Improving the efficiency of pig feed manufacturing and application of additives

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Co-operative Research Centre for High Integrity Australian Pork

By

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These appendices relate to part two of the project

Executive Summary

The project has demonstrated that processed feeds with similar ingredient mixtures differ widely between mills in throughput, energy use, pellet hardness and durability and nutritional quality for growing pigs. Preparation of grains before mixing, specific ingredients and additives used as well as the physical structure of the processing units all affect the cost of manufacture and physical and nutritional quality of the finished product.

Important findings from the project were:

- Characteristics of individual grains (wheat, barley, sorghum) that influence pellet quality and nutritional value are not additive when mixed in a diet with the characteristics of sorghum dominating.
- The size of grain particles, particularly the proportion of large particles, significantly affects RVA, *in vitro* starch digestion variables, pellet hardness and durability and the efficiency of feed use by young pigs. Regrinding large particles produced by normal hammer milling of grains increased the efficiency of feed use by young pigs by up to 22% with sorghum based diets and 15% with barley based diets.
- Low conditioning temperature for an extended period significantly increased the rate of *in vitro* starch digestion and efficiency of feed use by growing pigs.
- Addition of surfactants to diet mixtures produced small and variable results. At one mill, a surfactant significantly reduced retention time and pellet length, whereas there were no significant effects at the other mill.
- There were significant differences between soft, hard and naturally sprouted wheat samples in energy use during diet processing, pellet quality and efficiency of feed use by weaner pigs.
- Significantly more energy was used during diet processing and pellet formation (as measured by specific mechanical energy (SME, kJ/kg)) for diets based on soft wheat samples compared with diets based on either hard or naturally sprouted wheat.
- Pellets made from diets containing sprouted wheat were significantly softer and tended to be less durable (non-significant) than pellets made from diets containing either soft or hard wheat.
- Although feed consumption for pigs offered diets containing sprouted wheat was greater than for pigs offered diets containing either soft or hard wheat, efficiency of feed use was 16% less than weaner pigs given diets based on soft wheat. There was no difference between soft and hard wheat in efficiency of feed use.
- Increasing temperature of the conditioning period during feed processing from 60°C to 80°C significantly reduced the efficiency of feed use in weaner pigs by 16%.
- There was a strong relationship between several variables from the *in vitro* starch digestion assays (rate constant K, area under the curve and 240 min starch disappearance) and the efficiency of feed use by growing pigs. This observation needs strengthening with more data, but confirms that the *in vitro* assay is an excellent method for rapidly screening feed products for their nutritional value in pigs.
- Preliminary NIR calibrations developed from scans of the final pelleted product are encouraging for predicting faecal DE content, *in vitro* starch digestion variables and the physical and chemical characteristics of the product.

Sufficient information is now available from the experiments that have been conducted, literature results and theoretical information about factors that affect the efficiency of

processing measured by Specific Mechanical Energy (SME), pellet durability and nutritional value to develop a spreadsheet or other model that will allow optimisation of dietary ingredients and processing conditions for improving profitability of pig feed manufacture and nutritional quality of the feed.

A. Introduction

Differences in throughput rates and pellet quality are regularly observed in the manufacture of pig feed products and occur despite increasingly sophisticated procedures for process control and ingredient description and selection. Anecdotal reports suggest grain type and inclusion level, particle size and the conditioning and press settings of individual mills are contributing factors. Throughput and specific mechanical energy (SME) used are key measures of feed mill efficiency. Thus, feed mills direct considerable attention toward maximizing production rates due to their impact on overall mill costs and profitability. Pellet quality, that is product durability and absence of fines, is the most relevant measure of feed processing effectiveness in commercial stock feed manufacture. Few pig producers adequately record or monitor feed use efficiency so that feedback to mills on the performance of individual processing methods are generally restricted to complaints about the form and consistency of the pellet rather than any aspect of animal performance related to the nature of the feed supplied. Not surprisingly, variations in pellet quality are an ongoing source of frustration for both stock feed manufacturers and pig producers with potential implications for both mill efficiency and on-farm feed use efficiency.

Further complicating attempts to develop more consistent methods for feed manufacture is that the nutritional values of ingredients used in the preparation of diet formulations are generally developed from analysis of the unprocessed raw materials. Commercially prepared animal feeds are complex mixtures of heat-processed protein meals and minerals as well as grains and it is unclear how further processing during manufacture affects either the milling characteristics or nutritional value of the resulting pig feed products.

The extent to which processing conditions influence feed characteristics is also a consideration in the application of new technologies such as microwave and NIR spectroscopy (NIR) to the on-line control of feed manufacture systems. If processing alters the spectral profiles of individual grains and ration mixtures, there will be significant implications for the precision of individual nutrient calibrations and how readily such technology may be taken up by the wider stock feed industry.

The project aims to reduce production costs for high-quality pork through understanding how commercial processing conditions affect mill throughput, processing energy efficiency, product durability and the nutritional value of pig feed. This was achieved by investigating the effects of commercial processing conditions, grain characteristics, ingredient mixtures and additives on product throughput, energy efficiency of processing, durability of the product and the nutritional value of processed feed for pigs. Initially a survey of feeds collected from two commercial mills was conducted. The pig feeds collected varied in type and ingredient mixtures, as well as processing techniques. The feeds were subjected to *in vitro* starch digestion and RVA analysis as an assessment of likely differences in nutritional quality. Subsequently, an experiment investigating the effect on *in vitro* assays of sorghum content in the feed mix, hammer mill screen size, temperature of conditioning, use of surfactants and mill location was conducted. Finally, an experiment altering the amount of sorghum in the mixture, conditioning temperature and time was undertaken where both *in vitro* assays and growth performance for grower pigs were measured.

In addition, the potential was investigated for developing NIR calibrations and other tools which can be used by stock feed manufacturers to adjust processing conditions for specific

grain samples and ingredient mixtures to improve manufacturing efficiency, product durability and nutritional value for pigs.

Reliable NIR calibrations have been developed in projects 1B-101/104 to predict the energy value of cereal grains for pigs. These calibrations have proven to be extremely useful for reducing the cost of production of pigs offered meal or cold pelleted diets. The degree of starch gelatinisation within grains can substantially affect the rate of starch digestion by pigs and the energy value of feeds. The original NIR calibrations are therefore less reliable for predicting the energy value of feed pellets made by the conventional steam pellet press. A final objective of the project is to determine whether NIR calibrations of the final processed feed can be developed to reliably predict factors that may influence animal performance or directly predict animal performance.

The report is divided into two parts. Part One deals with commercial feed processing that examined the issues above. However, commercial mills are limited by their sizes, which restrict processing levels and settings, quantities of raw materials to process, possibly the grains to process because of locations, and the processed parameters to measure. With the global objective to understand material-process-property relationships, these limitations can affect the generation of baseline data that are valuable for commercial feed processing. A pilot-plant feed processing study is, therefore, required, and this was undertaken and forms Part Two of the report. Moreover, it is well recognised that Australian pig diets are grain-based with wheat, barley, sorghum, maize, triticale, and rice as the major cereals used. Wheat is the grain used predominantly for pig diets in most years in southern Australia. Both hard and soft wheat varieties are available and in some years, shot and sprung (sprouted) samples are common. These wheat types differ in their physical and chemical properties, as well as the concentration of naturally-occurring enzymes. For example, following rain damage, α-amylase will increase in sprouted wheat, while differences in fracturability can influence particle size distributions of milled soft and hard wheats. Previous Pork CRC and PGLP projects have shown that these variables influenced energy delivery and animal performance.

Hence, Part Two of the report details the following tasks:

- Sourcing soft, hard and sprouted wheats, and NIR-scanning of the wheats.
- Formulation of diets to fixed grain percentage, digestible energy and dry matter.
- Manufacturing pellet diets at different conditioning conditions while fixing other processing conditions in a pilot-scale feed mill.
- Establishing process response to changes in grain types and conditions.
- Assessing feed efficiency of the pellet diets with weaner pigs.
- Examination of pellet quality (hardness and durability).
- Analysing the physicochemical properties of the grains and diets.
- NIR-scanning of the diets.
- Investigating functional and digestibility properties of the diets.
- Examination of the relationships between *in vitro*, *in vivo* and NIR-predicted data to strengthen the NIRs calibrations.

PART ONE

COMMERCIAL FEED PROCESSING

B. Survey of Commercial Pig Feeds

The basic principle of manufacturing a pelleted product is to take a specified mixture of milled grains, protein meals and minerals from ambient temperature with moisture content of around 11% to approximately 15-17% moisture at the press to form the desired pelleted structure. The heat and excess moisture is then dissipated to produce a product that is sufficiently durable and stable to persist through transport, storage and delivery to the animal. The settings used in feed processing vary for each mill as does the nature of the final product.

Steam pelleting allows for the addition of moisture, needed to form or mould the pellet, in the presence of heat. The heat with moisture assists to gelatinise starch fractions within the grain component, effectively cooking the mixture and aiding to cement the mixture together. Through this process, steam pelleting assists to improve throughput while minimizing wear on mill equipment and reducing energy consumption at the press. Figure 1 provides an outline of a typical steam pelleting, feed manufacturing system.

B.1 Methods

Fifty samples (Table 1) were selected from a range of diets made by two feed mills (Riverina, formerly Better Blend, Oakey, Queensland, Mill A; Ridley AgriProducts, Toowoomba Queensland, Mill B) that involved different grains, at various stages of processing, with and without heat treatment. The samples consist of processed and unprocessed grains, all of which were ground in a laboratory hammer mill through a 1 mm retention sieve, to create sample material of a consistent size for analysis. To assess likely differences in the nutritional value of the samples, assays were conducted for *in vitro* starch digestion (Sopade and Gidley 2009) and RVA analyses (Mahasukhonthachat *et al.* 2010). The RVA variables listed below were obtained using the Thermocline for Windows$^{(R)}$ software. These measurements have been widely used to characterise pasting behaviours of human food and animal feed:

- Initial viscosity – Pasting viscosity at 1 min.
- Pasting temperature – Temperature of an appreciable change (>10%) change in viscosity
- Peak viscosity – Highest pasting viscosity prior to cooling the gelatinised starch dispersion
- Trough viscosity – Lowest pasting viscosity during holding the gelatinised starch dispersion at about 95^{o}C
- Final viscosity – Viscosity at the end of cooling and holding at 50^{o}C

For systems that exhibit monophasic starch digestograms during *in vitro* starch digestion, a modified first-order kinetic model (Eqn.[1]) can be used to describe the digestograms;

$$D_t = D_0 + D_{\infty-0} [1 - \exp (-K t)] \qquad \textit{Eqn. 1}$$

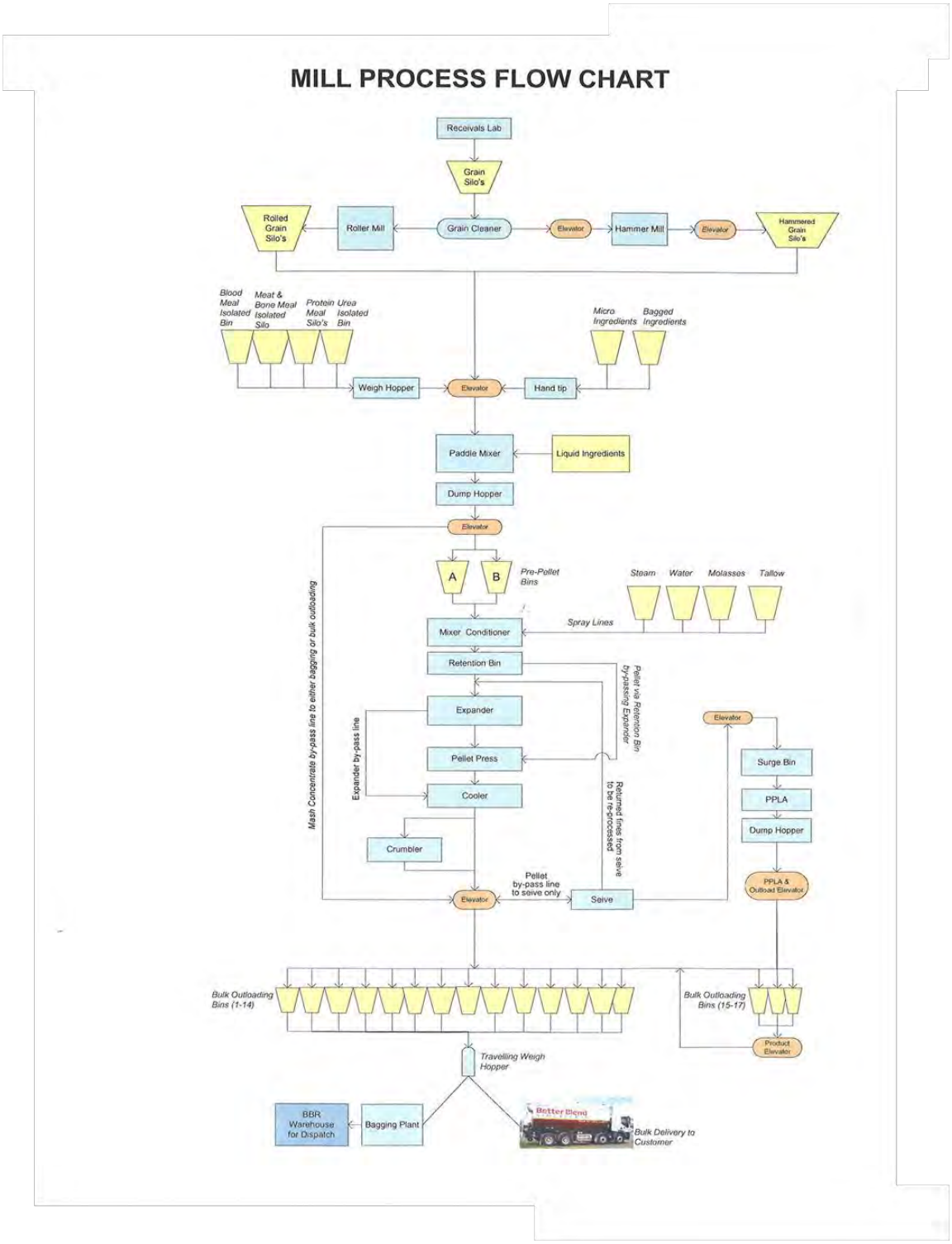


Figure 1. An approximation to the processing that is carried out at each mill
(Steam pelleting allows for the addition of moisture, needed to form or mould the pellet, in the presence of additional heat. Heat with moisture assists to gelatinise carbohydrates fractions within the grain component, effectively cooking the mixture and aiding to cement the mixture together. Through this process, steam pelleting assists to improve throughput while minimizing wear on mill equipment and reducing energy consumption at the press)

where, D_t = digested starch (g/100g dry starch) at time t (min), D_0 = digested starch (g/100g dry starch) at time t = 0, which is equivalent to gastric digestion, D_∞ = digested starch (g/100g dry starch) at infinite time ($t \rightarrow \infty$), which is equivalent to maximum digested starch, and K = rate of digestion (g/min). It has been widely estimated that digestion in the small intestine (pancreatic digestion) can be up to 4 h. Therefore, starch digestion at 240 min. (predicted, PreD240 and measured, ExpD240) as well as the area under the starch digestogram (g.min/100 g dry starch, AUC (Eqn. [2]), can be used as additional digestion variables, which estimate the amount of glucose released into the blood (glycemic index).

$$AUC = \left[(D_0 + D_{\infty-0})t + \frac{D_{\infty-0}}{K} \exp(-Kt) \right]_0^{240} \qquad \textit{Eqn. 2}$$

Hence, except when otherwise stated, six starch digestion parameters, D_0, D_∞, K, PreD240, ExpD240, and AUC were used to characterise the *in vitro* starch digestion of samples throughout the study.

For all analyses, samples were randomised and duplicated. The following hypotheses were investigated:

a. Grains behave differently when processed
b. End-use properties of grains are dependent on processing
c. Changes in product properties are progressive along the processing change

B.2 Results

Results are presented for 10 of the 50 samples examined. These samples demonstrate some of the major effects of processing on individual grains and feed mixtures. Figure 2 illustrates the effects of steam flaking either barley or sorghum on viscosity as measured with the RVA apparatus. Viscosity was substantially higher for barley grain (RID016, RID017) than for sorghum (RID014, RID015). However, for both grains, steam flaking increased viscosity, which possibly reflected better swelling behaviours of the flaked samples (RID016 cf. RID017 for barley and RID014 cf. RID015 for sorghum). Steam flaking is a heat-moisture treatment, which is expected to gelatinise starch and reduce RVA viscosity in the presence of other favourable conditions. However, the pasting behaviours of these samples indicate the flaking process did not appreciably gelatinise the starches in the barley and sorghum. This conclusion was reached because, apart from the pasting behaviours, microscopy (not shown) revealed many native starch granules (low gelatinisation) in the samples. Moreover, differential scanning calorimetry (not shown), which measures gelatinisation properties, showed evidence of incomplete gelatinisation in the samples. It is noteworthy that while the flaked (RID014) and raw (RID015) sorghum exhibited shear-thickening pasting behaviours, the flaked (RID016) and raw (RID017) barley showed shear-thinning behaviours. Mahasukhonthachat *et al.* (2010) proposed shear-thickening behaviours occur because of restricted swelling of sorghum starch due to protein matrix, but these starch-protein interactions can be disrupted by a careful choice of processing conditions. Such disruptions can enhance starch digestion and energy delivery from sorghum pellets as Mahasukhonthachat *et al.* (2010) measured shear-thinning pasting behaviours in extruded sorghum, which exhibited a substantial increase in *in vitro* starch digestion.

Table 1. Description of samples for the project (1 of 2)

S/No	Sample ID	Product Description	Other Details	Grain type	Company	Product	Form	Heat
1	ILS000941	Wheat	Composite of Received Samples - Grain for ILS000944 to ILS000949	Wheat	A	I	Hammer	No
2	ILS000942	Sorghum	Composite of Received Samples - Grain for ILS000944 to ILS000949	Sorghum	A	I	Hammer	No
3	ILS000943	Barley	Composite of Received Samples - Grain for ILS000944 to ILS000949	Barley	A	I	Hammer	No
4	ILS000944	WEANER 1 PELLET	Mill Profile - Post Mixer	Wheat,Sorghum	A	I	Mash	No
5	ILS000945	WEANER 1 PELLET	Mill Profile - Before RT Bin	Wheat,Sorghum	A	I	Mash	Yes
6	ILS000946	WEANER 1 PELLET	Mill Profile - Before Expander	Wheat,Sorghum	A	I	Mash	Yes
7	ILS000947	WEANER 1 PELLET	Mill Profile - Before Crusher	Wheat,Sorghum	A	I	Mash	Yes
8	ILS000948	WEANER 1 PELLET	Mill Profile - Before Press	Wheat,Sorghum	A	I	Mash	Yes
9	ILS000949	WEANER 1 PELLET	Mill Profile - After Cooler	Wheat,Sorghum	A	I	Pellet	Yes
10	ILS000956	DRY SOW PELLET	Mill Profile - Post Mixer	Wheat,Sorghum,Barley	A	II	Mash	No
11	ILS000957	DRY SOW PELLET	Mill Profile - Before RT Bin	Wheat,Sorghum,Barley	A	II	Mash	Yes
12	ILS000958	DRY SOW PELLET	Mill Profile - Before Expander	Wheat,Sorghum,Barley	A	II	Mash	Yes
13	ILS000959	DRY SOW PELLET	Mill Profile - Before Crusher	Wheat,Sorghum,Barley	A	II	Mash	Yes
14	ILS000960	DRY SOW PELLET	Mill Profile - Before Press	Wheat,Sorghum,Barley	A	II	Mash	Yes
15	ILS000961	DRY SOW PELLET	Mill Profile - After Cooler	Wheat,Sorghum,Barley	A	II	Pellet	Yes
16	ILS000962	Hammered Wheat	Mill Profile - Grain for ILS000950 to ILS000961	Wheat	A	II	Hammer	No
17	ILS000963	Hammered Sorghum	Mill Profile - Grain for ILS000950 to ILS000961	Sorghum	A	II	Hammer	No
18	ILS000972	LAC SOW 2 PELLET	Mill Profile - Post Mixer	Wheat,Sorghum	A	III	Mash	No
19	ILS000973	LAC SOW 2 PELLET	Mill Profile - Before RT Bin	Wheat,Sorghum	A	III	Mash	Yes
20	ILS000974	LAC SOW 2 PELLET	Mill Profile - Before Expander	Wheat,Sorghum	A	III	Mash	Yes
21	ILS000975	LAC SOW 2 PELLET	Mill Profile - Before Crusher	Wheat,Sorghum	A	III	Mash	Yes
22	ILS000976	LAC SOW 2 PELLET	Mill Profile - Before Press	Wheat,Sorghum	A	III	Mash	Yes
23	ILS000977	LAC SOW 2 PELLET	Mill Profile - After Cooler	Wheat,Sorghum	A	III	Pellet	Yes
24	ILS000978	Hammered Wheat	Mill Profile - Grain for ILS000972 to ILS000977	Wheat	A	III	Hammer	No
25	ILS000979	Hammered Sorghum	Mill Profile - Grain for ILS000972 to ILS000977	Sorghum	A	III	Hammer	No

Table 1. Description of samples for the project (2 of 2)

S/No	Sample ID	Product Description	Other Details	Grain type	Company	Product	Form	Heat
26	ILS000983	GROWER 2 PELLET	Mill Profile - Post Mixer	Wheat,Sorghum	A	IV	Mash	No
27	ILS000984	GROWER 2 PELLET	Mill Profile - Before RT Bin	Wheat,Sorghum	A	IV	Mash	Yes
28	ILS000985	GROWER 2 PELLET	Mill Profile - Before Expander	Wheat,Sorghum	A	IV	Mash	Yes
29	ILS000986	GROWER 2 PELLET	Mill Profile - Before Crusher	Wheat,Sorghum	A	IV	Mash	Yes
30	ILS000987	GROWER 2 PELLET	Mill Profile - Before Press	Wheat,Sorghum	A	IV	Mash	Yes
31	ILS000988	GROWER 2 PELLET	Mill Profile - After Cooler	Wheat,Sorghum	A	IV	Pellet	Yes
32	ILS000989	Hammered Wheat	Mill Profile - Grain for ILS000983 to ILS000988	Wheat	A	IV	Hammer	No
33	ILS000990	Hammered Sorghum	Mill Profile - Grain for ILS000983 to ILS000988	Sorghum	A	IV	Hammer	No
34	RID001	Ridley ExpandedSow	Ground wheat for ExpandedSow	Wheat	B	V	Hammer	No
35	RID002	Ridley ExpandedSow	Ground sorghum for ExpandedSow	Sorghum	B	V	Hammer	No
36	RID003	Ridley ExpandedSow	Ground barley for ExpandedSow	Barley	B	V	Hammer	No
37	RID004	Ridley ExpandedSow	ExpandedSow feed post mixer	Wheat,Sorghum,Barley	B	V	Mash	No
38	RID005	Ridley ExpandedSow	ExpandedSow feed post expansion	Wheat,Sorghum,Barley	B	V	Pellet	Yes
39	RID006	Ridley ExpandedSow	ExpandedSow finished feed	Wheat,Sorghum,Barley	B	V	Pellet	Yes
40	RID007	Ridley Starter	Starter feed post mixer	Wheat,Sorghum	B	VI	Mash	No
41	RID008	Ridley Starter	Starter feed post pelleting before crumbling	Wheat,Sorghum	B	VI	Pellet	Yes
42	RID009	Ridley Starter	Starter feed finished feed	Wheat,Sorghum	B	VI	Pellet	Yes
43	RID010	Ridley Grower	Grower feed post mixer	Wheat,Sorghum	B	VII	Mash	No
44	RID011	Ridley Grower	Grower feed post pelleting	Wheat,Sorghum	B	VII	Pellet	Yes
45	RID012	Ridley Grower	Grower finished feed	Wheat,Sorghum	B	VII	Pellet	Yes
46	RID013	Ridley Flaked	Finished feed steam flaked	Sorghum,Barley	B	VII	Pellet	Yes
47	RID014	Ridley Flaked	Steam flaked sorghum	Sorghum	B	VIII	Pellet	Yes
48	RID015	Ridley Flaked	Raw sorghum for flaking	Sorghum	B	VIII	Hammer	No
49	RID016	Ridley Flaked	Steam flaked barley	Barley	B	VIII	Pellet	Yes
50	RID017	Ridley Flaked	Raw barley for flaking	Barley	B	VIII	Hammer	No

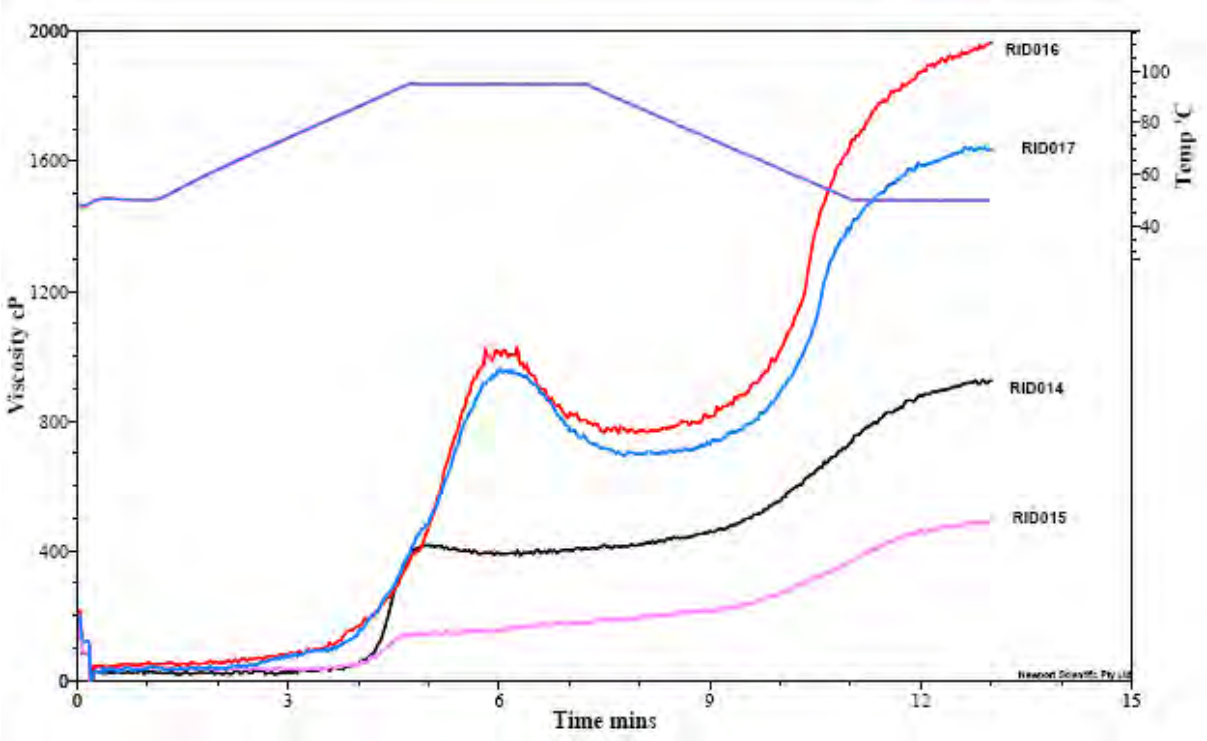


Figure 2. Pasting properties of the flaked and raw barley and sorghum

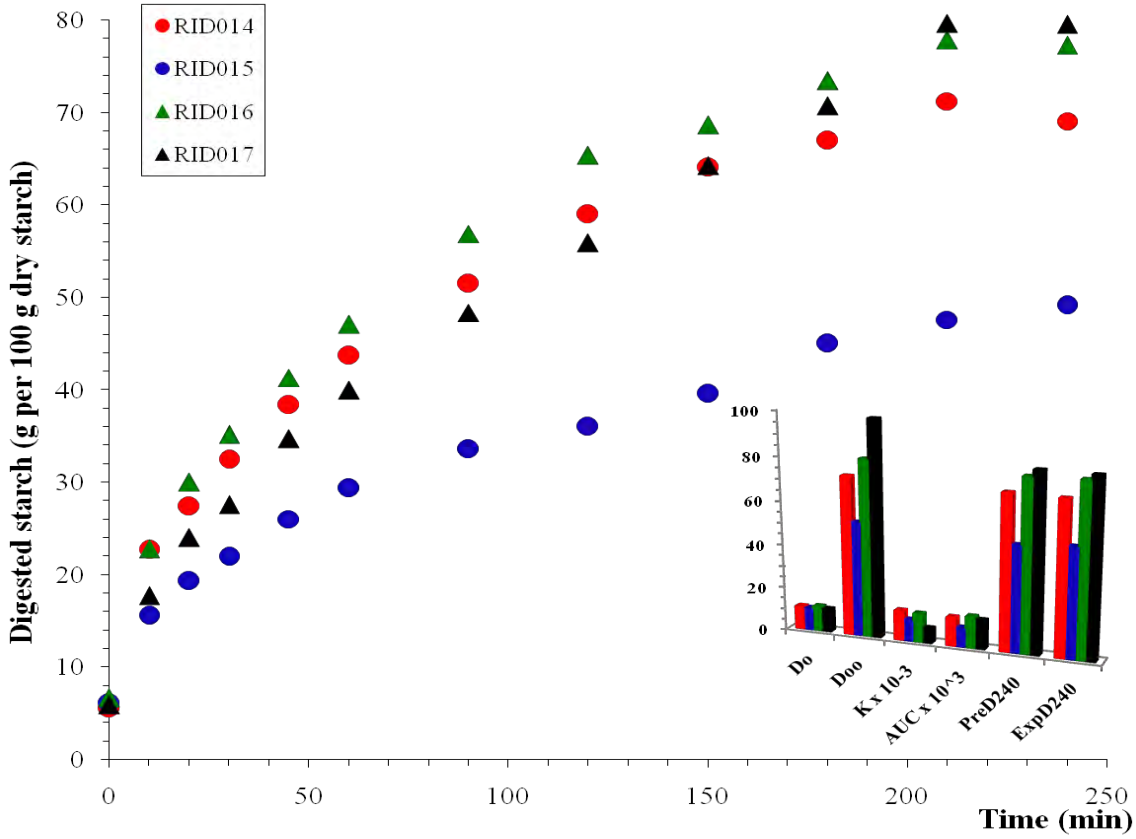


Figure 3. Starch digestograms of the flaked and raw barley and sorghum with the model parameters

The *in vitro* starch digestograms of the flaked and raw barley and sorghum are shown in Figure 3. The differences in the RVA behaviours are partially reflected in the *in vitro* starch digestibility of these samples. Possibly because of sorghum starch-protein interactions, the raw barley (RID017) was generally more digested than the raw sorghum (RID015). Steam flaking of both grains (barley RID016; sorghum RID014) increased the rate of starch digestion (K) compared with the raw grains, and sorghum was more affected (Figure 3, insert). Although substantial differences in the rate of starch digestion at 240 minutes remained for steam flaked sorghum compared with raw sorghum, there was little difference in the proportion of starch digested at this time between raw and steam-flaked barley (PreD240 and ExpD240).

Figure 4 shows the effect of processing whole diets containing a mixture of grains at different mills on pasting properties as measured by the RVA. There were marked differences in viscosity between sow diets containing wheat, sorghum and barley when prepared at mill A (ILS000959 & ILS000961) compared with mill B (RID004 & RID005). There were relatively small differences in viscosity measurements at each mill between the raw mixed mash and the finished pellet. However, at both mills, pelleting reduced viscosity. This was possibly because of an increase in starch gelatinisation. The marked differences in viscosity between the sow diets prepared at the different mills suggests that there were major differences in the treatment of grains prior to mixing and possibly processing conditions, rather than due to the processing techniques because both mills pelletised the feeds (ILS000961 and RID005). However, grower diets prepared at mill B (RID011) had similar viscosity profiles to the sow diets prepared at mill A (ILS000961). Pelleting the grower diet increased viscosity slightly compared with the mixed mash diet prior to pelleting, and this could be due to enhanced swelling, rather than gelatinisation.

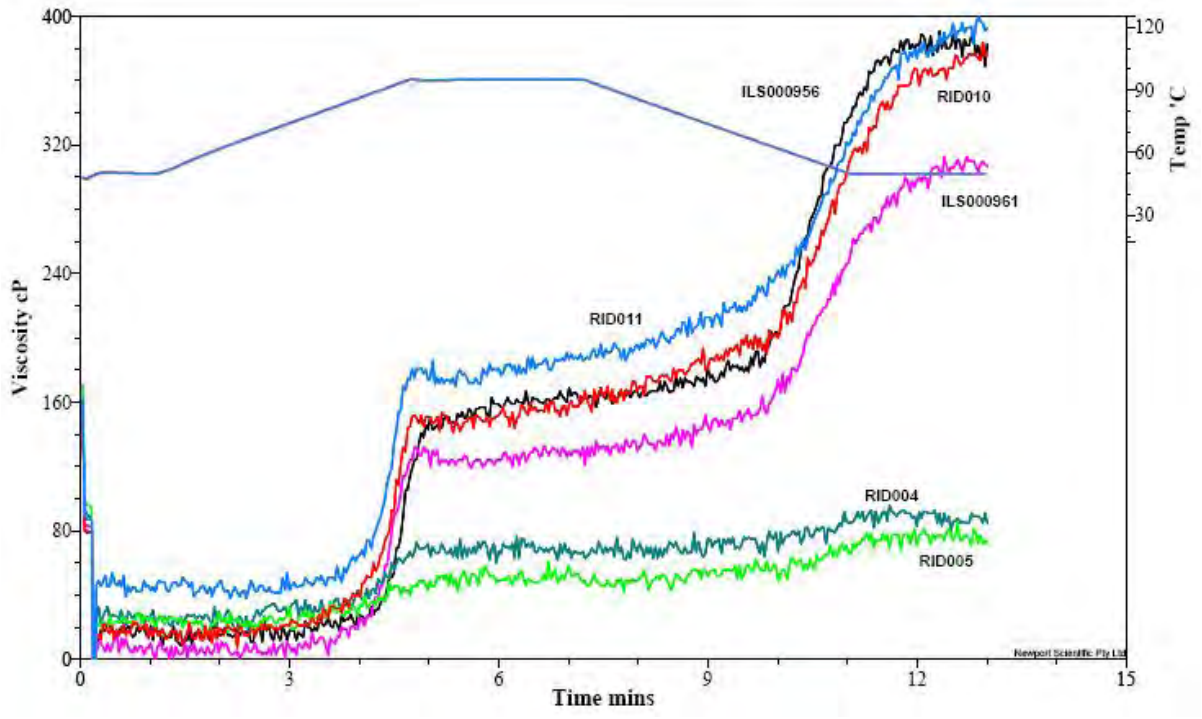


Figure 4. Pasting properties of the mixtures of grains from mills A and B

Figure 5 shows the *in vitro* starch digestograms for the mixed mash diets and pellets. Starch digestion was substantially faster for the sow diets prepared at mill B (RID005) compared with mill A (ILS000961) and reflects the differences seen in the viscosity measurements. Pelleting enhanced starch digestion for sow diets prepared by both mills. This is reflected by an increase (>10%) in all the digestion parameters compared to mash with the rate of digestion increasing by more than 200%. Similarly, starch digestion for grower diets prepared at mill B was substantially increased by pelleting (RID010 cf RID011). The increases observed were of the order as seen for the sow diets. .

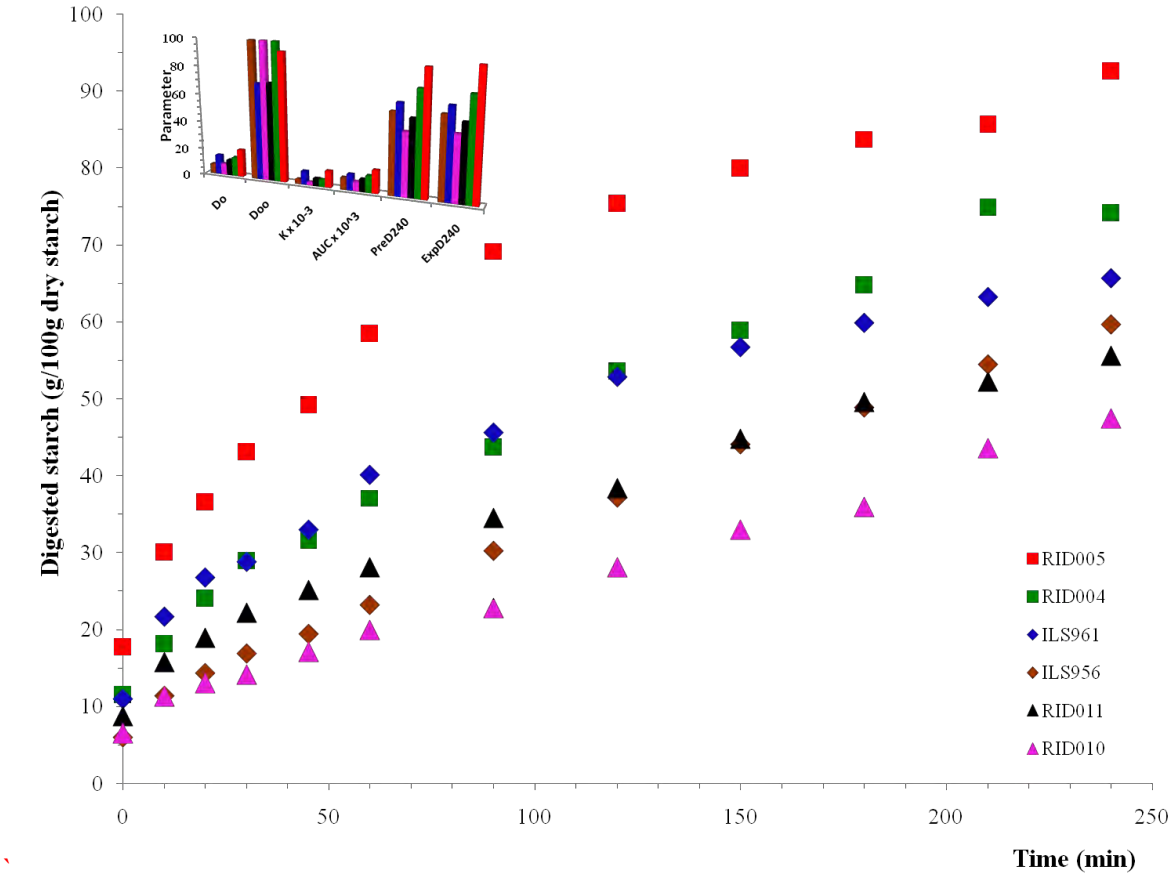


Figure 5. Starch digestograms for several diets from two mills

Although the overall effect of feed processing (mash and pellets) have been discussed, changes to feed properties can be progressive as the grains move through the processing chain. An understanding of these progressive changes can help identify the critical processes and operations that can be optimised for maximum pellet quality and animal performance. Figures 6A and 6B show how the variation in the pasting properties of typical samples from both mills change. It can be observed that, depending on the rations, when sorghum is mixed with wheat (or other cereals) the pasting behaviours of the mixture resemble sorghum behaviours. This suggests that while the other cereal(s) in the mixture may fully swell during the RVA analysis, the restricted swelling of sorghum starch due to its interaction with sorghum protein predominates. Differential scanning calorimetry (not shown) indicated that grains behave as individual grains in mixtures and grains in a mixture are not miscible. Hence, feed processors need to be aware of the characteristics of individual grains in a mixture. For example, sorghum has a higher pasting temperature than wheat (Table 2). Figure 6A shows steaming (during initial conditioning) reduced the RVA viscosity possibly because of partial gelatinisation, while expander (extended conditioning) caused the most

significant reduction in the RVA parameters. This slight increase in RVA viscosity occurred possibly because of post-process changes (structural and additives).

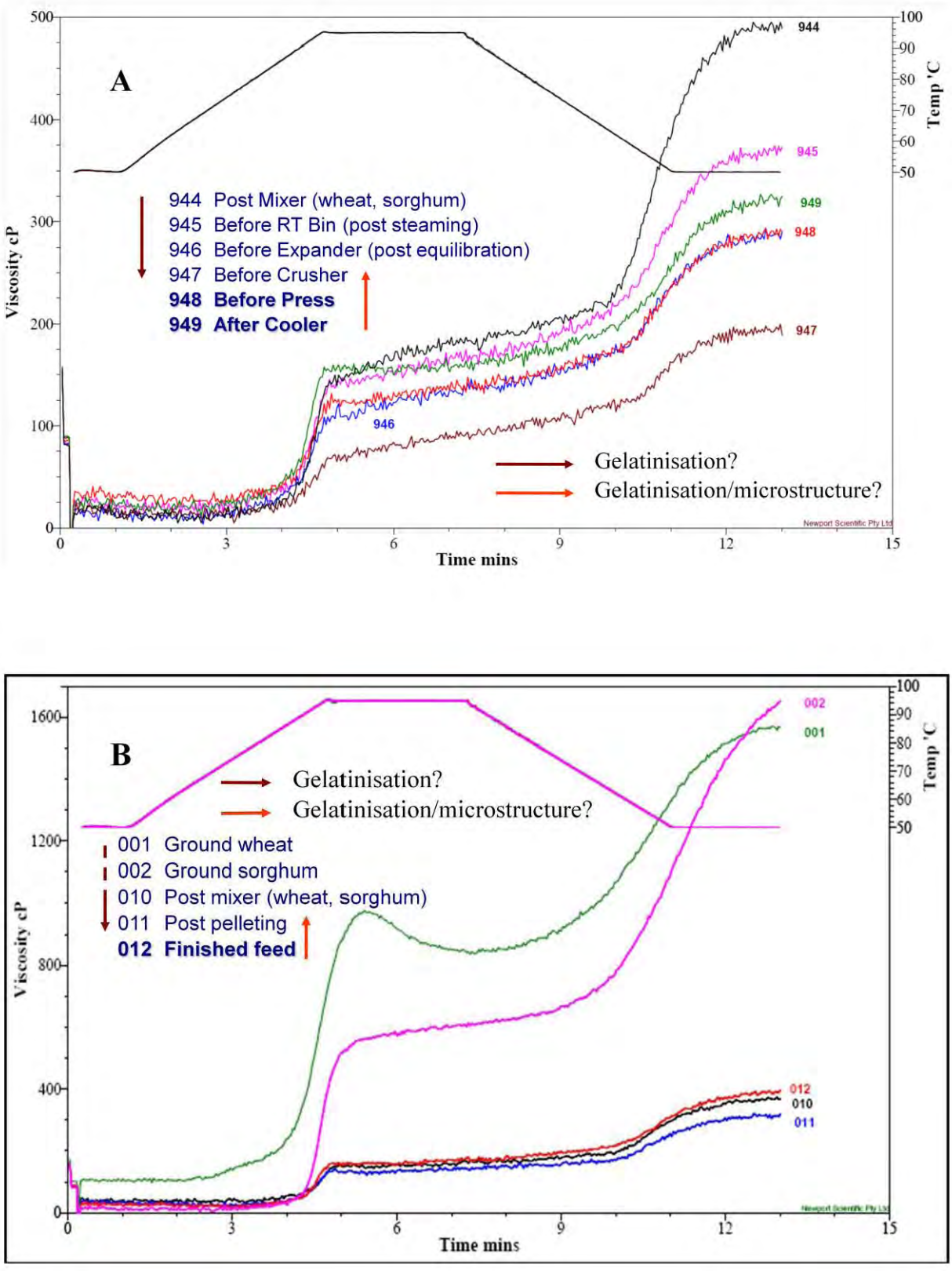


Figure 6. Progressive changes in the pasting properties (RVA) of typical samples – A (mill A), B (mill B)

Table 2. Changes in pasting properties along the processing chain as indicated by RVA variables[#]

Sample ID	Code	Material/Step	IV (cP)	PT (^{o}C)	PV (cP)	FV (cP)
Mill A						
ILS000941	941	Wheat	15.0^{a}	65.9^{a}	554.0^{a}	1181.5^{a}
ILS000942	942	Sorghum	25.0^{b}	86.2^{b}	121.5^{b}	318.0^{b}
ILS000944	944	Post Mixer	12.5^{a}	80.1^{c}	185.0^{c}	494.0^{c}
ILS000945	945	Before RT Bin (post steaming)	17.0^{ab}	74.2^{d}	169.5^{cd}	372.5^{d}
ILS000946	946	Before Expander (post equilibration)	18.0^{ab}	76.3^{d}	142.0^{b}	296.5^{b}
ILS000947	947	Before Crusher	36.0^{c}	78.4^{c}	104.0^{b}	208.0^{e}
ILS000948	948	Before Press	29.0^{bc}	75.5^{d}	145.0^{bd}	303.5^{b}
ILS000949	949	After Cooler	23.0^{ab}	75.2^{d}	172.0^{cd}	333.0^{b}
Mill B						
RID001	001	Wheat	109.5^{a}	66.0^{a}	937.0^{a}	1537.5^{a}
RID002	002	Sorghum	25.5^{b}	77.8^{b}	616.5^{b}	1628.0^{b}
RID010	010	Post Mixer	23.0^{b}	76.4^{b}	155.0^{c}	356.5^{c}
RID011	011	Post pelleting	40.5^{c}	75.4^{b}	178.5^{c}	377.5^{c}
RID012	012	Finished Feed	24.5^{b}	75.0^{b}	170.0^{c}	392.5^{c}

[#]In a column per mill, values with the same letters are not significantly ($p>0.05$) different; IV = initial viscosity, PT = pasting temperature, PV = peak viscosity, and FV = final viscosity

B.3 Conclusions

The survey of grains and diets taken from different places in the feed processing units confirmed that there are major effects of grain mixtures and mill processing techniques on pasting properties (RVA viscosity) and *in vitro* starch digestion. The *in vitro* assays provide a reflection of the likely differences in the nutritional quality of processed feeds. The survey showed that processed pellets of feeds with similar ingredient mixtures, for example sow feeds, can have widely different RVA and digestion characteristics when prepared by different mills. In addition, the survey suggested that the mixtures of grains when incorporated into a feed do not behave as would be expected by the sum of the *in vitro* characteristics of the individual grains.

Viscosity, as measured by the RVA, provides an assessment of the amount of starch in a feed that can be gelatinised. Potential gelatinisation increases as starch within the grain is made more accessible, for example, by fine grinding. However, viscosity will be reduced when starch has already been gelatinised prior to the RVA assay following, for example, wet heat treatment during processing. On the contrary, *in vitro* starch digestion assays indicate the accessibility of starch to amylase enzymes. Accessibility of starch would be expected to increase with fine grinding and also following gelatinisation, provided the starch has not retrograded. The rate of *in vitro* starch digestion has been shown in the Pork CRC project 1B-102 to be related to the proportion of dietary starch digested at the ileum.

