 'cross validation' of the calibration as determined by the NIR software
SECV	Standard error of cross validation – Standard error of the calibration when some observations are used for 'cross validation' of the calibration as determined by the NIR software
RPD	Ratio of Prediction to Deviation = SD/SECV an indication of the value of the calibration RPD < 1.5: calibration unsatisfactory RPD = 1.5 – 2.0: calibration can distinguish between high & low values (H-L) RPD = 2.0-2.5: calibration approximately quantitative RPD = 2.5-3.0: calibration predictions good RPD = > 3.0: calibration predictions excellent

[1] The abbreviations apply to following Tables

Tables 16 and 17 show, respectively, for whole and milled grain scans, the improvements in the NIR calibrations for predicting the Faecal DE content of cereal grains for pigs as results from the initial PGLP experiments and sequentially for the six Pork CRC experiments were added to the calibrations.

Adding grains to the calibration clearly improved the reliability for prediction of Faecal DE for unknown samples as shown by the increase in the RPD values and decrease in SECV values. The greatest improvements were made following inclusion of grains from experiment HR001. Addition of grains from the NG

experiments reduced the RPD value and did not lead to further improvement in SECV.

Table 16. Statistics for Faecal DE (MJ/kg as fed) NIR calibrations developed from whole grain scans as the number of samples used was increased from PGLP by adding results from six Pork CRC experiments.

Calibration	N	Mean	SD	RSQ	SEC	1-VR	SECV	RPD
PGLP – (1)	91	13.48	0.79	0.84	0.32	0.77	0.38	2.08
(1) + DS002/5 – (2)	121	13.68	0.73	0.85	0.29	0.82	0.31	2.35
(2) + DS004 – (3)	170	13.76	0.71	0.89	0.24	0.86	0.27	2.63
(3) + HR001 – (4)	219	13.71	0.71	0.90	0.23	0.87	0.26	2.76
(4) + NG001 - (5)	258	13.69	0.70	0.89	0.23	0.86	0.26	2.68
(5) + NG002 - (6)	288	13.68	0.69	0.89	0.23	0.86	0.26	2.65

Table 17. Statistics for Faecal DE (MJ/kg as fed) NIR calibrations developed from milled grain scans as the number of samples used was increased from PGLP by adding results from six Pork CRC experiments.

Calibration	N	Mean	SD	RSQ	SEC	1-VR	SECV	RPD
PGLP - (1)	92	13.52	0.80	0.85	0.31	0.80	0.36	2.22
(1) + DS002/5 - (2)	123	13.67	0.73	0.84	0.29	0.79	0.33	2.21
(2) + DS004 – (3)	170	13.76	0.71	0.90	0.22	0.84	0.28	2.54
(3) + HR001 – (4)	216	13.72	0.71	0.89	0.24	0.88	0.25	2.87
(4) + NG001 - (5)	253	13.69	0.70	0.88	0.24	0.87	0.25	2.80
(5) + NG002 - (6)	281	13.69	0.69	0.88	0.24	0.87	0.25	2.76

There are two reasons for the negative effects of adding results from the NG experiments to the calibrations. First, as explained above, the grains used in these experiments were selected because they were different and had different scans from those used in previous experiments. Many were identified as either GH and/or NH outliers. The selection of these grains was deliberate so the robustness of the calibrations could be improved to cover maize and grains with unusual characteristics. Second, the variance in Faecal DE values derived from the NG experiments was considerably greater than observed in previous experiments. For example, the mean standard error for grain Faecal DE for grains used in experiments up to and including HR001 was 0.101 (MJ/kg as fed). Addition of NG001 grains to those from previous experiments increased the mean standard error of the meta-analysis corrected values to 0.13 MJ/kg as fed. Addition of grains from NG002 further increased the mean standard error of the estimates of grain Faecal DE to 0.15 MJ/kg as fed. A major reason for the increase in variance of statistically corrected values was the variance found in AIA measurements made by the Symbio laboratory as discussed in section 3.1.

Calibrations with RPD values greater than 2.5 are considered good for predicting values for unknown samples. The addition of more grains, particularly maize samples and other grains selected because they are GH or NH outliers should further improve the RPD value for the calibrations. The target should be a RPD value greater than 3.0, which is considered excellent.

The SECV value, which provides an indication of the likely precision of prediction, decreased substantially from 0.38 for PGLP experiments to 0.26 for the final calibration. This result means that the sixth generation calibration developed can

predict with 95% confidence to within ± 0.52 MJ/kg as fed (0.26*2) of the actual Faecal DE value.

The best accuracy obtained by NIR calibrations (SECV) is thought to be a little less than twice the standard error of the measurements. Thus, the increase in variance for the statistically corrected Faecal DE values resulting from the addition of the results from the NG experiments to previous grains, means that the accuracy of NIR predicted values is unlikely to be improved greatly from the current value of ±0.26 MJ/kg as fed. However, addition of results from future experiments that show less variance, should reduce the overall standard errors of statistically corrected Faecal DE values and could allow further reduction in SECV values.