Although the survey of diets showed that there were differences in the nutritional quality of pig diets depending on the ingredient mixtures used and mill processing techniques, reasons for the differences could not be determined. Consequently, an experiment was designed to examine the effects of mill (three mills), grain mixture, grain grind size, temperature of conditioning, and addition of surfactants on the efficiency of feed processing, pellet quality and nutritional quality of the finished product.

C. Variation to Processing Conditions in Commercial Mills

C.1 Background

Particle size, grain type and moisture content and the nature of the other materials in the ration mixture will influence how quickly a given ration mixture will take up moisture either as steam or free water and so each have an influence on the nature of the material presented to the press for processing.

Thomas *et al*. (1997) categorised factors affecting feed quality according to the properties of:

- Raw materials, their chemical and physico-chemical characteristics, bulk density and particle size distribution
- Process variables including conditioning temperatures and pressure, press settings and cooling system
- System variables such as throughput and energy consumption
- Functional characteristics including starch hydration and gelatinisation, protein denaturation and fibre solubility and
- Optimisation objectives – nutritional quality, pellet durability and hardness and feed hygiene (bacteria control).

This experiment examines the effects of each of these properties of feed manufacture in relation to the particular challenges of producing quality pellets of high nutritional value from mixtures containing appreciable amounts of sorghum. Utilisation of sorghum in diets for pigs is particularly problematic because it is a small hard grain and not readily milled. Sorghum contains a high proportion of kafirin proteins that are poorly solubilised compared to other feed grains. The resulting low rates of starch hydration and solubilisation are thought to be major reasons for the lower feed conversion often observed in pigs fed sorghum-based diets. Anecdotal reports that extended conditioning and increased moisture addition into the ration mixture before processing can enhance the quality of sorghum-based rations was a further stimulus for the research. Industry experience and results from the survey suggest that different mills will process the same ration formulations (specifications and ingredients) differently, which leads to different nutritional outcomes and different rates of animal performance.

The three feed processing mills chosen for this study were Riverina, formerly Better Blend (BBS), Oakey Queensland; Rivalea, Corowa, NSW (QAF) and Ridley AgriProducts, Mooroopna, Victoria (RAP). These mills were chosen based on their willingness to participate in the study and because they have different processing techniques. Sorghum was chosen as a cereal of interest. Although the price of sorghum is frequently lower than for other cereal grains, it is not incorporated into pig diets at high proportions. Sorghum has a higher gelatinisation temperature (Table 2) than the other grains. Frequently the rate of throughput of manufactured feed and quality of the pellets are inferior when diets contain high proportions of sorghum compared with other cereal grains. A major objective of this experiment was to determine the effects of high sorghum inclusion rates in diets and how processing efficiency and pellet quality could be altered by specific changes in processing conditions.

C.2 Methods

C.2.1 Treatments

A pig grower feed formulated to contain similar raw materials and provide 13.8 MJDE/kg and 0.68 g available lysine per MJ DE was used at each mill. The ingredients used for the diets prepared at BBS are shown in Table 3.

Table 3. Ingredient inclusions (%) for rations manufactured under protocol BBS

Mill Nomenclature **Experimental diets**	**169464191** **PORK** **CRC MFP** **1 - 4 - 7**	**169464291** **PORK** **CRC MFP** **2 - 5 - 8**	**169464391** **PORK** **CRC MFP** **3 - 6- 9**	**169464491** **PORK** **CRC MFP** **10**	**169464591** **PORK** **CRC MFP** **11**	**169464691** **PORK** **CRC MFP** **12**
PHYZYME LITE	0.300	0.300	0.300	0.300	0.300	0.300
BARLEY FEED	35.0	20.0	3.80	21.0	21.0	21.0
SORGHUM RED FEED	0	40.9	60.0	40.0	40.0	40.0
WHEAT FEED	44.6	20.0	14.0	20.0	20.0	20.0
CANOLA MEAL 38%	2.00	4.10	5.90	4.30	4.30	4.30
SOYBEAN MEAL 47.5%	5.30	4.00	4.00	4.00	4.00	4.00
BLOOD MEAL 85%	0.500	0.909	1.58	1.52	1.52	1.52
MEAT MEAL 50%	4.60	4.10	3.00	3.00	3.00	3.00
TALLOW	1.70	0.500	0.500	0.500	0.500	0.500
MOLASSES	2.00	2.00	2.00	2.00	2.00	2.00
SURFACTANT LIQUID	2.00	2.00	2.00	0	0	0
BIOFOS 25KG	0	0	0	0.597	0.597	0.597
COPPER SULPHATE	0.055	0.055	0.055	0.054	0.054	0.054
DICALCIUM PHOSPHATE	0	0	0.827	1.20	1.20	1.20
LIMESTONE	1.20	0.376	1.20	0.748	0.748	0.748
SALT	0.250	0.234	0.250	0.250	0.250	0.250
LYSINE	0.274	0.330	0.322	0.302	0.302	0.302
METHIONINE LIQUID	0.062	0.082	0.076	0.076	0.076	0.076
THREONINE 98%	0.048	0.056	0.046	0.046	0.046	0.046
BETTER PIG PREMIX	0.100	0.100	0.100	0.100	0.100	0.100
TOTAL	100	100	100	100	100	100

Twelve treatments were made at the Better Blend mill, seven were made at the Rivalea mill and six at the Ridley AgriProducts mill. There were two treatments common to each mill. The treatments were composed of sorghum inclusion level, grind size, mill temperature, surfactant, Avizyme® and phytase. The treatments adopted at each mill are summarized in Tables 4. Samples were collected from several positions along the production line (Figure 1). Results are presented in this report for hammered grains and feed mixtures collected before (mash) and after (pellet) processing.

A separate randomisation was used at each mill providing an incomplete block design with at least two replicates. The data from each mill were analysed separately using a linear mixed model (GLM procedure, Minitab v11) with fixed effects for sample site, treatment and their interaction, and with random effects for replicate and day within replicate. Only main effects are reported.

Table 4. Manufacturing component (BBS, QAF, RAP) - Mill processing conditions

BBS DIETS	1	4	7	2	5	8	3	6	9	10	11	12
Sorghum inclusion (%)	0	0	0	40	40	40	60	60	60	40	40	40
Grind size (mm)	3	3	3	3	3	3	3	3	3	3	3	3
Surfactant	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N
Avizyme	N	N	N	N	N	N	N	N	N	N	Y	N
Steam temperature (^{o}C)	70	85	105	70	85	105	70	85	105	85	85	85
Steam pressure (kPa)	100	100	100	100	100	100	100	100	100	100	100	100
Batch size (Tonnes)	3	3	3	3	3	3	3	3	3	3	3	3
Throughput (tonnes per hour)	8	8	8	8	8	8	8	8	8	8	8	8
Expander temperature (^{o}C)	105	105	105	105	105	105	105	105	105	105	105	105

QAF DIETS	1	4	2	5	7	3	6
Sorghum inclusion (%)	0	0	40	40	40	60	60
Grind size (mm)	2	3	3	2	3	3	2
Avizyme	N	N	N	N	Y	N	N
Steam temperature (^{o}C)	85	85	85	85	85	85	85
Steam pressure (kPa)	68	68	68	68	68	68	68
Batch size (Tonnes)	3	3	3	3	3	3	3
Throughput (tonnes per hour)	10	10	10	10	10	10	10

RAP DIETS	1	2	3	4	5	6
Sorghum inclusion (%)	0	0	40	40	60	60
Grind size (mm)	3	3	3	3	3	3
Surfactant	N	Y	N	Y	N	Y
Steam temperature (^{o}C)	85	85	85	85	85	85
Steam pressure (kPa)	55.1	55.21	55.8	56.0	55.1	56.3
Batch size (Tonnes)	6	6	6	6	6	6
Throughput (tonnes per hour)	12	12	12	12	12	12

C.2.2 Laboratory procedures

Pellet samples obtained after processing were assessed for durability using a Holmen pellet tester (TekPro Limited Willow Park North Walsham Norfolk, UK). Laboratory analyses were undertaken using a NATA accredited laboratory (Symbio Alliance, Eight Mile Plains QLD 4113) using standard analytical procedures. Starch digestion was determined *in-vitro* by the method of Sopade and Gidley (2009). Water activity (a_w) was determined on a PAw kit analyser (Decagon Devices Inc., Pullman WA 99163, USA). Particle size distribution of both mash (post mixer) and pellet (post cooler) samples was obtained after the method described in ASABE (2008) following separation on a mechanical shaker. The percentage of fines was determined by passing a representative sample through a 2 mm sieve and calculating the percentage of the total sample that passed through the sieve. Pellet hardness was determined in duplicate on 10 pellets per sample using an Amandus Kahl Hardness tester. Pellet length was measured in duplicate on 10 pellets per sample using a micrometer. Bulk density was determined by weighing material in container of known volume.

C.2.3 Mill characteristics

Throughput at the pellet press was obtained from the respective mill batching systems. The capacities of individual mills are summarised in Table 5 and variables used to calculate retention times at the mixer-conditioner are given in Table 6. Specific Mechanical Energy used at the press (SMEp) was calculated (Eqn. 3) as the ratio of the measured press amp usage to maximum amp rating multiplied by the motor size (kW) divided by actual throughput (tonnes/hour):

$$\text{SMEp (kWh/tonne)} = \frac{Am_M \text{ X } P_R}{Am_{MAX} \text{ X } G} \qquad \textit{Eqn. 3}$$

where Am_M = measured press ampere usage (Amp), Am_{MAX} = maximum ampere rating of the press (Amp), P_R = maximum power rating of the motor (kW), and G = throughput (tonnes/hour)

C.2.4 RVA and in vitro *assays*

The RVA and *in vitro* starch digestion assays described above (B.1) were also performed on all diets made for the experiment.

C.3 Results

C.3.1 Mill and physical pellet characteristics

C.3.1.1 Effects of sorghum inclusion

Responses for some of the more important determinants of mill efficiency are shown in Table 7. Pellet durability and free water content, as determined by water activity (a_w), are critical measurements for pellet quality. Increasing the amount of sorghum in the diet showed markedly different effects between the mills. Pellet quality remained high as sorghum content was increased at the BBS mill, but not at either the QAF or RAP mills, where all diets containing sorghum had unsatisfactory pellet quality. A pellet is considered unsatisfactory or low in quality if durability is less than 90% and the hardness value is less than 7 kg. Although pellet durability was high for all sorghum treatments at BBS, water activity was higher than 0.65, where 0.7 is considered a critical value for microbial activity and may eventually lead to the formation of mould.

Mill throughput was substantially less at the BBS mill than at the other mills. Throughput was largely controlled at the mills, which were operated at different rates. Retention time permitted at the mixer conditioner is one of the key determinants of how much heat and moisture (conditioning) is introduced into the feed mixture. Retention time is generally reduced as throughput increases, but this effect can be offset by increasing the size of the mixer conditioner. Throughput is also affected by the bulk density of the feed mixture undergoing manufacture. The results suggest that bulk density and water activity of the mash may have contributed to variation between the mills. Although the amps used at the press were least for the BBS mill, the lower throughput resulted in the mill having the lowest overall efficiency as expressed by Specific Mechanical Energy.

Table 5. Feed manufacturing equipment and capacities

Components		BBS	QAF	RAP
BATCHING SYSTEM		Probatch Semi-automatic	Automatic	Automatic
MIXER	Type	Paddle shaft Horizontal		Paddle shaft Horizontal
	Capacity (tonnes)	3		3
	Amp Max	55		
	Amp	29		20
	kW	30		37
MIXER CONDITIONER	Diameter, ID	610		450
	Length m	3.28		2.3
	Amp Max	80		
	Amp	50		
	kW	45		
RETENTION BIN	Diameter (m)	2	na	na
	Amp Max	10		
	kW	10		
	Amp	8		
	kW	5.5		
EXPANDER	Amandus Kahl OE30		na	na
	Diameter mm	300		
	Length m	2.2		
	Amp Max	330		
	Amp	150		
	kW	250		
	TPH Max	22		
PRESS	Type	Munch	JayBee	Super Ace
	Diameter	520		500
	Holes	4.7		4
	Thickness	50		45
	Amp max	250		205
	kW	150		110
	TPH max	20		20
COOLER	Type	Counterflow		Counterflow
	Capacity (tonnes)	3		3
	Capacity max, TPH	20		15
	Discharge	Gravity		Pneumatic
	kW	55		30

Table 6. Variables used to calculate retention time at the mixer conditioner

Conditioning specifications	Units	BBS	RAP
Conditioner diameter	Metres	0.67	0.45
Conditioner length	Metres	2.800	2.300
Total conditioner volume	Metre³	0.987	0.366
Assumed fill		40%	40%
Working conditioner volume	Metre³	0.395	0.146
Mash bulk density	Tonnes/metre³	0.54	0.76
Stock in conditioner (i.e. 1 "fill")	Tonnes	0.213	0.111
Press throughput	Tonnes per hour	8	11.5
Number of "fills"/hour		37.5	103.4
Retention time	Seconds	96	35

Table 7. Processing conditions and responses to increasing sorghum inclusion at the BBS, QAF and RAP mills

Responses	BBS				QAF				RAP			
	0	40	60	sd	0	40	60	sd	0	40	60	sd
Amp @ press	142	137	142	9.1	206	207	205	3.3	163	159	159	2.1
Bulk density, mash (kg/m^3)	568	561	584	7	603	624	630	6	603	616	630	5
Bulk density, pellet (kg/m^3)	703	712	726	20	689	717	709	17	760	720	800	9
Water activity, mash	0.6	0.62	0.63	0.3	0.47	0.51	0.54	0.2	0.6	0.62	0.62	0.1
Water activity, pellet	0.66	0.68	0.68	0.2	0.52	0.53	0.54	0.1	0.63	0.63	0.66	0.2
Throughput (TPH)	7.7	7.5	7.5	0.4	9.2	9.7	9.3	0.6	12.1	12.1	11.6	0.4
Durability (%)	97	95.8	96.8	2.1	94.5	87.9	75.5	1.9	90	79	73.5	4.1
Retention time (sec)	113	111	113	2.4	94	94	98	4.5	76	76	82	2.7
SMEp[#] (kWh/Tonne)	12.2	11.5	12.1	0.5	10.3	10.2	10.5	0.6	7.9	7.7	8.2	0.3

[#]SMEp = Specific mechanical energy of the press, and it applies to all the tables it appears

C.3.1.2 Effects of conditioning temperature

The effects of conditioning temperature on mill and pellet characteristics were examined at BBS (Table 8). Increasing conditioning temperature significantly reduced the amps used at the press, throughput and SMEp, without affecting pellet quality. A closer evaluation of the effect of conditioning temperature is needed to determine the relative economic advantage of using less energy per tonne of feed mixed compared with the reduced throughput.

Table 8. Effect of conditioner temperature on mill and pellet characteristics, BBS

Characteristics	Temperature (^{o}C)				
	70	**85**	**105**	**sd**	**P**
Amp @ press	165	131	138	8.8	0.01
Moisture Mash %	12.8	12.0	12.8	0.46	NS
Bulk density Mash (kg/m^3)	565	571	578	20	NS
Bulk density Pellet (kg/m^3)	728	696	716	22	NS
Water activity (Mash)	0.59	0.61	0.58	0.03	0.05
Water activity (Pellet)	0.66	0.69	0.69	0.02	NS
Throughput (TPH)	7.7	7.1	7.0	0.27	0.1
Durability (%)	96.3	97.1	97.8	1.5	NS
Retention time (sec)	113	109	115	3.4	NS
SMEp (kWh/Tonne)	13.7	10.5	11.6	0.64	0.01

C.3.1.3 Effects of adding surfactants

Inclusion of surfactant at the BBS mill (Table 9) significantly reduced energy use (P<0.001, 139 v 151 amps) and retention time at the press (P<0.05, 106 v 118 sec) while increasing (P<0.05) a_w (0.63 v 0.53), moisture content (13.1 v 12.0 %) and throughput (7.6 v 7.0 t/h). Pellets produced with surfactant at the BBS mill tended (P=0.052) to be longer (13.7 mm) than those produced without surfactant (12.4 mm). In contrast, including a surfactant at the RAP mill (Table 10) had no statistically significant effect on these measures, despite numerically lower values recorded for durability, retention time and SMEp. Whether this inconsistency in results is a consequence of the different surfactants and application methods used at the two sites or some other factor is unclear.

Table 9. Processing conditions and responses to the inclusion of surfactant (KemWet LR^{TM}), BBS

Responses	No Surfactant	Surfactant	sd	P
Amp @ press	139	151	7.5	0.01
Moisture of the mash %	12.0	13.1	0.6	0.01
Bulk density, mash (kg/m^3)	580	560	0.01	NS
Bulk density, pellet (kg/m^3)	703	721	0.03	NS
Water activity, mash	0.53	0.63	0.02	0.05
Water activity, pellet	0.64	0.72	0.021	0.03
Throughput (TPH)	7.0	7.6	0.11	NS
Durability (%)	97.4	96.6	1.1	NS
Pellet length (mm)	12.4	13.7	0.6	0.05
Pellet hardness (kg)	12.2	13.6	1.2	NS
Geometric particle size, mash (mm)	0.504	0.520	0.027	NS
Geometric standard deviation, mash (mm)	2.18	2.14	0.047	NS
Geometric particle size, pellet (mm)	4.81	4.81	0.05	NS
Geometric standard deviation, pellet (mm)	1.16	1.17	0.05	NS
Retention time (sec)	119	106	3.0	0.02
SMEp (kWh/Tonne)	12.5	11.3	0.9	NS

Table 10. Processing conditions and responses to the inclusion of surfactant (Bredol), RAP

Responses	No Surfactant	Surfactant	sd	P
Amp @ press	156	157	2.2	NS
Bulk density, mash (kg/m^3)	613	619	5	NS
Bulk density, pellet (kg/m^3)	670	662	5	NS
Water activity, mash	0.61	0.61	0.01	NS
Water activity, pellet	0.64	0.65	0.01	NS
Throughput (TPH)	11.2	11.3	0.3	NS
Durability (%)	82.0	76.4	3.4	NS
Retention time (sec)	79	77	2.7	NS
SMEp (kWh/Tonne)	8.0	7.8	0.21	NS

C.3.1.4 Effect of grind size

Grind size showed significant effects on the initial bulk density of the mixture and the ultimate durability of the product (Table 11). The most striking effect of reducing the size of grain particles prior to mixing was on the durability of the pellet, which increased from 83 to 89% as the hammer mill screen size was reduced from 3 to 2 mm. Although not significant, a reduced grind size also tended to increase throughput and reduce the energy used during processing.

Table 11. Processing conditions and grind size responses, QAF

Responses	Grind Size mm			
	2	3	sd	P
Amp @ press	209	220	3.8	NS
Bulk density, mash (kg/m^3)	630	608	6	0.05
Bulk density, pellet (kg/m^3)	714	696	6	NS
Water activity, mash	0.49	0.49	0.03	NS
Water activity, pellet	0.53	0.52	0.06	0.1
Throughput (TPH)	9.5	9.1	0.57	NS
Durability (%)	88.9	83.0	1.9	0.001
Retention time (sec)	95	95	5.5	NS
SMEp (kWh/Tonne)	10.0	10.7	0.51	NS

C.3.1.5 Effects of sorghum inclusion and processing on pellet size and hardness

The particle size of the mixture processed at BBS (Table 12) showed a trend ($P<0.05$) to reduced particle size as sorghum inclusion increased. Pellet length was significantly ($P<0.001$) reduced in mixtures containing sorghum with relatively little effect on pellet hardness. Temperature had no statistically significant effect on either the particle size of the mixture to be processed or the physical characteristics of the pellet.

Table 12. Particle size of feed mixtures before (mash) processing and pellet characteristics in response to increasing sorghum inclusion and temperature, BBS

Characteristics	Sorghum inclusion (%)				Temperature (°C)				
	0	40	60	P	70	85	105	sd	P
D_{gw}, mash (mm)	0.535	0.531	0.484	0.05	0.532	0.510	0.508	0.02	NS
S_{gw}, mash (mm)	2.176	2.157	2.164	0.05	2.164	2.170	2.162	0.034	NS
Pellet length (mm)	15.0	12.0	12.2	0.001	12.6	13.1	13.4	0.45	NS
Pellet hardness (kg)	13.9	12.1	12.7	NS	12.8	12.8	13.0	0.95	NS
D_{gw}, pellet (mm)	4.82	4.84	4.77	NS	4.80	4.79	4.84	0.036	NS
S_{gw}, pellet (mm)	1.17	1.13	1.20	NS	1.17	1.18	1.14	0.038	NS

#D_{gw} = geometric particle size, S_{gw} = geometric standard deviation, and apply to all the tables where they appear

Reducing the screen size of the hammer mill used at QAF (Table 13) produced a numerical reduction in the particle size of the mixture to be processed but the difference was not statistically significant. The overall results from QAF indicate that grind size had relatively little effect on the physical characteristics of the pellet compared to the highly significant reductions in each measure of pellet quality with progressive increases in sorghum inclusion.

Table 13. Particle size of feed mixtures before (mash) processing and pellet characteristics in response to increasing sorghum inclusion and two grind sizes, QAF

Characteristics	Sorghum inclusion (%)				Grind size (mm)			
	0	40	60	P	2	3	sd	P
D_{gw}, mash (mm)	0.509	0.528	0.542	NS	0.513	0.540	0.017	NS
S_{gw}, mash (mm)	1.999	1.999	1.907	NS	1.955	1.981	0.016	NS
Pellet length (mm)	9.8	8.0	6.7	0.001	8.2	8.3	0.25	NS
Pellet hardness (kg)	6.5	5.0	3.9	0.001	5.0	5.3	0.15	NS
D_{gw}, pellet (mm)	4.55	4.13	3.81	0.01	4.17	4.16	0.09	NS
S_{gw}, pellet (mm)	1.38	1.61	1.75	0.01	1.57	1.59	0.05	NS

Increasing sorghum inclusion resulted in a numerical, but not statistically significant, increase in the particle size of the mash mixture at RAP, Table 14. This was broadly similar to the change in particle size with increasing sorghum inclusion observed at QAF and in contrast to that noted at BBS. The particle size of the mash mixture used at RAP (0.761mm) was generally coarser than that used at either BBS (0.512mm) or QAF (0.526mm). Increasing the amount of sorghum in the mixture to be pelleted resulted in highly significant reductions in pellet length and hardness following the general trend observed at the QAF. As noted previously for processing responses (Table 10), the use of surfactant at RAP had little effect on either particle size or pellet characteristics.

Table 14. Particle size of feed mixtures before (mash) processing and pellet characteristics in response to increasing sorghum inclusion and the use of a surfactant (Bredol), RAP

Characteristics	Sorghum inclusion %				Surfactant			
	0	40	60	P	No	Yes	sd	P
D_{gw}, mash (mm)	0.728	0.761	0.794	NS	0.750	0.772	0.036	NS
S_{gw}, mash (mm)	1.818	1.716	1.705	NS	1.734	1.759	0.026	NS
Pellet length (mm)	13.6	9.5	9.3	0.001	11.2	10.6	0.59	NS
Pellet hardness (kg)	9.7	8.8	7.3	0.02	9.0	8.3	0.55	NS
D_{gw}, pellet (mm)	4.867	4.689	4.619	NS	4.642	4.806	0.076	NS
S_{gw}, pellet	1.093	1.319	1.370	NS	1.344	1.178	0.071	NS

C.3.1.6 Effects of processing on particle size distribution

Manipulation of particle size is one of the most important methodologies in stock feed manufacture as it both enhances the uniformity of feed mixtures and increases the surface area of components available for hydration and absorption of sensible heat if undergoing further processing or if fed in mash form, the area available for enzyme digestion. The grains processed at QAF were passed through either a 2 or 3 mm hammer mill screen before being incorporated into diets. Figure 7 shows the retention of grains used in each study on sieves of 2mm, 1mm, 0.5mm, and 0.25mm diameter. The amount of fine particles (<0.25mm) in the grain components ranked around 20-25% for grains obtained from the BBS mill, 15-20% for those from the QAF mill and less than 15% for the RAP mill.

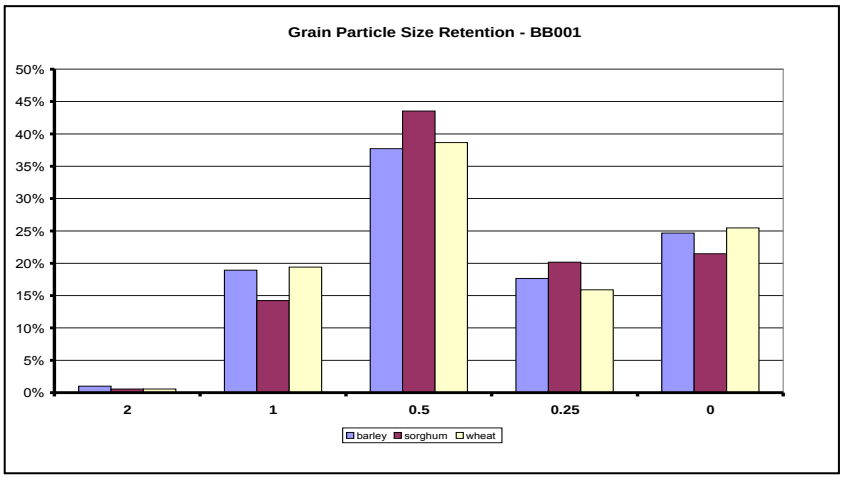
Similar rankings were observed for mash mixtures obtained following the addition of other ration components including protein meals and minerals, Figure 8. The distribution of fine particles (<0.25mm) in the mash mixtures ranged from around 25% for mixtures obtained from the BBS mill to around 15% for those from the QAF mill and approximately 10% for the RAP mill.

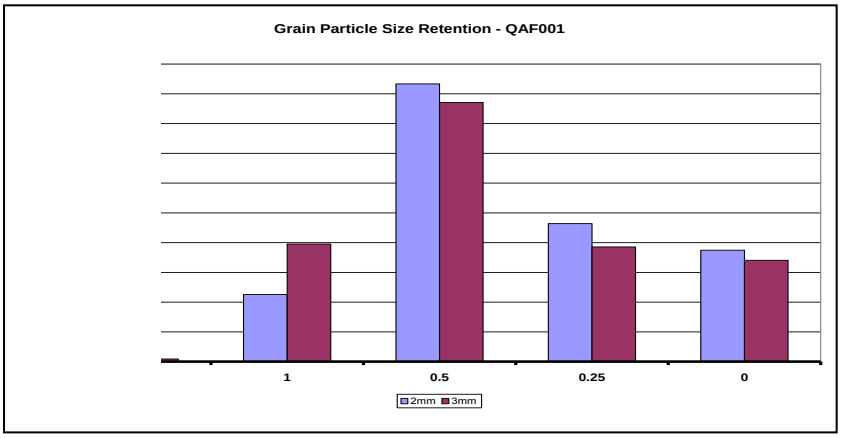
The results suggest that the greater proportion of fine particles in the feed mixtures at the BBS mill may also have contributed to the improvement in pellet durability observed at the BBS mill as the proportion of sorghum in the diet was increased compared with the other two mills.

C.3.2 Nutritional characteristics

C.3.2.1 Effects of processing on nutritional quality

The variables describing the *in vitro* digestion of starch for the diet that were common across the three mills are shown in Figure 9, while the net effect of processing of this diet on the variables is shown for the three mills in Figure 10. The insert in Figure 10 is the net effect of processing on the RVA parameters.





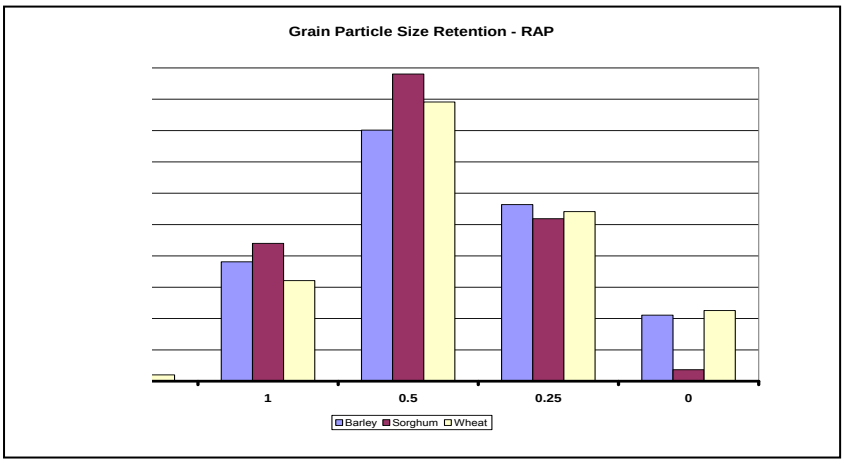


Figure 7. **Particle size distribution of barley, sorghum and wheat grains used in the manufacture component (sieve size 0 = pan)**

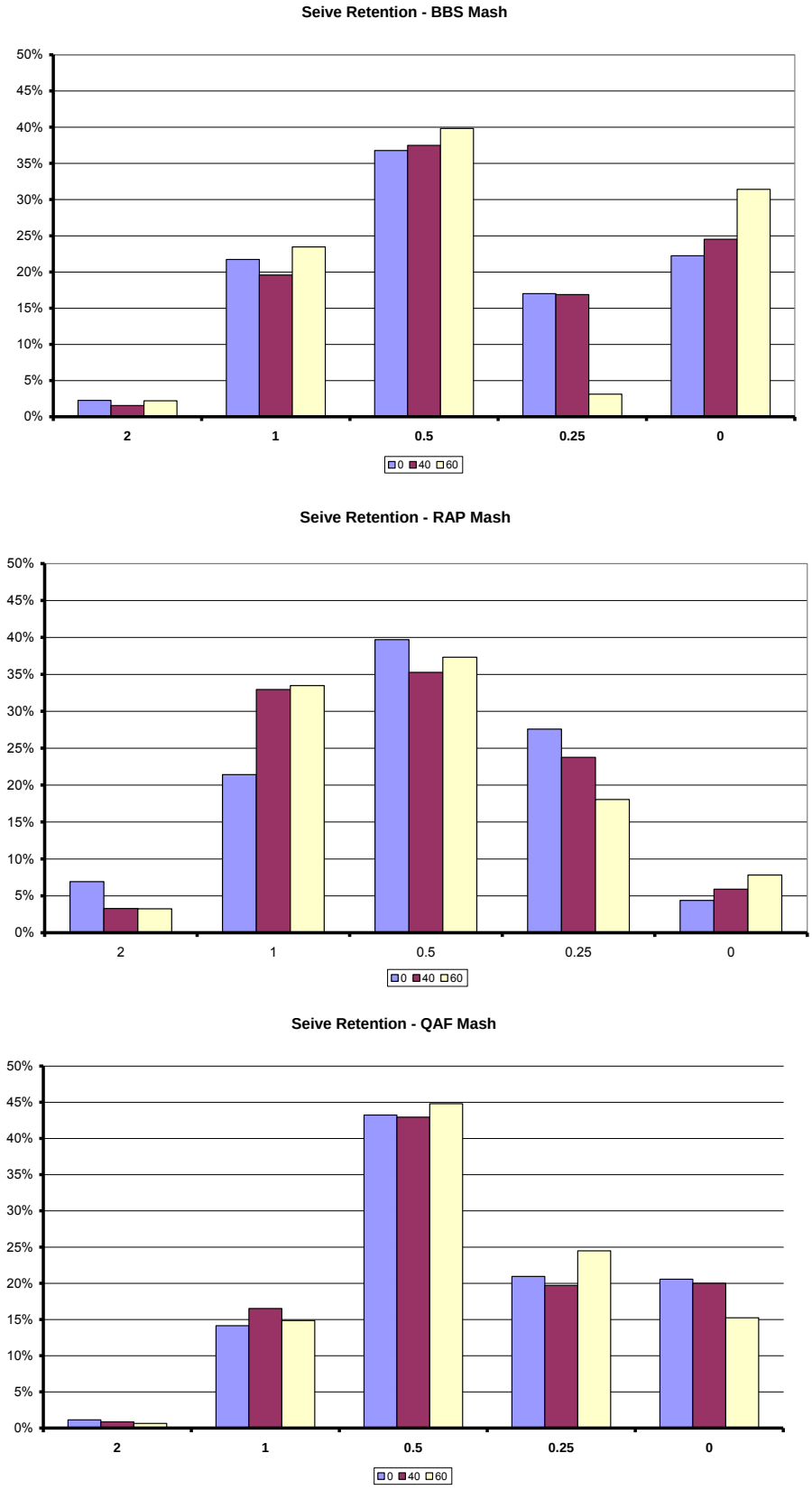


Figure 8. Particle size distribution of ration mixtures containing nil, 40% or 60 % sorghum as used in the manufacture component(sieve size 0 = pan).

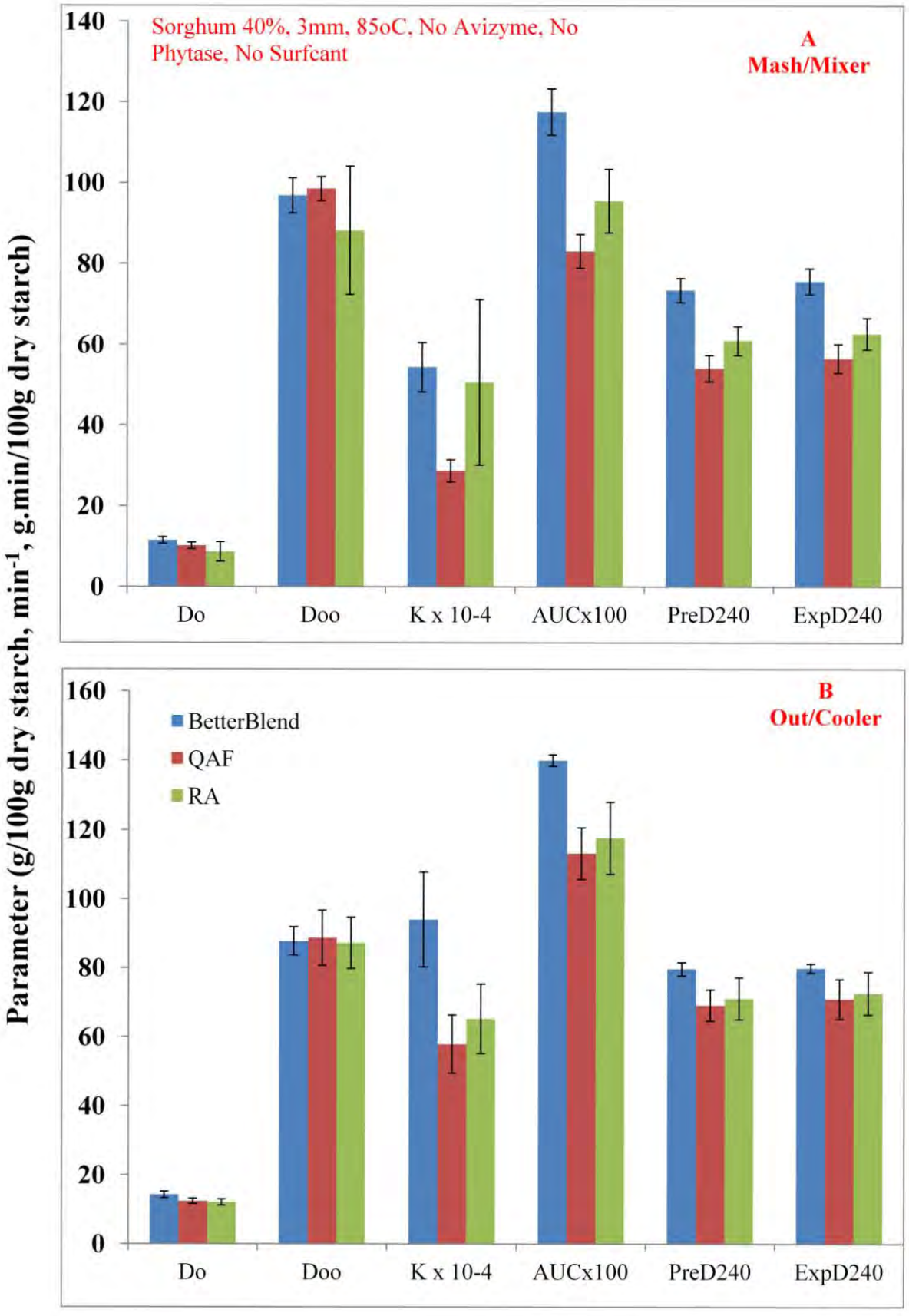


Figure 9. **Starch digestion variables of the mash and pellets from the diet common across the three mills.**

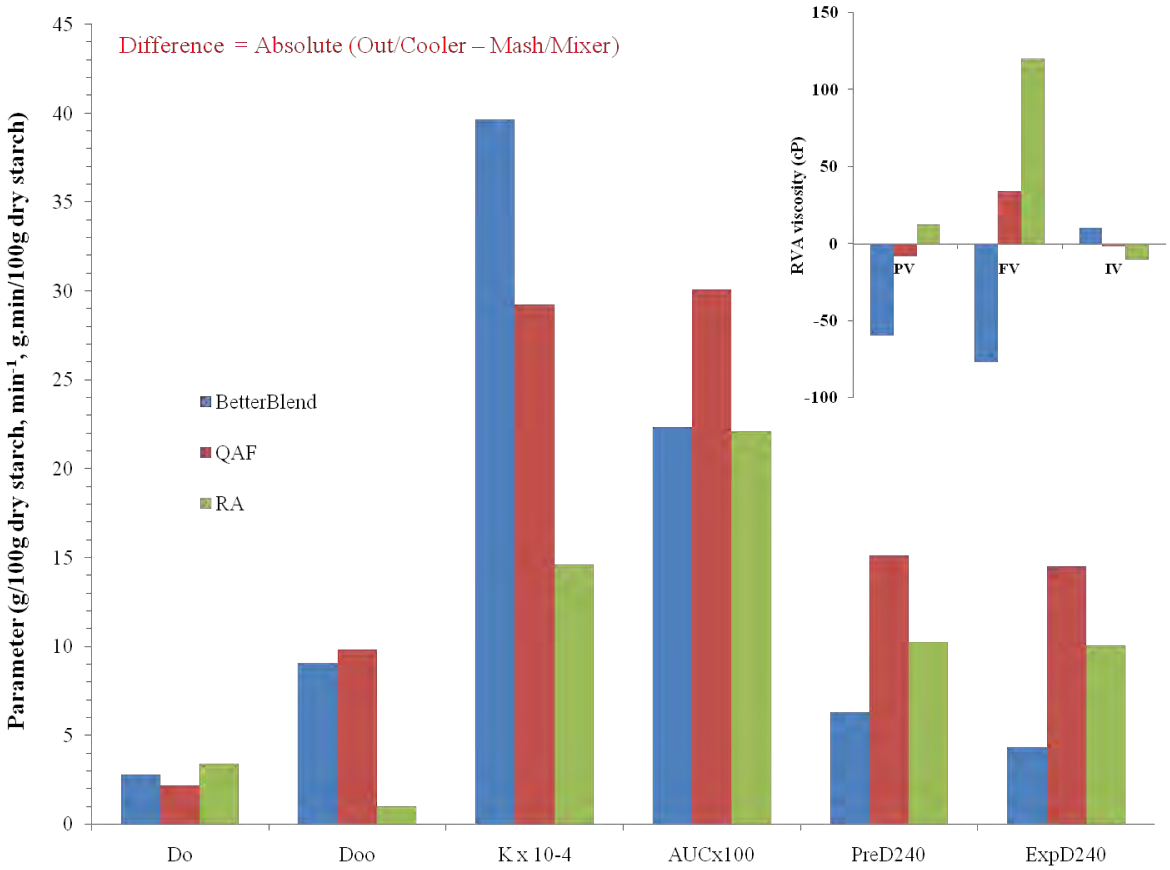


Figure 10. **Difference between mash and pellets in the variables describing starch digestion for the three mills (Insert, RVA parameters: PV = peak viscosity, FV = final viscosity, IV = initial viscosity).**

The results show that there were significant differences between the mills in the rate of digestion of starch in the mash diets before pelleting. The K (rate of digestion constant), D240 (digestion at 240 minutes) and AUC (area under the curve) values were all significantly higher for the diet prepared at BBS than when the same diet was prepared at QAF. The values for RAP were intermediate. These differences, particularly in the K and AUC values were maintained for the finished product. However, final D240 values were not significantly different for the pellets produced at the 3 mills. Processing had the largest net effect on the K value at BBS than the other mills. However, the QAF mill produced the greatest change in AUC and D240 following processing.

Apart from differences in the grains used, the measured differences in the nutritional quality of the mash from the 3 mills can be due to differences in the size distribution. As discussed above (C.3.1.6), BBS had the highest proportion of fines when the mixtures of grains were hammer-milled using a 3 mm-retention sieve. Research in the Pork CRC project 1B-102 has demonstrated the inverse relationship between particle size and digestibility with and without size fractionation. With the mash and pellets from QAF, where sieves 3 mm and 2 mm were used, the nutritional quality (including pasting properties) of the 2 mm sieve was nominally better (not shown). Generally, the finer the particles, the better are the nutritional properties because, as discussed above, the higher is the surface area for heat and mass transfer during processing. However, it should be stressed the optimum particle size depends on process economics, minimisation of fines and reducing deleterious effects on animal gut (erosion of gut wall) and digesta flow properties (viscosity-forming components).

Figure 9B shows that the pellets produced at BBS tended to have higher rates of *in vitro* starch digestion than the other mills. A higher rate of *in vitro* starch digestion at BBS compared with the other mills was also seen in the mash prior to processing (Figure 9A). Processing tended to cause less of a difference between the mash and pellets at BBS than the other mills in the starch digested *in vitro* at 240 minutes, area under the curve and peak RVA (Figure 10). These observations suggest that difference in treatment of the grains and other ingredients prior to mixing may have been the major cause of these differences in starch digestion rates. These differences in the nature of ingredients in the mash may also be responsible for the BBS mill producing the most durable pellets compared with the other mills. It has been stressed above (C.3.1.1) that the high SME in the BBS diets could indicate low RVA parameters and high starch digestibility. As can be seen in the RVA insert in Figure 10, BBS gave the highest drop in the peak and final viscosity, and increase in the initial viscosity. These trends indicate greater disruption to the starch structure, increased starch damage and greater gelatinisation of starch through the BBS mill, possibly as a consequence of the high SME.

C.3.2.2 Effects of processing on pellet composition

Tables 15, 16 and 17 show the effect of processing on the chemical composition of the diets manufactured at each of the 3 mills. There were generally only small effects of processing on the composition of the feeds. The moisture content of all mixtures increased following pelleting because of the addition of steam. The fat content of pellets made at BBS and RAP also increased because both mills added fat to the pellets post-processing. The greatest effect of processing was to increase the proportion of starch that was damaged. This is a measure of breakdown of starch granules and their potential susceptibility to amylase digestion. Damaged starch can also reflect the extent of gelatinisation in the diet.

Table 15. Processing effects on the chemical composition of a pig grower feed manufactured at BBS

Compositions	Before processing (Mash)			After processing (Pellet)		
	Sorghum inclusion (%)			Sorghum inclusion (%)		
	0	40	60	0	40	60
Protein (%)[#]	17.4	17.6	18.0	17.9	17.9	17.4
Fat (%)[#]	2.6	2.6	2.6	3.6	3.6	3.2
Moisture (%)	10.8	10.8	10.7	11.6	11.6	11.4
Ash (%)[#]	5.4	4.7	4.9	5.3	5.3	4.9
Crude fibre (%)	3.5	3.2	2.7	3.0	3.0	2.7
Nitrogen free extract (%)[#]	67.6	68.4	68.4	66.2	66.2	68.2
Starch (%)[#]	50.4	53.5	52.9	45.4	45.4	56.5
Phytate (%)[#]	1.2	1.2	1.1	1.1	1.1	1.1
Phosphorus (mg/kg)[#]	6787	6666	6692	6432	6432	6444
Damaged starch (g/100g starch	6.9	7.1	8.5	12.3	12.3	14.8

[#]Differences measured before and after processing could be due to the methods of analysis

Table 16. Processing effects on the chemical composition of a pig grower feed manufactured at QAF

Compositions	Before processing (Mash)			After processing (Pellet)		
	Sorghum inclusion (%)			Sorghum inclusion (%)		
	0	40	60	0	40	60
Protein (%)[#]	20.3	18.2	17.5	19.4	18.0	17.0
Fat (%)[#]	3.5	3.8	4.0	3.7	3.7	3.9
Moisture (%)	8.0	8.6	9.0	7.9	8.3	8.0
Ash (%)[#]	4.9	5.0	4.9	5.1	5.0	5.0
Crude fibre (%)	3.3	3.1	3.0	3.1	2.8	2.9
Nitrogen free extract (%)[#]	65.1	67.1	67.8	66.3	67.9	68.7
Starch (%)[#]	49.1	52.5	54.4	47.7	52.7	52.5
Phytate (%)[#]	1.2	1.3	1.3	1.3	1.4	1.4
Phosphorus (mg/kg)[#]	5455	5592	5653	5468	5850	5918
Damaged starch (g/100g starch	4.3	5.4	5.6	6.9	8.1	8.8

[#]Differences measured before and after processing could be due to the methods of analysis

Table 17. Processing effects on chemical composition of a pig grower feed manufactured at RAP

Compositions	Before processing (Mash)			After processing (Pellet)		
	Sorghum inclusion (%)			Sorghum inclusion (%)		
	0	40	60	0	40	60
Protein (%)[#]	17.7	16.2	16.3	17.8	16.4	16.1
Fat (%)[#]	3.2	3.4	3.5	4.1	4.4	3.9
Moisture (%)	10.4	10.2	10.2	10.3	10.2	10.4
Ash (%)[#]	4.6	6.1	8.3	4.7	6.1	7.4
Crude fibre (%)	2.7	2.4	2.9	4.0	3.0	2.1
Nitrogen free extract (%)[#]	68.5	68.8	65.6	65.9	66.7	67.1
Starch (%)[#]	42.0	44.4	44.2	41.8	43.7	44.8
Damaged starch (g/100g starch	2.1	3.5	3.7	3.3	4.5	4.9

[#]Differences measured before and after processing could be due to the methods of analysis

C.3.3 Grain composition and physical characteristics

Table 18 shows the chemical and particle size characteristics of the grains used at the three mills. As reported by van Barneveld (2000), there was a range in grain component concentrations both within and between mills. While each grain ranked in a similar order for individual components e.g. protein content higher in wheat than in barley or sorghum and fat higher in sorghum than in wheat or barley grain, there were sometimes marked differences between the grains used at each mill. Both starch and damaged starch content were high in sorghum that in either of the other grains. However, both measures varied substantially between sites. For example, sorghum starch content was 64% at BBS and 76% at QAF and 61% at RAP. Damaged starch in the same samples was 65, 58 and 79 g/kg starch, respectively.

Table 18. Chemical and physical characteristics of grains used in the manufacture of a pig grower feed at the mills

Characteristics	BBS				QAF				RAP			
	Barley	**Sorghum**	**Wheat**	**sd**	**Barley**	**Sorghum**	**Wheat**	**sd**	**Barley**	**Sorghum**	**Wheat**	**sd**
D_{gw} (mm)	0.514	0.511	0.511	0.017	0.508	0.565	0.529	0.028	0.592	0.656	0.540	0.016
S_{gw}	2.122	1.979	2.118	0.022	2.080	1.718	1.969	0.028	1.935	1.777	1.983	0.076
Protein (%)	12.1	10.8	15.4	0.73	14.4	9.9	13.6	0.58	13.5	9.3	14.1	0.99
Fat (%)	1.8	3.1	1.6	0.11	2.6	3.2	1.9	0.42	2.2	2.9	1.5	0.14
Moisture (%)	9.5	11.4	10.3	0.10	7.7	9.2	7.9	0.13	10.3	10.6	10.2	0.25
Ash (%)	2.3	1.5	2.0	0.19	1.7	1.4	1.8	0.15	1.9	1.3	1.8	0.06
Crude fibre (%)	4.7	2.1	2.4	0.16	2.6	1.6	2.9	0.27	4.2	1.4	2.5	0.57
Nitrogen free extract (%)	76.9	80.4	76.2	0.90	80.0	82.3	80.2	1.40	75.7	83.3	77.9	1.45
Starch (%)	52.4	64.1	55.1	1.45	64.9	76.5	61.6	1.82	47.7	61.6	52.3	1.88
Damaged starch (g/kg starch)	49	65	59	3.5	41	58	37	3.4	41	79	50	2.6
Phytate (%)	1.05	1.11	1.10	0.061	1.07	1.15	0.85	0.065	-	-	-	-
Phosphorus (mg/kg)	3457	2940	3353	129	2720	2698	2597	107	-	-	-	-
DEc[#] MJ/kg	16.4	16.8	17.4	0.41	17.6	17.7	17.3	0.2	17.0	17.7	17.4	0.20

[#]DEc = calculated digestible energy content after Noblet & Perez (1993), and applies to all the tables it appears

C.4 Conclusions

Depending on the type of process, pig feeds can vary in properties, which affect energy delivery from them. Maximising energy delivery from pig feeds demands a careful analysis of the process conditions and formulations, and how they impact on pellet properties and mill efficiency. Analysis of the mash and pellets from the three mills revealed differences in size distribution and how grains are milled, as well as the proportion of sorghum in the formulation. Although hammer mills were used by the mills, the size of the sieve is important to minimise large particles, and produce an optimum amount of fines thereby enhancing heat and mass transfer for destructurisation of starch and other components. Conditioning and pelleting are two processes identified as crucial to heat and mass transfer, and both were found to be different (specific mechanical energy and time) in the three mills. BBS had the longest conditioning time and highest specific mechanical energy, and its pellets, the most durable, exhibited the highest *in vitro* starch digestibility. However, pellet damaged starch was similar in the three mills. Irrespective of the mill, grain particle size, conditioning time and temperature, and proportion of sorghum were identified as important determinants of mill efficiency and pellet characteristics.

D. Processing Conditions and Animal Performance

Results from the survey and three-mill experiment suggest that the nutritional quality of pellets as judged by the rate of starch digestion *in vitro*, degree of gelatinisation and proportion of damaged starch, is affected by grain particle size, conditioning temperature and time, and the proportion of sorghum in the diet. Two experiments were undertaken to examine the effects of these processing characteristics on the performance of young pigs. One experiment examined the effects of altering the proportion of sorghum in the diet, conditioning temperature and conditioning time. The second experiment examined the effect of grain particle size, by collecting large particles on a sieve following hammer milling of the grain and recycling these particles through a mill with a smaller screen. The effects of the diets on growth and feed conversion efficiency were examined at two sites with weaner or grower pigs.

D.1 Effects of processing temperature, time and sorghum inclusion

D.1.1 Background

The study examined the effects of sorghum inclusion, temperature and conditioning time on the processing characteristics of a grower feed formulated to similar specifications as those used in the manufacturing component (C.2.1). The pellets were then fed to grower pigs for 28 days and effects on feed intake growth rate and efficiency of feed conversion (FCR) measured.

D.1.2 Methods

The diets were prepared at the BBS mill. Sorghum was included in the diet at 0 and 60%, temperature at the mixer conditioner was set to 80 ºC or 95 ºC and retention time was varied by bypassing the expander to simulate a conventional steam conditioning method (Conventional) or by utilizing the expander with 11 bar pressure at the cone (Extended). Table 19 indicates the operating conditions used.

Table 19. Processing conditions for experiment

Conditions	Diets							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sorghum Inclusion (%)	0	0	60	60	0	0	60	60
Steam Temperature (ºC)	80	80	95	95	80	80	95	95
Steam Pressure (kPa)	200	200	200	200	200	200	200	200
Batch Size (tonnes)	2	2	2	2	2	2	2	2
Throughput (t/h)	10	10	10	10	10	10	10	10
Expander pressure bar (kPa)	11	0	11	0	11	0	11	0

The RVA and *in vitro* starch digestion assays described above (B.1) were also performed on all diets made for the experiment.

An animal experiment was conducted at Wacol Pig Research Centre (DEEDI) to determine the effects of processing conditions on feed intake, growth rate and the efficiency of feed conversion to weight gain of young pigs growing from approximately 20 kg for 28 days. The design was a 2 x 2 x 2 x 2 non-resolvable incomplete block design. The experiment was

conducted in a series of runs, 10 in total. Each run involved the random allocation of the 8 diets to 16 pigs. There were therefore a total of 160 pigs used in this experiment. Each pig was given a 3-day acclimatisation period, and feed intake and growth rate were measured over the next 28 days. Feed intake and unconsumed feed (spilled feed and left overs) were recorded daily, and pig weights were recorded on days 0, 7 and 28. Pig start weight was measured and was used as a covariate in the statistical model used to analyse the results.

D.1.3 Results

D.1.3.1 Mill and pellet physical characteristics

The effects of sorghum inclusion as a proportion of the grain component of the diet, conditioning temperature and conditioning time on mill and pellet variables are shown in Table 20. The amount of energy expended at the press to process the feed mixtures, as indicated by press amps, was reduced by increasing sorghum inclusion, increased temperature at the mixer-conditioner and increased conditioning time. Water activity increased with increased sorghum inclusion and temperature but not by extended conditioning. Pellet quality characteristics were largely unaffected by either sorghum inclusion or temperature but showed some improvement with extended conditioning. Pellet length and hardness were increased with extended conditioning. None of the differences were large in absolute terms. The overall efficiency of processing, as measured by SME, was significantly improved by the inclusion of sorghum in the diet and by using the extended rather than short time conventional conditioning.

Table 20. Physical characteristics of feed components before (mash) and after processing (pellet)

Characteristics	Sorghum Inclusion		Temperature		Conditioning		sd	P
	0%	60%	80°C	95°C	Conven-tional	Exten-ded		
Bulk density, mash	600	600	610	580	600	590	0.07	NS
Water activity, mash	0.57	0.56	0.54	0.58	0.56	0.56	0.012	NS
D_{gw}, mash (mm)	0.57	0.56	0.56	0.58	0.57	0.57	0.005	NS
Press amps	128	113	127	114	123	118	3.1	NS
Water activity, pellet	0.68	0.70	0.68	0.70	0.69	0.68	0.009	NS
Bulk density, pellet	661	666	656	671	659	658	0.06	NS
Durability (%)	95.9	96.5	96.7	95.6	95.6	96.8	0.55	NS
Length (mm)	14.2	14.1	14.8	13.7	14.1	14.3	0.39	NS
Dgw, pellet (mm)	4.82	4.83	4.82	4.83	4.79	4.85	0.023	NS
Hardness (kg)	6.7	7.2	6.9	6.9	6.5	7.4	0.23	<0.01
Retention time (sec)	84.5	85.1	82.9	86.8	84.5	85.1	0.76	NS
SMEp	7.4	6.9	7.2	7.2	7.6	6.8	0.22	<0.05

D.1.3.2 Laboratory assessment of nutritional quality

The relationships between RVA viscosity and the main effects of conditioning, temperature and sorghum inclusion are shown in Figure 11. RVA viscosity increased as conditioning time, temperature and sorghum inclusion were increased, even if nominally. While an increase in conditioning time or temperature is expected to increase starch gelatinisation and lower

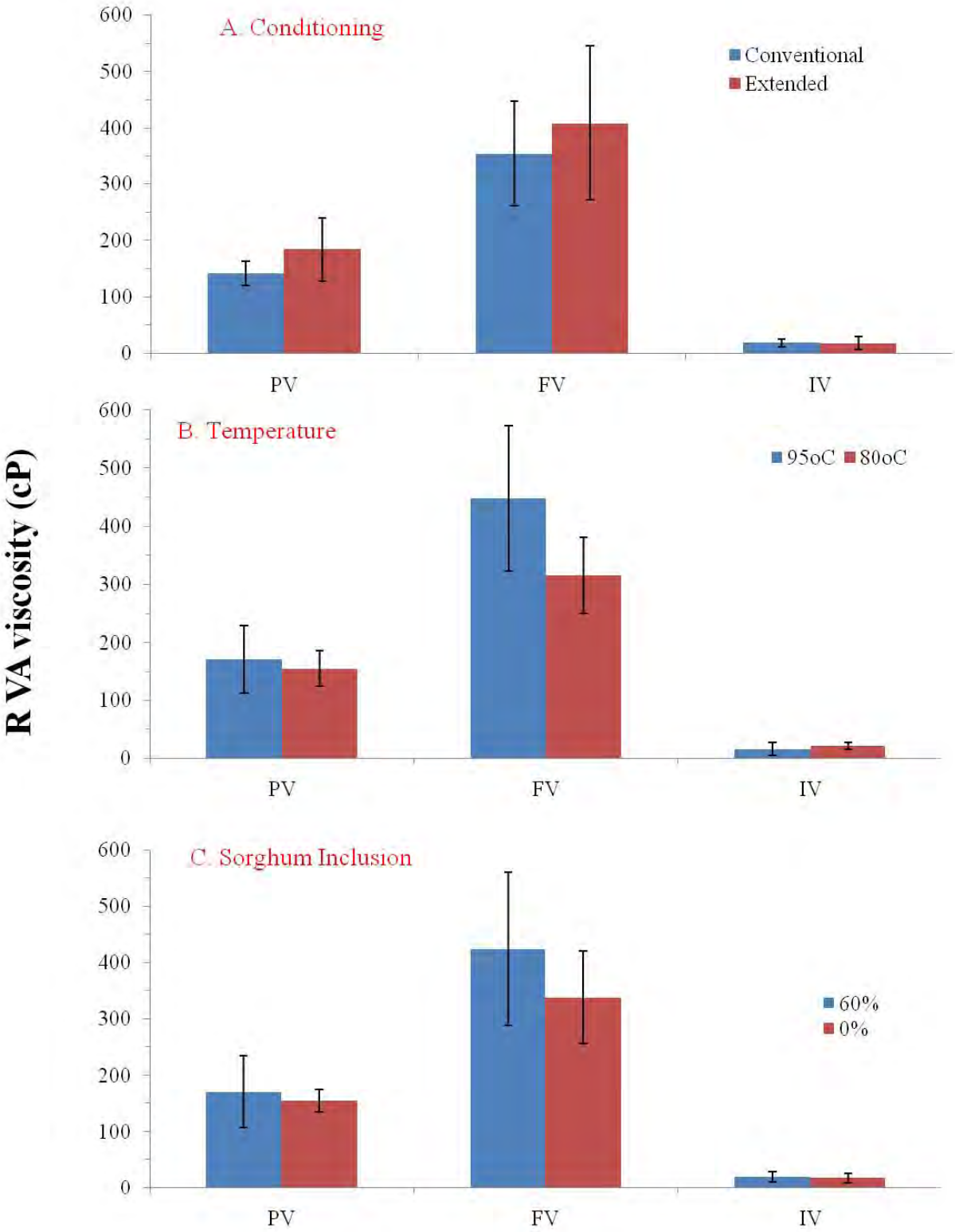


Figure 11. **Effects of process conditions and formulations on RVA parameters (PV = peak viscosity, FV = final viscosity and IV = initial viscosity)**

viscosity under favourable conditions, the measured effects indicate these process conditions and formulations enhanced swelling and hydration of the starch granules. This is expected, in sorghum particularly, if starch-protein interactions had been disrupted.

Generally, irrespective of the process conditions, pellets with no sorghum exhibited shear-thinning RVA behaviours, while shear-thickening behaviours were observed with 60%-sorghum pellets (not shown). Hence, since there was no substantial difference in steam conditioning at 80^{o} or 95^{o}C, but process cost might be lower at 80^{o}C, steam conditioning 60% sorghum at 80^{o}C and processing with expander would improve swelling and hydration of the starch granules with possible enhancement of digestibility.

In vitro starch digestibility varied with the pellets, the process conditions and formulations (Figure 12). The trends displayed with starch digestibility, even if nominally, are identical to the trends with the RVA viscosity with conditioning time and temperature, and sorghum inclusion enhancing *in vitro* starch digestibility. Hence, nutritional quality of pellets can increase if natural impediments (starch-protein interactions) to contacts of substrates with enzymes are destroyed or reduced. The conditions (60% sorghum, steam conditioning at 80^{o}C and expander) stated above could also enhance starch digestibility.

D.1.3.3 Effects of processing on chemical composition

The chemical composition of the mash and pellets are shown, respectively, in Tables 21 and 22. An examination of chemical components after processing (Table 22) shows some reduction in protein and fibre content at the higher processing temperature. While some of the protein sources used in the diet might volatilize, these results are difficult to explain because processing is not expected to change these components. However, the chemistry of the components might change because of the heat-moisture effects of processing, and the analytical techniques might not be sensitive to these changes. Starch damage generally increased with sorghum content, processing temperature and conditioning time, with the pellets having a higher damaged starch than the mash. Apart from the trend with sorghum, this observation is consistent with an increase in heat-moisture-time effects; the more extreme treatments roughly doubled the damaged starch content of the pellets. Since sorghum gelatinises at a higher temperature, some pre-pelleting conditions (not recorded) could have been adjusted upwards to produce acceptable pellets, even though the specific mechanical energy at the press was lower at 60% sorghum than at 0% sorghum (Table 20).

D.1.3.4 Results of animal studies

The results from the pig experiment were analysed over the full 28 days (0-28) and over the last 21 days (7-28). Starting weight was significant ($P < 0.005$) for feed intake (kg/d) and growth rate (kg/d) for both the full period and the last 21 days. Feed conversion ratio (FCR 0-28, feed/gain) was also significantly affected by conditioning temperature ($P = 0.005$). There were significant interactions for FCR between conditioning temperature and conditioning time for both the 0-28 day and 7-28 day periods (Tables 23 and 24).

The experiment showed that extending the time for conditioning at a low temperature before pelleting significantly improved the efficiency of feed use. This is consistent with the laboratory studies on the pasting behaviours and *in vitro* starch digestion (D.1.3.2), where these conditions were found to enhance (absolute values) swelling, hydration and digestion parameters.

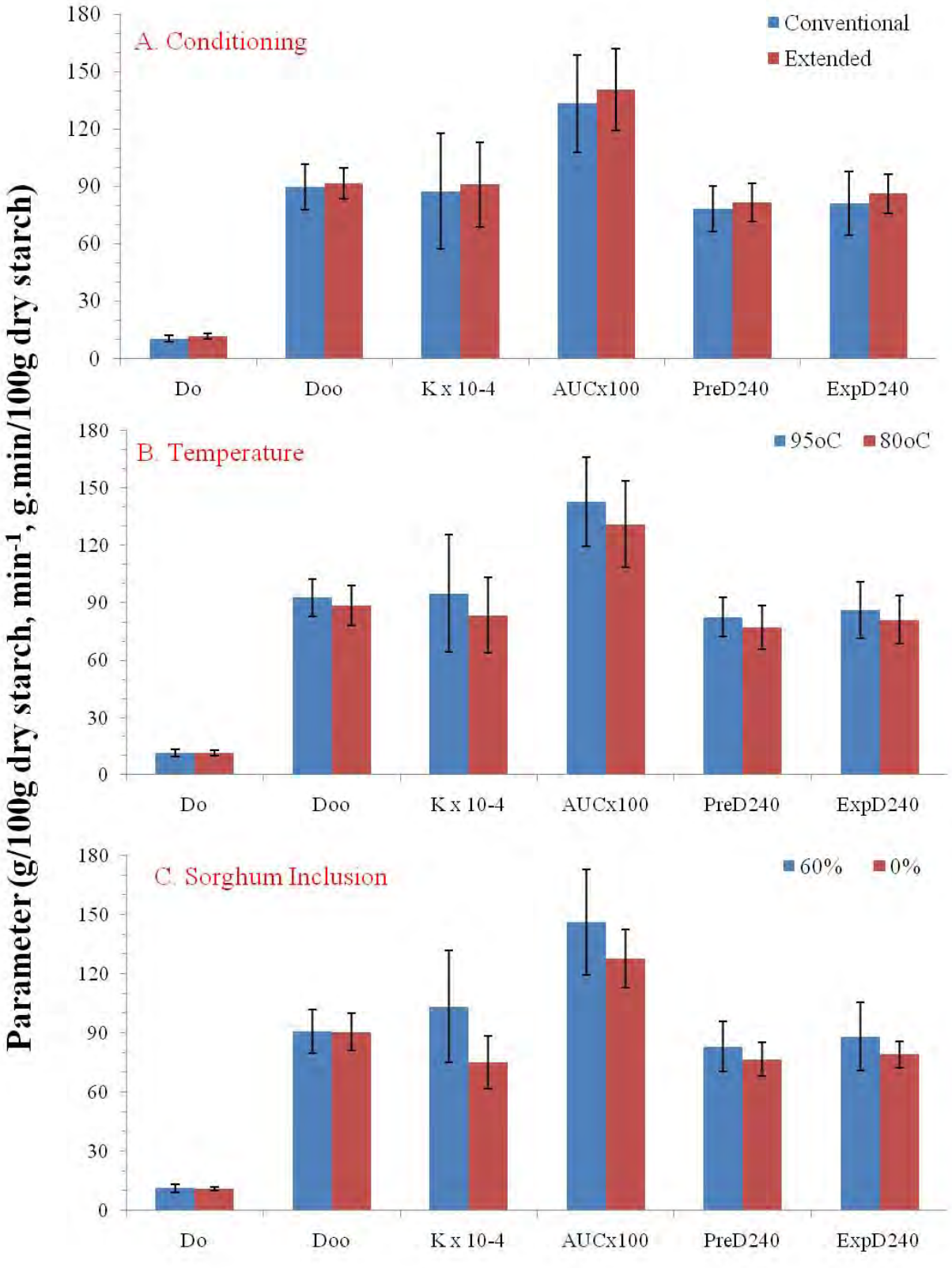


Figure 12. **Effects of process conditions and formulations on *in vitro* starch digestibility parameters**

Table 21. Chemical components of the mash[#]

Components	Sorghum Inclusion		Temperature		Conditioning		sd	P
	0%	60%	80°C	95°C	Conven-tional	Exten-ded		
Protein (%)	19.1	18.7	19.1	18.7	18.7	19.1	0.33	NS
Fat (%)	2.6	2.7	2.5	2.9	2.6	2.6	0.07	0.001
Fibre (%)	2.4	2.4	2.7a	2.2b	2.3	2.5	0.09	0.001
Ash (%)	6.7	6.6	6.7	6.7	6.6	6.7	0.07	NS
Moisture (%)	10.9[a]	10.7[b]	10.4[a]	11.2[b]	10.8	10.9	0.17	0.05
DEc (MJ/kg)	15.1	15.5	15.1	15.5	15.4	15.2	0.15	NS
Starch (%)	50.8	50.2	48.6	52.5	50.9	50.2	0.77	0.007
Damaged starch (g/kg)	52[a]	54.5[b]	42.6[a]	64.0[b]	55.0	51.5	1.56	0.001

[#]For each row per treatment, values with the same letters are not significantly different (P>0.05)

Table 22. Chemical components of the pellets

Components	Sorghum Inclusion		Temperature		Conditioning		sd	P
	0%	60%	80°C	95°C	Conven-tional	Exten-ded		
Protein (%)	18.7	18.9	19.1	18.5	18.7	18.9	0.5	0.014
Fat (%)	3.2	3.1	3.1	3.2	3.1	3.2	0.09	NS
Fibre (%)	2.4	2.3	2.6[a]	2.1[b]	2.3	2.4	0.06	0.001
Ash (%)	6.8	6.6	6.8	6.7	6.6	6.8	0.07	NS
Moisture (%)	12.2	12.0	12.2	12.0	12.0	12.3	0.17	NS
DEc (MJ/kg)	15.1[a]	15.4[b]	15.1	15.3	15.0[a]	15.4[b]	0.09	0.017
Starch (%)	47.7[a]	49.7[b]	46.5[a]	50.9[b]	48.3	49.0	0.37	0.37
Damaged starch (g/kg)	78[a]	104[b]	79[a]	103[b]	74[a]	109[b]	3.67	0.001

[#]For each row per treatment, values with the same letters are not significantly different (P>0.05)

Table 23. Predicted mean values for feed conversion ratio (feed:gain) over 0-28 days for the interaction between conditioning temperature (temp) and conditioning time (conditioning)

Temp	Conditioning	Predicted value	Standard error
High	Conventional	1.71[b]	0.016
High	Extended	1.74[b]	0.016
Low	Conventional	1.72[b]	0.016
Low	Extended	1.66[a]	0.016

[#]Values with the same letters are not significantly different (P>0.05)

Table 24. Predicted mean values for feed conversion ratio (feed:gain) over 7-28 days for the interaction between conditioning temperature time

Temp	Conditioning	Predicted value	Standard error
High	Conventional	1.77[b]	0.022
High	Extended	1.78[b]	0.022
Low	Conventional	1.79[b]	0.022
Low	Extended	1.72[a]	0.022

[#]Values with the same letters are not significantly different (P>0.05)

The poor feed conversion efficiency with high temperature conditioning is surprising because starch gelatinisation is expected to increase with temperature, although this, under favourable conditions, could increase the likelihood of starch retrogradation, which reduces digestion. However, the results from both the pasting and *in vitro* starch digestion did not suggest an increase in starch gelatinisation or pronounced effects of retrogradation at the high temperature conditioning. Based on the laboratory and animal studies, the beneficial effects of extended time of conditioning at low temperature possibly resulted from disruption of detrimental starch-protein interactions enabling easy access of liquid and digestive enzymes (*in vivo* and *in vitro*) to the starch substrate with a concomitant increase in the efficiency of feed use.

There was also a significant interaction between conditioning temperature and sorghum inclusion rate for FCR 7-28 (Table 25) with least favourable FCR and the most favourable FCR both observed at the higher sorghum inclusion.

Table 25. Predicted mean values for feed conversion ratio (feed:gain) over 7-28 days for the interaction between conditioning temperature and sorghum inclusion in the diet

Temp	Inclusion	Predicted value	Standard error
High	High	1.80[c]	0.022
High	Low	1.75[ab]	0.022
Low	High	1.73[ab]	0.022
Low	Low	1.78[bc]	0.022

[#]Values with the same letters are not significantly different (P>0.05)

D.1.3.5 In vitro starch digestion and feed conversion rate

Figure 13 shows the relationship between *in vitro* starch digestion parameters and feed conversion rate over 7-28 days. It can generally be observed that the higher the *in vitro* parameter, an indication of better starch digestibility, the higher is the efficiency of feed use (lower FCR728). The practical significance of this is that it appears animal performance can be predicted from *in vitro* digestion. However, more samples are required to establish this relationship. With the *in vitro* digestion assay as a part of quality control in a feed mill, feed manufacturers can process (conditions and formulations) for a pre-determined feed efficiency. The *in vitro* digestion assay is simple to use, and 10 samples (or 5 in duplicates) can be analysed within 6 hr to yield more than 12 data points. Private communications with a French stock feed manufacturer revealed the *in vitro* assay, fully described in the Pork CRC Project 1B-102, is now a valuable component of its feed control system, and it has been used to satisfactorily predict animal performance.

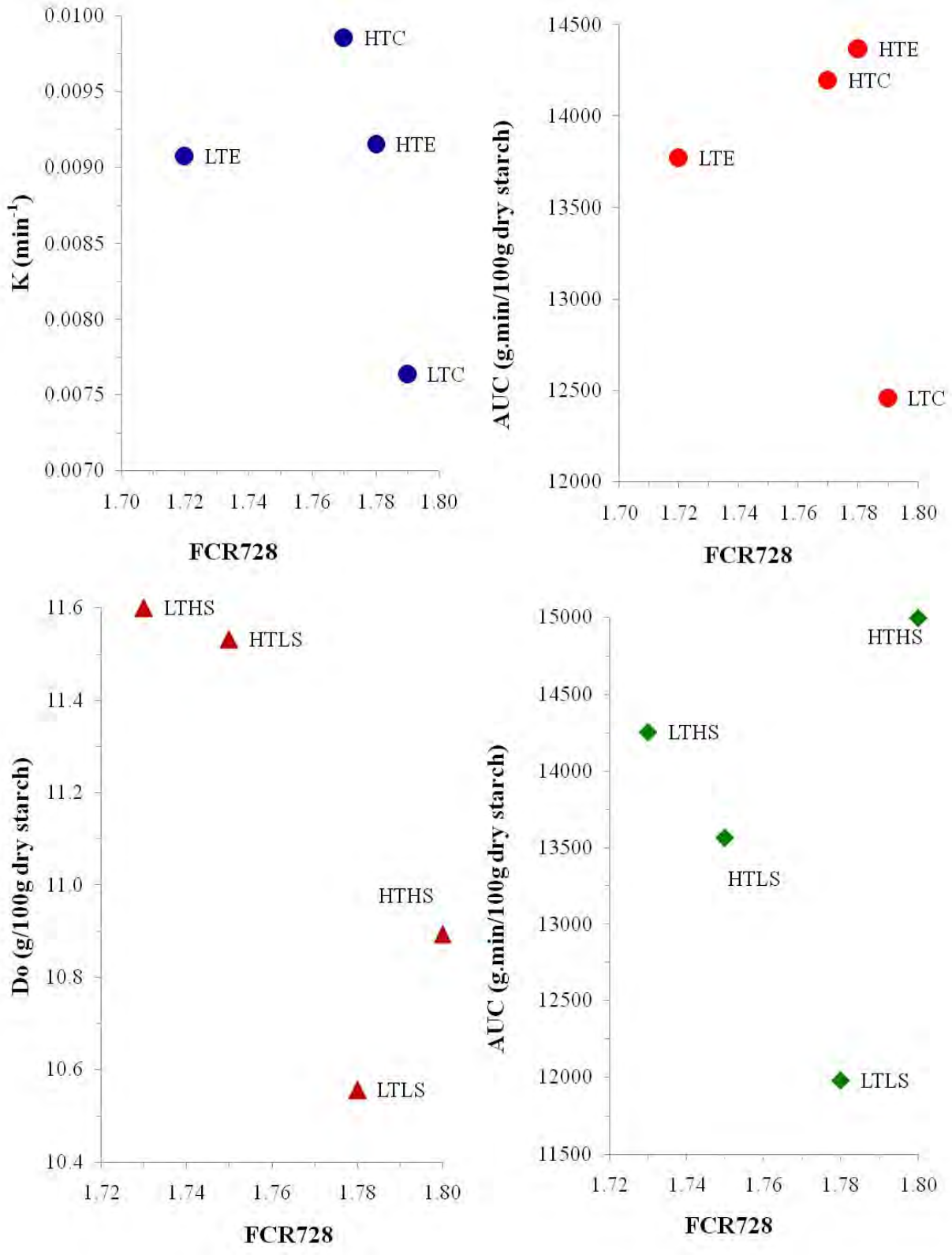


Figure 13. **Relationship between animal performance and *in vitro* digestion parameter (H = high, L = low, T = temperature, C = conventional conditioning, E = extended conditioning, S = sorghum inclusion)**

D.2 Effects of particle size and steam pelleting

Samples were obtained for laboratory analysis and animal performance trials, in collaboration with the stockfeed manufacturer, Better Blend Stockfeeds by (a) grinding each of sorghum and barley through a 4 mm hammer mill screen, (b) sieving the resultant material through a 1.8 mm (barley) or 0.9 mm (sorghum) sieve using a seed cleaner machine, (c) regrinding the captured particles through a 3.2 mm hammer mill screen, and adding these back to the particles that passed through the sieve after a first grind. This material is referred to as re-ground. A smaller sieve was selected for fractionating milled sorghum based on the slower rates of enzyme digestion compared with barley at the same particle size.

D.2.1 Mill and physical pellet characteristics

The grain inclusions and operating conditions used to prepare individual treatments are shown in Table 26. Unlike other experiments in this series, these diets were formulated with a single grain type to avoid the potential influence of other grain types on mill and animal performance. This is similar to the approach used in CRC studies for determination of input variables for NIRs calibrations including DS002 and AF004.

The physical characteristics of feed mixtures before and after processing are shown in Table 27. The bulk density and water activity (a_w) of the unprocessed mash was higher for sorghum than for barley. The reground material also showed a higher bulk density than that of the unfractionated control mixture although water activity was unchanged. Differences due to grain type and fractionation were also apparent in the pelleted material with the use of sorghum grain generally producing a denser, harder and slightly larger pellet than that produced on the barley mixtures. Fractionation and regrinding resulted in a softer and smaller pellet than that of the control treatments. The extent to which these measures of pellet quality were influenced by retention time or energy expenditure at the press is unclear. Sorghum diets showed greater SMEp despite a tendency to lower press amps than barley diets.

Table 26. Processing conditions for conventional and fractionated ration mixtures

Conditions	GA014 Diets							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sorghum inclusion (%)	72	72	72	72	0	0	0	0
Barley inclusion (%)	0	0	0	0	72	72	72	72
Form[#]	M	M	P	P	M	M	P	P
Grind type[†]	C	R	C	R	C	R	C	R
Steam temperature (^oC)	85	85	85	85	85	85	85	85
Steam pressure (kPa)	100	100	100	100	100	100	100	100
Batch size (Tonnes)	2	2	2	2	2	2	2	2
Throughput (Tonnes per hour, TPH)	15	15	15	15	15	15	15	15
Expander pressure (Bar)	-	-	11	11	-	-	11	11

[#]Form – M = mash, P = Pellet

[†]Grind type - C = conventional 3.8 mm hammer mill, R = fractionated and the large particles re-milled through a 3.2 mm screen prior to manufacture

Table 27. Physical components of conventional and fractionated ration mixtures before (mash) after (pellet) processing

Characteristics	Grain		Grind		sd	P
	Barley	Sorghum	Control	Regrind		
Bulk density, mash	535[a]	585[b]	540[a]	580[b]	3	P<0.001
Water activity, mash	0.53[a]	0.59[b]	0.56	0.56	0.025	NS
Moisture, mash (%)	11.9	12.5	12.4	12	0.08	P<0.01
Press amps	165[a]	150[b]	155	160	4.7	P<0.1
Water activity, pellet	0.68	0.7	0.69	0.68	0.009	NS
Bulk density, pellet	645[a]	718[b]	693	670	0.007	P<0.001
Dgw, pellet (mm)	2.69	2.77	2.88[a]	2.58[b]	0.008	P<0.001
Pellet length (mm)	10.4	11.3	12.2[a]	9.6[b]	0.18	P<0.001
Pellet hardness (kg)	4.8	5.6	5.9[a]	4.5[b]	0.33	P<0.05
Retention time (sec)	69	76	75	70	2.5	NS
SMEp	8.2[a]	9.3[b]	9	9.3	0.35	P<0.05

[#]For the grain or grind column, values with the same letters in a row are not significantly different (P>0.05)

D.2.2 Results of particle size analysis

The particle size distribution of milled and fractionated and reground grains is shown in Figure 14. Fractionation and re-grinding removed nearly all particles larger than 1.7 mm without generating many additional particles smaller than 0.125 mm. Screen size in hammer milling affects particle size distribution. The size distributions of the non-fractionated barley and sorghum milled through 3.2 mm screen and the resulting mixtures prepared for manufacture and subsequent use in feeding studies are shown in Figure 15.

The particle size distributions of milled and fractionated grains and feed mixtures shown in Figure 15 confirm that it was possible to separate grains using standard milling equipment to yield fractions that were physically and structurally distinct.

D.2.3 Chemical analyses and mill characteristics of ground grains and fractionated mixtures

Table 28 shows the chemical and physical characteristics of grains and grain fractions used in diet preparations. Regrinding the BIG barley particles through a 2.8 mm screen produced a similar mean particle size to that of SMALL particles obtained by fractionation over a 1.8 mm screen. For both grain types, these particles were slightly more than half the diameter of those found in the original sample milled through a 4 mm screen. Fractionation, regrinding and recombination lead to small but important differences between grain types. For sorghum, the SMALL particles tended to be higher in fibre and lower in protein than the original sample. This difference was not obvious for the barley grain.

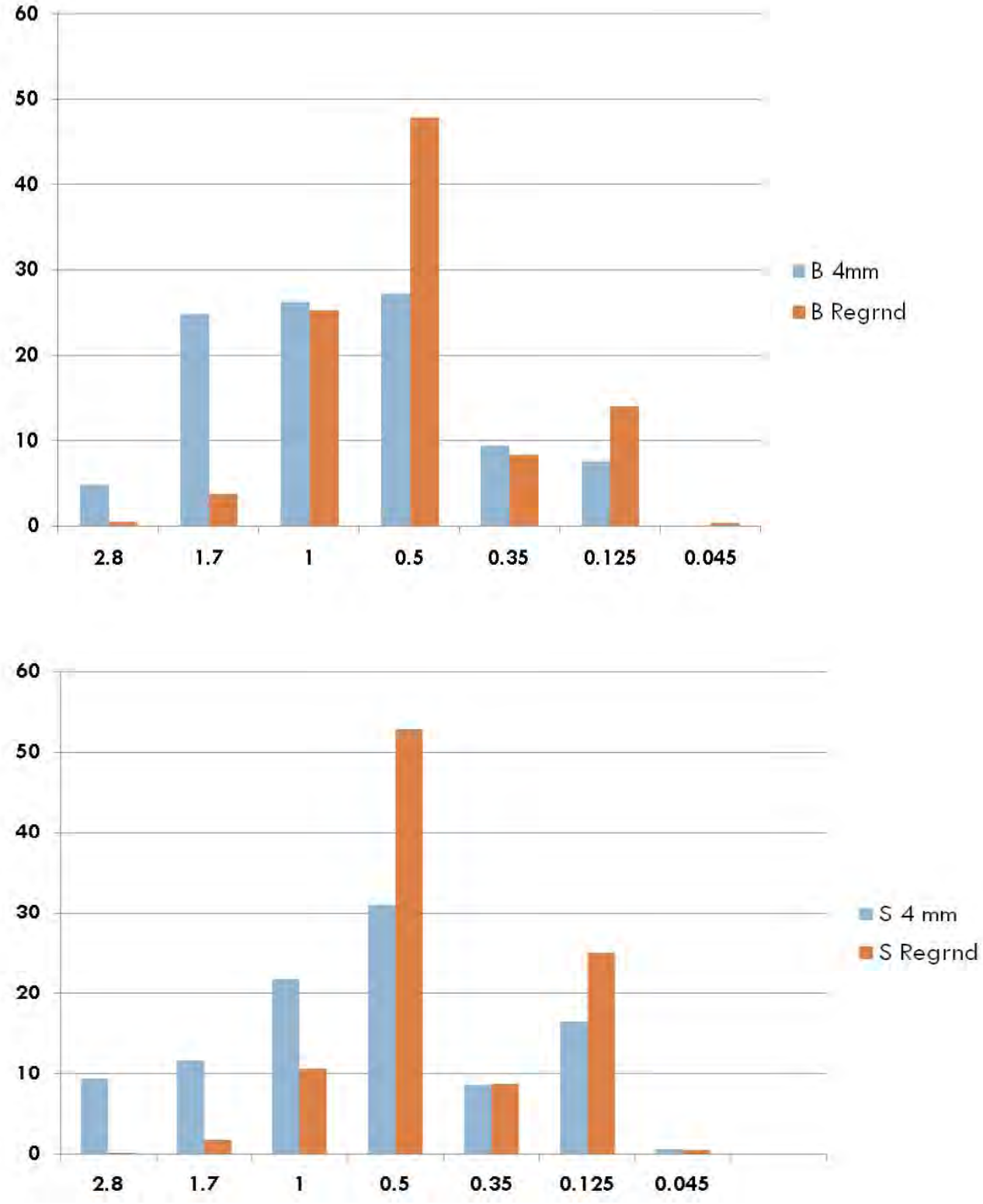


Figure 14. **Particle size distribution after a first hammer milling through a 4 mm screen and after re-grinding (3.2 mm screen) those particles captured on 1.8 mm (barley) and 0.9 mm (sorghum) screens**

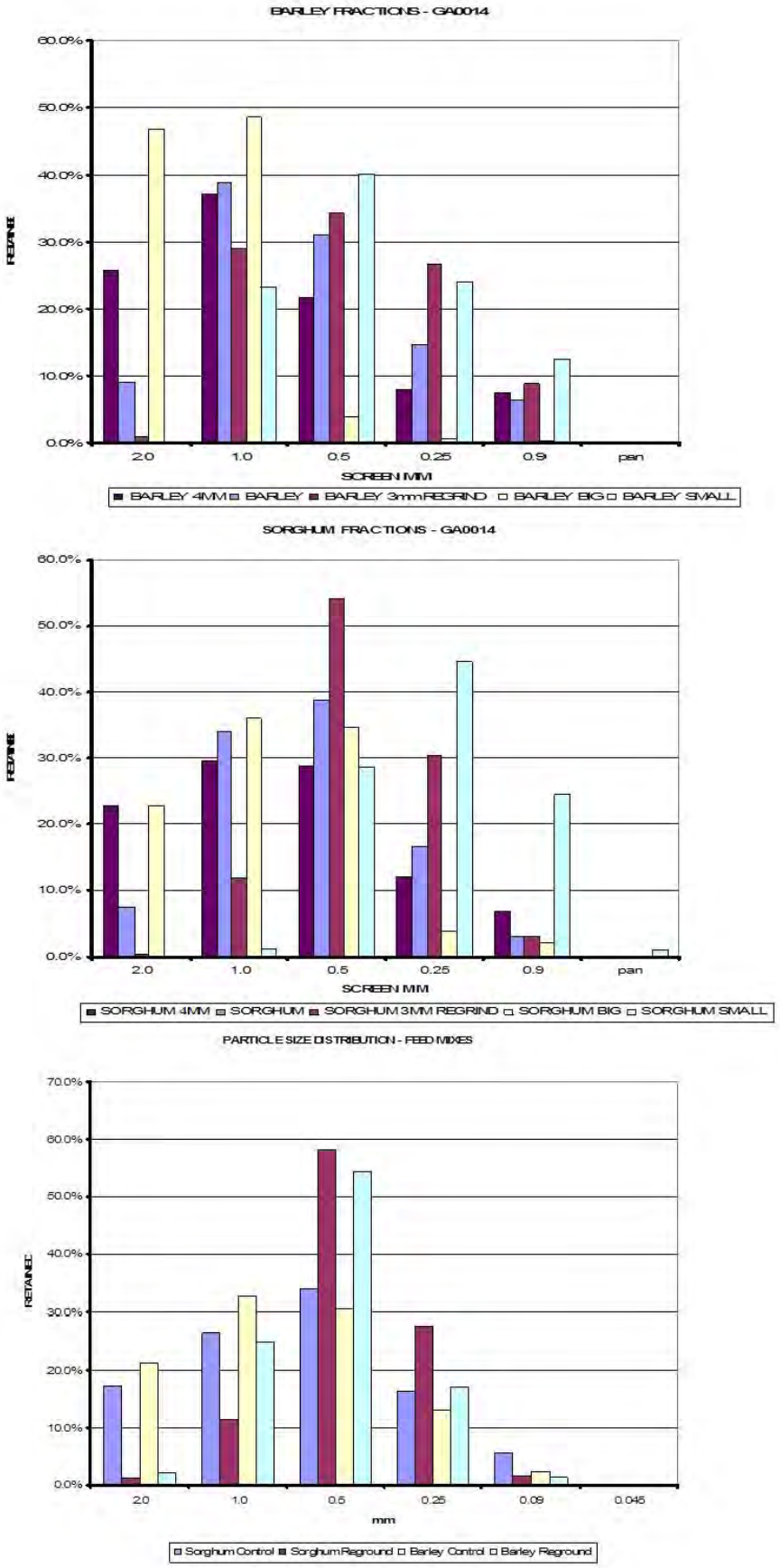


Figure 15. Particle size distribution of milled and fractionated grains and feed mixtures

Table 28. Chemical and physical characteristics of grains and grain fractions

Grain	Description	4 mm	BIG	SMALL	REGRIND	sd
Barley	Water activity	0.48	0.49	0.50	0.47	
	D_{gw}	1.210	1.800	0.670	0.700	
	S_{gw}	1.811	1.467	1.758	1.730	
	Protein (%)	14.1	13.8	15.3	14.2	0.81
	Fat (%)	1.9	1.6	3.1	2.0	0.21
	Moisture (%)	9.7	9.6	9.4	9.7	0.20
	Ash (%)	2.4	1.7	2.3	2.5	0.18
	Fibre (%)	4.8	2.4	5.9	5.8	0.6
	Starch (%)	46.3	-	-	-	0.30
	Damaged starch (g/kg)	49	-	-	-	0.8
Sorghum	Water activity	0.53	0.51	0.49	0.49	
	D_{gw}	1.040	1.080	0.470	0.620	
	S_{gw}	1.940	1.863	1.962	1.557	
	Protein (%)	11.6	11.3	9.2	14.2	0.81
	Fat (%)	3.1	3.3	4.9	3.1	0.21
	Moisture (%)	10.5	10.4	10.3	10.5	0.20
	Ash (%)	1.5	1.4	2.8	1.8	0.18
	Fibre (%)	2.1	2.0	4.8	5.8	0.6
	Starch (%)	58.1	-	-	-	0.30
	Damaged starch (g/kg)	72	-	-	-	0.8

In general, neither form nor grinding markedly altered the major chemical components (Table 29). The exception was damaged starch which was approximately doubled by pelleting but significantly reduced by regrinding. The most obvious differences in chemical components occurred between grains with sorghum diets showing lower protein, fibre and ash contents than the barley mixtures. Sorghum diets contained significantly higher ($P<0.05$) amounts of both starch and damaged starch than those using barley as the major grain.

Table 29. Chemical components of conventional and fractionated ration mixtures after processing[#]

Components	Grain		Form		Grind		sd	P
	Barley	Sorghum	Mash	Pellet	Control	Regrind		
Protein (%)	20.4[a]	17.7[b]	19	19.2	18.8	19.3	0.65	P<0.1
Fat (%)	4.5	4.1	4.4	4.2	3.6	5	0.69	NS
Moisture (%)	10	10.7	9.9[a]	10.8[b]	10.4	10.3	0.17	P<0.05
Ash (%)	7.6[a]	5.8[b]	6.6	6.8	6.5	7	0.35	P<0.05
Fibre (%)	5.1[a]	4.7[b]	4.9	4.6	4.6	4.9	0.15	P<0.05
Starch (%)	37.2[a]	49.1[b]	44.2	42	45.1	41.2	2	P<0.05
Damaged starch (g/kg)	59[a]	73[b]	45[a]	88[b]	73[a]	60[b]	1.8	P<0.05

[#]For the grain, form or grind column, values with the same letters in a row are not significantly different ($P>0.05$)

D.2.4 Animal studies

D.2.4.1 Methods

Re-ground and single grind samples of sorghum and barley were incorporated into a standard grower diet with grain representing 72% of the feed as shown in Table 26. Diets were fed either as a mash or after steam pelleting under typical commercial conditions. The diets were offered to young male weaner pigs at Rivalea or grower pigs at Wacol, Queensland.

The studies were undertaken using a 2 x 2 x 2 design with barley vs. sorghum, single grind vs. re-ground, and mash vs. pelleted as variables. In the Rivalea experiment, 20 pigs, 28 days of age were selected per treatment. Each pig was given a 5 day acclimatisation period and feed intake and growth rate were measured over the following 21 days. In the Wacol experiment, 12 pigs per treatment were selected in a randomized complete block design. The initial weight of the pigs was approximately 22 kg. The experiment was run for 28 days with intake and unconsumed feed (feed spilt on the floor and refused feed left in the trough) recorded daily, and pig weights recorded on days 0, 7, 21 and 28.

D.2.4.2 Results

When these diets were fed to weaner pigs (refer CRC Final Report 1B-113) only two traits had statistically significant treatment effects - FCR028 and FCR728. These were the only two of the 10 traits tested to show statistical significance and two traits where the covariate for start weight (STWT) was not statistically significant. The models fitted to both these traits contained terms that accommodated variance heterogeneity for runs.

A summary of the results is shown in Table 30 for the Rivalea weaner pig experiment and in Table 31 for the Wacol grower pig experiment.

Table 30. Rivalea weaner pig experiment. Effects of grain type, particle size and diet form on average daily intake (ADI), rate of gain (ROG) and feed conversion ratio (FCR) of pigs from 0-28 days[#]

Treatment*	ADI (± se mean) (kg/day as fed)	ROG(± se mean) (kg/day)	FCR(± se mean) (feed:gain)
BGM	$0.47^{a} \pm 0.027$	$0.23^{a} \pm 0.013$	$2.06^{b} \pm 0.094$
BGP	$0.47^{a} \pm 0.026$	$0.27^{b} \pm 0.013$	$1.79^{a} \pm 0.089$
BRM	$0.49^{a} \pm 0.027$	$0.27^{b} \pm 0.013$	$1.75^{a} \pm 0.094$
BRP	$0.45^{a} \pm 0.027$	$0.27^{b} \pm 0.013$	$1.73^{a} \pm 0.094$
SGM	$0.63^{b} \pm 0.026$	$0.27^{b} \pm 0.013$	$2.38^{c} \pm 0.091$
SGP	$0.50^{a} \pm 0.027$	$0.27^{b} \pm 0.013$	$2.06^{b} \pm 0.091$
SRM	$0.50^{a} \pm 0.028$	$0.25^{ab} \pm 0.013$	$1.85^{a} \pm 0.097$
SRP	$0.46^{a} \pm 0.028$	$0.23^{a} \pm 0.013$	$2.11^{b} \pm 0.096$

[#]For each column, values with the same letters are not significantly different (P>0.05)
*B = barley; S = sorghum; G = ground once; R = re-ground large fraction; M = mash; P = pellet

Table 31. Wacol grower pig experiment. Effects of grain type, particle size and diet form on average daily intake (ADI), rate of gain (ROG), and feed conversion ratio (FCR) of pigs from 0-28 days [#]

Treatment*	ADI (± se mean) (kg/day as fed)	ROG(± se mean) (kg/day)	FCR(± se mean) (feed:gain)
BGM	1.62^{a} ± 0.069	0.80^{a} ± 0.030	2.04^{a} ± 0.05
BGP	1.66^{a} ± 0.071	0.84^{a} ± 0.031	1.96^{ab} ± 0.051
BRM	1.60^{a} ± 0.071	0.86^{a} ± 0.032	1.88^{b} ± 0.052
BRP	1.63^{a} ± 0.077	0.85^{a} ± 0.035	1.90^{b} ± 0.055
SGM	1.86^{a} ± 0.074	0.85^{ab} ± 0.033	2.20^{a} ± 0.054
SGP	1.60^{b} ± 0.069	0.80^{b} ± 0.033	2.02^{b} ± 0.050
SRM	1.72^{ab} ±0.069	0.87^{a} ±0.030	1.98^{bc} ± 0.050
SRP	1.59^{b} ± 0.072	0.81^{ab} ± 0.031	1.92^{c} ± 0.052

[#]For each column, values with the same letters are not significantly different (P>0.05).
*B = barley; S = sorghum; G = ground once; R = re-ground large fraction; M = mash; P = pellet

Pelleting of diets containing re-ground grains did not improve the efficiency of use of either sorghum or barley based diets offered to grower pigs or barley diets offered to weaner pigs. However, pelleting of the re-ground sorghum diet resulted in a significant reduction in the efficiency of feed use by weaner pigs.