The NIR predicted Faecal DE values using the final calibration (6) based on whole grain scans compared with the meta-analysis statistically corrected measured values are shown in Figure 9.

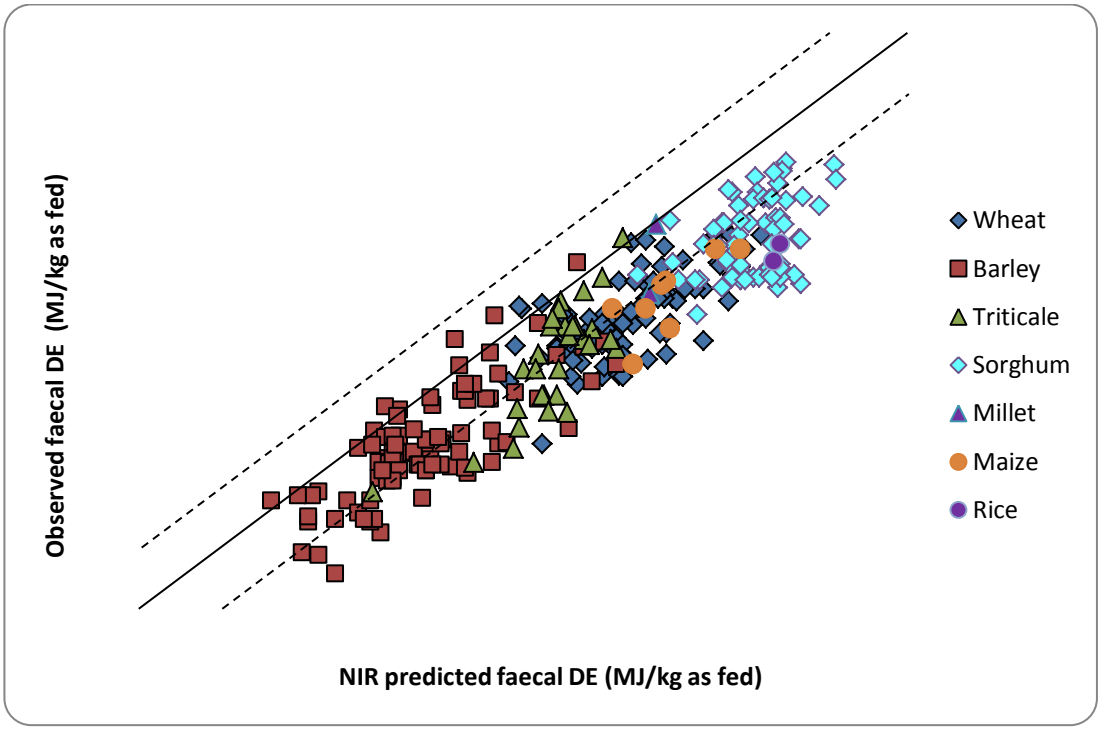

Figure 9. Relationship between Faecal DE predicted using the latest NIR calibraiton (6) based on whole grain scans and meta-analysis statistically corrected measured values for all PGLP and Pork CRC experiments. The solid line is the line of equivalence and the dotted lines (SECV*2) are 95% confidence limits.

3.4 Comparison of whole and ground grain scans

The statistics for NIR calibrations based on whole or milled grain scans are similar. For example, RSQ values were 0.89 and 0.88, respectively, for calibrations based on whole grain and milled grain scans. Similar comparison shows SECV values of 0.26 and 0.25 MJ/kg as fed, and RPD values of 2.65 and 2.76, respectively for calibrations based on whole and milled grain scans.

These calibration statistics suggest that similar results should be obtained for grain Faecal DE when calibrations are based on either whole grain scans milled grain scans. This assumption is reinforced when the latest (6) calibrations based on either whole grain or milled grain scans are used to predict Faecal DE values for all grains from the PGLP and Pork CRC experiments. The mean difference between predicted faecal DE using calibrations based on either whole grain or milled grain scans across all grains is only 0.01 MJ/kg as fed. However, such a comparison can be misleading. When predicted values for individual grains are compared, the difference between predictions based on NIR calibrations developed from whole grain or milled grain scans ranged from -0.53 to +0.65 MJ/kg as fed. The relationship between predicted Faecal DE (MJ/kg as fed) for each grain from the PGLP and Pork CRC experiments when the final (6) NIR calibration is based on whole or milled grain scans is shown in Figure 9. The R^2 for the relationship is only 0.92.

The difference in predicted values for individual grains is of major concern for the commercial use of AusScan because it means that those laboratories using calibrations based on milled grain scans will give different predicted values to their clients than would be obtained from calibrations based on whole grain scans.