Re-grinding to remove the large particles significantly reduced the intake of the sorghum based mash diets with little effect on growth rate for both the weaner and grower pigs. Consequently there was a substantial improvement in feed conversion efficiency.

Figure 16 shows that feed conversion ratio (FCR) values were lower (efficiency of feed use higher) for all comparisons of re-grinding with single grinds for all feeds except reground sorghum pellets fed to weaner pigs. Effects were particularly marked for mash feeds with re-grinding resulting in 22% and 10.5% improvement in the efficiency of feed use for sorghum offered, respectively, to weaner and grower pigs. Similarly re-grinding of barley fed as a mash resulted in 15% and 8.3% improvement in efficiency of feed use for weaner and grower pigs, respectively. Re-grinding of either sorghum or barley offered as a mash tended to result in a lower FCR than pelleting after a single grind, but this was only significant for weaner pigs fed sorghum based diets.

Pelleting of conventionally ground grain also resulted in significant improvements in feed conversion efficiency compared with the grain fed as mash for all comparisons except barley based diets offered to grower pigs. Pelleting of once ground sorghum grain diets resulted in 15% and 8% improvements in feed conversion efficiency compared with mash diets, respectively for weaner and grower pigs. Similarly, pelleting improved feed conversion efficiency by 13% compared with mash diets containing barley in weaner pigs

D.3 Conclusions

By manufacturing feed of a standard nutritional specification at 3 different mills it was possible to identify relationships between the chemical and physical characteristics of individual grains and feed mixtures and variations in process variables such as steam and water addition, system

variables (throughput and energy consumption) and functional changes in the final product. In doing so, this study has identified the relative contribution of a number of key processing and conditioning variables to the physical, nutritional and chemical quality of a pig grower feed.

Data is presented that confirms that processing conditions as well as formulations affect animal performance. Animal performance can be improved if starch in feed is accessible by liquid and digestive enzymes. This is critical in formulations involving sorghum, which gelatinises at a high temperature and whose starch is encapsulated by protein bodies. Extended conditioning of high sorghum mash, even at a low temperature, is beneficial to making the starch accessible for an improved feed conversion rate.

There were large effects of reducing the size of particles on the efficiency of feed use by young pigs offered either barley or sorghum based diets. These results are particularly important for pig enterprises offering mash feeds because removal of the large particles resulted in numerically better feed conversion efficiency than traditional milling and pelleting of the diets. Nevertheless, pelleting of diets containing conventionally milled grain resulted in significant improvements in feed conversion efficiency compared with mash diets for all comparisons except barley based diets fed to grower pigs. The results reported here indicate advantages for pellet quality but not necessarily animal performance for the use of the smallest economically-possible screen to reduce the average particle size of the mash when fed, and for better heat and mass transfer during feed manufacture.

These results of these studies should alert nutritionists, feed manufacturers and producers to a heightened awareness of how significantly the combination of process and system variables used in feed manufacture impact on pellet durability and feeding value. The differences identified in this series of studies have implications for how:

- Future NIRs calibrations are developed and applied to the prediction of feed intake and digestibility
- Mill operators should select processing conditions for sorghum-based feeds, and
- Nutritionists approach diet specification across different mill environments

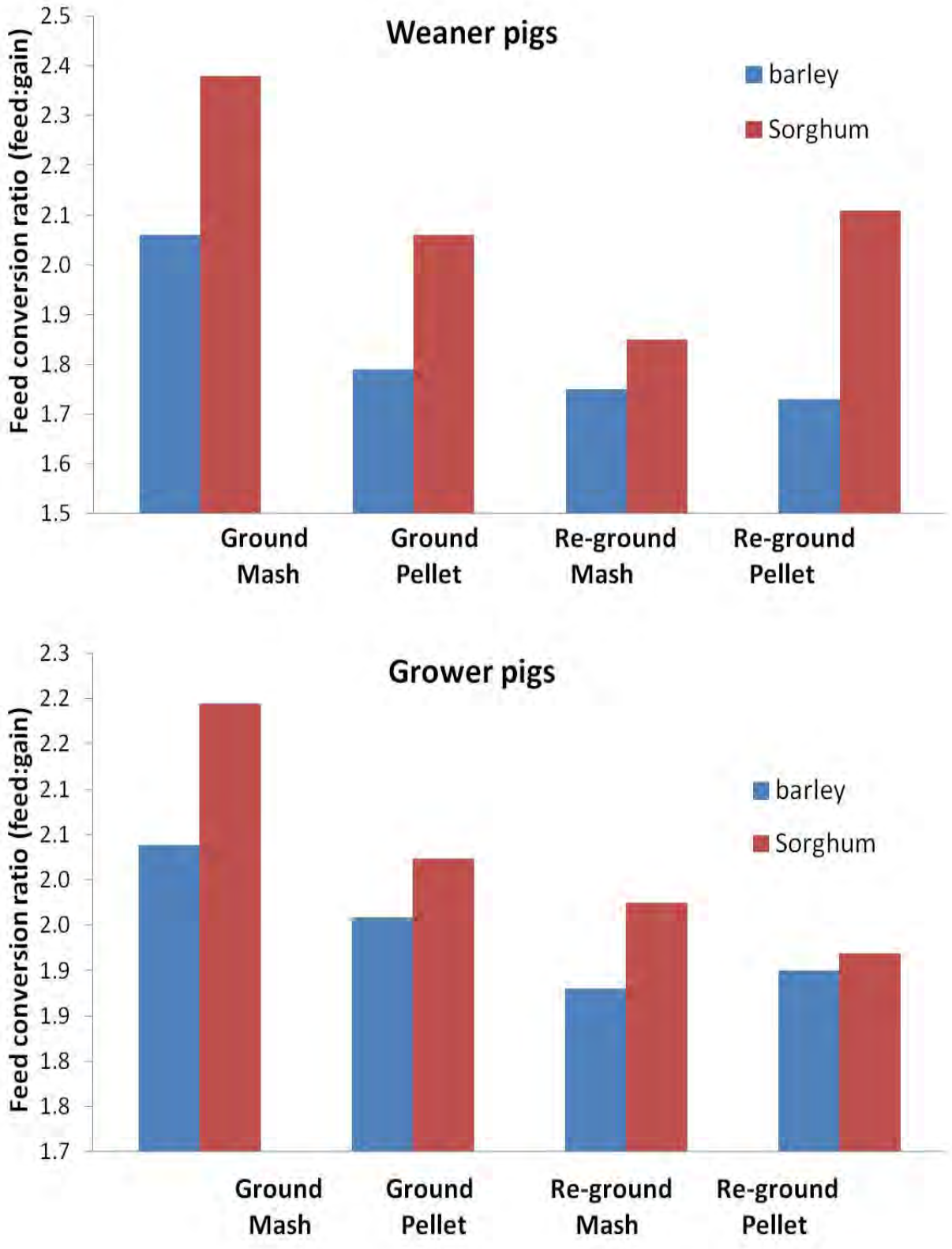


Figure 16. **Effect of re-grinding large particles fed either as mash or conventional pellets on feed conversion ratio for weaner and grower pigs**

E. Development of NIR Calibrations

E.1 Background

The Premium Grains for Livestock Program (PGLP), which was funded by the grains and livestock research organizations and Ridley AgriProducts, developed NIR calibrations for assessing rapidly the energy value of cereal grains for sheep, cattle, pigs and broilers. These calibrations have been extended and improved through further research supported by the Pork CRC, GRDC and the RIRDC Chicken Meat program. The calibrations have been licensed to feed testing laboratories, livestock integrators, stockfeed manufacturers, grain bulk handlers and others through the Pork CRC project, AusScan. The calibrations are anticipated to become the basis for trading grains for livestock in Australia, because research within PGLP showed the current methods based on test weight (kg/hl) and screenings percent do not represent well the energy value of grains for livestock.

Research within the Pork CRC project 1B-102 has shown that the NIR equations developed for predicting the energy value of cereal grains are satisfactory for use when diets undergo standard hammer milling and are fed as either a mash or when minimal heat-moisture treatments are applied like in mostly cold pelleting. However, the energy value of the grains can be changed substantially when the grain has a different particle size distribution or the diet is subjected to moist heat and is partially gelatinized as occurs during processing in many of the stock feed manufacturing plants.

Consequently, a major objective of this project was to develop NIR calibrations that can predict rapidly the likely nutritional value of processed diets at the site of their manufacture or use. In addition, the feasibility of developing NIR calibrations for assessing the durability of pellets and the efficiency of processing was investigated.

E.2 Methods

For all experiments conducted in project 1B-107, samples of mixed mash diets and of final products were scanned with a Foss 6500 instrument. For some experiments, samples were also taken along the processing stream and scanned. In addition, final pellets used in experiments in projects 1B-101 and 1B-104 to determine faecal DE content of grains fed to pigs, were scanned. The scans were analysed using principal component analyses (PCA) to determine whether differences between sampling sites and mills could be clearly identified. The scans were then used to develop NIR calibrations for a range of variables that may indicate the nutritional value of the final processed product and its durability.

E.3 Results

E.3.1 Effects of processing on NIR scans

The initial experiments where samples were provided from specific sampling stages of the stock feed processing from BBS, QAF and RAP show distinct clustering of the samples types as well as differences between processors. There appears to be no clear spectral effect of the various sorghum levels or other treatments in any of the mixes. It was important to build calibrations without bias toward a particular processing facility, processing stage or sample type.

Figures 17, 18 and 19 show a PCA of the various stages of processing from the two individual processors (BBS and QAF), as well as a comparison between BBS and QAF. The math treatment has removed particle size effects but clusters of the various processing stage, i.e. milling, ex-mash and pelleting, can be clearly identified in the spectral data.

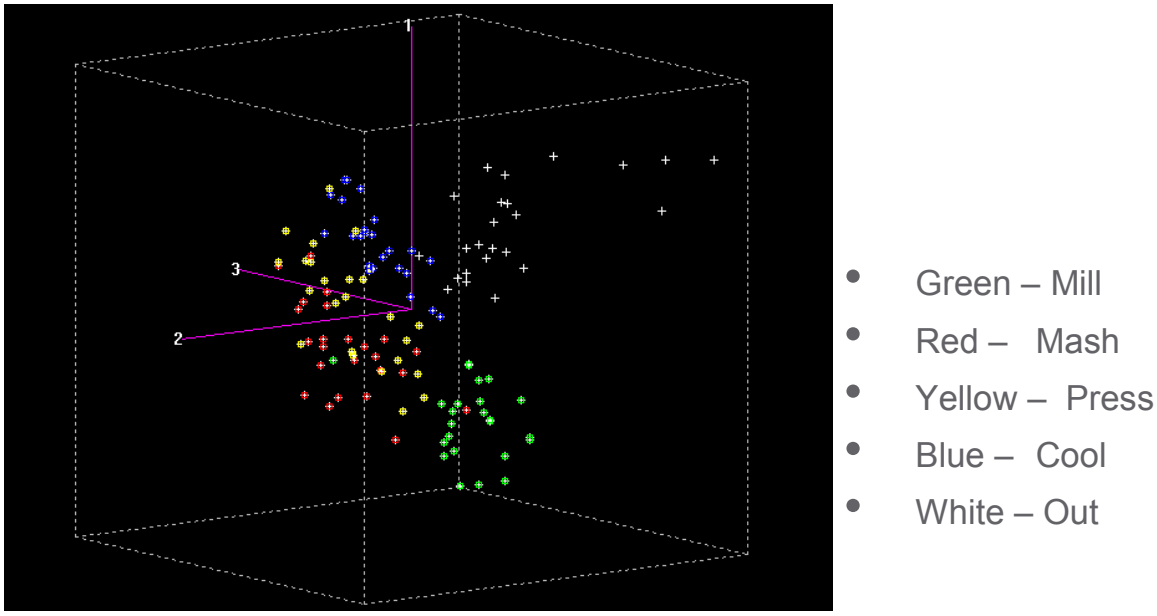


Figure 17. Spectral variation between manufacturing processes at BBS

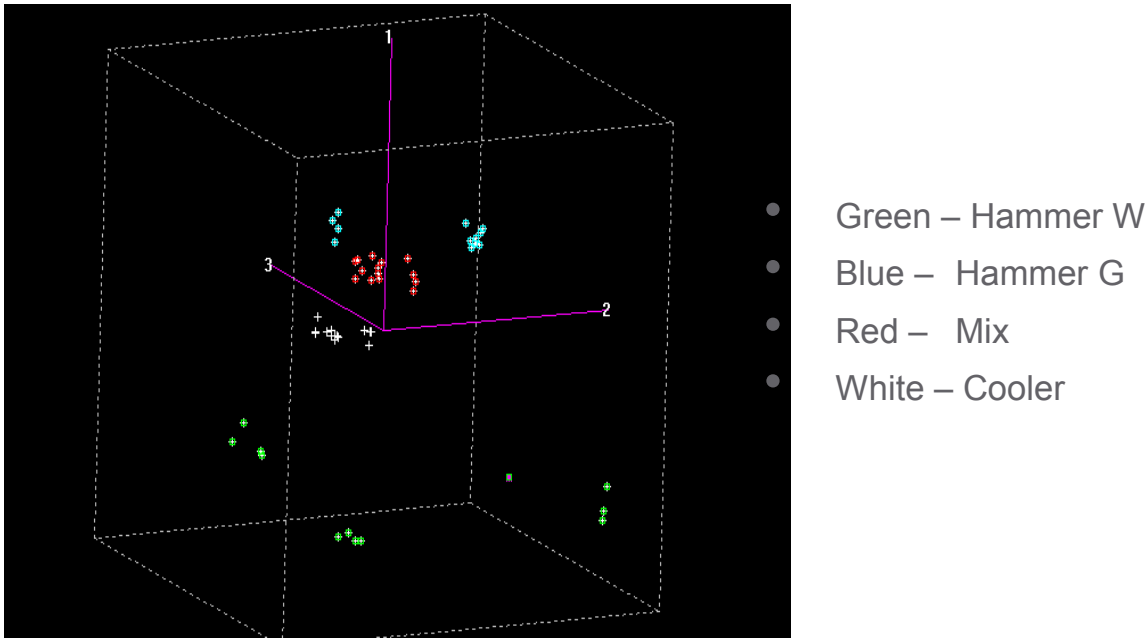


Figure 18. Spectral differences within an individual processor (QAF)

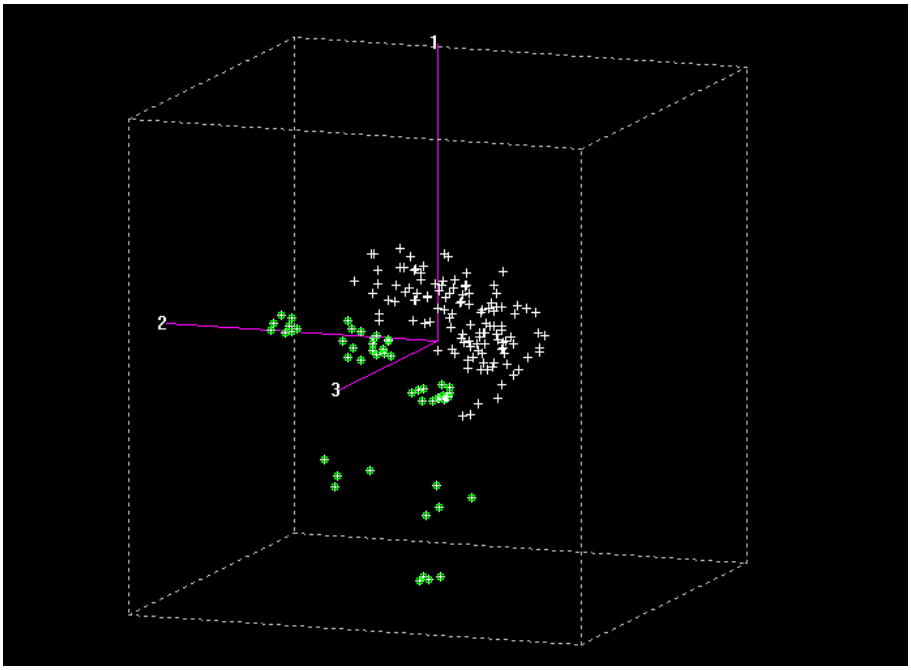


Figure 19. Example of spectral differences between two processors including all processing stages (BBS – white; QAF – green)

When all the spectra data was analysed to identify the chemical components explaining the variation in the spectra, the greatest effect was observed in one of starch regions (Figure 20). This would support already known data that starch, being the most plentiful source of energy in grains, is undergoing the most change during processing. There were other regions such as protein and lipid where there were spectra changes as well, albeit, not as strong as the main starch region.

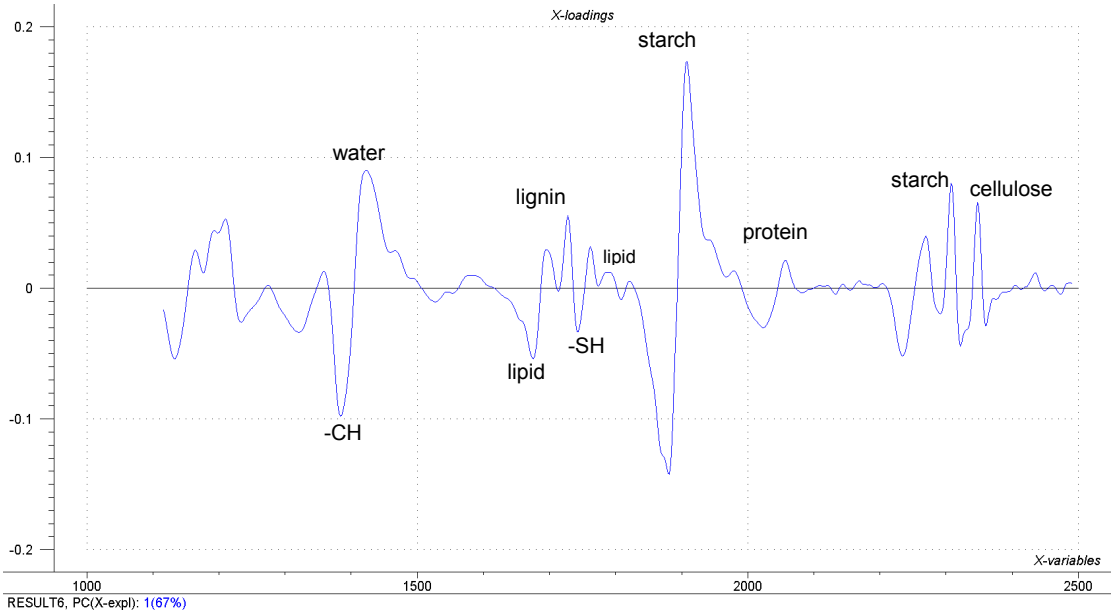


Figure 20. Loading plot of spectral regions associated with pellet quality

These differences in scans relating to where samples were taken in the processing chain, between mills and in chemical components suggest that robust NIR calibrations may be feasible for predicting the nutritional value of the finished product and other characteristics of product quality. The number of samples available for development of most of the calibrations is at the lower limit of that considered desirable for robust calibrations. However, they do provide evidence that robust calibrations may be developed with the addition of more samples.

E.3.2 Preliminary NIR calibrations

NIR calibrations were developed from scans on the finished processed pellet for a range of variables including those from *in vivo* faecal DE experiments, *in vitro* starch digestion assays, RVA assays, chemical analyses and pellet quality measurements.

The likely value of NIR calibrations can assessed in relation to the criteria shown in Table 32. 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 (Ratio of standard error of Prediction to standard Deviation of the prediction set)

One of the most critical parts of developing useful and applied NIR calibrations is to ensure the calibration models are used to estimate an unknown data set. This demonstrates the robustness of the calibrations. In this current study, the calibration set is built using a relatively small number of samples with no prediction of an unknown set. Given the variation in spectra between the processors (Figure 19), it would be inappropriate to use samples from one processor to build calibrations and predict samples from another processor. Hence, a combination of all processors is required for a useful calibration. However, additional samples are required to validate the accuracy of the calibrations and, as such a true RPD could be calculated rather than the ratio of SECV/SD.

The calibrations established for a range of variables are shown in Table 33. These calibrations are ranked in order of greatest robustness as indicated by RDP values. These preliminary calibrations are encouraging and suggest that, with more samples, robust calibrations for predicting the nutritional value of the final pelleted diet may be achievable.

The calibration for *in vivo* faecal DE was developed from pellets that were cold-pressed from experiments in projects 1B-101/104. These pellets would have varied only slightly in the extent of starch gelatinisation and particle size distribution. Hence, pellet samples with a considerably wider range in grain particle size distribution, ingredient mix and processing conditions would need to be fed to pigs to obtain faecal DE values, before the calibration could have wide application. The calibrations for both damaged starch and the *in vitro* starch digestion area under the curve (AUC) are considered to be potentially valuable for assessing the nutritional value of pellets. These calibrations were developed from a pellet samples that had been exposed to a wide range of grain particle sizes, ingredient mixtures and processing conditions. As discussed above (D.1.3.5), preliminary examination of animal feeding trial

results show that both AUC and K appear to be closely related to feed conversion efficiency measurements made when growing pigs were fed the diets (Figure 13).

Table 32. Criteria used to assess the value of NIR calibrations

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

E.4 Conclusions

There were large differences in the NIR spectra of feed as it passed through the pelleting process chain in individual mills. There were also large differences in the spectra between mills at the same point in the processing chain. Thus, universally effective NIR calibrations must be developed from product collected at specific points in the process and collected across a wide range of mills. The preliminary NIR calibrations based on scans of the final pelleted product suggest that commercially valuable calibrations can be developed to predict the faecal DE content and the *in vitro* starch digestion variables K and AUC, which were related to FCR measured in growing pigs. In addition, the preliminary calibrations predicted the moisture and chemical composition values of the pellets with reasonable accuracy.

The number of samples used to develop these calibrations would be regarded by NIR experts as being near the minimum and no validation of the calibrations was conducted. Hence, a wider range of final product samples from several mills and processing conditions is needed to first evaluate and then upgrade the calibrations to improve the accuracy and robustness of predictions.

Table 33. Statistics for NIR calibrations established for variables measured on final processed pellets

Variable[1]	N	Mean	Est. Max	Est. Min	RSQ	SECV	SD	RPD
In vivo	47	13.6	15.6	11.6	0.9576	0.18	0.66	3.7
Moisture	57	10.7	15.1	6.4	0.8966	0.5	1.70	3.2
starch damage	60	5.2	11.2	0.0	0.953	0.8	2.03	2.6
AUC	61	11275	13852	8699	0.614	1105	2464	2.2
K	53	8.04	12.98	3.09	0.8793	1.18	2.50	2.1
Ash	63	5.6	8.4	2.8	0.8684	0.5	0.95	2.1
peak time	75	5.3	7.3	3.3	0.771	0.3	0.69	2.1
Protein	60	17.6	21.2	14.0	0.8256	0.6	1.22	2.0
Fat	63	3.5	5.1	1.8	0.742	0.3	0.60	1.8
Durability	47	91.3	116.8	65.9	0.5718	6.2	10.9	1.7
Paste Temp	75	71.5	85.8	57.2	0.7749	2.8	4.78	1.7
Phytate	35	1.2	1.6	0.8	0.7612	0.1	0.14	1.7
NFE	47	67.5	71.9	63.2	0.7238	0.9	1.48	1.7
crude fibre	63	2.8	4.5	1.2	0.6456	0.4	0.60	1.6
D_∞	62	88.2	122.0	54.4	0.5181	10.5	16.7	1.6
ExpD240	61	76.7	114.9	38.5	0.6682	9.0	13.90	1.5
Starch	61	49.2	64.6	33.8	0.6989	3.3	5.08	1.5
Phosphorus	33	6212	7928	4495	0.533	429	636	1.5
Breakdown	73	39.4	94.0	0.0	0.5514	14.0	20.46	1.5
D_o	65	12.7	19.4	6.0	0.8139	1.6	2.2	1.4
PreD240	60	75.1	112.6	37.6	0.4326	10.0	13.60	1.4
Final V	73	415	948	0	0.4494	142	186	1.3
Setback	73	269.8	634.8	0.0	0.4333	99.3	130	1.3
Trough V	73	144.9	319.4	0.0	0.4008	49.2	59.50	1.2
Peak Visc	73	187.1	352.5	21.6	0.2447	50.6	58.10	1.1
Initial Visc	73	18.5	38.1	0.0	0.1135	6.6	7.37	1.1

[1]Key to abbreviations: *in vivo* is pig faecal DE value (MJ/kg as fed); D_o, D_∞, AUC, ExpD240, PreD240 are *in vitro* starch digestion variables; Breakdown, setback, Initial Visc(osity), Peak Visc(osity), Trough V(iscosity), Final V(iscosity), Paste Temp, peak Time are variables from the RVA analysis; moisture, starch damage, ash, protein, fat, phytate, NFE, crude fibre, starch, phosphorus are all chemical composition variables; durability is measured in pellets.

PART TWO

PILOT-PLANT FEED PROCESSING

F. Grain and Diet Formulation

F.1 Background

Wheat is a predominant feed grain, and like other grains, there are many varieties or genotypes, and during growth, there are environmental factors. These factors can include weather damage, for example, unexpected rains that can lead to sprouting or germination. Weather damaged and non-damaged grains are used for pig feeds, and analysing them helps to know if they can adequately supply the required nutrients for pigs. Such analysis helps to formulate diets to meet the required digestible energy, and to identify other grains and non-grain ingredients (additives) for appropriate pig nutrition.

F.2 Methods

F.2.1 Wheat

The soft wheat (1894) variety QAL2004 was harvested in late 2009 (unknown growing location) and sourced from AusGrains, Moree NSW. The hard wheat (1895) variety Sunvex, was harvested in late 2010 (unknown growing location) and sourced from Hart Bros. Seeds, Junee NSW. The sprouted wheat (1896) was an unknown variety, but was harvested in late 2010 (unknown growing location) and sourced from Michael White & Co. Wellington, NSW. About 6 tonnes of each grain was sourced and stored at ambient temperature in a shed at the University of Sydney, Plant Breeding Institute in Narrabri. The shed were bated for rodents and fumigated with aluminum phosphide every 8 weeks while in storage to kill any grain insects.

F.2.2 Feed formulation

The wheats were NIRs-scanned to predict their nutrients, and using the NIRs-predicted data, the diets were formulated to contain about 61.5% grain, 14.7 MJ/kg digestible energy (DE) and 90.2% dry matter.

F.2.3 Physicochemical property

The following physicochemical properties of the wheats were determined in randomised and duplicated experiments:

a. Major diameter, minor diameter, thickness, and 1000-grain count following standard procedures.
b. Proximate composition following methods, which are commonly used in Pork CRC projects:

    i. Moisture - About 2 g sample was dried in an oven for 3 hr at 135^{o}C.
    ii. Ash – About 2 g sample was ashed in a furnace at 600^{o}C for 4 hr.
    iii. Crude Protein – About 140 mg sample was combusted at 950^{o}C in a LECO analyser following the Dumas nitrogen method, and crude protein was calculated by multiplying the nitrogen value by 6.25.

iv. Crude Fat – About 2 g sample was analysed in a Soxhlet set-up using petroleum spirit.
v. Starch content – About 50 mg sample was analysed using the Megazyme™ dimethyl sulphoxide (DMSO)-amylase-amyloglucosidase procedure.

F.3 Results

Table 34 shows the physicochemical properties of the wheats. The sprouted wheat was slightly wetter than the other wheats, but the three wheats were within the safe moisture content (<12 - 14 g/100 g) for storage. As expected, the hard wheat had the highest measured and predicted protein contents, while the starch contents of the wheats were not materially different. From both laboratories, the hard and sprouted wheats had about the same kernel weight, which reflected in the calculated density. The bulk density or specific weight, however, reflected a different trend, while the sprouted wheat had the most broken kernels. With the physical dimensions of the wheats being larger than 2 mm on the average, broken kernels could have constituted most of the 5% fractions of the sprouted wheat that passed through the 2 mm-screen. This might be a measure of the fracturability of the wheats, and it is noteworthy than the hard wheat had negligible fractions through the 2-mm screen. Despite differences in the protein contents of the wheats, digestible energy was predicted to be about the same for the grains (ileal DE, 12 MJ/kg; faecal DE, 14 MJ/kg).

While differences were measured and predicted in some properties, the wheats had essentially the same values in certain properties. Differences and similarities in grain nutrients are usually taken into consideration in formulating diets with a view to delivering nutrients at a consistent level to pigs. NIRs and feed formulation packages have proved valuable in ensuring this consistency, and Tables 35 – 37 show the expected macro- and micro-nutrients in the formulated diets from the wheats to meet the requirements in F2.2. Apart from the proteins (21 – 25 g/100 g), nearly all the nutrients in the diets from the wheats were essentially the same. The difference of 4 g/100 g of proteins came about because the grain proportion was fixed. A follow-up study could be based on formulating with a fixed protein content. However, in the present study, the difference in the protein contents was thought not to be substantial enough to influence digestibility and animal performance, details of which are discussed below.

Table 34: Physicochemical (laboratory and NIR) properties of the wheats

Parameter	Soft wheat 1894	Hard wheat 1895	Sprouted wheat 1896
NIR-predicted values			
Protein (g/100 g)	10.3	17.1	10.9
Moisture (g/100 g)	10.5	10.6	12.3
Total Starch (g/100 g solids)	69.0	64.9	68.7
Crude fibre (g/100 g solids)	1.6	2.1	2.0
Acid detergent fibre (ADF) (g/100 g solids)	33	3.3	1.6
Neutral detergent fibre (NDF) (g/100 g solids)	17.0	17.6	11.2
Beta-glucan (g/100 g solids)	3.3	3.0	1.9
Insoluble arabinoxylan (g/100 g solids)	4.6	4.4	5.4
Total insoluble non-starch polysaccharides (NSP) (g/100 g solids)	6.2	6.0	5.9
Total soluble non-starch polysaccharides (NSP) (g/100 g solids)	1.3	1.8	1.9
Insoluble arabinoxylan (g/100 g solids)	4.6	4.4	5.4
Faecal digestible energy (DE), as fed (MJ/kg)	13.8	14.1	14.3
Ileal digestible energy (DE), as fed (MJ/kg)	11.9	12.8	12.0
Hydration capacity (g/100 g)	35.6	46.3	40.9
Laboratory measured values			
Specific weight (kg/hL)[a]	83.5	83.0	67.4
1000-grain weight (g)[a]	45.0	33.2	32.0
Screenings <2.0mm (g/100 g)[a]	3.0	0.2	5.0
Major diameter (mm)[b]	3.6	2.8	3.2
Minor diameter (mm)[b]	3.1	2.6	2.9
Thickness (mm)[b]	2.9	2.5	2.6
1000-grain weight[b]	48.6	34.6	36.4
Density (kg/m^3)[b,c]	360	446	406
Moisture (g/100 g)[b]	11.0	11.7	12.4
Fat (g/100 g)[b]	2.8	2.5	1.8
Ash (g/100 g)[b]	1.4	1.6	1.3
Protein (g/100 g)[b]	11.3	18.9	11.9
Total starch (g/100 g solids)[b]	62.5	52.8	57.5

[a]These values were obtained from The University of Sydney

[b]These values were obtained from The University of Queensland (Tan, 2012)

[c]This was calculated by assuming an ellipsoidal shape for wheat (volume = [4/3] $\pi\ x\ y\ z$; where x = major radius, y = minor radius and z = half of the thickness)

Table 35: Diet formulation for soft wheat 1894

```
================================================       Rivalea       (6198) ================================================
:     Single-Mix         (FM)       * Corowa *        {10} OCTOBER 2011               ALL DATA         16:50 18/10/11  0001 :
: 1027.1/2.11                       (    1)  Plant=1        David
:
=============================================================================================================================--
                                                Formula basic data
                    ------------------------------------------------------------------------------
                    Code      :       9797          Name      : WEANER 1 BASE DIET WHEAT 1 - WHEAT 1894

                    External reference: 6,P,W,W1
                    Script file name  :
                                                      Analysis
   ---------------------------------------   ---------------------------------------   ---------------------------------------
   [VOLUME]   %         :       100.0       SERINE     %         :         0.796659   LAYER:ME   KCALS/KG  :      3067.03897
   [DRYMAT]   %         :        90.224967  GLUTAMIN   %         :         1.691067   SULPHUR    %         :         0.203473
   DE_PIG     MJ/KG     :        14.53723   PROLINE    %         :         1.039703   COPPER     PPM       :        40.69862
   NE4G       MJ/KG     :        10.433837  OH_PROLI   %         :         0.137813   COBALT     PPM       :         0.0
   #ALY/NE4G  GM/MJ     :         0.128193  ASPARAG    %         :         1.23476    MANGANES   PPM       :        89.534665
   DEENZYME   MJ/KG     :        15.134173  #LYS/DE_   GM/MJ     :         0.100578   ZINC       PPM       :      2344.006418
   PROTEIN    %         :        20.743317  #ALY/DE_   GM/MJ     :         0.092008   IRON       PPM       :       273.1453
   FAT        %         :         6.186765  #MET/LYS   G/G       :         0.314438   IODINE     PPM       :         0.948335
   STARCH     %         :        38.46548   #M+C/LYS   G/G       :         0.541792   SELENIUM   PPM       :         0.637501
   FIBRE      %         :         1.724298  #THR/LYS   G/G       :         0.634529   #AME/ALY   G/G       :         0.297705
   ASH        %         :         5.085922  #ISO/LYS   G/G       :         0.521465   #ACY/ALY   G/G       :         0.166667
   CALCIUM    %         :         0.867046  #TRY/LYS   G/G       :         0.196711   #AM+/ALY   G/G       :         0.520135
   T:PHOS     %         :         0.654768  #VAL/LYS   G/G       :         0.693445   #ATH/ALY   G/G                 0.623855
   AV:PHOS    %         :         0.632697  AMETH      %         :         0.398194   #AIS/ALY   G/G       :         0.486149
   ENZAVPHOS  %         :         0.578553  AM+C       %         :         0.695705   #ATR/ALY   G/G       :         0.16889
   CAL:PHOS   G/G       :         1.324204  ATHREO     %         :         0.834435   #AVA/ALY   G/G       :         0.475205
   CAL:AVPHOS G/G       :         1.370397  AISOLEUC   %         :         0.650247   #ATH/DE_   GM/MJ     :         0.0574
   P:PHOS     %         :         0.159139  ATRYPTO    %         :         0.225898   ATYROSIN   %         :         0.3904
   CAL:ENZAVP G/G       :         1.498644  AVALINE    %         :         0.635608   AALANINE   %         :         0.623827
   LYSINE     %         :         1.462119  ACYSTINE   %         :         0.222925   AASPARTI   %         :         0.977527
   ALYSINE    %         :         1.337546  AP+T       %         :         0.86105    AASPARAG   %         :         1.08843
   METHION    %         :         0.459745  APHENYL    %         :         0.502943   AGLUTAMI   %         :         1.39158
   M+C        %         :         0.792164  ALEUCINE   %         :         0.93566    AGLUT:IN   %         :         1.422477
   THREO      %         :         0.927757  AHISTID    %         :         0.340819   AGLYCINE   %         :         0.66442
   ISOLEUC    %         :         0.762445  AARGININ   %         :         0.702621   ASERINE    %         :         0.44546
   TRYPTO     %         :         0.287615  SALT       %         :         0.795482   APROLINE   %         :         0.38402
   CYSTINE    %         :         0.337     %LEGUMES   %         :         9.0        LNAA       GM        :        19.005211
   VALINE     %         :         1.0139    ABC        MEQ/KG    :       569.819565   #TRY/LNA   G/G       :         0.048816
   HISTIDIN   %         :         0.544787  SODIUM     %         :         0.290208   BULKDENS   KG/HL     :        63.336668
   LEUCINE    %         :         1.501044  POTASS     %         :         0.570819   IONOPHORE  PPM       :         0.0
   PHENYLAL   %         :         0.869043  CHLORIDE   %         :         0.505356   W6 FA      %         :         0.0
   P+T        %         :         1.09629   MAGNES     %         :         0.148366   W3 FA      %         :         0.0
   ARGININE   %         :         1.106331  NA+K_CL    MEQ/KG    :       130.43282    W6:W3      G/G       :         0.0
   TYROSINE   %         :         0.460543  CHOLINE    MG/KG     :      1714.599313   SAT FA     %         :         0.0
   T:EAA      %         :         6.968253  LACTOSE    %         :         6.0        MONO FA    %         :         0.0
   ALANINE    %         :         1.046017  N:D:F:     %         :        10.83664    POLY FA    %         :         0.0
   ASPARTIC   %         :         1.599043  LINOLEIC   %         :         0.864032   ENDF       %         :         0.653822
   GLYCINE    %         :         1.138377  A:D:F:     %         :         2.950156
   GLUTAMIC   %         :         3.438392  RUMIN:ME   MJ/KG     :        12.682247

                          Raw material                      Available       %           [Kg]          Tonnes
                ------------------------------------------- --------- ------------ ------------ ----------------
                     300 CANOLA MEAL 36%                        [X]         4.0          120.0           6.0
                     325 SOYABEANMEAL-48%                       [X]         5.0          150.0           7.5
                     400 MEATMEAL                               [X]         4.266667     128.0           6.4
                     410 FISHMEAL 64%                           [X]         7.5          225.0          11.25
                     420 BLOODMEAL                              [X]         1.5           45.0           2.25
                     450 WHEY POWDER 11%                        [X]        10.0          300.0          15.0
                     500 WATER                                  [X]         1.0           30.0           1.5
                     502 NATUPHOS 5000                          [X]         0.01           0.3           0.015
                     504 TALLOW-ENZYME                          [X]         2.0           60.0           3.0
                     520 TALLOW-MIXER                           [X]         1.733333      52.0           2.6
                     551 SALT BIN ADD                           [X]         0.2            6.0           0.3
                     600 LYSINE-HCL                             [X]         0.416667      12.5           0.625
                     605 DL-METHIONINE                          [X]         0.086667       2.6           0.13
                     610 THREONINE                              [X]         0.183333       5.5           0.275
                     615 ISOLEUCINE H/A                         [X]         0.016667       0.5           0.025
                     620 TRYPTOPHAN H/A                         [X]         0.046667       1.4           0.07
                     650 ZINC OXIDE                             [X]         0.276667       8.3           0.415
                     700 QAF CREEP PMX                          [X]         0.166667       5.0           0.250001
                     770 ENDOX                                  [X]         0.02           0.6           0.03
                     989 RONOZYME                               [X]         0.03           0.9           0.045
                    1120 WHEAT 10.5%                            [X]        61.546666    1846.4          92.32
                                                                           ------------ ------------ ----------------
                                                                             100.0         3000.0         150.0
                                                                           ------------ ------------ ----------------
```

Table 36: Diet formulation for hard wheat 1895

```
================================================       Rivalea       (6198) ================================================
:     Single-Mix         (FM)       * Corowa *       {10} OCTOBER 2011              ALL DATA          16:50 18/10/11  0003
:
: 1027.1/2.11                       (    1)  Plant=1        David
:
================================================================================================================================
```

Formula basic data

Code : 9798 Name : WEANER 1 BASE DIET WHEAT 2 – WHEAT 1895

External reference: 6,P,W,W1

Script file name :

Analysis

[VOLUME]	%	:	100.0	SERINE	%	:	0.525853	LAYER:ME	KCALS/KG	: 1155.664103
[DRYMAT]	%	:	90.224967	GLUTAMIN	%	:	1.691067	SULPHUR	%	: 0.203473
DE_PIG	MJ/KG	:	14.783416	PROLINE	%	:	0.45501	COPPER	PPM	: 40.69862
NE4G	MJ/KG	:	10.587704	OH_PROLI	%	:	0.137813	COBALT	PPM	: 0.0
#ALY/NE4G	GM/MJ	:	0.13037	ASPARAG	%	:	1.23476	MANGANES	PPM	: 89.534665
DEENZYME	MJ/KG	:	15.134173	#LYS/DE_	GM/MJ	:	0.102233	ZINC	PPM	: 2344.006418
PROTEIN	%	:	24.74385	#ALY/DE_	GM/MJ	:	0.09337	IRON	PPM	: 273.1453
FAT	%	:	6.186765	#MET/LYS	G/G	:	0.328627	IODINE	PPM	: 0.948335
STARCH	%	:	36.2498	#M+C/LYS	G/G	:	0.463057	SELENIUM	PPM	: 0.637501
FIBRE	%	:	1.973562	#THR/LYS	G/G	:	0.687158	#AME/ALY	G/G	: 0.312022
ASH	%	:	5.085922	#ISO/LYS	G/G	:	0.589995	#ACY/ALY	G/G	: 0.161502
CALCIUM	%	:	0.879355	#TRY/LYS	G/G	:	0.214736	#AM+/ALY	G/G	: 0.566797
T:PHOS	%	:	0.688618	#VAL/LYS	G/G	:	0.776733	#ATH/ALY	G/G	: 0.643761
AV:PHOS	%	:	0.643775	AMETH	%	:	0.430691	#AIS/ALY	G/G	: 0.507201
ENZAVPHOS	%	:	0.597017	AM+C	%	:	0.782363	#ATR/ALY	G/G	: 0.174157
CAL:PHOS	G/G	:	1.276985	ATHREO	%	:	0.888596	#AVA/ALY	G/G	: 0.460479
CAL:AVPHOS	G/G	:	1.365935	AISOLEUC	%	:	0.7001	#ATH/DE_	GM/MJ	: 0.060108
P:PHOS	%	:	0.140675	ATRYPTO	%	:	0.240392	ATYROSIN	%	: 0.3904
CAL:ENZAVP	G/G	:	1.472914	AVALINE	%	:	0.635608	AALANINE	%	: 0.623827
LYSINE	%	:	1.511357	ACYSTINE	%	:	0.222925	AASPARTI	%	: 0.977527
ALYSINE	%	:	1.380321	AP+T	%	:	0.86105	AASPARAG	%	: 1.08843
METHION	%	:	0.496673	APHENYL	%	:	0.502943	AGLUTAMI	%	: 1.39158
M+C	%	:	0.699844	ALEUCINE	%	:	0.93566	AGLUT:IN	%	: 1.422477
THREO	%	:	1.038541	AHISTID	%	:	0.340819	AGLYCINE	%	: 0.66442
ISOLEUC	%	:	0.891693	AARGININ	%	:	0.702621	ASERINE	%	: 0.44546
TRYPTO	%	:	0.324543	SALT	%	:	0.795482	APROLINE	%	: 0.38402
CYSTINE	%	:	0.410856	%LEGUMES	%	:	9.0	LNAA	GM	: 19.066758
VALINE	%	:	1.173921	ABC	MEQ/KG	:	569.819565	#TRY/LNA	G/G	: 0.054469
HISTIDIN	%	:	0.397075	SODIUM	%	:	0.290208	BULKDENS	KG/HL	: 23.331335
LEUCINE	%	:	1.784159	POTASS	%	:	0.570819	IONOPHORE	PPM	: 0.0
PHENYLAL	%	:	0.592083	CHLORIDE	%	:	0.505356	W6 FA	%	: 0.0
P+T	%	:	1.09629	MAGNES	%	:	0.148366	W3 FA	%	: 0.0
ARGININE	%	:	1.272507	NA+K_CL	MEQ/KG	:	130.43282	W6:W3	G/G	: 0.0
TYROSINE	%	:	0.460543	CHOLINE	MG/KG	:	1714.599313	SAT FA	%	: 0.0
T:EAA	%	:	6.968253	LACTOSE	%	:	6.0	MONO FA	%	: 0.0
ALANINE	%	:	0.81214	N:D:F:	%	:	11.168992	POLY FA	%	: 0.0
ASPARTIC	%	:	1.279	LINOLEIC	%	:	0.864032	ENDF	%	: 0.653822
GLYCINE	%	:	0.873727	A:D:F:	%	:	2.950156			
GLUTAMIC	%	:	1.90588	RUMIN:ME	MJ/KG	:	12.682247			

	Raw material	Available	%	[Kg]	Tonnes
300	CANOLA MEAL 36%	[X]	4.0	120.0	6.0
325	SOYABEANMEAL-48%	[X]	5.0	150.0	7.5
400	MEATMEAL	[X]	4.266667	128.0	6.4
410	FISHMEAL 64%	[X]	7.5	225.0	11.25
420	BLOODMEAL	[X]	1.5	45.0	2.25
450	WHEY POWDER 11%	[X]	10.0	300.0	15.0
500	WATER	[X]	1.0	30.0	1.5
502	NATUPHOS 5000	[X]	0.01	0.3	0.015
504	TALLOW-ENZYME	[X]	2.0	60.0	3.0
520	TALLOW-MIXER	[X]	1.733333	52.0	2.6
551	SALT BIN ADD	[X]	0.2	6.0	0.3
600	LYSINE-HCL	[X]	0.416667	12.5	0.625
605	DL-METHIONINE	[X]	0.086667	2.6	0.13
610	THREONINE	[X]	0.183333	5.5	0.275
615	ISOLEUCINE H/A	[X]	0.016667	0.5	0.025
620	TRYPTOPHAN H/A	[X]	0.046667	1.4	0.07
650	ZINC OXIDE	[X]	0.276667	8.3	0.415
700	QAF CREEP PMX	[X]	0.166667	5.0	0.250001
770	ENDOX	[X]	0.02	0.6	0.03
989	RONOZYME	[X]	0.03	0.9	0.045
1121	WHEAT 1895	[X]	61.546666	1846.4	92.32
			100.0	3000.0	150.0

Table 37: Diet formulation for sprouted wheat 1896

```
================================================       Rivalea       (6198): ============================================

Single-Mix         (FM)       * Corowa *        {10} OCTOBER 2011              ALL DATA            16:50 18/10/11  0005 :
: 1027.1/2.11                       (    1)  Plant=1        David
:=============================================================================================================================
                                                Formula basic data
                    ------------------------------------------------------------------------------
                    Code      :       9799          Name      : WEANER 1 BASE DIET SHOT AND SPRUNG – WHEAT 1896_SPROUT

                    External reference: 6,P,W,W1
                    Script file name  :
                                                      Analysis
   ---------------------------------------   ---------------------------------------   ---------------------------------------
   [VOLUME]   %         :       100.0       SERINE     %         :         0.888979   LAYER:ME   KCALS/KG  :      3067.03897
   [DRYMAT]   %         :        90.224967  GLUTAMIN   %         :         4.202171   SULPHUR    %         :         0.301948
   DE_PIG     MJ/KG     :        14.820344  PROLINE    %         :         1.267426   COPPER     PPM       :         5.191527
   NE4G       MJ/KG     :        10.341517  OH_PROLI   %         :         0.950229   COBALT     PPM       :         0.061547
   #ALY/NE4G  GM/MJ     :         0.129692  ASPARAG    %         :         1.628659   MANGANES   PPM       :        11.691465
   DEENZYME   MJ/KG     :        15.134173  #LYS/DE_   GM/MJ     :         0.099778   ZINC       PPM       :       367.271058
   PROTEIN    %         :        21.05105   #ALY/DE_   GM/MJ     :         0.090498   IRON       PPM       :       273.1453
   FAT        %         :         5.755938  #MET/LYS   G/G       :         0.316315   IODINE     PPM       :         1.563802
   STARCH     %         :        38.711666  #M+C/LYS   G/G       :         0.551519   SELENIUM   PPM       :         0.699048
   FIBRE      %         :         2.096655  #THR/LYS   G/G       :         0.65029    #AME/ALY   G/G       :         0.296943
   ASH        %         :         5.085922  #ISO/LYS   G/G       :         0.520184   #ACY/ALY   G/G       :         0.280585
   CALCIUM    %         :         0.879355  #TRY/LYS   G/G       :         0.199495   #AM+/ALY   G/G       :         0.482738
   T:PHOS     %         :         0.688618  #VAL/LYS   G/G       :         0.711458   #ATH/ALY   G/G       :         0.627498
   AV:PHOS    %         :         0.643775  AMETH      %         :         0.398262   #AIS/ALY   G/G       :         0.493394
   ENZAVPHOS  %         :         0.597017  AM+C       %         :         0.647452   #ATR/ALY   G/G       :         0.159264
   CAL:PHOS   G/G       :         1.276985  ATHREO     %         :         0.841606   #AVA/ALY   G/G       :         0.595999
   CAL:AVPHOS G/G       :         1.365935  AISOLEUC   %         :         0.661744   #ATH/DE_   GM/MJ     :         0.056787
   P:PHOS     %         :         0.177603  ATRYPTO    %         :         0.213607   ATYROSIN   %         :         0.607044
   CAL:ENZAVP G/G       :         1.472914  AVALINE    %         :         0.799359   AALANINE   %         :         0.867551
   LYSINE     %         :         1.478737  ACYSTINE   %         :         0.376324   AASPARTI   %         :         1.324157
   ALYSINE    %         :         1.341208  AP+T       %         :         1.407584   AASPARAG   %         :         1.435061
   METHION    %         :         0.467746  APHENYL    %         :         0.789135   AGLUTAMI   %         :         3.601352
   M+C        %         :         0.815552  ALEUCINE   %         :         1.368432   AGLUT:IN   %         :         3.632248
   THREO      %         :         0.961608  AHISTID    %         :         0.494495   AGLYCINE   %         :         0.95689
   ISOLEUC    %         :         0.769215  AARGININ   %         :         1.017556   ASERINE    %         :         0.76501
   TRYPTO     %         :         0.295     SALT       %         :         0.795482   APROLINE   %         :         1.098946
   CYSTINE    %         :         0.354233  %LEGUMES   %         :         9.0        LNAA       GM        :        20.568497
   VALINE     %         :         1.052059  ABC        MEQ/KG    :       569.819565   #TRY/LNA   G/G       :         0.046751
   HISTIDIN   %         :         0.573714  SODIUM     %         :         0.290208   BULKDENS   KG/HL     :        63.336668
   LEUCINE    %         :         1.548435  POTASS     %         :         0.570819   IONOPHORE  PPM       :         0.0
   PHENYLAL   %         :         0.899816  CHLORIDE   %         :         0.505356   W6 FA      %         :         0.0
   P+T        %         :         1.722835  MAGNES     %         :         0.148366   W3 FA      %         :         0.0
   ARGININE   %         :         1.169109  NA+K_CL    MEQ/KG    :       130.43282    W6:W3      G/G       :         0.0
   TYROSINE   %         :         0.70673   CHOLINE    MG/KG     :      1714.599313   SAT FA     %         :         0.0
   T:EAA      %         :         9.61476   LACTOSE    %         :         6.0        MONO FA    %         :         0.0
   ALANINE    %         :         1.0891    N:D:F:     %         :         8.8056     POLY FA    %         :         0.0
   ASPARTIC   %         :         1.672899  LINOLEIC   %         :         0.864032   ENDF       %         :         3.608062
   GLYCINE    %         :         1.206079  A:D:F:     %         :         2.106967
   GLUTAMIC   %         :         4.416984  RUMIN:ME   MJ/KG     :        12.559153
                          Raw material                        Available       %           [Kg]          Tonnes
          ------------------------------------------------- --------- ------------ ------------ ----------------
                300 CANOLA MEAL 36%                          [X]         4.0          120.0           6.0
                325 SOYABEANMEAL-48%                         [X]         5.0          150.0           7.5
                400 MEATMEAL                                 [X]         4.266667     128.0           6.4
                410 FISHMEAL 64%                             [X]         7.5          225.0          11.25
                420 BLOODMEAL                                [X]         1.5           45.0           2.25
                450 WHEY POWDER 11%                          [X]        10.0          300.0          15.0
                500 WATER                                    [X]         1.0           30.0           1.5
                502 NATUPHOS 5000                            [X]         0.01           0.3           0.015
                504 TALLOW-ENZYME                            [X]         2.0           60.0           3.0
                520 TALLOW-MIXER                             [X]         1.733333      52.0           2.6
                551 SALT BIN ADD                             [X]         0.2            6.0           0.3
                600 LYSINE-HCL                               [X]         0.416667      12.5           0.625
                605 DL-METHIONINE                            [X]         0.086667       2.6           0.13
                610 THREONINE                                [X]         0.183333       5.5           0.275
                615 ISOLEUCINE H/A                           [X]         0.016667       0.5           0.025
                620 TRYPTOPHAN H/A                           [X]         0.046667       1.4           0.07
                650 ZINC OXIDE                               [X]         0.276667       8.3           0.415
                700 QAF CREEP PMX                            [X]         0.166667       5.0           0.250001
                770 ENDOX                                    [X]         0.02           0.6           0.03
                989 RONOZYME                                 [X]         0.03           0.9           0.045
               1122 SHOT AND SPRUNG WHEAT 1896               [X]        61.546666    1846.4          92.32
                                                                      ------------ ------------ ----------------
                                                                        100.0         3000.0         150.0
                                                                      ------------ ------------ ----------------
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G. Manufacture of Pellet Diets

G.1 Background

Grains and non-grain materials (additives) undergo heat-moisture treatments to make pellets. The steps involved in pellet manufacture involve milling the base grains, mixing with additives and conditioning the mixture before pelleting, cooling and distributing to piggeries. Depending on location or end use, grains, additives and mill types and settings can vary, so do process times and temperatures. These conditions affect pellet quality, and availability of its nutrients, and they influence process response such as energy consumption. Establishing material-process-property relationships is paramount to optimising pellet manufacture for maximum desirable pellet properties.

G.2 Methods

G.2.1 Milling

The wheats were ground in a Ripple Mill^{TM} (Figure 21) at a fixed setting. The additives that were required from the diet formulation (Tables 35 – 37) were sourced and mixed at Rivalea Australia, Corowa NSW and supplied as ready-to-use.

G.2.2 Particle size distribution

The particle size distribution of the wheats were analysed following standard procedures (ASABE, 2008), and D_{gw} and S_{gw} were obtained and used to characterise the distributions as before. Also, the particle size distribution of the additives was analysed from representative samples of three batches.

G.2.3 Experimental design

The pellets were manufactured at the University of Sydney, Camden using a pilot-scale pellet mill (Fig. 22). After preliminary runs for stable processing, the conditions stated below were used to statistically design an experiment with 1⅓ replicates to yield 24 batches of pellets (Table 38).

- Wheat: Soft, Hard and Sprouted; 3 levels
- Conditioning temperature (^{o}C): 60, 70 and 80
- Conditioning time (sec.): 7 and 14
- Total batches = 3 x 3 x 2 x 1⅓ = 24

The pellets were manufactured in a random order (Table 38), and samples (3 x 1 kg each) were taken for storage and laboratory analyses.

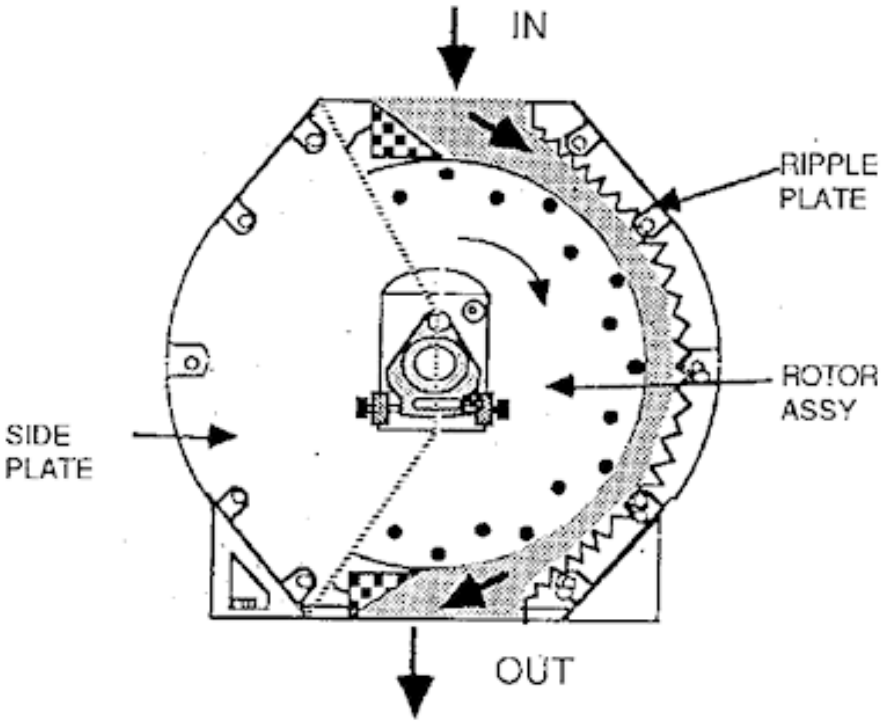

Figure 21: **A schematic diagram of a ripple mill (Source: http://www.satake.com.au/size_reduction/Ripple_Mill.htm; accessed 14 January 2013)**

Figure 22: **The pellet mill (Courtesy - Palmer Milling Engineering, Griffith NSW 2680)**

Table 38: Processing and replicated conditions for the pellets

Diet No.	Combination	Conditioning		Grain ID
		Temp(^{o}C)	Speed (sec)	
1	t_80^{o}C_s_7 sec_g_1894	80	7	1894
2	t_60^{o}C_s_7 sec_g_1894	60	7	1894
3	t_80^{o}C_s_14 sec_g_1896_SPROUT	80	14	1896_SPROUT
4	t_70^{o}C_s_14 sec_g_1896_SPROUT	70	14	1896_SPROUT
5	t_70^{o}C_s_7 sec_g_1896_SPROUT	70	7	1896_SPROUT
6	t_80^{o}C_s_7 sec_g_1896_SPROUT	80	7	1896_SPROUT
7	t_70^{o}C_s_14 sec_g_1895	70	14	1895
8	t_60^{o}C_s_14 sec_g_1895	60	14	1895
9	t_70^{o}C_s_7 sec_g_1895	70	7	1895
10	t_60^{o}C_s_14 sec_g_1895	60	14	1895
11	t_80^{o}C_s_14 sec_g_1894	80	14	1894
12	t_60^{o}C_s_7 sec_g_1894	60	7	1894
13	t_70^{o}C_s_14 sec_g_1894	70	14	1894
14	t_70^{o}C_s_7 sec_g_1894	70	7	1894
15	t_80^{o}C_s_7 sec_g_1895	80	7	1895
16	t_80^{o}C_s_14 sec_g_1895	80	14	1895
17	t_60^{o}C_s_7 sec_g_1896_SPROUT	60	7	1896_SPROUT
18	t_80^{o}C_s_14 sec_g_1896_SPROUT	80	14	1896_SPROUT
19	t_70^{o}C_s_14 sec_g_1894	70	14	1894
20	t_60^{o}C_s_14 sec_g_1894	60	14	1894
21	t_80^{o}C_s_7 sec_g_1895	80	7	1895
22	t_60^{o}C_s_7 sec_g_1895	60	7	1895
23	t_60^{o}C_s_14 sec_g_1896_SPROUT	60	14	1896_SPROUT
24	t_70^{o}C_s_7 sec_g_1896_SPROUT	70	7	1896_SPROUT

G.2.4 Process parameters

The temperatures of the conditioned (post-conditioner) samples were periodically recorded as well as the power (current, Amp) usage of the pellet press. The specific mechanical energy (SME) consumption during pelletisation was calculated as (Eqn. 3; C2.3):

$$SME_{pelletiser} = \frac{Am \times P_R}{Am_{MAX} \times G}$$

where, P_R = power rating of the pellet press (30 kW), G = mass flow rate (kg/hr), Am = operating current (ampere) of the pellet press, Am_{MAX} = Maximum current (ampere) of the pellet press (53 A).

G.3 Results

Figure 23 shows the particle size distributions and parameters of the wheats and additives. Although there were differences in their physicochemical properties (Table 34), the wheats ground to essentially the same particle size characteristics in the Ripple Mill^{TM}. In view of the influence of particle size on functional and digestibility properties of diets, it is unlikely that particle size would exercise substantial between-wheat effects on the properties of the diets being studied. The additive batches were mixed and packaged to be essentially the same, and even though there were batch differences on the weight retained on sieves 1.00, 0.75 and 0.50 mm, the particle size parameters did not reflect any marked batch differences (Fig. 23B). In previous projects, particle size was demonstrated to markedly influence functional, digestibility and animal performance. However, the results obtained with the wheats and additives show that any differences measured in the properties of the diets or process response could not be due to particle size effects.

Amongst others, temperature and energy consumption are important process responses during pellet manufacture. Unfortunately, there were logistic problems that prevented all the planned data to be collected and collated. Because of the missing data, the measured temperature and specific mechanical energy data were not subjected to any rigorous statistical analysis. The means and standard errors were calculated, and their nominal values were compared for likely trends.

Figure 24 shows that irrespective of the wheats and conditioning time, the conditioned samples reached the set temperatures (coefficient of variation ≈ 1%) before pelletisation. However, it appears there were processing effects on the SME (Table 39). Possibly because of the inverse relationships between temperature and viscosity, more energy was used during pelletisation as the conditioning temperature was reduced. In studies with extrusion, the viscosity of materials being processed (melt viscosity) predominantly controls SME. With more endogenous enzymes (e.g. amylases), the sprouted wheat was expected to have the lowest viscosity, and, consequently the lowest SME amongst the wheats. This is reflected in Table 39. Also, melt viscosity is generally affected by starch (positive) and fat (negative). Even though there were nominal differences in the composition of the wheats (Table 34), the diets were formulated to essentially the same starch and fat contents (Tables 35 – 37). While this suggests the rheological behaviours are expected to be identical, there might be non-nutrient differences and/or heat-induced nutrient differences. Rheological properties of samples have proved useful in explaining SME in processes like extrusion. Logistic problems prevented conditioned samples being obtained, and consequently analysed, in the present study. Hence, there are no immediate reasons for the SME differences in the soft and hard wheats, but other properties of the diets discussed below might be useful in explaining these differences. However, it seems understanding feed processing demands rheological characterisation of conditioned samples, and experiments in future studies could be planned along these lines.

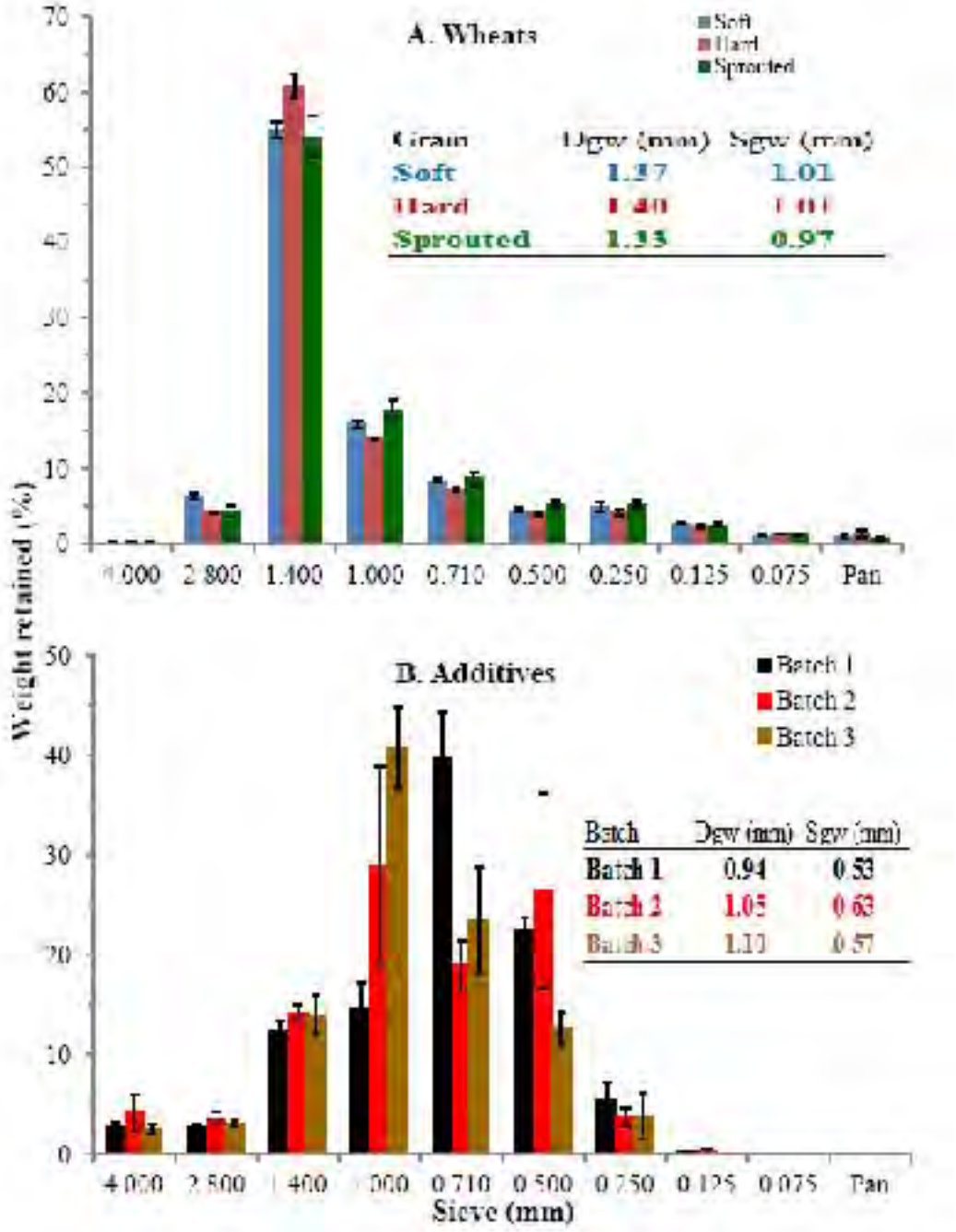

Figure 23: **Particle size distributions and parameters. A – wheats, B – additives (errors bar are sd)**

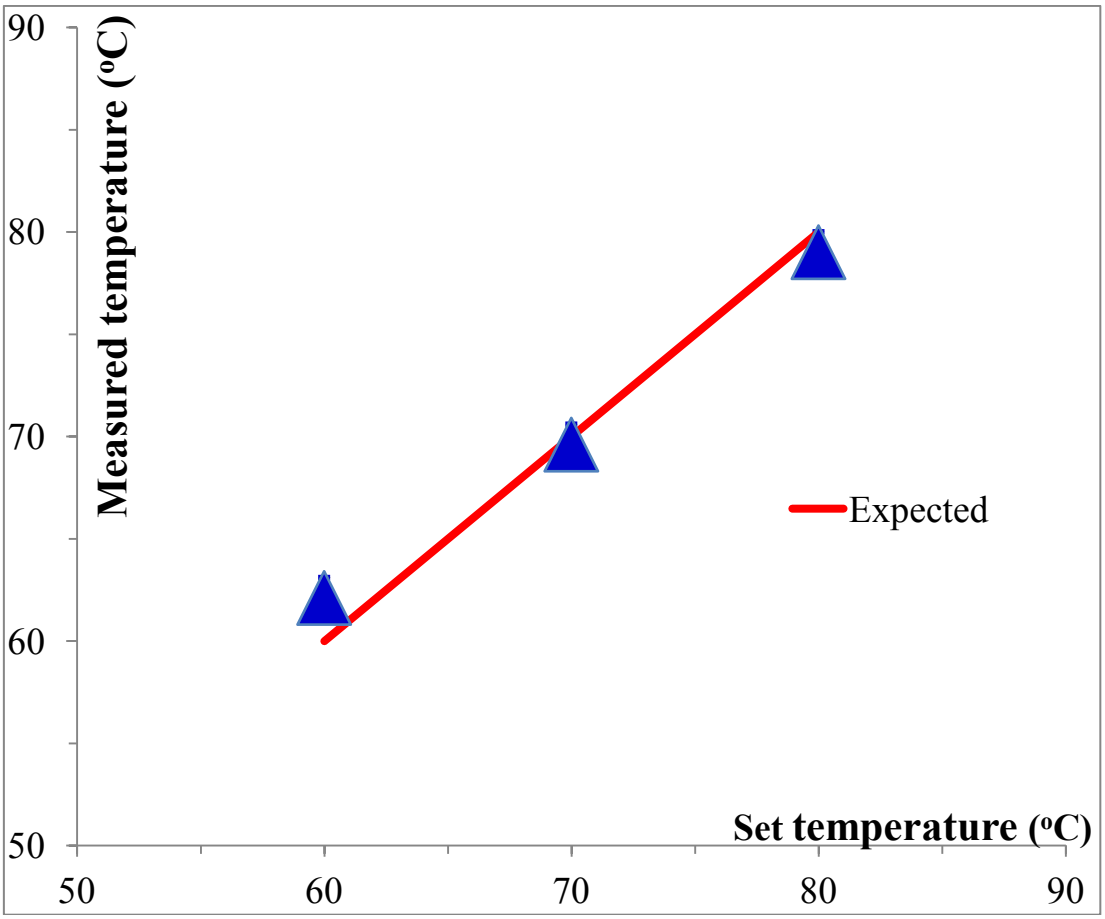

Figure 24: **The measured temperature of the conditioned samples against the set temperature**

Table 39: **The main effects of the processing conditions on the specific mechanical energy (SME)**

Main effect	SME (kJ/kg)*
Grain	
Soft wheat 1894	36.3 ± 0.64
Hard wheat 1895	31.3 ± 1.81
Sprouted wheat 1896	32.1 ± 1.35
Temperature (°C)	
60	35.6 ± 0.97
70	33.4 ± 1.65
80	31.8 ± 1.56
Time (sec)	
7	33.1 ± 1.21
14	33.8 ± 1.35

*Values are means ± Standard Error of Mean (SEM)

H. Proximate Composition of the Pellet Diets

H.1 Background

As detailed in Tables 35 – 37, the diets were formulated based on NIRs predictions. Examining the actual composition of the diets is beneficial in ascertaining the predicted nutrients, putting the actual contents in functional and digestibility properties that need these and strengthening relevant NIRs calibrations. Experiments were statistically designed to analyse the proximate composition of the diets.

H.2 Methods

The moisture, total starch, protein, fat and ash contents of the pellet diets were determined following standard procedures (F.2.3).

H.3 Results

Table 40 shows the measured and NIRs-predicted values, and Appendices II – VI are the statistical reports on the moisture, starch, protein, fat, and ash contents respectively. The R-package ASREML was used for analysing the laboratory data for these reports. In addition, a t-test (Minitab$^{(R)}$ ver. 16) was performed to examine significant differences between the measured and predicted values.

The diets were formulated (Tables 35 – 37) for different protein contents, but for essentially the same dry matter (90.2%), ash (5.1%), fat (5.8 – 6.2%), and starch (36.2 – 38.7%) contents. However, the mash and/or pellets were significantly ($p<0.05$) lower in the measured dry matter and starch contents than formulated, while the ash, fat and protein contents were significantly higher than formulated (Table 40). There were no grain effects on the ash and fat contents of the diets (Appendices V – VI), but grain effects were measured on the dry matter and starch contents (Appendices II – III). With reference to the dry matter, the pellets (87.8%) were significantly wetter than the mash (89.0%), and there were conditioning temperature and time effects. Contrary to expectations, the higher the conditioning temperature, the wetter were the pellets. It is expected that with more heat, more water would be evaporated as temperatures dropped to ambient. However, with more heat, the various physicochemical transformations (e.g. starch gelatinisation) in mash would be enhanced thereby increasing water-binding ability that could reduce pellet dry matter. Water-binding abilities are also expected to be enhanced when residence time in conditioners increases, and hydrophilic components and accompanying transformations have time to bind water. These will reduce evaporated water, increase moisture content or decrease dry matter of pellets. However, the dry matter at the two conditioning times of 7 sec. (87.7%) and 14 sec. (87.9%) were essentially the same.

The starch content was significantly ($p<0.05$) grain-dependent (Appendix III), with the soft wheat having the highest (38.6 g/100 g solids) starch content, while both the hard (33.4 g/100 g solids) and sprouted (35.7 g/100 g solids) wheats were not significantly ($p>0.05$) different in starch content. This trend follows the starch content of the grains (Table 34), and as highlighted in C.3, the starch content can explain the specific mechanical energy (SME) consumption when the pellets were processed. The soft wheat, with the highest

Table 40: Proximate composition of the pellet diets

Composition (g/100 g solids)[#]	Soft wheat – 1894		Hard wheat – 1895		Sprouted wheat – 1896	
	Predicted[†]	Measured*	Predicted[†]	Measured*	Predicted[†]	Measured*
Dry matter	90.2	87.6 - 90.1	90.2	87.3 – 89.4	90.2	86.1 – 88.9
Protein	22.9	22.1 – 24.6	27.4	27.7 – 29.4	23.4	22.6 – 26.4
Fat	6.9	7.4 – 9.0	6.9	7.3 – 8.0	6.4	7.0 – 9.1
Total starch	42.7	33.6 – 42.2	40.1	27.3 – 36.1	42.9	29.6 – 39.8
Fibre	1.9	nd[‡]	2.2	nd	2.3	nd
Ash	5.6	6.4 – 8.0	5.6	6.7 – 7.6	5.6	6.2 – 8.4

[#]Dry matter was expressed as g/100 g (wet basis)
[†]Diet formulations from NIR predictions (Table 1)
[*]Wet chemistry and predictions from statistical analysis
[‡]nd = not applicable or determined

starch content, had the highest SME, while the hard and sprouted wheats with similar starch contents had essentially the same SME. With the limited data available, there seems to be a direct relationship between starch content and SME during pelletisation. More data are, however, required for the exact relationship, but it is worth stating that, in addition to starch content (quantity), starch properties or quality (amylose content, granule size, degree of polymerisation and branching, crystallinity pattern, etc.) will define melt rheology during processing.

Despite being wheats, the present study has shown that there can be differences due to variety or pre-harvest conditions experienced by grains. Differences have been measured in the chemical composition of the resulting pellets. Moreover, these differences can extend to the quality and other properties of the pellets, which can also depend on process conditions.

I. Pellet Quality: Hardness and Durability

I.1 Background

Hardness and durability of pellets are important mechanical and textural properties, and amongst others, they determine pellet quality. These properties influence the stability of pellets during handling and transporting, and how pellets disperse in gastrointestinal fluids with consequence for feed intake, feed flow, digestibility, and animal performance. As stated in Part One of this report (C.3.1.1.) pellet is considered unsatisfactory or low in quality if durability is less than 90% and hardness is less than 7 kg, but values less than these have been reported (Zimonja & Svihus, 2009). Previous Pork CRC project revealed how these properties are affected by raw materials and feed process conditions, but the specific trends with grain varieties, and process temperature and time were not clearly defined. Moreover, more data are required to strengthen the prediction of pellet durability and hardness from material and process data as expatiated in section L below.

I.2 Methods

The 24 pellets described in G.2.3 were analysed for their durability (Holmen pellet tester) and hardness (Amandus Kahl tester) using standard procedures. The hardness analysis was done in triplicate, while the durability analysis was not replicated because of limited sample size. The data were analysed using the GLM model of Minitab ver. 16, and results are only presented as means and standard errors.

I.3 Results

Irrespective of the wheat, and conditioning temperature and time, the hardness of the pellets was less than 7 kg (Table 41). However, from the durability values of 90% and above, the pellets can be classified as acceptable. There were no effects of grain, and conditioning temperature and time, and their interactions on the pellet durability, but a significant grain effect was obtained on the hardness. The pellets from the sprouted wheat were significantly the softest (4.0 kg), while the pellets from the soft wheat (5.1 kg) were not significantly harder than those from the hard wheat (4.9 kg).

Because of their importance in pellet quality, various studies have been conducted on pellet durability and hardness, and their dependence on feed processing. Contrary to the wheat effects in the present study, Jha *et al.* (2011) did not obtain a significant varietal effect from 12 Canadian wheats, despite compositional differences. Possibly because of different heat-moisture-shear treatments during cold-pelletisation (CP), steam-pelletisation (SP) and extrusion (EX), Zimonja & Svihus (2009) observed that heat addition to wheat- and oat-based diets increased pellet durability by about 30%, and almost doubled the hardness. Because of partial starch gelatinisation and protein denaturation in the same study, cold pelletisation yielded pellets of about 80% durability and 25 N (2.6 kg) hardness. Contrary to the non-significant effects of conditioning temperature (60 – 80^{o}C) in the present study, Lundblad *et al.* (2011) reported an increase in pellet durability by doubling the conditioning temperature of their wheat-based diets.

Table 41: Predicted hardness and durability of the 18 pellet treatments*

Grain	Conditioning temperature (°C)	Conditioning time (sec)	Hardness (kg)		Durability (%)	
			Value[#]	SE[†]	Value	SE
Soft	60	7	4.5	0.44	92.2	3.35
Soft	60	14	5.0	0.63	92.6	4.74
Soft	70	7	5.0	0.63	94.0	4.74
Soft	70	14	5.5	0.44	93.4	3.35
Soft	80	7	5.0	0.63	94.2	4.74
Soft	80	14	5.7	0.63	91.9	4.74
Hard	60	7	4.3	0.63	90.4	4.74
Hard	60	14	5.0	0.44	91.2	3.35
Hard	70	7	4.7	0.63	89.1	4.74
Hard	70	14	4.3	0.63	94.8	4.74
Hard	80	7	5.2	0.44	92.1	3.35
Hard	80	14	6.0	0.63	96.4	4.74
Sprouted	60	7	5.0	0.63	91.6	4.74
Sprouted	60	14	4.3	0.63	84.3	4.74
Sprouted	70	7	3.8	0.44	86.6	3.35
Sprouted	70	14	3.3	0.63	93.0	4.74
Sprouted	80	7	3.3	0.63	89.6	4.74
Sprouted	80	14	4.0	0.44	89.3	3.35

[#]Value = predicted mean from the Minitab ver. 16 General Linear Model procedure
[†]SE = standard error

As expected, Lundblad *et al.* (2011) also measured an increase in starch gelatinisation of the diets at the higher conditioning temperature. However, the measured degrees of starch gelatinisation (24 – 77 g/100 g) in the expanded and extruded diets are not commensurate with the durability (90 – 91%) of the pellets. While gelatinised starch binds pellet particles and improves durability and hardness, denatured proteins were thought to bind the particles in starch-free pellets (Zimonja & Svihus, 2009). Hence, chemical compositions of the raw materials are important. However, in the present study, the starch and protein contents of the wheats were not substantially different to explain the trends with the pellet hardness reported above. Perhaps, starch and protein states in the pellets, discussed below, can explain the measured pellet quality. It is also relevant to explore how pellet durability and hardness influence animal performance.

J. Pasting Properties of the Pellets

J.1 Background

Through hydrogen bonding, ingredients in feeds swell to various extents in water or aqueous media. The swelling behaviours are mainly defined by starch, and in processed feeds, by any heat-moisture treatments that starch had been subjected to. When heated during swelling in excess water, as in Rapid Visco-Analyser (RVA), starch pastes, and the changes in its viscosity are measures of its pasting properties. In previous projects, the importance of pasting properties, which are functional attributes, in evaluating the quality of pig diets, and consequently animal performance, was discussed. The various grain and processing factors that affect these properties were highlighted. In the present project, changes to the pasting properties of mash and pellets were investigated.

J.2 Methods

The twenty-four (24) pellet samples produced as in Table 38 were collected with the corresponding mash, and ground in a laboratory hammer mill through a 1 mm retention sieve, to create samples of a consistent size for analysis. Both the milled and non-milled samples were chilled-stored prior to analysis. As in previous projects (Gidley *et al.*, 2010), Rapid Visco-Analyser (RVA) was used with 10% solids in each 25 g-dispersion, and standard profile 1. The variables below were obtained using the RVA software:

- Initial viscosity – Pasting viscosity at 1 min.
- Pasting temperature – Temperature of an appreciable change (>10%) change in viscosity
- Peak viscosity – Highest pasting viscosity prior to cooling the gelatinised starch dispersion
- Trough viscosity – Lowest pasting viscosity during holding the gelatinised starch dispersion at about 95^{o}C
- Final viscosity – Viscosity at the end of cooling and holding at 50^{o}C

The pasting properties of the mash and pellet samples were statistically analysed, and the change (mash-pellet) in the pasting parameters were computed to understand the contributions of the processing conditions.

J.3 Results

Figure 25 shows that the grains pasted differently, with the pellets from the sprouted wheat being the most shear-thinned, while those from the hard wheat were the least shear-thinned. With endogenous enzymes, amylolysis combined with swelling and gelatinisation in the sprouted wheat to reduce the paste viscosity of the sprouted-wheat diets (and mash). The high proteins in the hard wheat could have led to relatively high starch-protein interactions, which possibly reduced swelling and gelatinisation during pasting. However, with the soft wheat pellets, in which endogenous enzymes or restricted swelling were relatively absent, the pasting behaviours were typical of regular wheats.

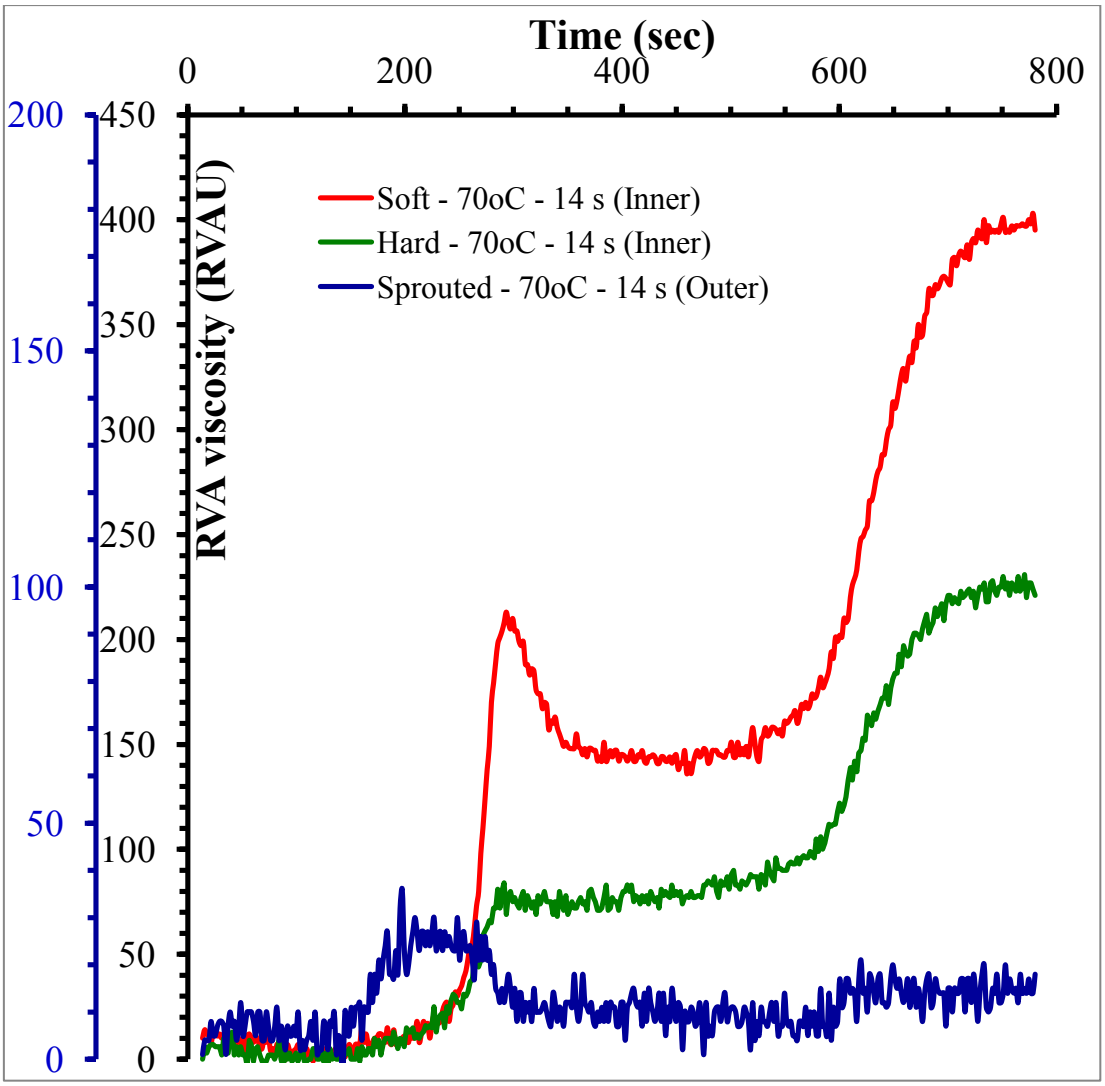

Figure 25: Typical viscograms of the pellets showing grain effects

Statistical analysis of the pasting behaviours of the mash and pellets (Appendix VII) revealed that the pellets generally pasted at the same temperature as the mash because the main effect of type was not significant (Table 42). However, Table 42 shows the type of grain (soft, hard or sprouted) significantly ($p < 0.05$) affected all the pasting parameters, apart from the initial viscosity. In addition to the significant main effects, there were significant interactions effects, and the predicted and least significant difference (LSD) values are summarised in Tables 43 and 44. It can be seen that, apart from differences amongst the grains, the conditioning temperature and time further defined the differences between diets.

Although values were predicted for the mash at different conditioning temperatures and times for each grain (Table 44), it is worth noting that the mash was not subjected to these conditions. Hence, the effects of processing are better visualised with the pellets (Table 44), and the changes (mash minus pellet) to the pasting parameters of the mash brought about by processing are summarised in Figure 26. The conditioning changed the time for the hard wheat to swell and reach its peak viscosity more than the other wheats, and the

Table 42: Summary of statistical analysis (p-values) of the RVA parameters as affected by the processing conditions

Term	Pasting temperature	Initial viscosity	Peak time	Peak viscosity	Breakdown	Trough viscosity	Final viscosity	Setback
Type (mash-pellet)	0.202	0.024	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Grain	0.013	0.974	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Temp	0.693	0.384	0.748	0.171	0.043	0.023	0.752	0.349
Time	0.494	0.377	0.579	0.001	0.018	0.001	0.708	0.944
Type x Grain	0.283	0.120	0.015	<0.001	<0.001	<0.001	<0.001	<0.001
Type x Temp	0.258	0.850	0.836	0.353	0.915	0.437	0.799	0.847
Grain x Temp	0.236	0.711	<0.001	0.024	0.152	0.015	0.056	0.036
Type x Time	0.864	0.865	0.026	0.112	0.531	0.101	0.558	0.731
Grain x Time	0.573	0.611	0.357	0.135	0.019	0.005	0.177	0.057
Temp x Time	0.911	0.709	0.036	0.315	0.046	0.077	0.177	0.229
Type x Grain x Temp	0.557	0.665	0.005	0.023	0.178	0.026	0.016	0.018
Type x Grain x time	0.144	0.965	0.929	0.009	0.854	0.040	0.064	0.044
Type x Temp x Time	0.994	0.051	0.656	0.016	0.787	0.073	0.870	0.665
Grain x Temp x Time	0.045	0.006	<0.001	0.001	0.202	0.004	0.082	0.009
Type x Grain x Temp x Time	0.478	0.473	<0.001	0.026	0.029	0.075	0.030	0.217

Table 43: Predicted RVA parameter values of the three-level interactions

Grain	Conditioning		Pasting Temp. (°C)	Initial viscosity (RVAU)#	Trough viscosity (RVAU)	Setback (RVAU)
	Temp. (°C)	Time (sec)				
Soft	60	7	63.6	11.6	85.7	14.1
Soft	60	14	61.3	13.9	108.5	14.2
Soft	70	7	73.4	14.3	107.8	16.3
Soft	70	14	71.5	13.3	111.3	14.7
Soft	80	7	60.9	20.3	63.3	9.4
Soft	80	14	80.3	18.4	95.6	14.8
Hard	60	7	69.2	11.1	62.3	12.0
Hard	60	14	67.9	15.3	60.9	11.5
Hard	70	7	65.6	12.9	39.3	10.3
Hard	70	14	67.9	13.7	60.5	10.8
Hard	80	7	70.0	18.8	73.9	11.5
Hard	80	14	67.7	9.7	61.5	10.9
Sprouted	60	7	60.2	24.4	NA†	3.0
Sprouted	60	14	68.6	6.6	NA	2.0
Sprouted	70	7	61.4	17.1	5.6	3.0
Sprouted	70	14	63.4	11.1	6.2	3.4
Sprouted	80	7	64.6	7.8	3.4	4.4
Sprouted	80	14	58.5	16.6	22.1	3.4
*LSD (5%)**			*11.85*	*10.34*	*[1.14]*	*[2.29]*

#1 RVAU = 12 cP.

†NA = Not available because values are either outliers or zeros.

*LSD (5%) = Least significant difference at 5% level, values in [ ] are for the non-transformed predicted values.

These apply to all tables where they appear.

effects were more at 70°C and 14 sec., and at 60°C and 7 sec. (Figure 26). The latter conditions (60°C and 7 sec.) also decreased the peak time for the sprouted wheat, while the former conditions (70°C and 14 sec.) increased the peak time for both the soft and sprouted wheats. The sprouted-wheat diets were, however, more affected. A reduction in the peak time could indicate an open structure or disruption of anti-swelling components or interactions in a material, thereby enhancing hydration and pasting, and potentially digestibility. However, irrespective of the wheat and conditioning conditions, the change in the peak time was less than 2 min., and it is doubtful if this indicates a measurable openness of the pellet structure or disruption of anti-swelling components. Comparatively, the processing conditions affected the peak and final viscosities of the wheats along the trend – soft > hard > sprouted (Figure 26). The peak and final viscosities of the pelleted sprouted wheat were generally less than the mash to suggest partial gelatinisation of the pellets during processing. On the other hand, these viscosities were higher in the pelleted soft and hard wheats than the mash. While this does not indicate gelatinisation, it possibly shows a destructurisation effect that enhanced swelling and pasting. At any of the

Table 44: Predicted RVA parameter values of the four-level interactions

Type	Grain	Conditioning temperature (^{o}C)	Conditioning time (sec)	Peak time (min)	Peak viscosity (RVAU)	Breakdown (RVAU)	Final viscosity (RVAU)
Mash	Soft	60	7	4.8	101.9	27.7	247.1
Mash	Soft	60	14	4.8	99.3	17.9	235.3
Mash	Soft	70	7	4.9	127.5	25.6	343.0
Mash	Soft	70	14	4.8	155.5	46.6	319.7