A strong recommendation from these comparisons is that calibrations based on milled grain scans are no longer made available commercially. The measured differences between predictions from NIR calibrations bases on whole or milled grains scans could be expected. Grains differ in their fracturability, and with mill differences, particle size distributions can vary between grains milled with the same mill, and within a grain milled in different mills. Particle size distributions of grains milled in disc-, hammer- and roller-mills are discussed in project 4B-107, and infra-red spectroscopy has revealed differences in samples milled to different particle sizes. However, there are no detailed studies on NIRS-particle size relationships. Since milling differences cannot be controlled, the use of whole grains scans for developing NIR calibrations will minimise the variations that are inherent with milling grains. Whole grain scans are faster and eliminates the cost of milling.

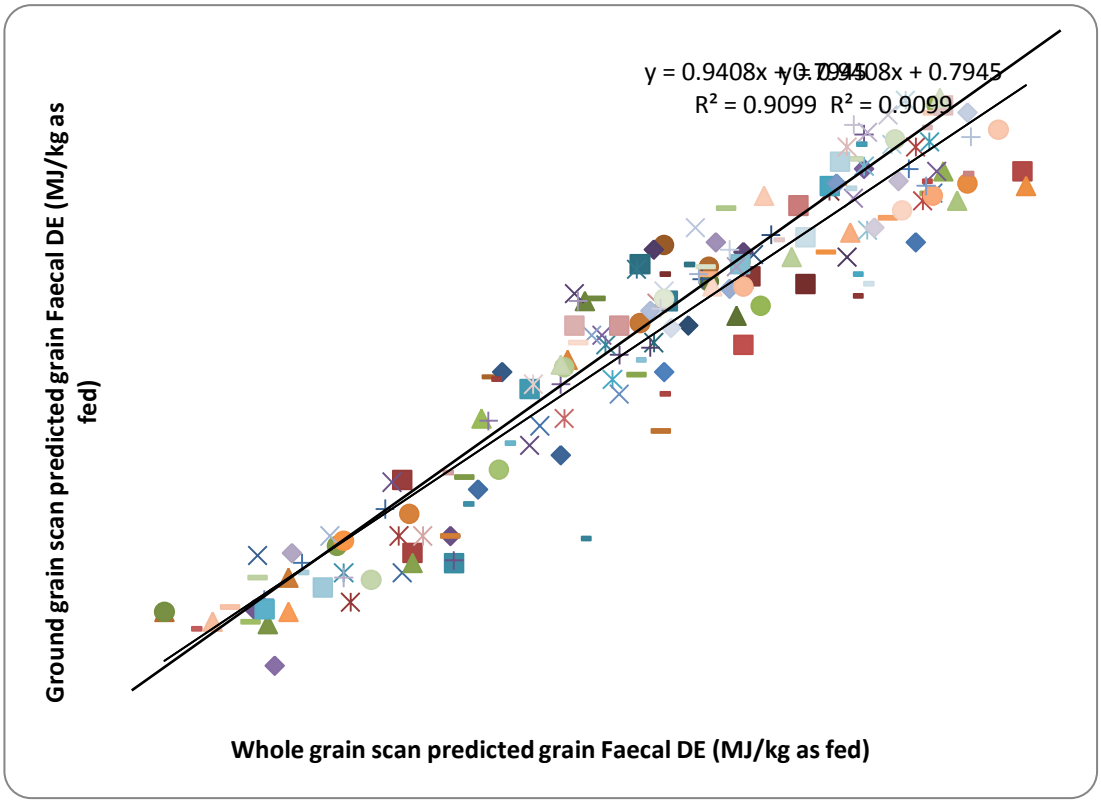

Figure 9. Relationship between predicted Faecal DE values derived from NIR calibrations based on either whole grain scans and ground grain scans for all PGLP and Pork CRC experimental grains included in the latest NIR calibrations. R^2 = 0.92.

4. Application of Research

The results from this project can be applied directly to the grain and pig industries through an upgrade of the NIR calibrations released by AusScan to its licensees. Furthermore, the Pork CRC is in negotiations with a major global company to make the AusScan calibrations available worldwide through a web based system that accepts NIR instrument scans from anywhere around the world and provides a rapid turnaround of predicted values.

Accurate estimation of the energy value of cereal grain used by pig producers can have a considerable effect on profitability. Previous assessments by three Australian pig nutrition consultants have agreed that using cereal grains with 1 MJ/kg as fed difference in DE content is worth approximately $18/tonne when wheat is $250/tonne. AUSPIG simulations (APL Priority Workshop) predicted that a difference of $50/tonne in feed price was worth $0.19/kg carcass. Thus, a saving of $18/tonne could be worth up to 7 cents/kg carcass.

5. Conclusion

Adding 47 new grains from the two experiments in this project to previous grains used to develop NIR calibrations for pig Faecal DE did not improve the calibration statistics. The lack of improvement was due to the grains used in these experiments being selected because they were different and had different scans

from those used in previous experiments and to the variance in measured Faecal DE values being considerably greater than observed in previous experiments. Nevertheless, the selection of the different grains used in the experiments was deliberate so the robustness of the calibrations could be improved to cover maize and grains with unusual characteristics.

More experiments need to be conducted with both 'normal' and unusual grains, particularly maize samples and other grains selected because they are GH or NH outliers. Selection of these grains should further improve the RPD value for the calibrations, with a target for a RPD value greater than 3.0. A NIR validation experiment would then be needed with a wide range of maize and 'normal' samples included.