Mash	Soft	80	7	4.8	87.0	28.2	134.0
Mash	Soft	80	14	4.8	100.5	29.9	270.6
Mash	Hard	60	7	6.4	63.9	10.0	154.7
Mash	Hard	60	14	6.3	81.2	14.5	188.2
Mash	Hard	70	7	6.5	37.2	12.3	124.4
Mash	Hard	70	14	6.4	68.4	17.1	123.7
Mash	Hard	80	7	6.7	70.6	8.9	174.4
Mash	Hard	80	14	6.6	57.1	13.1	154.5
Mash	Sprouted	60	7	4.2	38.6	24.1	23.6
Mash	Sprouted	60	14	3.4	20.9	14.0	2.0
Mash	Sprouted	70	7	3.3	28.1	22.1	19.8
Mash	Sprouted	70	14	3.2	45.2	32.8	14.6
Mash	Sprouted	80	7	3.1	39.0	31.6	22.0
Mash	Sprouted	80	14	3.5	54.7	25.5	29.6
Pellet	Soft	60	7	4.9	148.2	51.3	316.2
Pellet	Soft	60	14	4.9	211.2	76.8	371.5
Pellet	Soft	70	7	4.9	191.5	68.6	399.8
Pellet	Soft	70	14	4.9	190.4	74.1	339.0
Pellet	Soft	80	7	4.8	136.9	75.0	166.0
Pellet	Soft	80	14	4.9	204.7	69.7	389.9
Pellet	Hard	60	7	4.8	89.0	10.8	254.8
Pellet	Hard	60	14	6.7	81.4	14.9	221.9
Pellet	Hard	70	7	6.7	61.1	11.6	223.4
Pellet	Hard	70	14	4.8	98.4	20.0	227.3
Pellet	Hard	80	7	6.7	95.2	10.3	249.6
Pellet	Hard	80	14	6.9	76.6	15.0	219.2
Pellet	Sprouted	60	7	3.5	39.1	34.4	9.4
Pellet	Sprouted	60	14	3.6	31.4	24.2	6.0
Pellet	Sprouted	70	7	3.7	30.9	31.7	10.1
Pellet	Sprouted	70	14	3.5	45.6	36.7	13.8
Pellet	Sprouted	80	7	3.9	31.5	28.7	16.3
Pellet	Sprouted	80	14	3.9	50.7	30.9	18.2
LSD (5%)				*0.44*	*30.09*	*11.17*	*[3.30]*

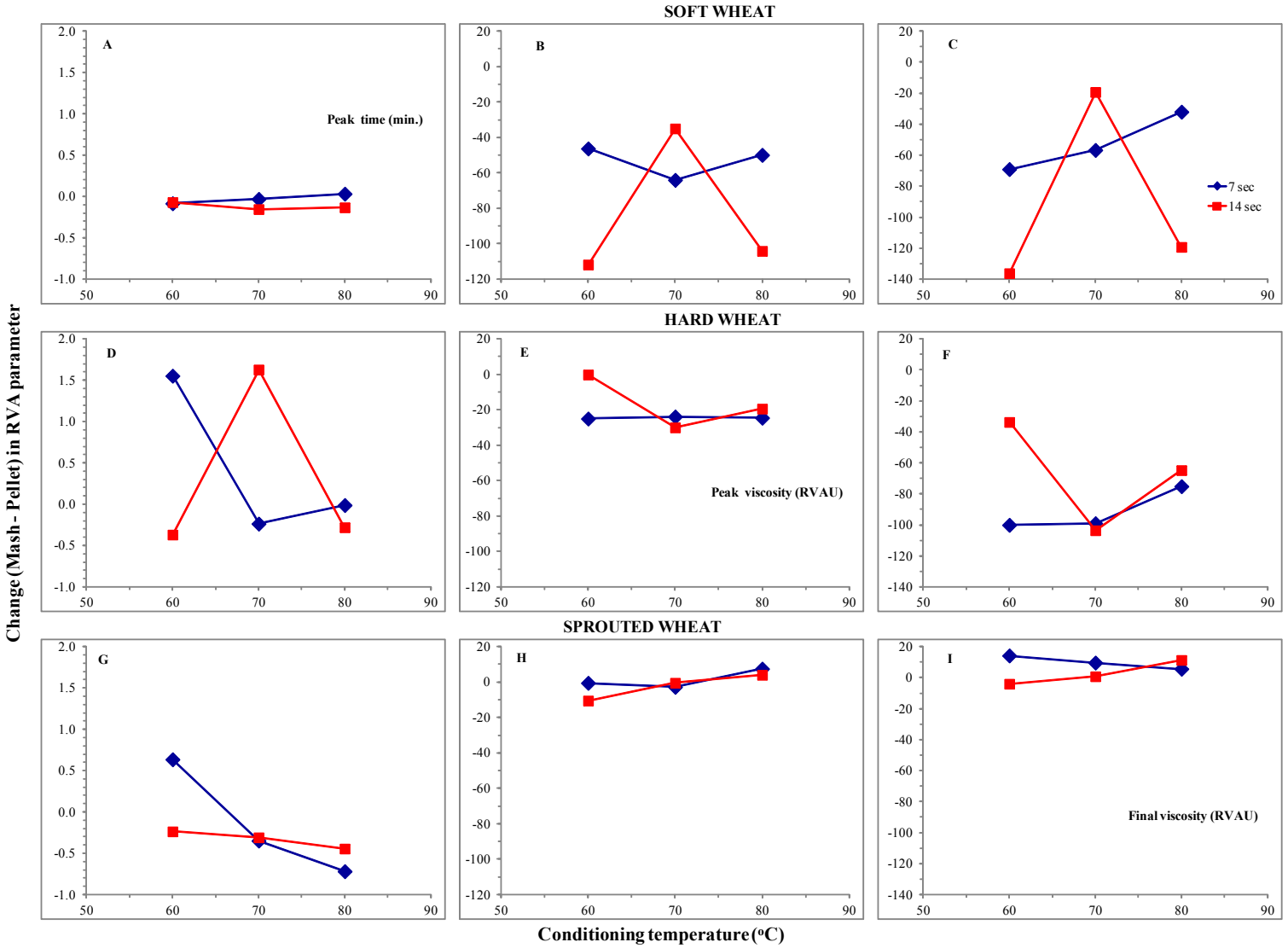

Figure 26: Dependence of the changes (mash-pellet) in pasting parameters of the mash on the grain, and conditioning temperature and time

conditioning temperature, the long conditioning time was beneficial to the soft and hard wheat, while the short conditioning time benefited the sprouted wheat, and the additional naturally-occurring enzymes in the latter were possibly responsible for the trend.

As expected, the time-temperature combinations during the conditioning process influenced the main pasting component (starch) in the pellet diets. The variety of the grain and the extent of weather damage affected the response of the diets due to structural differences or presence of naturally-occurring enzymes. It appears that while a long conditioning time at preferably a high conditioning temperature will aid desirable changes to the pasting properties of hard- or soft-wheat during feed manufacture, a short conditioning time at a low conditioning temperature will complement the actions of endogenous enzymes for desirable structural changes in pellet diets from sprouted wheat.

K. *In vitro* Starch Digestion of the Pellets

K.1 Background

Starch is the main energy-yielding component of grain-based pig feeds, and its digestibility is dependent on grain genotype and processing conditions because these factors affect its physicochemical transformations (e.g. gelatinisation, retrogradation and destructurisation). Starch digestion can be studied by *in vitro* and *in vivo* (animal) techniques, which have been reasonably correlated in many studies. Although kinetics of *in vivo* starch digestion can be investigated, time-course *in vitro* starch digestion is common, and the digestion parameters (e.g. rate of digestion, gastric digested starch and maximum digestible starch) used to understand processing and material effects.

K.2 Methods

The milled pellet diets (J.2) were analysed using the rapid *in vitro* starch digestion procedure of Sopade & Gidley (2009). For monophasic starch digestograms, the modified first-order kinetic model (Eqn. 1 in B.1) was used to describe the digestograms, and the following starch digestion parameters were also used (Sopade *et al.*, 2011) in characterizing the samples:

- Gastric digested starch (D_o, g/100 g dry starch)
- Measured or experimental digested starch ($ExpD_{240}$, g/100 g dry starch) at 4 hr
- Predicted digested starch (PreD240, g/100 g dry starch) at 4 hr using the modified first-order kinetic model
- Rate of starch digestion (min^{-1})
- Area under the starch digestogram (AUC, g.min/100 g dry starch
- Maximum digestible starch ($D_\infty = D_o + D_{\infty\text{-}o}$, g/100g dry starch)

K.3 Results

Irrespective of the grain and processing condition, the pellets exhibited monophasic digestograms (Figure 27). The grain types significantly affected the starch digestion parameters (Appendix VIII), and neither the conditioning time nor temperature significantly affected how starch digested in the pellets when the modified first-order kinetic model was used. The predicted parameters for the 18 pellet treatments are summarised in Table 45.

The time-course starch digestion data were also analysed using a linear mixed model incorporating cubic smoothing splines. The details of this approach with its statistics are in Appendix VIII.

Structure, gelatinisation and/or swelling ability affect(s) how starch digests in materials, in addition to the activity of endogenous enzymes. As identified in J.3, these factors are most likely responsible for the measured digestibility of the pellets.

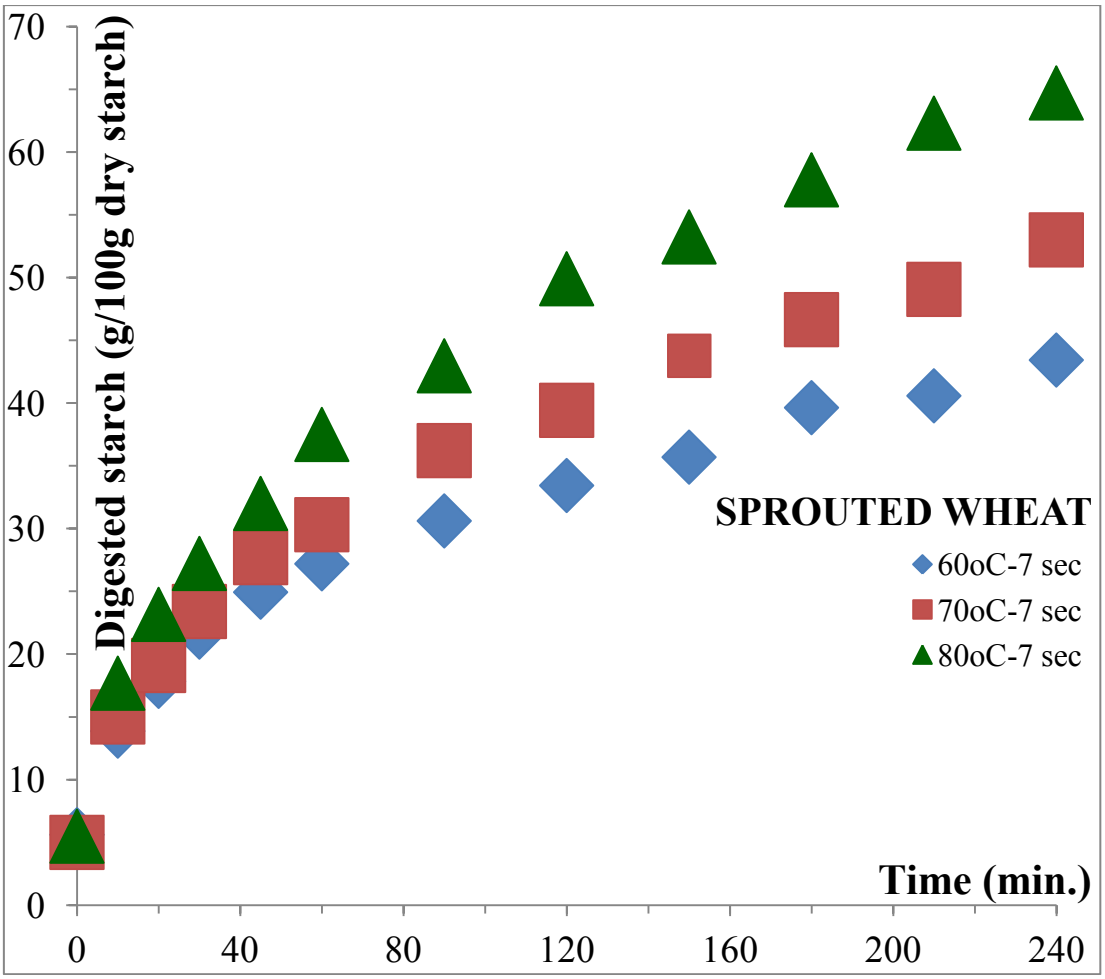

Figure 27: **Typical starch digestograms of the pellets showing the temperature effects**

Table 45: Predicted starch digestion parameters for the 18 pellet treatments using the modified first-order kinetic model*

Grain	Conditioning Temperature	Conditioning time	D_o		D_∞		K x 10^{-3}		AUC x 10^3		Experimental D_{240}		Predicted D_{240}	
	(^oC)	(sec)	Value[#]	SE[†]	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE
Soft	60	7	7.8	0.74	40.1	3.74	16.5	2.14	7.5	0.54	41.1	3.22	39.1	2.96
Soft	60	14	5.6	1.06	30.6	5.03	18.9	2.83	6.2	0.76	31.5	4.45	30.8	4.15
Soft	70	7	5.1	1.06	29.8	5.01	20.5	2.80	6.0	0.76	31.8	4.44	29.8	4.15
Soft	70	14	5.8	0.75	36.4	3.61	17.0	2.01	6.9	0.54	37.0	3.17	35.7	2.94
Soft	80	7	5.9	1.06	28.5	4.99	20.9	2.79	6.2	0.76	31.5	4.43	30.2	4.14
Soft	80	14	5.9	1.06	37.7	5.14	15.2	2.99	6.7	0.76	37.2	4.49	34.5	4.16
Hard	60	7	6.5	1.06	38.5	5.01	11.6	2.80	6.7	0.76	39.0	4.44	36.5	4.15
Hard	60	14	7.2	0.76	36.4	3.67	17.0	2.08	6.9	0.54	38.2	3.19	35.6	2.95
Hard	70	7	5.9	1.06	34.3	4.99	16.2	2.79	6.3	0.76	35.6	4.43	33.1	4.14
Hard	70	14	5.8	1.05	31.4	4.98	17.1	2.77	5.8	0.76	32.1	4.43	29.6	4.14
Hard	80	7	5.5	0.75	33.3	3.66	18.7	2.07	6.6	0.54	35.7	3.19	33.6	2.94
Hard	80	14	6.3	1.06	33.9	5.01	17.0	2.80	6.4	0.76	34.5	4.44	32.7	4.15
Sprouted	60	7	8.7	1.06	49.0	4.99	12.8	2.78	8.6	0.76	43.6	4.44	47.0	4.14
Sprouted	60	14	8.3	1.06	52.7	4.99	11.0	2.78	9.0	0.76	50.6	4.44	50.1	4.15
Sprouted	70	7	8.6	0.75	53.6	3.63	12.5	2.03	9.1	0.54	53.0	3.18	50.3	2.94
Sprouted	70	14	7.2	1.06	36.7	5.05	18.3	2.85	7.1	0.76	38.9	4.45	36.0	4.15
Sprouted	80	7	9.9	1.06	68.1	5.00	9.8	2.79	11.1	0.76	64.3	4.44	63.0	4.15
Sprouted	80	14	7.3	0.75	58.3	3.72	9.9	2.12	8.9	0.54	54.1	3.21	50.9	2.96

*The digestion parameters are as defined in K.2.
[#]Value = predicted value
[†]SE = standard error

L. Starch Gelatinisation Characteristics of the Pellets

L.1 Background

Feed processing involves heat-moisture treatments, and with starch in the mash, gelatinisation can occur, and this is suspected to partly define the pasting (J.3) and starch digestibility (K.3) behaviours of the pellets. Differential Scanning Calorimetry (DSC) is a common technique for investigating starch gelatinisation characteristics, and the resulting gelatinisation temperature and enthalpy from DSC, add to the overall understanding of the status of starch in materials.

L.2 Methods

The milled pellet and mash samples (J.2) were analysed in a TA DSC Q2000 differential scanning calorimeter (TA Instruments, Lukens Drive, New Castle, DE 19720, USA) as described before (Gidley *et al.*, 2010); total weight of 20–25 mg, sample:water ratio of about 1:4, rehydration for about 1 h at room temperature, isothermal at 30^{o}C for 5 min., scanning to 120^{o}C at 10^{o}C min^{-1}, empty pan served as the reference. The TA Universal Analysis^{TM} software was used to analyse the thermograms for the onset, peak and end temperatures, and enthalpy of gelatinisation.

L.3 Results

All the pellet and mash diets exhibited similar thermograms (Figure 28) akin to a single-stage endothermic behaviour, which is consistent with starch gelatinisation in excess water. Some samples exhibited endotherms at temperatures greater than 80^{o}C (not shown) that were most likely from non-starch components (e.g. protein denaturation). There was significant grain main effect across the gelatinisation parameters (Appendix IX), and, amongst the diets, the hard wheat-based diets gelatinised at the highest temperature (Figure 29A). Possibly because of desirable granular destructurisation with temperatures, the pellets gelatinised at temperatures that were inversely proportional to the conditioning temperature (Figure 29A).

The sample type x grain x temperature x time interactions significantly affected the gelatinisation enthalpy (Appendix IX), and the predicted values were used to calculate the degree of starch gelatinisation (DG) in the pellets:

$$DG = 100 - \frac{100 \times \Delta H_{gel,pellet}}{\Delta H_{gel,mash}}$$

where, $\Delta H_{gel,pellet}$ = enthalpy of gelatinisation of the pellet, and $\Delta H_{gel,mash}$ = enthalpy of gelatinisation of the mash. The trends of DG with grain, temperature and time are summarised in Figure 29A – 29D. The degree of starch gelatinisation of the sprouted grain at 80^{o}C and 7 sec. was about 60%, and this was considered an outlier (not shown).

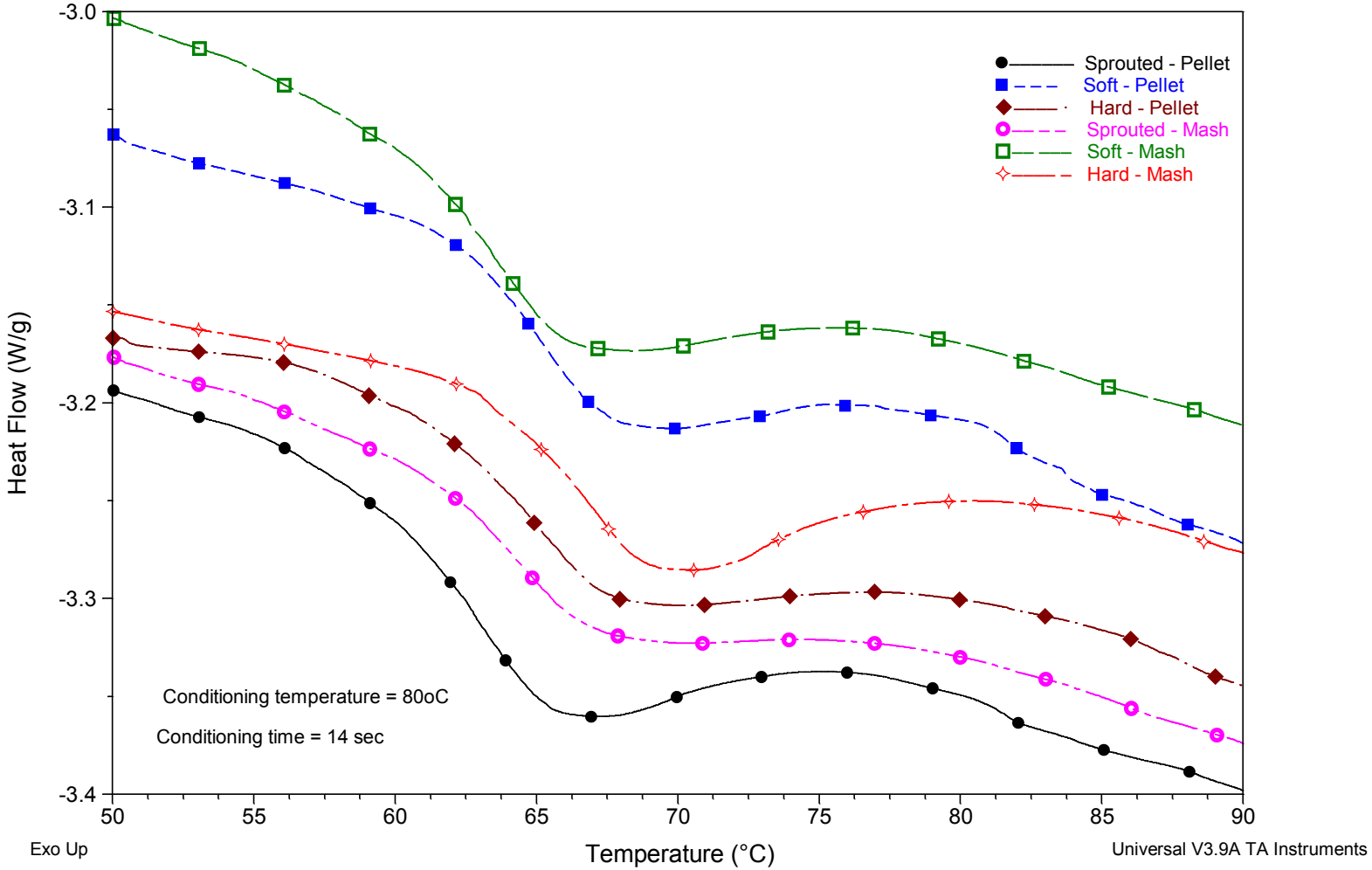

Figure 28: Typical thermograms of the pellets and corresponding mash

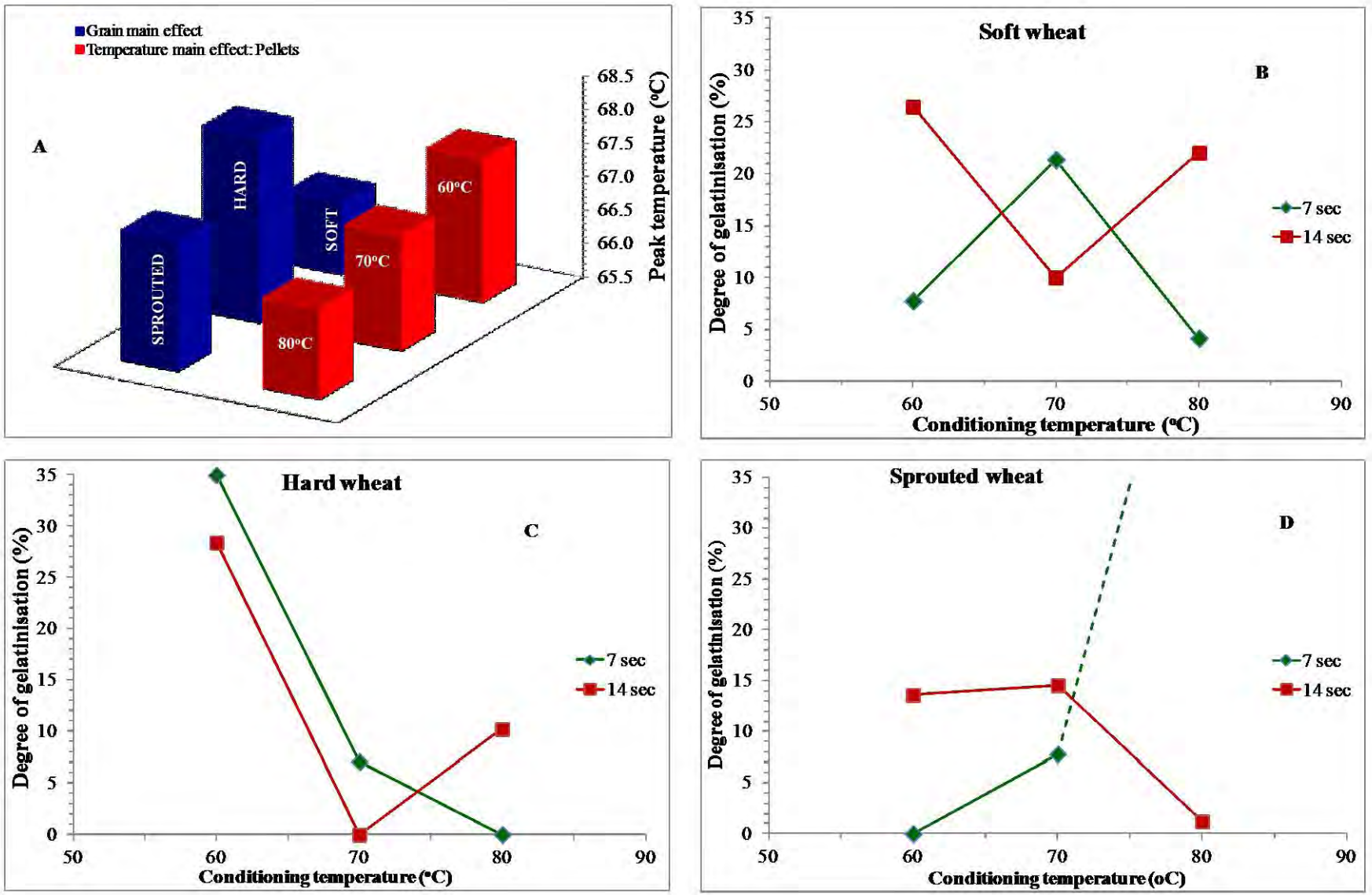

Figure 29: **Gelatinisation characteristics of the pellets showing the grain and conditioning temperature main effects, and the grain x conditioning temperature x conditioning time interactions. A - Peak temperature, B, C, D - Degree of starch gelatinisation**

Irrespective of the grain and processing, the degree of starch gelatinisation was less than 35%, with the sprouted wheat pellets generally exhibiting the lowest DG amongst the grains. The hard wheat diets showed the highest DG at the conditioning temperature of 60^{o}C, while the soft wheat diets had the highest DG at the other temperatures at any of the conditioning times. With its high protein content, the hard wheat was expected to produce pellets that would be least gelatinised. This is because the high protein content could lead to starch-protein interactions, or a hard-to-cook character that is typified in pulses or legume (Tinus *et al.*, 2012). Such could have been responsible for the high gelatinisation temperature in the hard wheat (Figure 29A). Moreover, contrary to expectations, conditioning at the highest temperature of 80^{o}C did not give the highest DG in the pellets.

Pig diets are not necessarily gelatinised completely, but high DG (up to 80%) in Australian commercial pellets had been measured (Gidley *et al.*, 2010). The low DG measured in the pellets agrees with the observations on the pasting behaviours of the pellets (J.3), where the peak viscosity of the mash, for example, was generally less than that of the pellets (Figure 26).

The DG of the diets was also investigated with a colourimetry technique, based on differential solubility of amylose when the molarity of a strong alkali is varied. This technique, which is widely used in the literature, has not been reported in any Pork CRC projects. It was, therefore, investigated in this project with the aims of introducing it as a simpler technique than calorimetry, and to ascertain the low DG of the pellet diets in the present study.

The technique works on the assumption that amylose, the linear fraction of starch, solublises in an alkali medium. Upon reacting with iodine, in the presence of iodide, the solubilised amylose forms a blue-black complex, whose colouration is quantified spectrophotometrically as a measure of the degree of starch gelatinisation (Birch & Priestley, 1973; Cai & Diosady, 1993). The procedure is summarized in Fig. 10, and its major limitations are the possibility of interference from non-amylose solubles and differences in solubility of amylose in materials. Hence, comparisons need to be cautiously made, but it is widely used for different and diverse materials for their degrees of starch gelatinisation, it uses more test material than calorimetry, and it is a simple, fast and relatively inexpensive technique that can be adapted for quality control in feed processing, if needs be.

As a supportive analysis on the status of starch, the degree of starch gelatinisation in the milled pellet diets (J.2) were analysed in triplicates in a random order following the procedure in Figure 30. Generally, the degree of starch gelatinisation was low (7 - 12%) in the pellet diets (Figure 31), as revealed by the DSC, although higher values were obtained in the latter. However, as indicated by the DSC, the hard wheat pellet diets were the most gelatinised, while the sprouted and soft wheat pellets were gelatinised to about the same extent. Also, like the DSC analysis, there were no consistent effects of conditioning time on the degree of gelatinisation of the pellets from the amylose solubility procedure (Figure 31). However, in the present study, there was no significant correlation ($p > 0.05$) between the DG values from the two methods.

Irrespective of the processing conditions, specifically conditioning temperature and time, it appears that the type of wheat being processed is the most significant factor in defining the

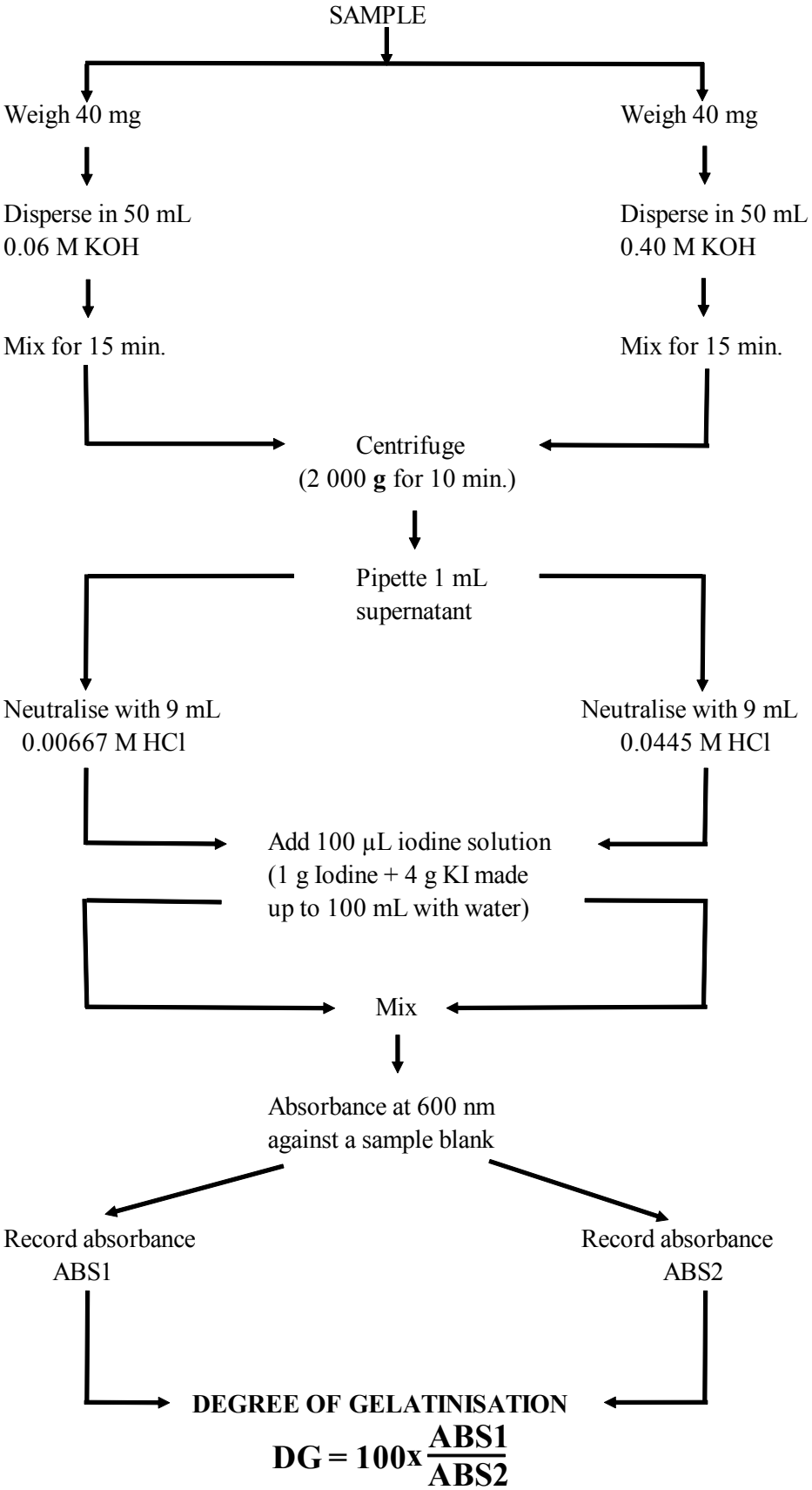

Figure 30: Flowchart of the procedure for degree of starch gelatinisation by the amylose solubility method

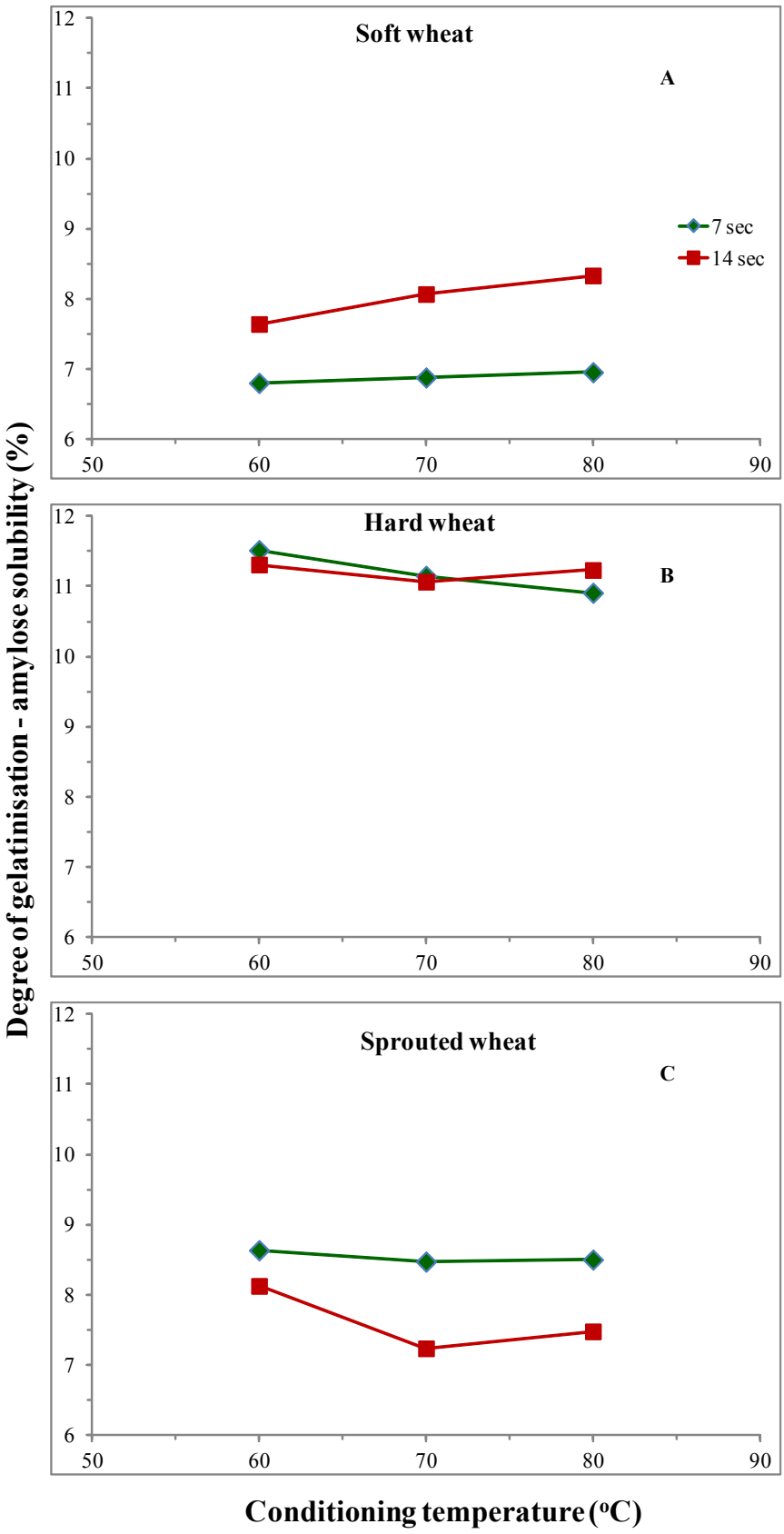

Figure 31: **Grain x conditioning temperature x conditioning time interactions (nominal values) on degree of starch gelatinisation by the amylose solubility method**

degree of gelatinisation of the pellets investigated. High temperatures, with more thermal energy, or long conditioning times, with increased residence time, were expected to produce high gelatinisation. However, the reverse was generally measured. Possibly, the range of temperature or time investigated was not wide enough for measurable differences. It is noteworthy that, in the absence of experimental errors, the pasting and gelatinisation behaviours at 70^{o}C were generally different from those at the other two temperatures (60^{o} and 80^{o}C). The measured behaviours at the former temperature were expected to be intermediate between the latter temperatures. With some pasting and gelatinisation parameters, a contrary trend was obtained, and non-starch components (e.g. proteins) could have played a significant role at this temperature. Or, there could have been unavoidable processing problems at the 70^{o}C conditioning temperature.

M. *In vitro* Protein Digestion of the Pellets

M.1 Background

Another energy-yielding component of pig feeds is proteins, whose monomers, amino acids, are important in growth and body building. Materials and processing conditions affect protein digestibility, and for maximum feed efficiency, high protein digestibility is desirable, in the absence of any deleterious implications. Optimising feed processing for maximum feed efficiency demands an understanding of what feed processing parameters affect protein digestion.

M.2 Methods

The milled pellet samples (J.2) were analysed using a pH-drop *in vitro* protein digestion procedure that is detailed in Tinus *et al.* (2012). The details of this procedure are also available in the final report of Project 4B-107 (Sopade *et al.*, 2011). *In vitro* protein digestibility (IVPD) was defined as:

$$IVPD = 65.66 + 18.10\ \Delta pH_{10\ min}$$

where $\Delta pH_{10\ min}$ is the change in pH of the digesta after 10 min. from an initial value of about 8.0.

M.3 Results

Figure 32 shows the typical protein digestogram of the pellet, a monophasic configuration resulting from a time-dependent reduction in pH as amino acids and/or peptides were released during protein digestion. Statistical analysis revealed the effects of grains ($p = 0.035$) and grains x temperature ($p = 0.039$) significantly affected IVPD (Appendix IX). For both the soft and hard wheats, IVPD was enhanced as the conditioning temperature increased, while the reverse was obtained for the sprouted wheat (Fig. 33). As obtained for starch, the proteins in the hard wheat pellets were the most digestible at effectively all the temperatures, while above 60^{o}C, the proteins in the pellets from the sprouted wheat were the least digestible.

Proteins, including those from wheat, are denatured from 50 – 80^{o}C (Schofield *et al.*, 1983; Redl *et al.*, 2003; Falcão-Rodrigues *et al.*, 2005), and the changes brought about by denaturation, in the absence of any adverse cross-linking or molecular transformations, can enhance protein digestion. Hence, the direct relationship between the conditioning temperature and IVPD for the soft and hard wheats is expected. In addition to amylases, sprouted grains generally have endogenous proteases, as well as lipoxygenase, catalase, peroxidase, and phenol oxidase (Edwards *et al.*, 1989). Being proteins themselves, these enzymes can be denatured or inactivated above 50^{o}C. If not inactivated or denatured, these enzymes could complement exogenous proteases in the *in-vitro* procedure to possibly increase protein digestion when the conditioning temperature was increased. However, the inverse IVPD-temperature observed with the sprouted wheat is contrary to this, and could suggest a temperature-driven inhibition to protein digestion in the wheat.

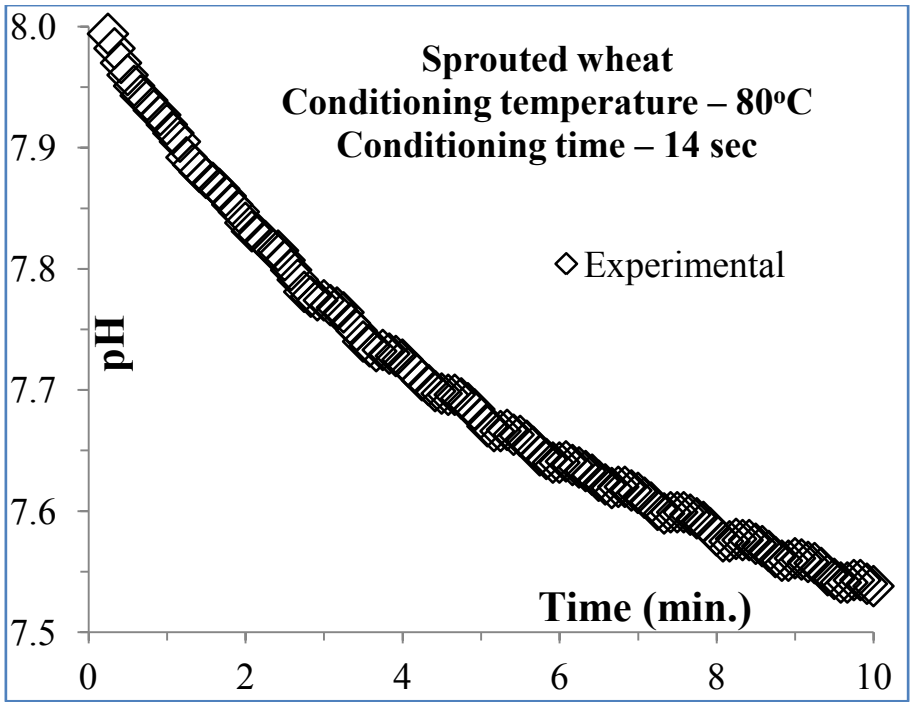

Figure 32: **Typical protein digestogram of the pellets**

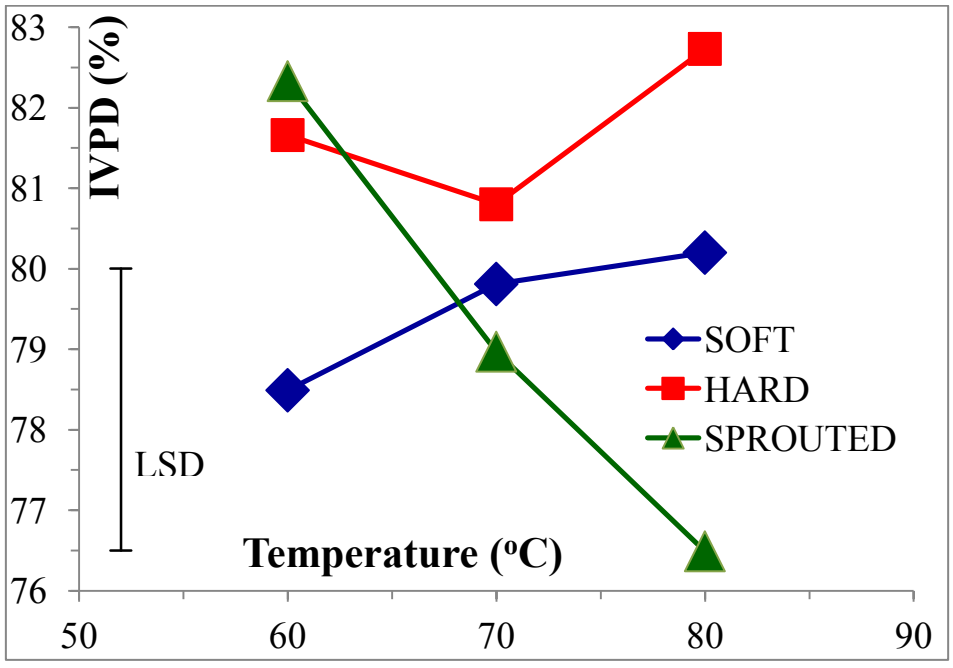

Figure 33: **Grain x temperature effects on *in-vitro* protein digestibility of the pellets (LSD = least significant difference at 5%)**

Although protein digestion is expected to be dependent on material and processing, the observation with the sprouted wheat shows material-specific processing requirements. A processing condition that favours digestion in one grain, might adversely affect digestion in another grain. Irrespective of the component being digested, the status or structure of the component in the processed material (e.g. pellets) can provide an insight into the susceptibility of the component for digestion.

N. Fourier Transform Infra-Red Spectroscopy (FTIR) on the State of Starch and Protein in the Pellets

N.1 Background

Infra-red spectroscopy is widely used in food and animal industries, with near infra-red spectroscopy (NIRs), discussed below (L.), being an outstanding one in feed manufacture for grain quality, and in assessing animal performance from predicted digestibility of feed. Depending on the wavelength (or wavenumber) of analysis, there are near-, mid- and far-infra-red regions with broad wavenumbers (cm^{-1}) of 14,000 – 4,000, 4,000 – 400 and 400 – 10 respectively. FTIR falls in the mid-infra-red region, and like other spectroscopic technique, FTIR is used to identify organic and inorganic chemicals depending on the response of the inherent bonds to applied energy. FTIR is used for quantitative analysis to an accurate extent because the response of chemical bonds in a material is proportional to their concentrations. Specifically, FTIR has been used to investigate crystalline and amorphous regions in starch, and amide regions or secondary structure of proteins (van Soest *et al.*, 1995; Htoon *et al.*, 2009; Chen & Sopade, 2013; Chen *et al.*, 2013; Miller *et al.*, 2013). Hence, FTIR can reveal structure of material components, and how this is affected by treatments, as indicated by changes to the constituent bonds. Both heat-moisture-shear driven starch gelatinisation and protein denaturation manifest as chemical changes to the major components.

N.2 Methods

FTIR spectroscopy was done using the Spectrum FTIR 100 spectrometer (Perkin Elmer, Waltham, Mass. 02451, USA) equipped with a deuterated triglycine sulphate (DTGS) detector, a universal attenuated total reflectance (ATR) single reflectance cell and a diamond crystal. Triplicate test samples of each of the milled pellet diets (F2.), in a randomised order, were placed on the crystal, gently pressed (optimum force of 100 units) before scanning (32 times; ≈ 22^{o}C; 4 cm^{-1} resolution) from 2,000 - 800 cm^{-1}, which are C-C, C-O and N-H stretching regions, where starch and protein structures are sensitive and can be studied (van Soest *et al.*, 1995; Chen & Sopade, 2013; Miller *et al.*, 2013). A background spectrum (no test sample) was subtracted from all spectra, which were baseline-corrected with the equipment software.

N.3 Results

The spectra from the pellet diets are typified by Fig. 34 for the starch (A) and protein regions (B). For starch, intensities around 1045 and/or 995 cm^{-1} are related to the crystalline phase, while those around 1022 cm^{-1} are linked to the amorphous phase, with ratios 995/1022 and 1045/1022 as arbitrary estimates of starch structural and molecular orderliness or crystallinity, and possibly inversely related (van Soest *et al.*, 1995; Htoon *et al.*, 2009; Chen & Sopade, 2013). Hence, a change in the ratios would indicate changes to the crystalline or amorphous region of the starch molecule. This can explain some starch-related behaviours (pasting, digestibility or gelatinisation) mentioned above.

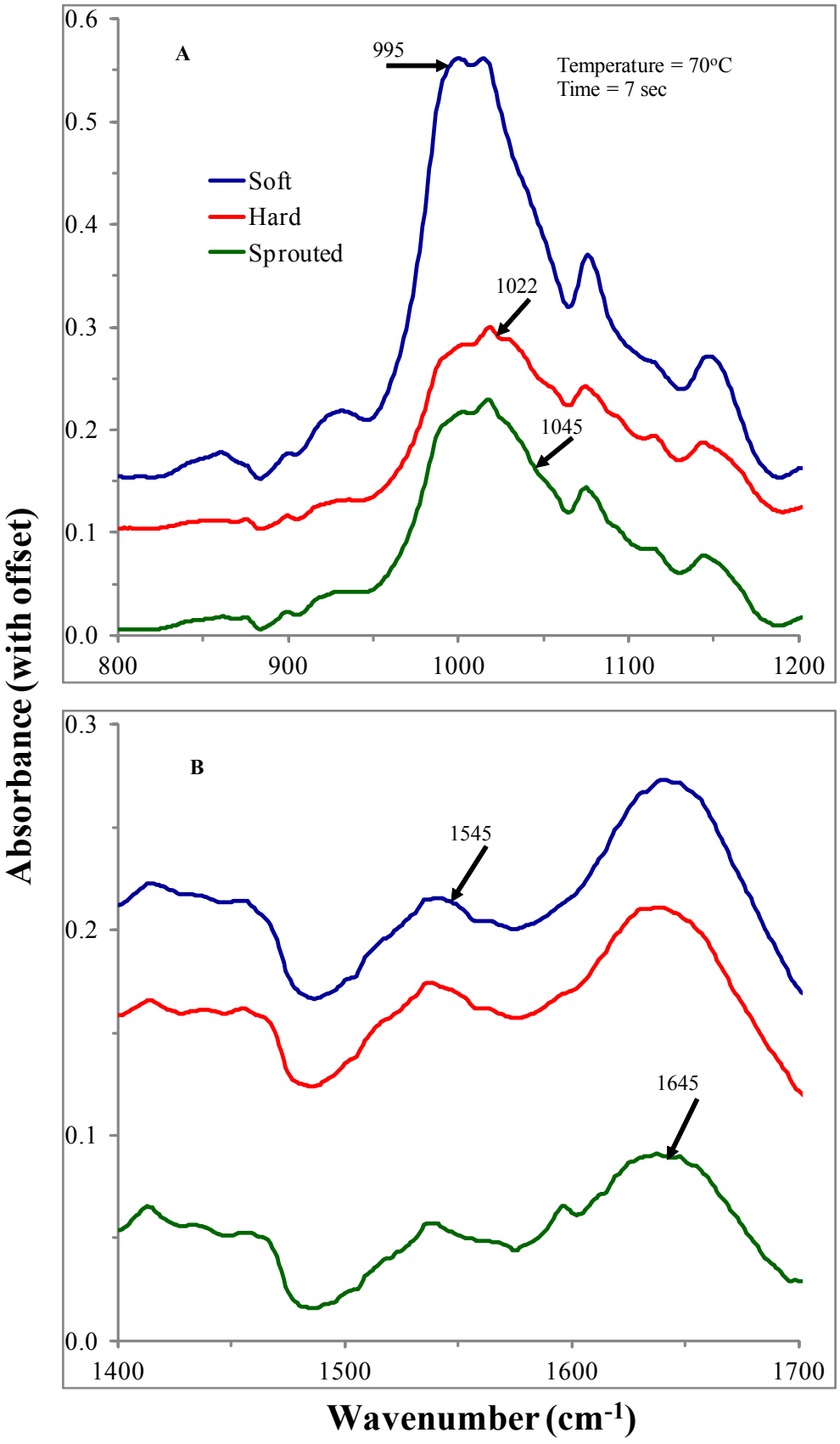

Figure 34: **Typical spectra of the pellet diets**

The 995/1022 ratio of the pellets ranged from 0.89 – 1.05, while the 1045/1022 ratio was from 0.71 – 0.81 (Figure 35A - C). ANOVA from Minitab^{TM} ver. 16 revealed conditioning time and grain effects in the following order:

995/1022: 14 sec > 7 sec
995/1022: SOFT > SPROUTED > HARD

1045/1022 – HARD > SPROUTED > SOFT

Even though the range was not wide, the trend with the 1045/1022 ratio follows the trends with the degree of starch gelatinisation measured by both the DSC and amylose solubility method (L.3.), while opposite to the trend with the 995/1022 ratio. In the absence of pronounced retrogradation, starch gelatinisation in the present study possibly increased the amorphous region (and reduced the crystalline region) of the starch to increase the 1045/1022 ratio and reduce the 995/1022 ratio. These FTIR ratios are related to the degree of starch gelatinisation from the amylose solubility method as below, but Pearson's correlation analysis did not show any significant relationships with the DSC degree of gelatinisation:

FTIR (995/1022) = 1.17 – 0.02 AMY-DG
$r^2 = 0.644$, $p < 0.001$

FTIR (1045/1022) = 0.61 + 0.02 AMY-DG
$r^2 = 0.675$, $p < 0.001$

With respect to proteins, wavenumbers 1545 and 1645 cm^{-1} are the amide regions II and I respectively, and are usually analysed separately (Barth & Zscherp, 2002; Miller *et al.*, 2013). The amide I region (≈ 1650 cm^{-1}) is mainly due to the C==O stretching vibration with minor contributions from the C-N stretching vibration, C-C-N deformation and N-H in-plane bend. The amide region I is most commonly used to analysis protein secondary structure. On the other hand, the amide region II (≈ 1550 cm^{-1}) is mainly from the out-of-phase combination of the N-H in-plane bend and the C-N stretching vibration with smaller contributions from the C-O in-plane bend and C-C and N-C stretching vibrations. However, unlike amide I, amide II is less correlated with protein secondary structure, and the former has been successfully related to *in-vitro* protein digestion of diverse plant and animal protein materials after deconvolution and differentiation of the spectra into various bands (Carbonaro *et al.*, 2012). While the mathematical exercise is valuable, it was thought that like starch, the ratio of amide II:amide I regions (1545/1645 ratio), could be a coefficient of the status of protein secondary structure in the pellets. ANOVA (Minitab^{TM} ver. 16) did not reveal any significant effects when the regions were considered separately, but there were grain and conditioning time effects, when the ratio, which ranged from 0.55 - 0.72 (Fig. 15D - F), was used:

1545/1645 – 7 sec > 14 sec
1545/1645 - HARD > SPROUTED > SOFT

We are not aware of any report on the significance of the 1545/1645 ratio, but it is noteworthy that the *in vitro* protein digestion (I3.) revealed a significant grain effect, with the hard wheat pellets having the highest *in vitro* protein digestibility (HARD, 81.7%;

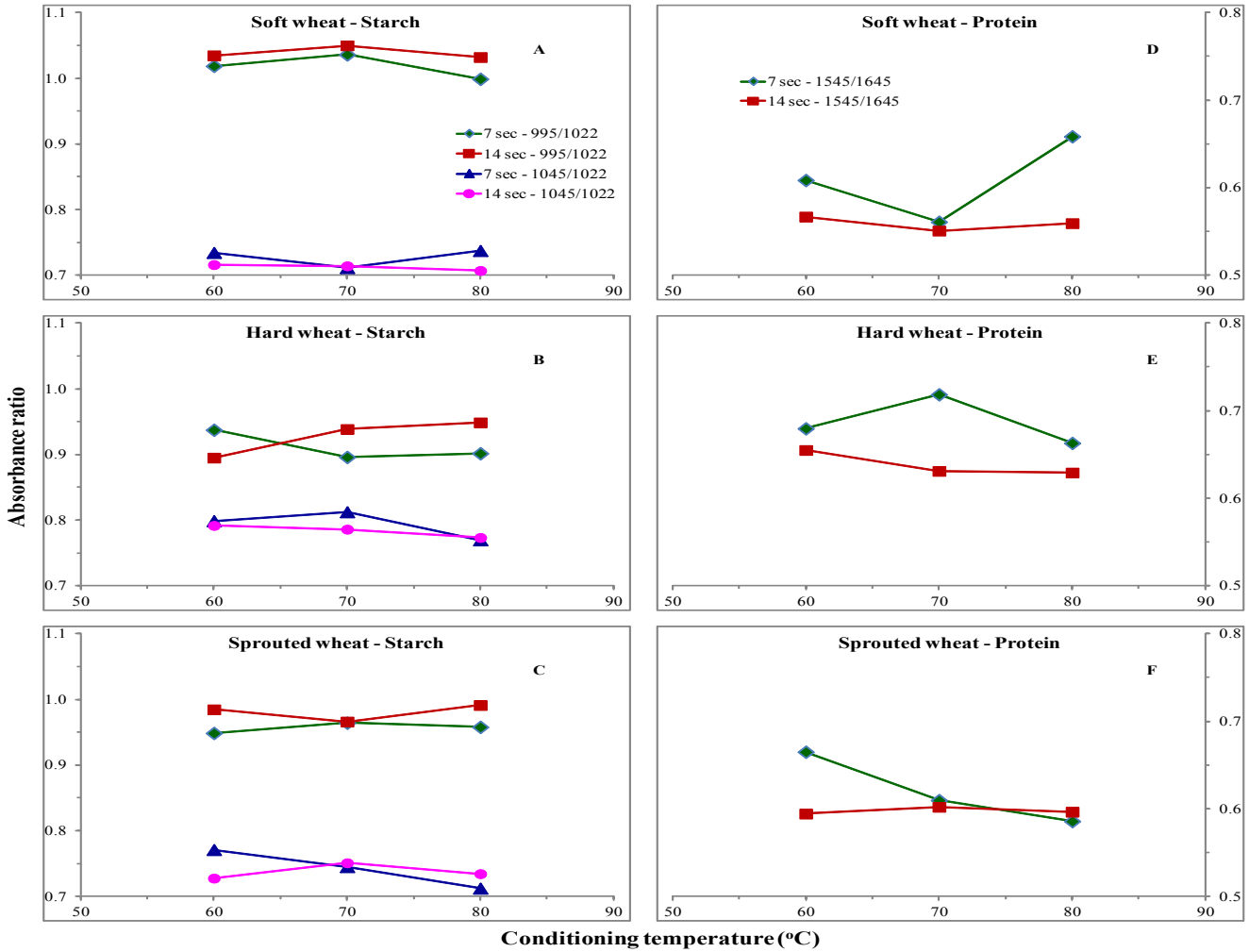

Figure 35: Effects of grain x conditioning temperature x conditioning time on the FTIR absorbance ratios

SOFT, 79.5%; SPROUTED, 79.3%). A linear regression analysis revealed a significant relationship between the amide ratio and the *in vitro* protein digestibility (IVPD):

FTIR (1545/1645) = 0.01 IVPD – 0.24
$r^2 = 0.235$, $p = 0.04$

Even though this might be a curve fitting exercise with no particularly strong correlation, the relationships of the FTIR ratios with starch and protein characteristics indicate, as demonstrated for other samples, the usefulness of FTIR to probe structures of animal feeds, and, if a detailed analysis is required, it complements techniques such as X-ray diffraction and Nuclear Magnetic Resonance (NMR). The latter techniques were not considered in the present study, but the information and trends from the FTIR analysis are encouraging, and can complement NIRs. The spectra obtained were sensitive to grain type and processing conditions. With minimum sample preparation, the FTIR-ATR technique opens an avenue to understand, predict and possibly optimize feed processing for maximum animal performance.

O. Animal Performance – Feed Efficiency with Weaners

O.1 Background

Pellet quality varies among commercial feed manufacturers, and this has been discussed in various Pork CRC projects to be due to operational differences and raw material properties, which can be location-specific. The measured properties above (F – N) lend credence to the roles of grain and process conditions in defining pellet properties. The variation in pellet properties can influence feed wastage and growth performance of pigs. Part One of this report mainly concentrated on laboratory (*in vitro*) analysis. Hence, in order to optimize commercial processing procedures, an understanding of how processing conditions define animal performance, is required. There is also the need to predict animal performance by examining how pellet properties relate to this.

O.2 Methods

O.2.1 Animals and treatments

A total of 597 male weaners were weaned at an average age of 28 days (average weight = 6.2 ± 0.05 kg) and transferred into individual weaner pens. Pigs were selected in three replicates:

- Replicate 1 - 200 pigs selected on 28 March 2012
- Replicate 2 - 200 pigs selected 2 May 2012
- Replicate 3 - 197 pigs selected 6 June 2012

At the conclusion of the acclimatisation period (described below), 192 pigs per replicate were allocated to one of the 24 diets (G.2.3).

O.2.2 Husbandry and management

Pigs were weaned at an average age of 28 days and transferred into individual pens in a climate controlled weaner facility. All weaners were individually weighed at entry and randomly allocated to pen within the shed. All pigs were offered a commercial starter diet for 5 - 6 days prior to the start of the test period (day 0) at which time all animals were weighed again, and 192 pigs selected to start the test period. The choice of which pigs to omit was based on initial weight, with lighter pigs or those deemed unwell typically excluded from the trial. Within replicate, pigs were randomised to the 24 diets using the statistical computing environment R (R Core Team, 2012) using the od package (Butler, 2011). Pigs were offered the test diets *ad libitum* for the entire test period, while water was freely available via a single nipple drinker separate to the feeders. All animals were individually weighed weekly and feed intake determined by feed disappearance.

All procedures carried out in this investigation were undertaken in accordance to the Rivalea Standard Operating Procedure for the Individual Weaner Facility (SOP-025) and under the direction of the Rivalea Animal Care and Ethics Committee (Appendix XI).

O.3 Results

The animal performance on the pellet diets were assessed over 21 days, and Table 46 summarises the statistics (Appendix XII) on this. Only the main effects of grain, and conditioning temperature and time were significant, and the trends displayed by these variables on the performance parameters are as in Figure 36. Although the pigs consumed more of the sprouted-wheat diets, these diets were the least efficient, while both the soft-wheat and hard-wheat diets had comparable feed efficiency and feed intake. Starch and proteins are the main energy and growth components in a diet, and as discussed above (H.3), the hard wheat had the highest protein content, but a lower starch content than the soft wheat. The sprouted wheat, with its endogenous enzymes, also had a lower starch content than the soft wheat, even though their protein contents were comparable.

Various studies have been conducted on the dependence of animal performance on sprouted grains. While sprouting is beneficial in increasing endogenous enzymes as pointed out before (K.3.), it can reduce component-component interactions to enhance digestibility , as reported for sorghum (Balogun *et al.*, 2006). Also, the loss of dry matter during sprouting can lead to more feed consumption and higher feed intake (Peer and Lesson, 1985). However, possibly because of compositional differences, the higher feed intake does not necessarily translate to higher weight gain, and consequently, higher feed efficiency. Although contrary to the result in the present study (Fig. 16), sprouting, either naturally- or artificially-induced, has been shown not to affect the feed value of animal diets (Peer & Leeson, 1985; Rule *et al.*, 1986; Shem *et al.*, 1990; Skiba *et al.*, 2002). This has also been demonstrated in PGLP and various Pork CRC projects.

Figure 36 shows that low temperature and extended conditioning enhanced pig growth and feed efficiency. As reported above (L.3.), the high conditioning temperature did not give a high degree of starch gelatinisation even at the highest conditioning time. It could be that the conditioning times were too short for the wet heat to penetrate the grains. The positive effects of conditioning time are, however, expected because, even though not clearly reflected in the starch gelatinisation, and *in vitro* digestion of starch and protein of the diets, starch and protein are expected to be more destructurised with processing time. It appears that any one of the wheats can be used with low temperature and extended conditioning for a high efficiency of feed use. Although processed under the same conditions and no sprouted wheat was used, Jha *et al.* (2011) did not find any effects on feed efficiency from 12 wheat varieties that included hard and soft wheats.

A major objective of the present study was to understand how processing influences animal performance, and the various analyses done on the mash and pellet diets are to explore the processing-property relationships of the pellets. The analyses were also done to gain an insight into how animal performance can be predicted from pellet properties. Table 47 shows the Pearson's correlation coefficients of the various properties of the diets. Diets with high peak and final viscosities, and gelatinisation showed a high efficiency of feed use. With a generally low degree of starch gelatinisation, this trend could have resulted from destructurisation or open-structure effects that did not materialize in high

Table 46: Summary of statistical analysis (p-values) of the animal performance parameters as affected by the processing conditions*

Term	Weight gain/ Rate of Growth	Feed intake/ Average Daily Feed Intake	Feed Conversion Ratio
Grain	0.616	<0.001	<0.001
Temp	<0.001	0.100	<0.001
Time	0.055	0.001	0.038
Grain x Temp	0.269	0.687	0.427
Grain x Time	0.642	0.399	0.141
Temp x Time	0.102	0.339	0.092
Grain x Temp x Time	0.072	0.221	0.188

*Abbreviation: Rate of Growth (kg/day) = RoG, Average Daily Feed Intake (kg) = ADFI. Feed Conversion Ratio = FCR

gelatinisation. The strong inverse relationship between four *in-vitro* starch digestion parameters (D_o, D_∞, AUC, and $PreD_{240}$) and pasting properties (peak and final viscosities), is contrary to expectations, and suggests that high digestibility could be inimical to high efficiency of feed use. However, Table 47 also shows that a high rate of starch digestion yielded a high efficiency of feed use (low FCR).

Various studies have related animal performance and *in-vitro* starch digestion, with the general consensus being that the latter reasonably predicts animal performance (Drew *et al.*, 2012; Giuberti *et al.*, 2012; Montoya & Leterme, 2012). Incidentally, the bulk of the studies was conducted on single-point measurements of *in-vitro* starch digestion, and it is now realized that time-course starch digestion, from which the rate of starch digestion is obtainable, is more useful (Sopade & Gidley, 2009). A high rate of starch digestion is reported (Giuberti *et al.*, 2012) to lead to high ileal digestibility, influence feed intake, avoid 'ileal brake', and reduce ileal starch fermentation amongst others. Although more studies with diverse (starch- and protein-containing) materials are required, the present study shows the usefulness of the rate of *in-vitro* starch digestion in predicting the efficiency of feed use ($r = -0.589$, $p = 0.01$). The *in-vitro* protein digestograms of the pellets were also described (not shown) using the modified first kinetic equation in G.2 (Tinus *et al.*, 2012). Like the starch digestion, an inverse relationship was obtained between the feed conversion ratio (FCR) and the rate of protein digestion ($r = -0.25$, $p = 0.31$), and this was not significant.

On the textural and mechanical properties of the pellets, only durability-feed intake (inverse) and hardness-FCR (inverse) were significant (Table 47). In the absence of mitigating factors, a durable pellet would have less fines on being handled/transported to enhance feed intake, while a hard pellet might not disperse easily in the gastrointestinal fluid for enzyme diffusion thereby reducing efficiency of feed use or increasing FCR. However, opposite trends were obtained in the present study, and this could be due to the generally low hardness (≈ 5 kg) and durability (≈ 91%) of the pellets as highlighted in E.3. It is noteworthy that the *in-vitro* protein digestibility of the pellets positively corrected with their hardness, which together with the durability, positively correlated with the rate of starch digestion (Table 47).

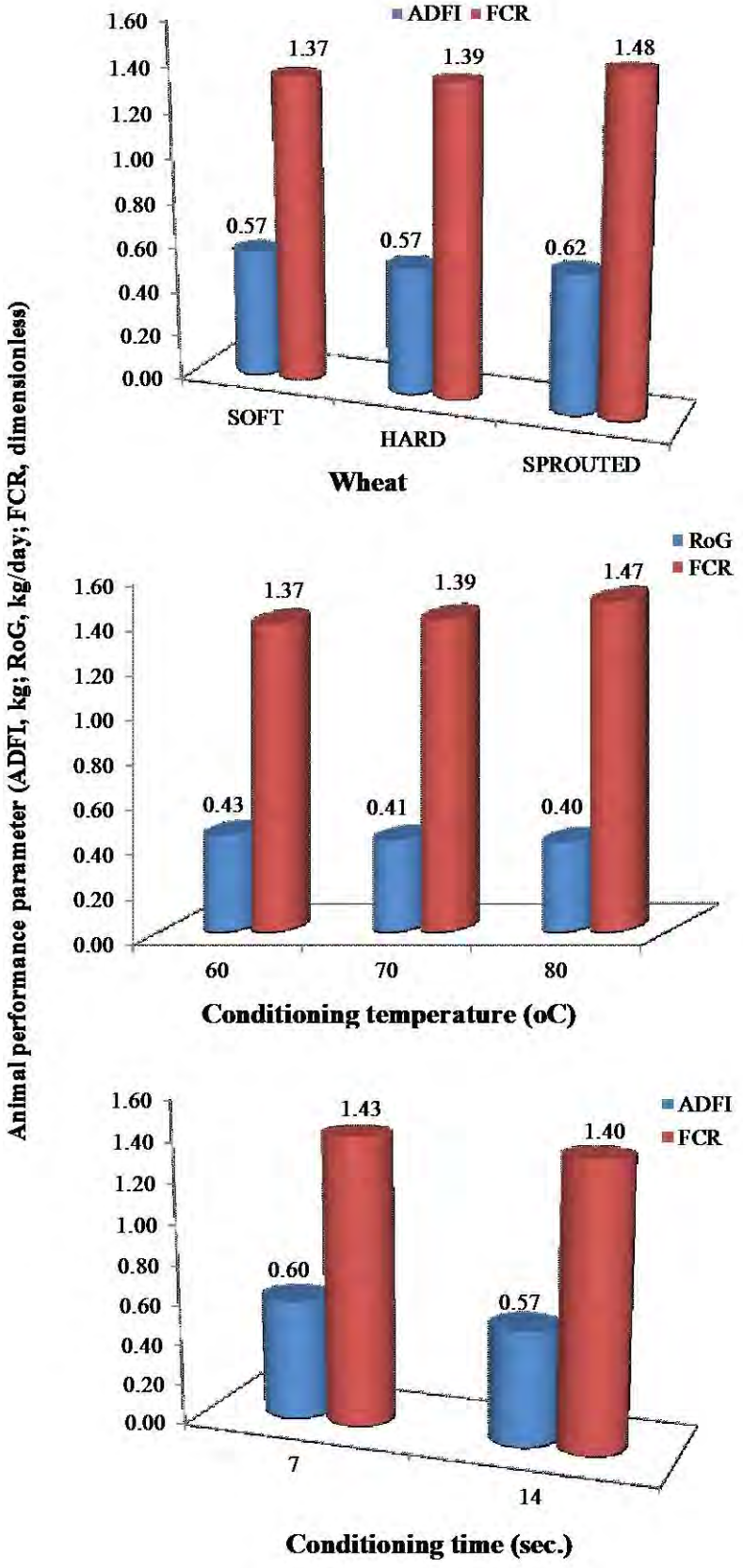

Figure 36: **Animal performance parameters of the pellet diets as affected by the processing condition**

Table 47: Pearson's correlation analysis of the key parameters of the pellets†

Parameter	PeakVisc	FinalVisc	PeakTime	InitialVisc	IVPD	1545/1645	Amy-DG	DSC-DG	995/1022	1045/1022	RoG	ADFI	FCR	D_o	D_∞	K	AUC	$PreD_{240}$	Hard-ness	Durab-ility
PeakVisc	1.000																			
FinalVisc	0.940 ***	1.000																		
PeakTime	0.316 NS	0.572 *	1.000																	
InitialVisc	0.320 NS	0.382 NS	0.318 NS	1.000																
IVPD	-0.116 NS	0.005 NS	0.522 *	0.061 NS	1.000															
1545/1645	-0.413 NS	-0.210 NS	0.485 *	0.208 NS	0.484 *	1.000														
Amy-DG	-0.225 NS	0.069 NS	0.721 ***	0.243 NS	0.503 *	0.646 **	1.000													
DSC-DG	0.373 NS	0.413 NS	0.229 NS	0.213 NS	0.000 NS	-0.191 NS	0.103 NS	1.000												
995/1022	0.620 **	0.367 NS	-0.459 NS	-0.058 NS	-0.413 NS	-0.826 ***	-0.802 ***	0.142 NS	1.000											
1045/1022	-0.398 NS	-0.131 NS	0.562 *	0.196 NS	0.505 *	0.897 ***	0.822 ***	-0.063 NS	-0.865 ***	1.000										
RoG	-0.036 NS	-0.060 NS	-0.207 NS	0.344 NS	0.026 NS	0.091 NS	-0.092 NS	0.255 NS	-0.044 NS	0.056 NS	1.000									
ADFI	-0.526 *	-0.582 *	-0.461 NS	0.196 NS	-0.029 NS	0.140 NS	-0.131 NS	-0.173 NS	-0.174 NS	-0.041 NS	0.563 *	1.000								
FCR	-0.567 *	-0.621 **	-0.382 NS	-0.238 NS	-0.135 NS	-0.001 NS	-0.134 NS	-0.570 *	-0.099 NS	-0.156 NS	-0.455 NS	0.456 NS	1.000							
D_o	-0.722 ***	-0.769 ***	-0.571 *	-0.237 NS	-0.268 NS	-0.023 NS	-0.151 NS	-0.212 NS	-0.179 NS	-0.089 NS	0.313 NS	0.670 **	0.448 NS	1.000						
D_∞	-0.660 **	-0.746 ***	-0.604 **	-0.332 NS	-0.271 NS	-0.157 NS	-0.220 NS	-0.233 NS	-0.039 NS	-0.262 NS	0.111 NS	0.686 **	0.639 **	0.873 ***	1.000					
K	0.727 ***	0.732 ***	0.514 *	0.254 NS	0.102 NS	-0.049 NS	-0.004 NS	0.193 NS	0.179 NS	-0.029 NS	-0.157 NS	-0.700 ***	-0.589 **	-0.816 ***	-0.891 ***	1.000				
AUC	-0.637 **	-0.732 ***	-0.596 **	-0.327 NS	-0.301 NS	-0.175 NS	-0.247 NS	-0.243 NS	-0.042 NS	-0.295 NS	0.156 NS	0.685 **	0.600 **	0.905 ***	0.986 ***	-0.834 ***	1.000			
$PreD_{240}$	-0.654 **	-0.743 ***	-0.601 **	-0.331 NS	-0.283 NS	-0.159 NS	-0.225 NS	-0.241 NS	-0.046 NS	-0.271 NS	0.138 NS	0.692 ***	0.622 **	0.894 ***	0.997 ***	-0.874 ***	0.995 ***	1.000		
Hardness	0.645 **	0.663 **	0.583 *	0.473 *	0.468 *	-0.003 NS	0.221 NS	0.086 NS	0.173 NS	0.014 NS	0.003 NS	-0.360 NS	-0.473 *	-0.610 **	-0.604 **	0.597 **	-0.578 *	-0.595 **	1.000	
Durability	0.510 *	0.535 *	0.367 NS	0.320 NS	-0.133 NS	-0.060 NS	0.056 NS	-0.044 NS	0.162 NS	0.036 NS	-0.366 NS	-0.678 **	-0.293 NS	-0.543 *	-0.702 ***	0.721 ***	-0.658 **	-0.688 **	0.498 *	1.000

†Level of significance: *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$, NS = non-significance ($p>0.05$)

P. Enhancement of the NIR calibrations

P.1 Background

Over a number of years, initially through the PGLP and then through successive Pork CRC projects, NIR calibrations have been developed in grains to assess potential energy value for animals. Within the pork industry where grains and other raw materials are processed into pellets, calibrations were developed for energy and other traits. However, additional grains were required to provide more robust calibration models. In this project, additional grains including sprouted grains were incorporated. This objective was important to ensure any NIR calibrations were 'familiar' in seeing sprouted grains as these types of grains are common in the grain industry due to pre-harvest sprouting events resulting in premium grains (wheat and barley) being downgraded to feed.

One of the key objectives of this project was to expand the previous NIR calibrations to included sprouted grains, which had been through a number of processing and diet treatments.

P.2 Methods

The experiment process for the NIR analysis is as in E.2 using the Foss 6500 instrument using WinISI software. The spectra were analysed using principal component analyses (PCA) to determine whether differences between the new samples and the previous calibration samples could be identified. The scans were then used to develop NIR calibrations for a range of variables that may indicate the nutritional value of the final processed product and its durability.

P.3 Results

P.3.1 Effects of processing on NIR scans

PCA is a dimensional reduction technique whereby multiple dimensions (in the case of NIR 1000 wavelengths) are reduced to few components (spectral regions) that explain most of the variation in the spectra between samples. In using PCA, E.3 (Part One) shows the various stages of processing from BBS and QAF, as well as a comparison between BBS and QAF (Figures 17 – 19). The 24 samples in Part Two (Table 38) are represented by new cluster in the PCA model (Figure 37), which indicates variations in some regions of the spectra. These spectral variations are useful in improving the current set of calibrations.

P.3.2 Preliminary NIR calibrations

As shown above the reference values for the new samples were for most traits outside the range of the previous sample set. This additional range in values strengthens the calibrations as well as providing potential future values which commercial calibrations could utilise.

NIR calibrations were developed from scans on the finished processed pellet for a range of variables including those from *in vivo* faecal DE experiments, *in vitro* starch digestion assays (K), RVA assays (J), chemical analyses (H), and pellet quality measurements (I).

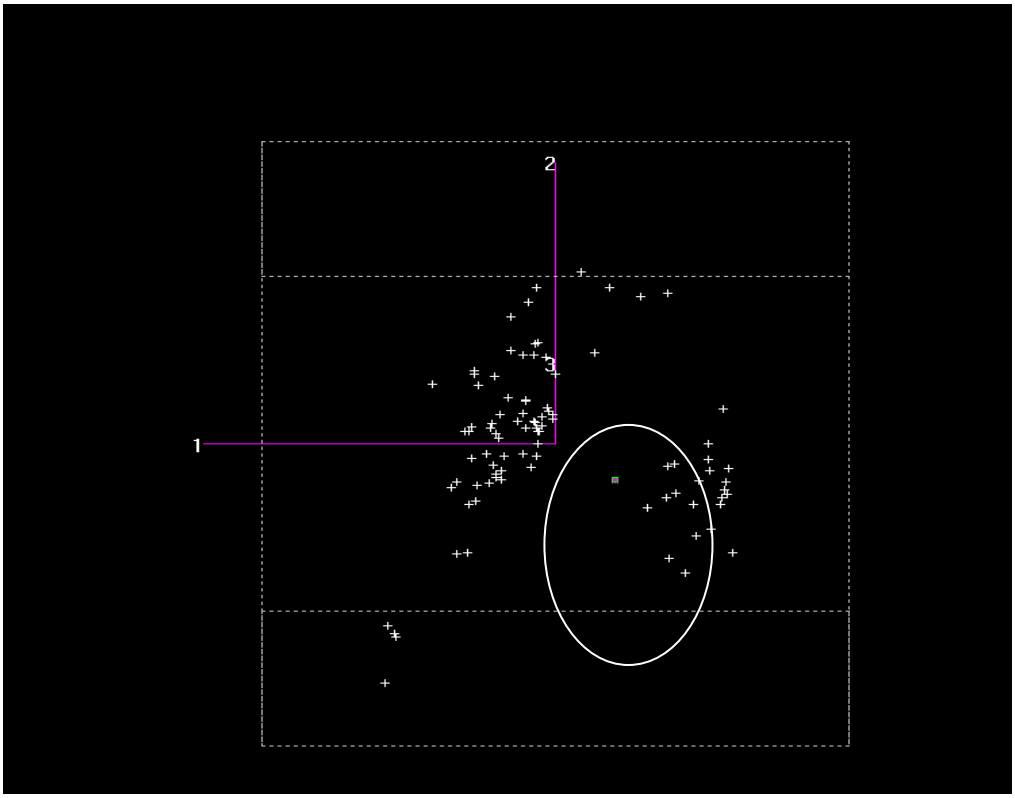

Figure 37. PCA showing new samples (circled) clustering away from the previous samples used in the calibrations

The likely value of NIR calibrations can assessed in relation to the criteria shown in Table 48. The three most important criteria for judging the suitability of a calibration as discussed before (E.3.2) 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 (Ratio of standard error of Prediction to standard Deviation of the prediction set)

Table 48 shows the comparison between the previous calibration statistics and the new calibration statistics that incorporate the new samples in Table 38. In most traits, there was an improvement with the new samples. This was generally because of an extension in the range of values of these traits, but not in all cases. The previous calibrations included other traits such as phosphorus and phytate but these analyses were not included in the Part Two of the project, and phosphorus and phytate were, therefore, not compared.

An improvement would be considered where there is an increase in the R^2, a decrease in the SECV and an increase in the RPD. For protein, fat and starch, there were improvements in the calibrations, although for starch the RPD is still less than 2.0. Durability also showed improvement with an RPD of 2.0, suggesting this model is potentially useful in ranking pellet

durability. However, for moisture, ash and fibre there were no improvements in the calibration models. For the *in vitro* starch digestibility traits (K), there were improvements in the D_∞, ExpD240 and PreD240, while the remaining starch digestibility traits showed no improvements. A number of the traits associated with starch quality as measured using the RVA (J) also showed improved calibrations. These were peak viscosity, trough viscosity, final viscosity, setback, and peak time. These traits all had RPD greater than 2.0 suggesting these calibrations are potentially useful in screening pellet quality. For the remaining RVA parameters, there were no improvements. The calibration for initial viscosity was again very poor. The RVA initial viscosity, using the standard procedure 1, is usually measured after 1 min. of standing at 50^{o}C upon mixing at 160 rpm. The rehydration characteristics of samples will affect this, and the poor predictions could have resulted from this, whereby some samples rehydrated well, while other samples poorly rehydrated. Perhaps, a measure of the initial viscosity upon complete rehydration after standing overnight could improve predictions of initial viscosity.

Table 48. Comparison of calibration statistics from previous calibrations and the new calibrations

Variable	Previous			New		
	RSQ	SECV	RPD	RSQ	SECV	RPD
Moisture	0.8966	0.5	3.2	0.7789	0.6	2.1
Ash	0.8684	0.5	2.1	0.8553	0.7	1.9
Protein	0.8256	0.6	2.0	0.9785	0.7	5.1
Fat	0.742	0.3	1.8	0.9654	0.5	4.2
Starch	0.6989	3.3	1.5	0.7611	4.0	1.8
Crude fibre	0.6456	0.4	1.6	0.6812	0.5	1.6
Durability	0.5718	6.2	1.7	0.7214	6.5	2.0
AUC	0.614	1105	2.2	0.8044	1650	2.2
K	0.8793	1.18	2.1	0.8521	1.3	1.9
D_0	0.8139	1.6	1.4	0.8051	1.8	1.7
D_∞	0.5181	10.5	1.6	0.9122	8.4	2.5
ExpD240	0.6682	9.0	1.5	0.8432	10.8	2.1
PreD240	0.4326	10.0	1.4	0.8478	8.5	2.6
Peak Visc	0.2447	50.6	1.1	0.5089	52.6	2.2
Trough V	0.4008	49.2	1.2	0.8918	22.7	2.1
Breakdown	0.5514	14.0	1.5	0.5219	11.9	1.4
Final V	0.4494	142	1.3	0.9289	104	2.3
Setback	0.4333	99.3	1.3	0.9038	71	2.4
Peak Time	0.7710	0.3	2.1	0.8750	0.2	2.5
Paste Temp	0.7749	2.8	1.7	0.7384	2.9	1.6
InitalVisc	0.1135	6.6	1.1	0.1232	7.0	1.1

Key to abbreviations: *in vivo* is pig faecal DE value (MJ/kg as fed); D_o, D_∞, AUC, ExpD240, PreD240 are *in vitro* starch digestion variables; Breakdown, setback, Initial Visc(osity), Peak Visc(osity), Trough V(iscosity), Final V(iscosity), Paste Temp, Peak Time are variables from the RVA analysis; moisture, starch damage, ash, protein, fat, crude fibre, starch are all chemical composition variables; durability is measured in pellets.

The addition of new samples to the previous calibration models has generally shown an improvement in most traits tested. There were improvements in three chemical traits, three *in vitro* starch digestibility traits and a number of starch viscosity and pasting traits. Close selection of new sampling using PCA, identifying samples which would extend the range of

values, provided these values would be seen in commercial reality, as well as selecting samples with chemical properties that lie within the current ranges, would continue to enhance the NIR calibrations for the benefits of the pig industry in Australia and globally.

Q. Research Needs and Recommendations

Q.1 Further research

The research conducted to date within this project suggests there two areas for continued activity that are needed to provide outcomes that will be of high value to both stockfeed manufacturers and pig producers. These continuing activities are:

(a) Development of a spreadsheet or other computerised model for optimising feed ingredient-additive mixtures and mill processing conditions to produce pellets of high durability and nutritional quality most energy efficiently,
(b) Evaluation and upgrading of the preliminary NIR calibrations to provide inputs for the proposed processing model and to predict the likely mill energy requirements, chemical composition and nutritional value for pigs of the finished product.

Q.1.1 Processing model

There is sufficient information from the experiments that have been conducted, literature results and theoretical information about factors that affect the efficiency of processing measured by Specific Mechanical Energy (SME), pellet durability and nutritional value. The vast information from this project will be used for a robust and versatile new model that predicts pellets properties from material and processing conditions.

This new model could be used to determine the optimal processing conditions, such as particle grind size, water addition, conditioning temperature, conditioning time and throughput, depending on the ingredients included in a diet and the structure of the conditioning-pelleting equipment, while predicting the effects on SME, pellet durability and likely nutritional value of the finished product. An optimisation routine could possibly be added to determine the best ratio of ingredients (grain types, protein sources etc.) and processing conditions to minimise costs of production and maximise product quality depending on the relative costs of ingredients and processing energy.

With further refinement, the model could also be used to design processing facilities given specific sets of ingredient inclusion. It may also be possible to expand the model to include the effects of conditioning variables on the availability of lysine through predicting changes in reactive lysine.

Initially, the model will be developed from an understanding of the physical, chemical and rheological interactions that occur at each stage of the feed processing chain. Mathematical relationships and parameters for the algorithms will be derived from theoretical knowledge and information published in the literature and derived from the experiments conducted in this project.

Q.1.2 Enhancing NIR calibrations

NIR always benefits from more samples to enhance its calibrations. Although many and diverse materials were investigated in this project, more samples, when available, would be useful. Also, more pellet quality parameters (e.g. degree of starch gelatinisation and *in vitro* protein digestion) could be explored for NIR calibrations.

Q.2 Recommendations

The project achieved its set objectives by studying the effects of pilot and commercial pig feed processing conditions on feed properties. The information available, particularly the usefulness of *in vitro* starch digestion parameters in predicting animal performance, will benefit the stockfeed manufacturing and pig production industries to screen pellets. A follow-up project will develop a versatile and robust model to optimise ingredient mixtures and processing conditions.

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APPENDIX I

EXPERIMENTAL DESIGN CODES FOR PART TWO OF THE PROJECT

Code	Title
DH001	Processing of the pellet batches
PS027	Manufacture of diets with differences in grain and conditioning
PS028	Particle size distribution of three wheats for pig feed
PS029	Moisture content of mash and pellets from wheat
PS030	Starch content of wheat pellets
PS031	Nitrogen content of wheat pellets
PS032	Crude fat content of wheat pellets
PS033	Ash content of wheat pellets
PS034	Gelatinisation characteristics of mash and pellets from wheat
PS035	Rapid *in-vitro* starch digestibility of pellets from wheat
PS036	*In-vitro* protein digestibility of pellets from wheat using the pH-drop method
PS037	Differential scanning calorimetry (DSC) studies on mash and pellets from wheat
11N093C	Influence of grain and processing conditions on pig growth (Rivalea)

APPENDIX II

STATISTICAL REPORT ON MOISTURE CONTENT OF THE PELLET DIETS

GRDC Grains Research & Development Corporation

Pork CRC

UNIVERSITY OF WOLLONGONG

Statistical Consulting Report

PS029 - Moisture content of mash and pellet diets derived from wheat

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July 24, 2012

Table of Contents

1 Introduction

This report has been compiled for Dr Peter Sopade, a senior research fellow with the University of Queensland. The protocol for this experiment has already been described in the document titled *Moisture content of mash and pellets from wheat.* There are two aims to this experiment. These are stated in the next section after first considering the experimental design.

2 Experimental design

In this report the term "diet" is synonymous with "pellet batch" or "batch" for short. A total of 24 batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "speed" (7, 14) where the bracketed text contains the levels of each factor. Batch number and treatment are provided in Table 2.1.

In this experiment samples to be analysed in the laboratory for moisture content were taken before and after the pelleting process. The "type" of sample collected has been classifed as either "mash" or "pellet". Two duplicates of both types of sample were collected. Therefore, the experiment comprises a total of 98 samples (2 duplicates $\times$ 2 types $\times$ 24 batches) to be analysed in the laboratory for moisture content. An experimental design based on 8 runs of 12 samples, where runs 1-4 contain the first duplicate of 48 samples comprising the combination of type and batch and runs 5-8 contain the second duplicate of 48 samples comprising the combination of type and batch, was generated in the R (R Development Core Team, 2011) statistical computing environment using the R package `od` (Butler, 2011).

There are two aims of this experiment. The first is to form predictions of moisture content for the 48 combinations of batch and type. These predictions are later used to derive other quantities of interest. The second aim is to determine if the main effects and interactions of type, grain, temperature, and speed affect moisture content. The statistical methods used to meet these aims are discussed

in the next section.

Table 2.1: List of batch numbers and treatments. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Batch (Diet) No.	Grain	Temp.	Speed
1	1894	80	7
2	1894	60	7
3	1896_SPROUT	80	14
4	1896_SPROUT	70	14
5	1896_SPROUT	70	7
6	1896_SPROUT	80	7
7	1895	70	14
8	1895	60	14
9	1895	70	7
10	1895	60	14
11	1894	80	14
12	1894	60	7
13	1894	70	14
14	1894	70	7
15	1895	80	7
16	1895	80	14
17	1896_SPROUT	60	7
18	1896_SPROUT	80	14
19	1894	70	14
20	1894	60	14
21	1895	80	7
22	1895	60	7
23	1896_SPROUT	60	14
24	1896_SPROUT	70	7

3 Statistical Methods

The two aims necessitate the fitting of two different linear mixed models to the moisture content data. The reason linear mixed model technology is being used is that the linear mixed model can be formulated in such a way that it is analagous 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. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). Unlike ANOVA, the linear mixed model has the ability to accomodate missing values in the response and explanatory variables and a wide range of variance models.

To meet the first aim, i.e., form predictions of moisture content for the 48 combinations of batch and type, the following model (in symbolic notation) was fitted to the moisture content data,

$$\text{MC} \sim 1 + \text{type:batch} + \mathbf{rblock} + \mathbf{run} + \mathbf{units} \tag{3.1}$$

where 1 is the overall mean, and type, batch, and run have been described previously. The terms in bold type are fitted as random effects and a variance component is associated with each of these terms. The term "rblock" refers to the two blocks of runs, i.e., runs 1-4 and runs 5-8. The term "units" represents random error. The estimated variance component associated with this term is referred to as the residual variance.

To meet the second aim the following model (in symbolic notation) was fitted to the moisture content data,

$$\begin{aligned} \text{MC} \sim & 1 + \text{type} + \text{grain} + \text{temp} + \text{speed} \\ & + \text{type:grain} + \text{type:temp} + \text{type:speed} \\ & + \text{grain:temp} + \text{grain:speed} + \text{temp:speed} \\ & + \text{type:grain:temp} + \text{type:grain:speed} \\ & + \text{grain:temp:speed} + \text{type:grain:temp:speed} \\ & + \mathbf{rblock} + \mathbf{run} + \mathbf{units} \end{aligned} \tag{3.2}$$

where the terms 1, type, grain, temp, speed, rblock, run, and units have been defined previously.

4 Results

Predictions based on the model presented in (3.1) for the combinations of batch and type are listed in Table 4.1.

Table 4.1: Predicted values and standard errors for type and batch combinations.

type/batch	predicted.value	standard.error
m1	11.13	0.229
m10	10.71	0.229
m11	10.46	0.229
m12	10.65	0.229
m13	9.93	0.229
m14	10.75	0.229
m15	11.14	0.229
m16	11.15	0.229
m17	11.26	0.229
m18	11.79	0.229
m19	10.03	0.229
m2	11.45	0.229
m20	10.34	0.229
m21	10.73	0.229
m22	11.00	0.229
m23	11.15	0.229
m24	11.53	0.229
m3	11.91	0.229
m4	11.56	0.229
m5	11.28	0.229
m6	12.26	0.229
m7	11.26	0.229

m8	10.94	0.229
m9	10.60	0.229
p1	12.36	0.229
p10	11.60	0.229
p11	11.91	0.229
p12	11.05	0.229
p13	11.41	0.229
p14	11.36	0.229
p15	11.65	0.229
p16	11.95	0.229
p17	12.71	0.229
p18	12.75	0.229
p19	10.76	0.229
p2	12.25	0.229
p20	10.96	0.229
p21	12.19	0.229
p22	12.70	0.229
p23	13.09	0.229
p24	12.57	0.229
p3	13.40	0.229
p4	12.66	0.229
p5	13.00	0.229
p6	13.90	0.229
p7	12.46	0.229
p8	11.97	0.229
p9	11.91	0.229

Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2) are presented in Table 4.2. The three-way interaction of grain, temperature, and time is statistically significant ($P < 0.05$) and is the appropriate level to form predictions. These are presented in Table 4.3.

Table 4.2: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2).

Term	DF	F.con	P-value
type	1	209.80	<0.001
grain	2	81.10	<0.001
temp	2	9.73	<0.001
time	1	9.88	0.003
type:grain	2	3.61	0.034
type:temp	2	0.06	0.939
grain:temp	4	3.77	0.009
type:time	1	0.22	0.640
grain:time	2	5.10	0.009
temp:time	2	2.53	0.089
type:grain:temp	4	1.82	0.139
type:grain:time	2	1.08	0.346
type:temp:time	2	0.11	0.900
grain:temp:time	4	2.79	0.035
type:grain:temp:time	4	0.79	0.539

Table 4.3: Predicted values and standard errors for the three-way interaction of grain, temperature, and speed based on the model presented in (3.2). The average standard error of difference (avsed) can be multiplied by 2 to give an approximate least significant difference at the 5% level.

grain	temp	speed	predicted.value	standard.error
1894	60	7	11.35	0.136
1894	60	14	10.65	0.193
1894	70	7	11.05	0.193
1894	70	14	10.54	0.136
1894	80	7	11.75	0.193
1894	80	14	11.18	0.193
1895	60	7	11.85	0.193
1895	60	14	11.30	0.136
1895	70	7	11.25	0.193
1895	70	14	11.85	0.193
1895	80	7	11.44	0.136
1895	80	14	11.55	0.193
1896_SPROUT	60	7	11.98	0.193
1896_SPROUT	60	14	12.12	0.193
1896_SPROUT	70	7	12.10	0.136
1896_SPROUT	70	14	12.10	0.193
1896_SPROUT	80	7	13.08	0.193
1896_SPROUT	80	14	12.46	0.136
avsed			0.249	

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APPENDIX III

STATISTICAL REPORT ON STARCH CONTENT OF THE PELLET DIETS

Statistical Consulting Report

PS030 - Starch content of wheat pellets

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August 17, 2012

Table of Contents

1 Introduction

This report has been compiled for Dr Peter Sopade, a senior research fellow with the University of Queensland. The protocol for this experiment has already been described in the document titled *Starch content of wheat pellets*. The trait considered in this report is **starch content (% of dry matter)** which is a function of a number of other variables described in the experimental protocol. There are two aims to this experiment. These are stated in the next section after first considering the experimental design.

2 Experimental design

In this report the term "diet" is synonymous with "pellet batch" or "batch" for short. A total of 24 batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "speed" (7, 14) where the bracketed text contains the levels of each factor. Batch number and treatment are provided in Table 2.1.

Two duplicates from each batch are analysed in the laboratory for starch content. Therefore, the experiment comprises a total of 48 samples (2 duplicates $\times$ 24 batches). An experimental design based on 8 runs of 6 samples, where runs 1-4 and runs 5-8 comprise a blocking factor with each containing one duplicate of every batch listed in Table 2.1, was generated in the R (R Development Core Team, 2011) statistical computing environment using the R package `od` (Butler, 2011).

There are two aims of this experiment. The first is to form predictions of starch content for each batch, and the second is to determine if the main effects and interactions of grain, temperature, and speed affect starch content. The statistical methods used to meet these aims are discussed in the next section.

Table 2.1: List of batch numbers and treatments. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Batch (Diet) No.	Grain	Temp.	Speed
1	1894	80	7
2	1894	60	7
3	1896_SPROUT	80	14
4	1896_SPROUT	70	14
5	1896_SPROUT	70	7
6	1896_SPROUT	80	7
7	1895	70	14
8	1895	60	14
9	1895	70	7
10	1895	60	14
11	1894	80	14
12	1894	60	7
13	1894	70	14
14	1894	70	7
15	1895	80	7
16	1895	80	14
17	1896_SPROUT	60	7
18	1896_SPROUT	80	14
19	1894	70	14
20	1894	60	14
21	1895	80	7
22	1895	60	7
23	1896_SPROUT	60	14
24	1896_SPROUT	70	7

3 Statistical Methods

The two aims necessitate the fitting of two different linear mixed models to the starch content data. The linear mixed model can be formulated in such a way that it is analagous 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. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). Unlike ANOVA, the linear mixed model has the ability to accommodate missing values in the response and explanatory variables and can accommodate a wide range of variance models. The R package `asreml` (Butler et al., 2007) was used to fit the two models presented below.

To meet the first aim, i.e., form predictions of starch content for each batch, the following model (in symbolic notation) was fitted to the starch content data,

$$\text{SC} \sim 1 + \text{batch} + +\mathbf{rblock} + \mathbf{run} + \mathbf{units} \tag{3.1}$$

where 1 is the overall mean, and batch and run have been described previously. The term "rblock" is a two level blocking factor comprising runs 1-4 and runs 5-8. The terms in bold type are fitted as random effects and a variance component is associated with each of these terms. The term "units" represents random error and is not explicitly fitted in the R call to `asreml`. The estimated variance component associated with this term is referred to as the residual variance.

To meet the second aim the following model (in symbolic notation) was fitted to the starch content data,

$$\begin{aligned} \text{SC} \sim & 1 + \text{grain} + \text{temp} + \text{speed} \\ & + \text{grain:temp} + \text{grain:speed} + \text{temp:speed} \\ & + \text{grain:temp:speed} + \mathbf{rblock} + \mathbf{run} + \mathbf{units} \end{aligned} \tag{3.2}$$

where the terms 1, grain, temp, speed, rblock, run, and units have been defined previously.

An assumption of both these models is that the residuals are normally distributed and have constant variance. This assumption was satisfactorily met in both models.

4 Results

Predictions for each batch based on the model presented in (3.1) are listed in Table 4.1.

Table 4.1: Starch content predicted values and standard errors for batch.

batch	predicted.value	standard.error
1	39.02	1.480
2	33.62	1.480
3	39.76	1.480
4	35.60	1.473
5	36.51	1.480
6	29.56	1.480
7	36.06	1.473
8	33.40	1.473
9	33.41	1.480
10	27.32	1.480
11	39.12	1.480
12	36.17	1.480
13	37.78	1.480
14	39.26	1.480
15	33.68	1.473
16	34.11	1.473
17	35.90	1.473
18	37.68	1.480
19	40.02	1.480
20	42.18	1.473
21	33.89	1.480
22	35.41	1.473
23	36.91	1.480
24	34.84	1.480

Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2) are presented in Table 4.2. The three-way interaction of grain, temperature, and speed is statistically significant ($P < 0.01$) and is therefore the appropriate level at which to form predictions. These are presented in Table 4.3.

Table 4.2: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2).

Term	DF	F.con	P-value
grain	2	19.81	<0.001
temp	2	0.88	0.427
speed	1	6.75	0.015
grain:temp	4	1.54	0.217
grain:speed	2	4.72	0.017
temp:speed	2	2.36	0.113
grain:temp:speed	4	6.50	0.001

Table 4.3: Predicted values and standard errors for the three-way interaction of grain, temperature, and speed based on the model presented in (3.2). The average standard error of difference (avsed) can be multiplied by 2 and 2.8 to give an approximate least significant difference at the 5% level and 1% level respectively.

grain	temp	speed	predicted.value	standard.error
1894	60	7	35.17	1.204
1894	60	14	42.50	1.633
1894	70	7	39.35	1.633
1894	70	14	38.95	1.204
1894	80	7	39.05	1.633
1894	80	14	39.45	1.633
1895	60	7	34.95	1.633
1895	60	14	30.30	1.204
1895	70	7	33.50	1.633
1895	70	14	35.85	1.633
1895	80	7	34.12	1.204
1895	80	14	34.25	1.633
1896_SPROUT	60	7	36.20	1.633
1896_SPROUT	60	14	36.80	1.633
1896_SPROUT	70	7	35.67	1.204
1896_SPROUT	70	14	35.60	1.633
1896_SPROUT	80	7	28.80	1.633
1896_SPROUT	80	14	38.23	1.204
avsed			2.014	

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APPENDIX IV

STATISTICAL REPORT ON PROTEIN CONTENT OF THE PELLET DIETS

Statistical Consulting Report

PS031 - Protein content of wheat pellets

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July 25, 2012

Table of Contents

1 Introduction

This report has been compiled for Dr Peter Sopade, a senior research fellow with the University of Queensland. The protocol for this experiment has already been described in the document titled *Nitrogen content of wheat pellets*. The trait considered in this report is **protein percentage on a dry basis** which is a function of moisture and nitrogen content. There are two aims to this experiment. These are stated in the next section after first considering the experimental design.

2 Experimental design

In this report the term "diet" is synonymous with "pellet batch" or "batch" for short. A total of 24 batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "speed" (7, 14) where the bracketed text contains the levels of each factor. Batch number and treatment are provided in Table 2.1.

Two duplicates from each batch are analysed in the laboratory for protein content. Therefore, the experiment comprises a total of 48 samples (2 duplicates $\times$ 24 batches). An experimental design based on 2 runs of 24 samples, where a run is used as a blocking factor and contains one duplicate of every batch listed in Table 2.1, was generated in the R (R Development Core Team, 2011) statistical computing environment using the R package `od` (Butler, 2011).

There are two aims of this experiment. The first is to form predictions of protein content for each batch, and the second is to determine if the main effects and interactions of grain, temperature, and speed affect protein content. The statistical methods used to meet these aims are discussed in the next section.

Table 2.1: List of batch numbers and treatments. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Batch (Diet) No.	Grain	Temp.	Speed
1	1894	80	7
2	1894	60	7
3	1896_SPROUT	80	14
4	1896_SPROUT	70	14
5	1896_SPROUT	70	7
6	1896_SPROUT	80	7
7	1895	70	14
8	1895	60	14
9	1895	70	7
10	1895	60	14
11	1894	80	14
12	1894	60	7
13	1894	70	14
14	1894	70	7
15	1895	80	7
16	1895	80	14
17	1896_SPROUT	60	7
18	1896_SPROUT	80	14
19	1894	70	14
20	1894	60	14
21	1895	80	7
22	1895	60	7
23	1896_SPROUT	60	14
24	1896_SPROUT	70	7

3 Statistical Methods

The two aims necessitate the fitting of two different linear mixed models to the protein content data. The reason linear mixed model technology is being used is that the linear mixed model can be formulated in such a way that it is analagous 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. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). Unlike ANOVA, the linear mixed model has the ability to accommodate missing values in the response and explanatory variables and a wide range of variance models. The R package `asreml` (Butler et al., 2007) was used to fit the two models presented below.

To meet the first aim, i.e., form predictions of protein content for each batch, the following model (in symbolic notation) was fitted to the protein content data,

$$\text{Prot.} \sim 1 + \text{batch} + \mathbf{run} + \mathbf{units} \tag{3.1}$$

where 1 is the overall mean, and batch and run have been described previously. The terms in bold type are fitted as random effects and a variance component is associated with each of these terms. The term "units" represents random error and is not explicitly fitted in the R call to `asreml`. The estimated variance component associated with this term is referred to as the residual variance.

To meet the second aim the following model (in symbolic notation) was fitted to

the protein content data,

$$\begin{aligned} \text{Prot} \sim & 1 + \text{grain} + \text{temp} + \text{speed} \\ & + \text{grain:temp} + \text{grain:speed} + \text{temp:speed} \\ & + \text{grain:temp:speed} + \mathbf{run} + \mathbf{units} \end{aligned} \tag{3.2}$$

where the terms 1, grain, temp, speed, run, and units have been defined previously.

An assumption of both these models is that the residuals are normally distributed and have constant variance. This assumption was satisfactorily met in both models. A centred linear covariate representing sample order in a run was icluded in both models. This term was found to be statistically significant ($P < 0.001$) for the first run in both models.

4 Results

Predictions for each batch based on the model presented in (3.1) are listed in Table 4.1.

Table 4.1: Protein content predicted values and standard errors for batch.

batch	predicted.value	standard.error
1	24.02	0.204
2	24.60	0.209
3	22.58	0.205
4	23.48	0.210
5	23.48	0.215
6	26.39	0.206
7	28.36	0.213
8	29.35	0.204
9	28.59	0.212
10	29.07	0.215
11	23.67	0.207
12	23.83	0.207
13	23.07	0.205
14	23.01	0.206
15	28.07	0.210
16	27.74	0.206
17	23.85	0.204
18	23.94	0.213
19	22.09	0.204
20	24.01	0.206
21	28.40	0.209
22	28.11	0.212
23	23.28	0.204
24	24.66	0.204

Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2) are presented in Table 4.2. The three-way interaction of grain, temperature, and speed is statistically significant ($P < 0.05$) and is the appropriate level to form predictions. These are presented in Table 4.3.

Table 4.2: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2).

Term	DF	F.con	P-value
grain	2	432.50	<0.001
temp	2	6.27	0.005
speed	1	14.68	0.001
grain:temp	4	8.18	<0.001
grain:speed	2	11.39	<0.001
temp:speed	2	9.95	0.001
grain:temp:speed	4	2.96	0.036

Table 4.3: Predicted values and standard errors for the three-way interaction of grain, temperature, and speed based on the model presented in (3.2). The average standard error of difference (avsed) can be multiplied by 2 to give an approximate least significant difference at the 5% level.

grain	temp	speed	predicted.value	standard.error