Comparison of calibrations based on whole grain scans with those based on milled grain scans suggest there is little difference in calibration statistics or the mean values predicted by both calibrations for all grains used in PGLP and Pork CRC experiments. However, the predicted values for individual grains varied from - 0.53 to + 0.65, with a R^2 of the relationship being only 0.92. Thus, for most grains different values would be predicted from calibrations based on whole grain scans compared with those form the milled grain calibration.

Differences of this magnitude between the calibrations are unacceptable for commercial application. It is recommended strongly that milled grain calibrations are removed from the AusScan suite.

Nevertheless, it is recognised that milled grains are still used in some instances worldwide for developing NIR calibrations. Hence, in order to make AusScan acceptable to the global NIRs users and enhance its versatility it is important to understand the reasons for the whole-milled grain differences in predicted values as recorded in this project. Milling increases the surface area and can present more grain components to infrared rays for a possibly deeper component-spectra interactions. However, there is little knowledge of the underlying relationships between particle size and NIR calibration outputs. Fourier transform infrared spectroscopy that is based on the mid-infrared has revealed differences in particle size and milling actions. An understanding of the NIR-particle size relationship might widen the use of AusScan.

6. Limitations/Risks

There is little risk to these updated calibrations being commercialised through the existing AusScan project. However, there is a risk that values for individual grains will not be predicted accurately, either because they are identified by the NIR software as being GH or NH outliers or their characteristics are not identified by the NIR instrument scanning process.

7. Recommendations

7.1. More experiments need to be conducted with both 'normal' and unusual grains, particularly maize samples and other grains selected because they are GH or NH outliers to further validate and upgrade the NIR

calibrations for Faecal and Ileal DE. Selection of these grains should further improve the RPD value for the calibrations, with a target for a RPD value greater than 3.0.

7.2. NIR calibrations based on milled grain scans are removed from the AusScan suite of commercially available calibrations because the values predicted for individual grains differs widely depending whether the calibration is developed using whole grain or milled grain scans.

8. References

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Appendix – Experiment NG001

Experimental design and allocation of diets to pigs in experiment NG001.

NG001 Experimental Design

Spare Pig information

Pig No.	Pig ID
101	7426
201	7427
301	7428
401	7429

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
1	38	36	7887_SPROUT_MED	sorghum	1	1	7430	1	1	1841
2	50	30	7887_RAW0	sorghum	2	2	7431	1	1	1842
3	29	22	3932_RAW0	barley	3	3	7432	1	1	1843
4	48	6	1766_RAW0	wheat	4	4	7433	1	1	1844
5	45	25	6830_RAW0	triticale	5	5	7434	1	1	1845
6	18	13	3924_RAW0	barley	6	6	7435	1	1	1846
7	35	8	1887_RAW0	wheat	7	7	7436	1	1	1847
8	23	24	3875_RAW0	barley	8	8	7437	1	1	1848
9	6	11	3874_RAW0	barley	9	9	7438	1	1	1849
10	20	35	7855_RAW0	sorghum	10	10	7439	1	1	1850
11	10	28	6850_RAW0	triticale	11	11	7440	1	1	1851
12	39	29	6859_RAW0	triticale	12	12	7441	1	1	1852

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
13	15	14	3927_RAW0	barley	13	13	7442	1	1	1853
14	27	4	1884_RAW0	wheat	14	14	7443	1	1	1854
15	10	28	6850_RAW0	triticale	1	1	7430	2	1	1855
16	14	20	3928_RAW0	barley	2	2	7431	2	1	1856
17	9	27	6849_RAW0	triticale	3	3	7432	2	1	1857
18	25	38	8082_RAW0	maize	4	4	7433	2	1	1858
19	2	17	3929_RAW0	barley	5	5	7434	2	1	1859
20	3	18	3926_RAW0	barley	6	6	7435	2	1	1860
21	1	15	3871_RAW0	barley	7	7	7436	2	1	1861
22	37	32	7869_RAW0	sorghum	8	8	7437	2	1	1862
23	33	23	3931_RAW0	barley	9	9	7438	2	1	1863
24	26	3	1763_RAW0	wheat	10	10	7439	2	1	1864
25	22	16	3923_RAW0	barley	11	11	7440	2	1	1865
26	20	35	7855_RAW0	sorghum	12	12	7441	2	1	1866
27	17	20	3928_RAW0	barley	13	13	7442	2	1	1867
28	7	21	3930_RAW0	barley	14	14	7443	2	1	1868
29	41	2	1883_RAW0	wheat	1	1	7430	3	1	1869
30	47	5	1886_RAW0	wheat	2	2	7431	3	1	1870
31	16	10	3897_RAW0	barley	3	3	7432	3	1	1871
32	27	4	1884_RAW0	wheat	4	4	7433	3	1	1872
33	7	21	3930_RAW0	barley	5	5	7434	3	1	1873
34	23	24	3875_RAW0	barley	6	6	7435	3	1	1874
35	42	7	1770_RAW0	wheat	7	7	7436	3	1	1875
36	36	31	7887_SPROUT_HIGH	sorghum	8	8	7437	3	1	1876
37	46	8	1887_RAW0	wheat	9	9	7438	3	1	1877
38	11	34	7886_RAW0	sorghum	10	10	7439	3	1	1878
39	40	27	6849_RAW0	triticale	11	11	7440	3	1	1879
40	37	32	7869_RAW0	sorghum	12	12	7441	3	1	1880
41	31	18	3926_RAW0	barley	13	13	7442	3	1	1881

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
42	43	9	1888_RAW0	wheat	14	14	7443	3	1	1882
43	19	33	7885_RAW0	sorghum	1	1	7430	4	1	1883
44	6	11	3874_RAW0	barley	2	2	7431	4	1	1884
45	18	13	3924_RAW0	barley	3	3	7432	4	1	1885
46	32	19	3873_RAW0	barley	4	4	7433	4	1	1886
47	5	40	8084_RAW0	maize	5	5	7434	4	1	1887
48	34	1	1882_RAW0	wheat	6	6	7435	4	1	1888
49	36	31	7887_SPROUT_HIGH	sorghum	7	7	7436	4	1	1889
50	26	3	1763_RAW0	wheat	8	8	7437	4	1	1890
51	38	36	7887_SPROUT_MED	sorghum	9	9	7438	4	1	1891
52	8	16	3923_RAW0	barley	10	10	7439	4	1	1892
53	50	30	7887_RAW0	sorghum	11	11	7440	4	1	1893
54	44	26	6831_RAW0	triticale	12	12	7441	4	1	1894
55	49	34	7886_RAW0	sorghum	13	13	7442	4	1	1895
56	12	33	7885_RAW0	sorghum	14	14	7443	4	1	1896
57	2	17	3929_RAW0	barley	1	1	7430	5	1	1897
58	41	2	1883_RAW0	wheat	2	2	7431	5	1	1898
59	24	39	8083_RAW0	maize	3	3	7432	5	1	1899
60	28	9	1888_RAW0	wheat	4	4	7433	5	1	1900
61	15	14	3927_RAW0	barley	5	5	7434	5	1	1901
62	47	5	1886_RAW0	wheat	6	6	7435	5	1	1902
63	44	26	6831_RAW0	triticale	7	7	7436	5	1	1903
64	29	22	3932_RAW0	barley	8	8	7437	5	1	1904
65	1	15	3871_RAW0	barley	9	9	7438	5	1	1905
66	21	12	3922_RAW0	barley	10	10	7439	5	1	1906
67	4	38	8082_RAW0	maize	11	11	7440	5	1	1907
68	16	10	3897_RAW0	barley	12	12	7441	5	1	1908
69	13	37	7857_RAW0	sorghum	13	13	7442	5	1	1909
70	30	23	3931_RAW0	barley	14	14	7443	5	1	1910

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
71	34	1	1882_RAW0	wheat	1	1	7444	6	2	1911
72	16	10	3897_RAW0	barley	2	2	7445	6	2	1912
73	50	30	7887_RAW0	sorghum	3	3	7446	6	2	1913
74	27	4	1884_RAW0	wheat	4	4	7447	6	2	1914
75	37	32	7869_RAW0	sorghum	5	5	7448	6	2	1915
76	6	11	3874_RAW0	barley	6	6	7449	6	2	1916
77	7	21	3930_RAW0	barley	7	7	7450	6	2	1917
78	42	7	1770_RAW0	wheat	8	8	7451	6	2	1918
79	2	17	3929_RAW0	barley	9	9	7452	6	2	1919
80	39	29	6859_RAW0	triticale	10	10	7453	6	2	1920
81	24	39	8083_RAW0	maize	11	11	7454	6	2	1921
82	21	12	3922_RAW0	barley	12	12	7455	6	2	1922
83	36	31	7887_SPROUT_HIGH	sorghum	13	13	7456	6	2	1923
84	48	6	1766_RAW0	wheat	14	14	7457	6	2	1924
85	41	2	1883_RAW0	wheat	1	1	7444	7	2	1925
86	15	14	3927_RAW0	barley	2	2	7445	7	2	1926
87	17	20	3928_RAW0	barley	3	3	7446	7	2	1927
88	39	29	6859_RAW0	triticale	4	4	7447	7	2	1928
89	11	34	7886_RAW0	sorghum	5	5	7448	7	2	1929
90	4	38	8082_RAW0	maize	6	6	7449	7	2	1930
91	18	13	3924_RAW0	barley	7	7	7450	7	2	1931
92	5	40	8084_RAW0	maize	8	8	7451	7	2	1932
93	12	33	7885_RAW0	sorghum	9	9	7452	7	2	1933
94	1	15	3871_RAW0	barley	10	10	7453	7	2	1934
95	32	19	3873_RAW0	barley	11	11	7454	7	2	1935
96	35	8	1887_RAW0	wheat	12	12	7455	7	2	1936
97	9	27	6849_RAW0	triticale	13	13	7456	7	2	1937
98	46	8	1887_RAW0	wheat	14	14	7457	7	2	1938
99	32	19	3873_RAW0	barley	1	1	7444	8	2	1939

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
100	25	38	8082_RAW0	maize	2	2	7445	8	2	1940
101	23	24	3875_RAW0	barley	3	3	7446	8	2	1941
102	2	17	3929_RAW0	barley	4	4	7447	8	2	1942
103	13	37	7857_RAW0	sorghum	5	5	7448	8	2	1943
104	7	21	3930_RAW0	barley	6	6	7449	8	2	1944
105	44	26	6831_RAW0	triticale	7	7	7450	8	2	1945
106	38	36	7887_SPROUT_MED	sorghum	8	8	7451	8	2	1946
107	40	27	6849_RAW0	triticale	9	9	7452	8	2	1947
108	34	1	1882_RAW0	wheat	10	10	7453	8	2	1948
109	45	25	6830_RAW0	triticale	11	11	7454	8	2	1949
110	47	5	1886_RAW0	wheat	12	12	7455	8	2	1950
111	30	23	3931_RAW0	barley	13	13	7456	8	2	1951
112	50	30	7887_RAW0	sorghum	14	14	7457	8	2	1952
113	29	22	3932_RAW0	barley	1	1	7444	9	2	1953
114	43	9	1888_RAW0	wheat	2	2	7445	9	2	1954
115	42	7	1770_RAW0	wheat	3	3	7446	9	2	1955
116	22	16	3923_RAW0	barley	4	4	7447	9	2	1956
117	27	4	1884_RAW0	wheat	5	5	7448	9	2	1957
118	10	28	6850_RAW0	triticale	6	6	7449	9	2	1958
119	6	11	3874_RAW0	barley	7	7	7450	9	2	1959
120	28	9	1888_RAW0	wheat	8	8	7451	9	2	1960
121	16	10	3897_RAW0	barley	9	9	7452	9	2	1961
122	49	34	7886_RAW0	sorghum	10	10	7453	9	2	1962
123	33	23	3931_RAW0	barley	11	11	7454	9	2	1963
124	3	18	3926_RAW0	barley	12	12	7455	9	2	1964
125	26	3	1763_RAW0	wheat	13	13	7456	9	2	1965
126	8	16	3923_RAW0	barley	14	14	7457	9	2	1966
127	44	26	6831_RAW0	triticale	1	1	7444	10	2	1967
128	19	33	7885_RAW0	sorghum	2	2	7445	10	2	1968

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
129	29	22	3932_RAW0	barley	3	3	7446	10	2	1969
130	5	40	8084_RAW0	maize	4	4	7447	10	2	1970
131	24	39	8083_RAW0	maize	5	5	7448	10	2	1971
132	23	24	3875_RAW0	barley	6	6	7449	10	2	1972
133	31	18	3926_RAW0	barley	7	7	7450	10	2	1973
134	20	35	7855_RAW0	sorghum	8	8	7451	10	2	1974
135	14	20	3928_RAW0	barley	9	9	7452	10	2	1975
136	45	25	6830_RAW0	triticale	10	10	7453	10	2	1976
137	21	12	3922_RAW0	barley	11	11	7454	10	2	1977
138	38	36	7887_SPROUT_MED	sorghum	12	12	7455	10	2	1978
139	48	6	1766_RAW0	wheat	13	13	7456	10	2	1979
140	15	14	3927_RAW0	barley	14	14	7457	10	2	1980
141	48	6	1766_RAW0	wheat	1	1	7458	11	3	1981
142	36	31	7887_SPROUT_HIGH	sorghum	2	2	7459	11	3	1982
143	2	17	3929_RAW0	barley	3	3	7460	11	3	1983
144	25	38	8082_RAW0	maize	4	4	7461	11	3	1984
145	23	24	3875_RAW0	barley	5	5	7462	11	3	1985
146	21	12	3922_RAW0	barley	6	6	7463	11	3	1986
147	50	30	7887_RAW0	sorghum	7	7	7464	11	3	1987
148	13	37	7857_RAW0	sorghum	8	8	7465	11	3	1988
149	34	1	1882_RAW0	wheat	9	9	7466	11	3	1989
150	39	29	6859_RAW0	triticale	10	10	7467	11	3	1990
151	1	15	3871_RAW0	barley	11	11	7468	11	3	1991
152	26	3	1763_RAW0	wheat	12	12	7469	11	3	1992
153	41	2	1883_RAW0	wheat	13	13	7470	11	3	1993
154	16	10	3897_RAW0	barley	14	14	7471	11	3	1994
155	39	29	6859_RAW0	triticale	1	1	7458	12	3	1995
156	17	20	3928_RAW0	barley	2	2	7459	12	3	1996
157	47	5	1886_RAW0	wheat	3	3	7460	12	3	1997

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
158	42	7	1770_RAW0	wheat	4	4	7461	12	3	1998
159	12	33	7885_RAW0	sorghum	5	5	7462	12	3	1999
160	37	32	7869_RAW0	sorghum	6	6	7463	12	3	2000
161	7	21	3930_RAW0	barley	7	7	7464	12	3	2001
162	32	19	3873_RAW0	barley	8	8	7465	12	3	2002
163	24	39	8083_RAW0	maize	9	9	7466	12	3	2003
164	8	16	3923_RAW0	barley	10	10	7467	12	3	2004
165	30	23	3931_RAW0	barley	11	11	7468	12	3	2005
166	18	13	3924_RAW0	barley	12	12	7469	12	3	2006
167	38	36	7887_SPROUT_MED	sorghum	13	13	7470	12	3	2007
168	10	28	6850_RAW0	triticale	14	14	7471	12	3	2008
169	11	34	7886_RAW0	sorghum	1	1	7458	13	3	2009
170	45	25	6830_RAW0	triticale	2	2	7459	13	3	2010
171	42	7	1770_RAW0	wheat	3	3	7460	13	3	2011
172	6	11	3874_RAW0	barley	4	4	7461	13	3	2012
173	44	26	6831_RAW0	triticale	5	5	7462	13	3	2013
174	14	20	3928_RAW0	barley	6	6	7463	13	3	2014
175	9	27	6849_RAW0	triticale	7	7	7464	13	3	2015
176	43	9	1888_RAW0	wheat	8	8	7465	13	3	2016
177	46	8	1887_RAW0	wheat	9	9	7466	13	3	2017
178	29	22	3932_RAW0	barley	10	10	7467	13	3	2018
179	20	35	7855_RAW0	sorghum	11	11	7468	13	3	2019
180	47	5	1886_RAW0	wheat	12	12	7469	13	3	2020
181	22	16	3923_RAW0	barley	13	13	7470	13	3	2021
182	13	37	7857_RAW0	sorghum	14	14	7471	13	3	2022
183	19	33	7885_RAW0	sorghum	1	1	7458	14	3	2023
184	6	11	3874_RAW0	barley	2	2	7459	14	3	2024
185	15	14	3927_RAW0	barley	3	3	7460	14	3	2025
186	26	3	1763_RAW0	wheat	4	4	7461	14	3	2026

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
187	33	23	3931_RAW0	barley	5	5	7462	14	3	2027
188	34	1	1882_RAW0	wheat	6	6	7463	14	3	2028
189	21	12	3922_RAW0	barley	7	7	7464	14	3	2029
190	50	30	7887_RAW0	sorghum	8	8	7465	14	3	2030
191	28	9	1888_RAW0	wheat	9	9	7466	14	3	2031
192	40	27	6849_RAW0	triticale	10	10	7467	14	3	2032
193	31	18	3926_RAW0	barley	11	11	7468	14	3	2033
194	45	25	6830_RAW0	triticale	12	12	7469	14	3	2034
195	37	32	7869_RAW0	sorghum	13	13	7470	14	3	2035
196	35	8	1887_RAW0	wheat	14	14	7471	14	3	2036
197	3	18	3926_RAW0	barley	1	1	7458	15	3	2037
198	27	4	1884_RAW0	wheat	2	2	7459	15	3	2038
199	32	19	3873_RAW0	barley	3	3	7460	15	3	2039
200	24	39	8083_RAW0	maize	4	4	7461	15	3	2040
201	15	14	3927_RAW0	barley	5	5	7462	15	3	2041
202	4	38	8082_RAW0	maize	6	6	7463	15	3	2042
203	49	34	7886_RAW0	sorghum	7	7	7464	15	3	2043
204	20	35	7855_RAW0	sorghum	8	8	7465	15	3	2044
205	36	31	7887_SPROUT_HIGH	sorghum	9	9	7466	15	3	2045
206	41	2	1883_RAW0	wheat	10	10	7467	15	3	2046
207	10	28	6850_RAW0	triticale	11	11	7468	15	3	2047
208	16	10	3897_RAW0	barley	12	12	7469	15	3	2048
209	18	13	3924_RAW0	barley	13	13	7470	15	3	2049
210	5	40	8084_RAW0	maize	14	14	7471	15	3	2050
211	34	1	1882_RAW0	wheat	1	1	7472	16	4	2051
212	20	35	7855_RAW0	sorghum	2	2	7473	16	4	2052
213	39	29	6859_RAW0	triticale	3	3	7474	16	4	2053
214	46	8	1887_RAW0	wheat	4	4	7475	16	4	2054
215	2	17	3929_RAW0	barley	5	5	7476	16	4	2055

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
216	7	21	3930_RAW0	barley	6	6	7477	16	4	2056
217	29	22	3932_RAW0	barley	7	7	7478	16	4	2057
218	5	40	8084_RAW0	maize	8	8	7479	16	4	2058
219	18	13	3924_RAW0	barley	9	9	7480	16	4	2059
220	42	7	1770_RAW0	wheat	10	10	7481	16	4	2060
221	41	2	1883_RAW0	wheat	11	11	7482	16	4	2061
222	21	12	3922_RAW0	barley	12	12	7483	16	4	2062
223	27	4	1884_RAW0	wheat	13	13	7484	16	4	2063
224	26	3	1763_RAW0	wheat	14	14	7485	16	4	2064
225	48	6	1766_RAW0	wheat	1	1	7472	17	4	2065
226	35	8	1887_RAW0	wheat	2	2	7473	17	4	2066
227	32	19	3873_RAW0	barley	3	3	7474	17	4	2067
228	18	13	3924_RAW0	barley	4	4	7475	17	4	2068
229	23	24	3875_RAW0	barley	5	5	7476	17	4	2069
230	42	7	1770_RAW0	wheat	6	6	7477	17	4	2070
231	49	34	7886_RAW0	sorghum	7	7	7478	17	4	2071
232	24	39	8083_RAW0	maize	8	8	7479	17	4	2072
233	14	20	3928_RAW0	barley	9	9	7480	17	4	2073
234	34	1	1882_RAW0	wheat	10	10	7481	17	4	2074
235	30	23	3931_RAW0	barley	11	11	7482	17	4	2075
236	22	16	3923_RAW0	barley	12	12	7483	17	4	2076
237	19	33	7885_RAW0	sorghum	13	13	7484	17	4	2077
238	27	4	1884_RAW0	wheat	14	14	7485	17	4	2078
239	17	20	3928_RAW0	barley	1	1	7472	18	4	2079
240	7	21	3930_RAW0	barley	2	2	7473	18	4	2080
241	16	10	3897_RAW0	barley	3	3	7474	18	4	2081
242	10	28	6850_RAW0	triticale	4	4	7475	18	4	2082
243	28	9	1888_RAW0	wheat	5	5	7476	18	4	2083
244	41	2	1883_RAW0	wheat	6	6	7477	18	4	2084

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
245	48	6	1766_RAW0	wheat	7	7	7478	18	4	2085
246	50	30	7887_RAW0	sorghum	8	8	7479	18	4	2086
247	33	23	3931_RAW0	barley	9	9	7480	18	4	2087
248	11	34	7886_RAW0	sorghum	10	10	7481	18	4	2088
249	5	40	8084_RAW0	maize	11	11	7482	18	4	2089
250	36	31	7887_SPROUT_HIGH	sorghum	12	12	7483	18	4	2090
251	47	5	1886_RAW0	wheat	13	13	7484	18	4	2091
252	44	26	6831_RAW0	triticale	14	14	7485	18	4	2092
253	38	36	7887_SPROUT_MED	sorghum	1	1	7472	19	4	2093
254	24	39	8083_RAW0	maize	2	2	7473	19	4	2094
255	31	18	3926_RAW0	barley	3	3	7474	19	4	2095
256	25	38	8082_RAW0	maize	4	4	7475	19	4	2096
257	8	16	3923_RAW0	barley	5	5	7476	19	4	2097
258	3	18	3926_RAW0	barley	6	6	7477	19	4	2098
259	2	17	3929_RAW0	barley	7	7	7478	19	4	2099
260	1	15	3871_RAW0	barley	8	8	7479	19	4	2100
261	13	37	7857_RAW0	sorghum	9	9	7480	19	4	2101
262	45	25	6830_RAW0	triticale	10	10	7481	19	4	2102
263	37	32	7869_RAW0	sorghum	11	11	7482	19	4	2103
264	12	33	7885_RAW0	sorghum	12	12	7483	19	4	2104
265	29	22	3932_RAW0	barley	13	13	7484	19	4	2105
266	4	38	8082_RAW0	maize	14	14	7485	19	4	2106
267	43	9	1888_RAW0	wheat	1	1	7472	20	4	2107
268	40	27	6849_RAW0	triticale	2	2	7473	20	4	2108
269	36	31	7887_SPROUT_HIGH	sorghum	3	3	7474	20	4	2109
270	21	12	3922_RAW0	barley	4	4	7475	20	4	2110
271	9	27	6849_RAW0	triticale	5	5	7476	20	4	2111
272	13	37	7857_RAW0	sorghum	6	6	7477	20	4	2112
273	37	32	7869_RAW0	sorghum	7	7	7478	20	4	2113

PIG_DIET	PROCESS BATCH	CULTIVAR_LABEL	sample ID + info code	grain type	Pig No.	Cage No.	Pig ID	TIME_PERIOD	RUN	faecal sample ID
274	47	5	1886_RAW0	wheat	8	8	7479	20	4	2114
275	39	29	6859_RAW0	triticale	9	9	7480	20	4	2115
276	10	28	6850_RAW0	triticale	10	10	7481	20	4	2116
277	6	11	3874_RAW0	barley	11	11	7482	20	4	2117
278	15	14	3927_RAW0	barley	12	12	7483	20	4	2118
279	1	15	3871_RAW0	barley	13	13	7484	20	4	2119
280	38	36	7887_SPROUT_MED	sorghum	14	14	7485	20	4	2120