1894	60	7	24.21	0.242
1894	60	14	24.06	0.346
1894	70	7	22.97	0.345
1894	70	14	22.59	0.243
1894	80	7	24.01	0.343
1894	80	14	23.61	0.347
1895	60	7	28.02	0.353
1895	60	14	29.17	0.246
1895	70	7	28.68	0.353
1895	70	14	28.45	0.355
1895	80	7	28.31	0.253
1895	80	14	27.78	0.345
1896_SPROUT	60	7	23.83	0.343
1896_SPROUT	60	14	23.29	0.343
1896_SPROUT	70	7	24.12	0.248
1896_SPROUT	70	14	23.40	0.351
1896_SPROUT	80	7	26.34	0.346
1896_SPROUT	80	14	23.20	0.250
avsed			0.450	

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APPENDIX V

STATISTICAL REPORT ON FAT CONTENT OF THE PELLET DIETS

Statistical Consulting Report

PS032 - Crude fat content of wheat pellets

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July 25, 2012

Table of Contents

1 Introduction

This report has been compiled for Dr Peter Sopade, a senior research fellow with the University of Queensland. The protocol for this experiment has already been described in the document titled *Crude fat content of wheat pellets*. The trait considered in this report is **crude fat percentage on a dry basis** which is a function of moisture content. There are two aims to this experiment. These are stated in the next section after first considering the experimental design.

2 Experimental design

In this report the term "diet" is synonymous with "pellet batch" or "batch" for short. A total of 24 batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "speed" (7, 14) where the bracketed text contains the levels of each factor. Batch number and treatment are provided in Table 2.1.

Two duplicates from each batch are analysed in the laboratory for crude fat content. Therefore, the experiment comprises a total of 48 samples (2 duplicates $\times$ 24 batches). An experimental design based on 4 runs of 12 samples, where runs 1-2 and runs 3-4 comprise a blocking factor with each containing one duplicate of every batch listed in Table 2.1, was generated in the R (R Development Core Team, 2011) statistical computing environment using the R package `od` (Butler, 2011).

There are two aims of this experiment. The first is to form predictions of crude fat content for each batch, and the second is to determine if the main effects and interactions of grain, temperature, and speed affect crude fat content. The statistical methods used to meet these aims are discussed in the next section.

Table 2.1: List of batch numbers and treatments. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Batch (Diet) No.	Grain	Temp.	Speed
1	1894	80	7
2	1894	60	7
3	1896_SPROUT	80	14
4	1896_SPROUT	70	14
5	1896_SPROUT	70	7
6	1896_SPROUT	80	7
7	1895	70	14
8	1895	60	14
9	1895	70	7
10	1895	60	14
11	1894	80	14
12	1894	60	7
13	1894	70	14
14	1894	70	7
15	1895	80	7
16	1895	80	14
17	1896_SPROUT	60	7
18	1896_SPROUT	80	14
19	1894	70	14
20	1894	60	14
21	1895	80	7
22	1895	60	7
23	1896_SPROUT	60	14
24	1896_SPROUT	70	7

3 Statistical Methods

The two aims necessitate the fitting of two different linear mixed models to the crude fat content data. The linear mixed model can be formulated in such a way that it is analagous 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. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). Unlike ANOVA, the linear mixed model has the ability to accommodate missing values in the response and explanatory variables and can accommodate a wide range of variance models. The R package `asreml` (Butler et al., 2007) was used to fit the two models presented below.

To meet the first aim, i.e., form predictions of crude fat content for each batch, the following model (in symbolic notation) was fitted to the crude fat content data,

$$\text{Fat} \sim 1 + \text{batch} + + \mathbf{rblock} + \mathbf{run} + \mathbf{units} \tag{3.1}$$

where 1 is the overall mean, and batch and run have been described previously. The term "rblock" is a two level blocking factor comprising runs 1-2 and runs 3-4. The terms in bold type are fitted as random effects and a variance component is associated with each of these terms. The term "units" represents random error and is not explicitly fitted in the R call to `asreml`. The estimated variance component associated with this term is referred to as the residual variance.

To meet the second aim the following model (in symbolic notation) was fitted to the crude content data,

$$\begin{aligned} \text{Fat} \sim & 1 + \text{grain} + \text{temp} + \text{speed} \\ & + \text{grain:temp} + \text{grain:speed} + \text{temp:speed} \\ & + \text{grain:temp:speed} + \mathbf{rblock} + \mathbf{run} + \mathbf{units} \end{aligned} \tag{3.2}$$

where the terms 1, grain, temp, speed, rblock, run, and units have been defined previously.

An assumption of both these models is that the residuals are normally distributed and have constant variance. This assumption was satisfactorily met in both models.

4 Results

Predictions for each batch based on the model presented in (3.1) are listed in Table 4.1.

Table 4.1: Crude fat content predicted values and standard errors for batch.

batch	predicted.value	standard.error
1	7.86	0.228
2	8.99	0.228
3	6.96	0.228
4	7.29	0.228
5	7.79	0.228
6	9.09	0.228
7	7.54	0.228
8	8.04	0.228
9	7.74	0.228
10	7.94	0.228
11	7.69	0.228
12	7.64	0.228
13	7.69	0.228
14	7.71	0.228
15	7.29	0.228
16	7.26	0.228
17	7.36	0.228
18	7.06	0.228
19	7.36	0.228
20	7.71	0.228
21	7.56	0.228
22	7.91	0.228
23	7.36	0.228
24	7.86	0.228

Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2) are presented in Table 4.2. The three-way interaction of grain, temperature, and speed is statistically significant ($P < 0.01$) and is therefore the appropriate level at which to form predictions. These are presented in Table 4.3.

Table 4.2: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2).

Term	DF	F.con	P-value
grain	2	1.60	0.220
temp	2	1.84	0.176
speed	1	18.65	<0.001
grain:temp	4	3.92	0.012
grain:speed	2	5.68	0.008
temp:speed	2	3.19	0.056
grain:temp:speed	4	5.00	0.003

Table 4.3: Predicted values and standard errors for the three-way interaction of grain, temperature, and speed based on the model presented in (3.2). The average standard error of difference (avsed) can be multiplied by 2 and 2.8 to give an approximate least significant difference at the 5% level and 1% level respectively.

grain	temp	speed	predicted.value	standard.error
1894	60	7	8.34	0.194
1894	60	14	7.61	0.267
1894	70	7	7.77	0.267
1894	70	14	7.53	0.191
1894	80	7	7.76	0.267
1894	80	14	7.63	0.267
1895	60	7	7.97	0.267
1895	60	14	8.09	0.197
1895	70	7	7.68	0.267
1895	70	14	7.48	0.267
1895	80	7	7.43	0.191
1895	80	14	7.32	0.267
1896_SPROUT	60	7	7.26	0.267
1896_SPROUT	60	14	7.26	0.267
1896_SPROUT	70	7	7.90	0.194
1896_SPROUT	70	14	7.39	0.267
1896_SPROUT	80	7	9.19	0.267
1896_SPROUT	80	14	6.91	0.197
avsed			0.335	

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APPENDIX VI

STATISTICAL REPORT ON ASH CONTENT OF THE PELLET DIETS

Statistical Consulting Report

PS033 - Ash content of wheat pellets

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Table of Contents

1 Introduction

This report has been compiled for Dr Peter Sopade, a senior research fellow with the University of Queensland. The protocol for this experiment has already been described in the document titled *Ash content of wheat pellets*. The trait considered in this report is **ash percentage on a dry basis** which is a function of moisture content. There are two aims to this experiment. These are stated in the next section after first considering the experimental design.

2 Experimental design

In this report the term "diet" is synonymous with "pellet batch" or "batch" for short. A total of 24 batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "speed" (7, 14) where the bracketed text contains the levels of each factor. Batch number and treatment are provided in Table 2.1.

Two duplicates from each batch are analysed in the laboratory for ash content. Therefore, the experiment comprises a total of 48 samples (2 duplicates $\times$ 24 batches). An experimental design based on 4 runs of 12 samples, where runs 1-2 and runs 3-4 comprise a blocking factor with each containing one duplicate of every batch listed in Table 2.1, was generated in the R (R Development Core Team, 2011) statistical computing environment using the R package `od` (Butler, 2011).

There are two aims of this experiment. The first is to form predictions of ash content for each batch, and the second is to determine if the main effects and interactions of grain, temperature, and speed affect ash content. The statistical methods used to meet these aims are discussed in the next section.

Table 2.1: List of batch numbers and treatments. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Batch (Diet) No.	Grain	Temp.	Speed
1	1894	80	7
2	1894	60	7
3	1896_SPROUT	80	14
4	1896_SPROUT	70	14
5	1896_SPROUT	70	7
6	1896_SPROUT	80	7
7	1895	70	14
8	1895	60	14
9	1895	70	7
10	1895	60	14
11	1894	80	14
12	1894	60	7
13	1894	70	14
14	1894	70	7
15	1895	80	7
16	1895	80	14
17	1896_SPROUT	60	7
18	1896_SPROUT	80	14
19	1894	70	14
20	1894	60	14
21	1895	80	7
22	1895	60	7
23	1896_SPROUT	60	14
24	1896_SPROUT	70	7

3 Statistical Methods

The two aims necessitate the fitting of two different linear mixed models to the ash content data. The linear mixed model can be formulated in such a way that it is analagous 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. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). Unlike ANOVA, the linear mixed model has the ability to accommodate missing values in the response and explanatory variables and can accommodate a wide range of variance models. The R package `asreml` (Butler et al., 2007) was used to fit the two models presented below.

To meet the first aim, i.e., form predictions of ash content for each batch, the following model (in symbolic notation) was fitted to the ash content data,

$$\text{Ash} \sim 1 + \text{batch} + +\mathbf{rblock} + \mathbf{run} + \mathbf{units} \tag{3.1}$$

where 1 is the overall mean, and batch and run have been described previously. The term "rblock" is a two level blocking factor comprising runs 1-2 and runs 3-4. The terms in bold type are fitted as random effects and a variance component is associated with each of these terms. The term "units" represents random error and is not explicitly fitted in the R call to `asreml`. The estimated variance component associated with this term is referred to as the residual variance.

To meet the second aim the following model (in symbolic notation) was fitted to the ash content data,

$$\begin{aligned} \text{Ash} \sim & 1 + \text{grain} + \text{temp} + \text{speed} \\ & + \text{grain:temp} + \text{grain:speed} + \text{temp:speed} \\ & + \text{grain:temp:speed} + \mathbf{rblock} + \mathbf{run} + \mathbf{units} \end{aligned} \tag{3.2}$$

where the terms 1, grain, temp, speed, rblock, run, and units have been defined previously.

An assumption of both these models is that the residuals are normally distributed and have constant variance. This assumption was satisfactorily met in both models.

4 Results

Predictions for each batch based on the model presented in (3.1) are listed in Table 4.1.

Table 4.1: Ash content predicted values and standard errors for batch.

batch	predicted.value	standard.error
1	7.20	0.175
2	8.00	0.175
3	6.15	0.175
4	6.85	0.175
5	7.15	0.175
6	8.40	0.175
7	7.15	0.175
8	7.55	0.175
9	7.15	0.175
10	7.45	0.175
11	7.00	0.175
12	7.25	0.175
13	6.80	0.175
14	6.65	0.175
15	6.75	0.175
16	7.05	0.175
17	7.20	0.175
18	6.75	0.175
19	6.40	0.175
20	7.05	0.175
21	6.70	0.175
22	6.80	0.175
23	6.60	0.175
24	7.00	0.175

Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2) are presented in Table 4.2. The three-way interaction of grain, temperature, and speed is statistically significant ($P < 0.01$) and is therefore the appropriate level at which to form predictions. These are presented in Table 4.3.

Table 4.2: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2).

Term	DF	F.con	P-value
grain	3	1.13	0.352
temp	2	4.77	0.016
speed	1	12.72	0.001
grain:temp	4	5.19	0.003
grain:speed	2	19.70	<0.001
temp:speed	2	4.17	0.026
grain:temp:speed	4	6.15	0.001

Table 4.3: Predicted values and standard errors for the three-way interaction of grain, temperature, and speed based on the model presented in (3.2). The average standard error of difference (avsed) can be multiplied by 2 and 2.8 to give an approximate least significant difference at the 5% level and 1% level respectively.

grain	temp	speed	predicted.value	standard.error
1894	60	7	7.62	0.152
1894	60	14	7.02	0.209
1894	70	7	6.68	0.209
1894	70	14	6.60	0.152
1894	80	7	7.21	0.209
1894	80	14	7.03	0.209
1895	60	7	6.79	0.209
1895	60	14	7.45	0.175
1895	70	7	7.16	0.209
1895	70	14	7.12	0.209
1895	80	7	6.74	0.153
1895	80	14	7.08	0.209
1895.0	60	7	NA	NA
1895.0	60	14	7.61	0.292
1895.0	70	7	NA	NA
1895.0	70	14	NA	NA
1895.0	80	7	NA	NA
1895.0	80	14	NA	NA
1896_SPROUT	60	7	7.17	0.209
1896_SPROUT	60	14	6.59	0.209
1896_SPROUT	70	7	7.10	0.153
1896_SPROUT	70	14	6.82	0.209
1896_SPROUT	80	7	8.41	0.209
1896_SPROUT	80	14	6.44	0.153
avsed			0.227	

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APPENDIX VII

STATISTICAL REPORT ON PASTING BEHAVIOURS OF THE MASH AND PELLETS

Statistics for the Australian Pork Industry Technical Report Series

PS034 - Wheat mash and pellet gelatinisation characteristics

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February 21, 2013

Contents

1 Introduction

This report has been compiled for Dr Peter Sopade. The protocol for this experiment has already been described in the document titled PS034 - Gelatinisation characteristics of mash and pellets from wheat. A number of traits are considered in this report. They are listed in Table 1.1.

Table 1.1: Traits analysed in this report, their unit of measurement, and abbreviation. The unit of measurement cP stands for centipoise; a measurement of dynamic viscosity.

Trait (unit of measurement)	Abbreviation
pasting temperature ($^{\circ}C$)	ptemp
peak time (min.)	ptime
peak viscosity (cP)	peak
trough viscosity (cP)	trough
breakdown (cP)	brk
final viscosity (cP)	fvisc
setback (cP)	sb
initial viscosity (cP)	iv

The aim of this experiment is to investigate the effects of grain, conditioning temperature, and conditioning time on each of the traits listed above. A number of these traits are highly correlated (Table 1.2).

Table 1.2: Raw correlations between the 8 traits analysed in this report.

	peak	trough	brk	fvisc	sb	ptime	ptemp	iv
peak	1.000	0.934	0.756	0.929	0.916	0.118	0.301	-0.119
trough	0.934	1.000	0.471	0.984	0.966	0.372	0.364	-0.078
breakdown	0.756	0.471	1.000	0.487	0.489	-0.389	0.078	-0.150
fvisc	0.929	0.984	0.487	1.000	0.996	0.351	0.385	-0.176
sb	0.916	0.966	0.489	0.996	1.000	0.337	0.391	-0.222
ptime	0.118	0.372	-0.389	0.351	0.337	1.000	0.165	-0.226
ptemp	0.301	0.364	0.078	0.385	0.391	0.165	1.000	0.035
iv	-0.119	-0.078	-0.150	-0.176	-0.222	-0.226	0.035	1.000

2 Experimental design

In this report the term "diet" is synonymous with "batch". A total of 24 batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "time" (7, 14) where the bracketed text contains the levels of each factor. Batch number and treatment are provided in Table 2.1.

Two duplicates of the 24 pelleted batches and 2 duplicates of mash samples derived from the 24 batches were analysed in the laboratory for gelatinisation characteristics. Therefore, the experiment comprises a total of 96 lab samples. An experimental design based on 6 runs of 10 samples and 4 runs of 9 samples (runs 4,5,6, and 10) with runs 1-5 and runs 6-10 comprising a blocking factor, referred to as dblock, with each containing one mash and pellet duplicate of every batch listed in Table 2.1. A design was generated in the R (R Development Core Team, 2011) statistical computing environment using the R package `od` (Butler, 2011).

2 Experimental design

Table 2.1: List of batch numbers and treatments. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Batch (Diet) No.	Grain	Temp.	Time
1	1894	80	7
2	1894	60	7
3	1896_SPROUT	80	14
4	1896_SPROUT	70	14
5	1896_SPROUT	70	7
6	1896_SPROUT	80	7
7	1895	70	14
8	1895	60	14
9	1895	70	7
10	1895	60	14
11	1894	80	14
12	1894	60	7
13	1894	70	14
14	1894	70	7
15	1895	80	7
16	1895	80	14
17	1896_SPROUT	60	7
18	1896_SPROUT	80	14
19	1894	70	14
20	1894	60	14
21	1895	80	7
22	1895	60	7
23	1896_SPROUT	60	14
24	1896_SPROUT	70	7

3 Statistical Methods

For each trait linear mixed model technology was used to fit a statistical model to the data. The linear mixed model 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. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). Unlike ANOVA, the linear mixed model has the ability to accommodate missing values in the response and explanatory variables and can accommodate a wide range of variance models. The R package `asreml` (Butler et al., 2007) was used to fit all models.

To meet the aim of the experiment the following model, using the symbolic notation described by Wilkinson and Rogers (1973), was fitted to each trait,

$$\begin{aligned} \text{trait} \quad \sim \quad & 1 + \text{type*grain*temp*time} \\ & + \mathbf{dblock} + \mathbf{batch} + \mathbf{pelletday} + \mathbf{pelletorder} + \mathbf{pelletsession} \\ & + \mathbf{labday} + \mathbf{laborder} + \mathbf{units} \end{aligned} \tag{3.1}$$

where 1 is the overall mean, type, grain, temp, and time represent the treatment factors type (pellet or mash), grain, conditioning temperature, and conditioning time. Terms in bold font are fitted as random effects. For each random term there is an associated variance component. The term units represents random error and is not explicitly fitted in the R call to `asreml`. A single estimated variance component associated with this term would be referred to as the residual variance. In the case of a single estimated variance component associated with the vector of residuals there is an assumption that the residuals are normally distributed with constant variance. The traits trough viscosity, final viscosity, and setback (which are highly correlated) required a square root transformation to better satisfy this assumption.

A separate variance structure for each grain type was included in the model fitted to peak

time and breakdown. A centred linear covariate associated with the order samples were processed in the laboratory was fitted as a fixed effect in the models for peak viscosity and breakdown ($P < 0.05$ and $P < 0.01$ respectively). The later also included a centred linear covariate associated with the order diets were pelleted ($P < 0.01$). All traits except pasting temperature and peak time had non-treatment variation that could be attributed to the pelleting process in some way, such as variation between pelleting days, variation between pelleting orders, variation between pelleting sessions, and variation between pelleting batches. This reinforces the need to have an experimental design which can accommodate these potential sources of variation.

There were a small number of outliers and a number of "zero" data points. Both types were declared "NA" or missing (see Table 3.1).

Table 3.1: Number of outliers and zero data points for particular traits.

Trait	Number of Outliers	Number of zero data points
ptime	3	NA
trough	1	14
fvisc	NA	4
sb	NA	4
iv	NA	2

Wald type F-statistics for each trait are presented in Section 4. Section 4 has been partitioned into traits which have been analysed on their natural scale and those that have been analysed on a (square root) transformed scale. For each trait predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level have been provided in *.csv* files.

4 Results

4.1 Traits analysed on their natural scale

Wald type F-statistics and associated p-values for the terms fitted as fixed effects in model (3.1) for the traits pasting temperature, peak time, peak viscosity, breakdown, and initial viscosity are presented in Table 4.1. The four-way interaction of type, grain, conditioning temperature, and conditioning time is statistically significant for the traits peak time, peak viscosity, and breakdown. The three-way interaction of grain, conditioning temperature, and conditioning time was statistically significant for the traits initial viscosity and pasting temperature. The three-way interaction of type, conditioning temperature and conditioning time was borderline significant for the trait initial viscosity and predicted values have been produced for this interaction as well as the others.

Table 4.1: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the models for the traits peak temperature, peak time, peak viscosity, breakdown, and initial viscosity.

		Pasting temperature		Peak time		Peak viscosity		Breakdown		Initial viscosity	
Term	Df	F-statistic	P-value	F-statistic	P-value	F-statistic	P-value	F-statistic	P-value	F-statistic	P-value
type	1	1.74	0.202	23.04	<0.001	141.00	<0.001	49.56	<0.001	5.93	0.024
grain	2	5.42	0.013	861.20	<0.001	509.30	<0.001	241.90	<0.001	0.03	0.974
temp	2	0.37	0.693	0.29	0.748	1.93	0.171	3.71	0.043	1.00	0.384
time	1	0.49	0.494	0.32	0.579	13.63	0.001	6.68	0.018	0.82	0.377
lin(laborder)	1	NA	NA	NA	NA	5.12	0.016	11.00	0.001	NA	NA
lin(pelletorder)	1	NA	NA	NA	NA	NA	NA	7.12	0.005	NA	NA
type:grain	2	1.34	0.283	5.25	0.015	69.05	<0.001	85.63	<0.001	2.37	0.120
type:temp	2	1.45	0.258	0.18	0.836	1.17	0.353	0.01	0.915	0.16	0.850
grain:temp	4	1.51	0.236	8.42	<0.001	5.96	0.024	2.07	0.152	0.54	0.711
type:time	1	0.03	0.864	5.74	0.026	2.45	0.112	0.65	0.531	0.03	0.865
grain:time	2	0.57	0.573	1.09	0.357	2.21	0.135	3.81	0.019	0.50	0.611
temp:time	2	0.09	0.911	3.93	0.036	1.27	0.315	3.62	0.046	0.35	0.709
type:grain:temp	4	0.77	0.557	5.20	0.005	4.60	0.023	1.89	0.178	0.60	0.665
type:grain:time	2	2.14	0.144	0.07	0.929	6.02	0.009	0.33	0.854	0.04	0.965
type:temp:time	2	0.01	0.994	0.43	0.656	3.97	0.016	0.43	0.787	3.47	0.051
grain:temp:time	4	2.97	0.045	9.31	<0.001	7.71	0.001	1.74	0.202	5.05	0.006
type:grain:temp:time	4	0.91	0.478	13.95	<0.001	5.80	0.026	5.50	0.029	0.92	0.473

4.2 Traits analysed on a (square root) transformed scale

Wald type F-statistics and associated p-values for the terms fitted as fixed effects in model (3.1) for the traits trough viscosity, final viscosity and setback are presented in Table 4.2. The four-way interaction of type, grain, conditioning temperature, and conditioning time was statistically significant for the trait final viscosity. The three way interactions of grain, conditioning temperature, and conditioning time; type, grain, and conditioning time; and type, grain, and conditioning temperature were statistically significant for the traits trough viscosity and setback.

Table 4.2: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the models for the traits trough viscosity, final viscosity, and setback.

		Trough viscosity		Final viscosity		Setback	
Term	Df	F-statistic	P-value	F-statistic	P-value	F-statistics	P-value
type	1	49.29	<0.001	68.50	<0.001	132.40	<0.001
grain	2	412.80	<0.001	287.60	<0.001	409.70	<0.001
temp	2	4.58	0.023	0.29	0.752	1.11	0.349
time	1	16.87	0.001	0.14	0.708	0.01	0.944
type:grain	2	18.30	<0.001	37.39	<0.001	25.11	<0.001
type:temp	2	0.86	0.437	0.23	0.799	0.17	0.847
grain:temp	4	4.04	0.015	2.77	0.056	3.17	0.036
type:time	1	2.95	0.101	0.35	0.558	0.12	0.731
grain:time	2	7.13	0.005	1.89	0.177	3.31	0.057
temp:time	2	2.92	0.077	1.89	0.177	1.59	0.229
type:grain:temp	3	3.49	0.026	3.98	0.016	3.85	0.018
type:grain:time	2	3.81	0.040	3.17	0.064	3.66	0.044
type:temp:time	2	2.99	0.073	0.14	0.870	0.42	0.665
grain:temp:time	3	5.40	0.004	2.42	0.082	4.60	0.009
type:grain:temp:time	3	2.50	0.075	3.33	0.030	1.58	0.217

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APPENDIX VIII

STATISTICAL REPORT ON *IN VITRO* STARCH DIGESTION OF THE PELLET DIETS

Statistics for the Australian Pork Industry Technical Report Series

PS035 - Rapid in-vitro starch digestibility of wheat pellets

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Contents

1 Introduction

This report has been compiled for Dr Peter Sopade. The protocol for this experiment has already been described in the document titled PS035 - *in-vitro* starch digestion. The trait considered in this report is **digested starch content**. There are two aims to this experiment. The first is to investigate the effects of grain, conditioning temperature, and conditioning time on *in-vitro* starch digestion. The second is to provide predicted values (sometimes referred to as reference values in the context of NIR calibration) for use in a NIR calibration.

2 Experimental design

In this report the term "diet" is synonymous with "pellet batch" or "batch" for short. A total of 24 batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "time" (7, 14) where the bracketed text contains the levels of each factor. Batch number and treatment are provided in Table 2.1.

Two duplicates from each batch are analysed in the laboratory for digested starch content. Therefore, the experiment comprises a total of 48 samples (2 duplicates $\times$ 24 batches). An experimental design based on 6 runs of 8 samples with runs 1-3 and runs 4-6 comprising a blocking factor with each containing one duplicate of every batch listed in Table 2.1. A design was generated in the R (R Development Core Team, 2011) statistical computing environment using the R package `od` (Butler, 2011). For each of the 48 samples digested starch content measurements are taken at 12 time periods, which are 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 minutes.

2 Experimental design

Table 2.1: List of batch numbers and treatments. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Batch (Diet) No.	Grain	Temp.	Time
1	1894	80	7
2	1894	60	7
3	1896_SPROUT	80	14
4	1896_SPROUT	70	14
5	1896_SPROUT	70	7
6	1896_SPROUT	80	7
7	1895	70	14
8	1895	60	14
9	1895	70	7
10	1895	60	14
11	1894	80	14
12	1894	60	7
13	1894	70	14
14	1894	70	7
15	1895	80	7
16	1895	80	14
17	1896_SPROUT	60	7
18	1896_SPROUT	80	14
19	1894	70	14
20	1894	60	14
21	1895	80	7
22	1895	60	7
23	1896_SPROUT	60	14
24	1896_SPROUT	70	7

3 Statistical Methods

The two aims necessitate the fitting of two models. We consider each in turn.

3.1 Statistical model to investigate the effects of grain, conditioning temperature, and conditioning time

To meet the first aim we have used a linear mixed model incorporating cubic smoothing splines (Verbyla et al., 1999) to model *in-vitro* digested starch over time. Using this technology we are able to model the non-linear relationship of digested starch over time, use Wald type F-statistics to test the significance of the main effects and interactions between grain, conditioning temperature and conditioning time, and can account for non-treatment sources of variation.

The following model (using the symbolic notation described by Wilkinson and Rogers (1973) except we have used the ":" notation in place of the "·" notation) was fitted to the *in-vitro* digested starch data,

$$\begin{aligned} \text{DS} \sim\ & 1 + \text{grain*ctemp*ctime*lin(ltime)} \\ & + \mathbf{PelletBatch} + \mathbf{PelletDay} + \mathbf{PelletOrder} \\ & + \mathbf{PelletSession} + \mathbf{LabBlock} + \mathbf{LabRun} + \mathbf{LabRun{:}LabBottle} \\ & + \mathbf{spl(ltime)} + \mathbf{(grain*ctemp*ctime){:}spl(ltime)} \\ & + \mathbf{dev(ltime)} + \mathbf{(grain*ctemp*ctime){:}dev(ltime)} + \mathbf{units} \end{aligned} \tag{3.1}$$

where 1 is the overall mean, grain, ctemp, and ctime represent the treatment factors grain, conditioning temperature, and conditioning time. The term lin(ltime) represents linear trend over the 12 times at which *in-vitro* digested starch was measured in the laboratory. The terms in bold are fitted as random effects and have an associated variance component.

The names of most terms are self explanatory. However, a short description of spl(ltime), dev(ltime), and units follows.

The term spl(ltime) is used to model curvature and the term dev(ltime) represents random deviations from the fitted cubic smoothing spline. The term units represents random error and is not explicitly fitted in the R call to `asreml`. A single estimated variance component associated with this term would be referred to as the residual variance. In the case of a single estimated variance component associated with the vector of residuals there is an assumption that the residuals are independent and identically distributed (iid). For longitudinal (or repeated measures) data this assumption is not realistic. A more realistic assumption would be that each of the 12 time points at which digested starch was measured had its own variance and that these time points were correlated. In particular, time points that are closer together will be more correlated than time points further apart. For the variance matrix associated with the vector of residuals we have considered a series of antedependence variance models. The improvements in the residual log-likelihood and the p-value associated with a residual maximum likelihood ratio test (REMLRT) of this improvement is provided in Table 3.1.

Table 3.1: Series of variance models considered for the variance matrix associated with the vector of residuals, the number of parameters for each variance model, each models residual log-likelihood (LL) at convergence, and p-values associated with residual maximum log-likelihood ratio tests (REMLRT) of one model versus another.

Model No.	Variance model	No. parameters	LL	Test & p-value
(1)	iid	1	-602.37	
(2)	ante,1	23	-380.79	(2) v (1) < 0.001
(3)	ante,2	33	-362.00	(3) v (2) < 0.001
(4)	ante,3	42	-356.53	(4) v (3) 0.27

An antedependence model of order 2 was the final variance model chosen for the variance matrix associated with the vector of residuals. The Wald type F-statistics associated with the fixed effects in model (3.1) are presented in section 4.

3.2 Statistical model for providing predicted values to be used in a NIR calibration

To meet the second aim we have also used a linear mixed model incorporating cubic smoothing splines (Verbyla et al., 1999) to model *in-vitro* digested starch over time. However, since the aim is to provide predicted values for pellet batches the model is different

to the model in (3.1). For the purposes of providing pellet batch predicted values (or reference values) to be used in a NIR calibration we have used the following model,

$$\begin{aligned} \text{DS} \sim\ & 1 + \text{PelletBatch} + \text{lin(ltime)} + \text{PelletBatch:lin(ltime)} \\ & + \mathbf{LabBlock} + \mathbf{LabRun} + \mathbf{LabRun:LabBottle} \\ & + \mathbf{spl(ltime)} + \mathbf{PelletBatch:spl(ltime)} \\ & + \mathbf{dev(ltime)} + \mathbf{PelletBatch:dev(ltime)} + \mathbf{units}. \end{aligned} \tag{3.2}$$

The final variance model associated with the vector of residuals was, like model (3.1), an antedependence variance model of order 2. We will refer to this model as LMM-CSS.

Another popular model for modelling *in-vitro* digested starch is the first order kinetic model (FOKM). In section 5 we provide a graph of the observed data with both the LMM-CSS and FOKM fitted values. We also calculate the mean square error of prediction (MSEP), defined as the average of the sum of the squared differences between fitted values and observed data, for each model. A short discussion on the implications of the later is provided in section 6.

4 Results

Conditional F-statistics and associated p-values for the terms fitted as fixed effects in model (3.1) are presented in Table 4.1. The four-way interaction of grain, conditioning temperature, conditioning time, and laboratory time is statistically significant ($P < 0.01$). Requested predicted values have been provided to the lead researcher in *.csv* files.

Table 4.1: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in model (3.1).

Term	DF	F.con	P-value
grain	2	8.35	0.007
temp	2	5.12	0.029
ctime	1	0.10	0.762
lin(ltime)	1	2546.00	<0.001
grain:temp	4	1.47	0.283
grain:ctime	2	2.69	0.116
temp:ctime	2	0.41	0.675
grain:lin(ltime)	2	96.17	<0.001
temp:lin(ltime)	2	15.36	0.001
ctime:lin(ltime)	1	10.80	0.008
grain:temp:ctime	4	0.74	0.588
grain:temp:lin(ltime)	4	11.35	0.001
grain:ctime:lin(ltime)	2	4.94	0.032
temp:ctime:lin(ltime)	2	1.60	0.249
grain:temp:ctime:lin(ltime)	4	10.94	0.001

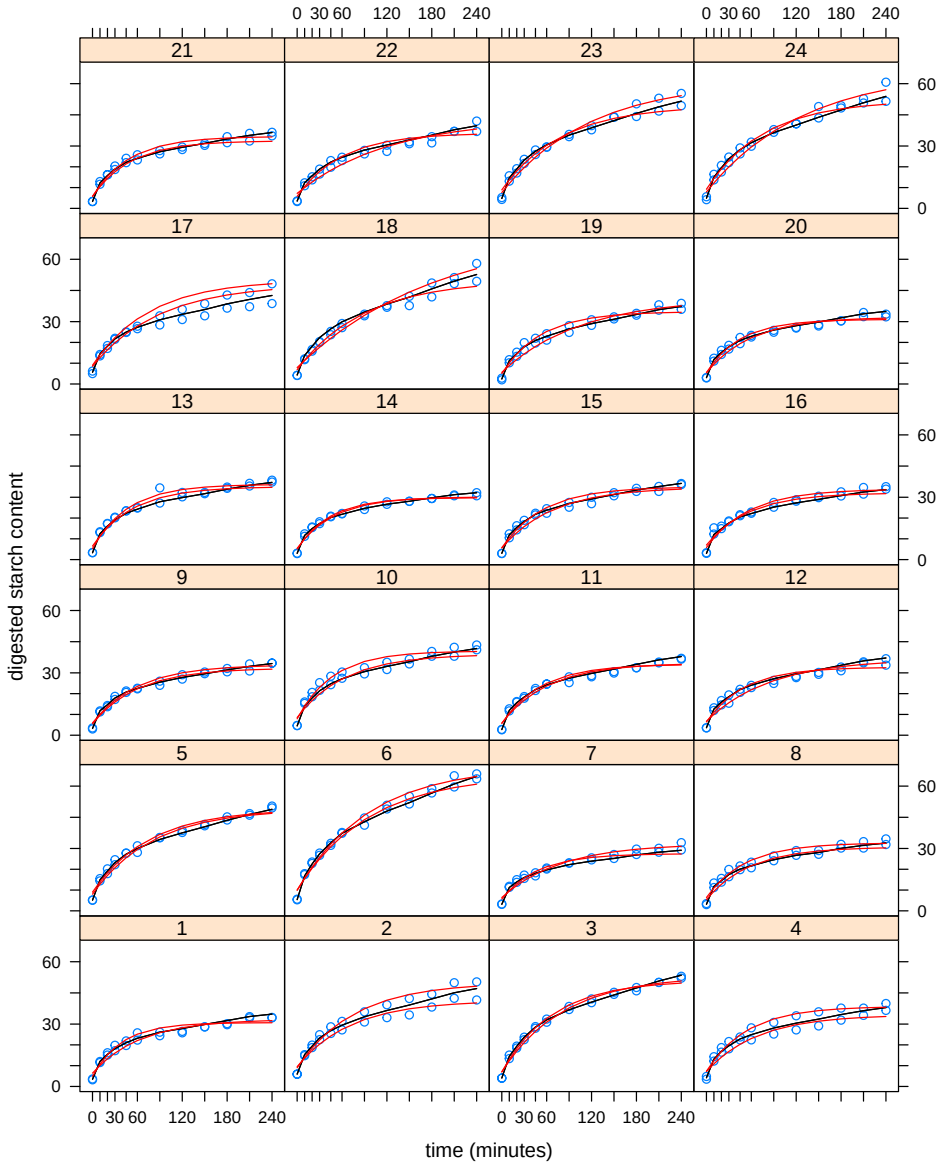
5 Model comparisons

A graph of LMM-CSS and FOKM fitted values superimposed on the observed data is provided in Figure 5.1. From this graph we would conclude that the FOKM provides a satisfactory fit to the majority of the observed data. A question of interest is how much better is the fit when using the linear mixed model incorporating cubic smoothing splines. As an objective measure we have considered the mean square error of prediction (MSEP) for both models (Table 5.1).

Table 5.1: Mean square error of prediction (MSEP) for LMM-CSS and FOKM.

Model	MSEP
LMM-CSS	2.55
FOKM	4.13

Figure 5.1: Graph of LMM-CSS (black lines) and FOKM (red lines) fitted values against observed data for each of the 24 pellet batches. Note that only 1 line is visible for LMM-CSS as there is no variation between pellet batch duplicates so the fitted values are the same, i.e. it looks like 1 line but is in fact 2 lines on top of each other.



6 Conclusions

For the purposes of providing pellet batch predicted values to be used in a NIR calibration the linear mixed model incorporating cubic smoothing (LMM-CSS) should be the preferred option. The mean square error of prediction (MSEP) when using this model is approximately 38% less than that when using a first order kinetic model (FOKM). It should be noted that when using LMM-CSS derived quantities of interest such as area under the curve (AUC) and rate of change (K) can be numerically calculated if required.

If the aim is to consider the main effects and interactions of grain, conditioning temperature, and conditioning time, then the same technology (but different model) can be used. This technology can accommodate both treatment and non-treatment effects, as well as a wide range of variance models. This results in more reliable estimates of treatment effects compared to the alternative of fitting a first order kinetic model to each pellet batch laboratory duplicate, computing a derived quantity, then fitting another model analogous to ANOVA to determine treatment effects.

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Results follow:
BEGIN

	Do	Doo	K	AUC	Exp240	Pre240
Do	1.00	0.74	-0.62	0.82	0.77	0.80
Doo	0.74	1.00	-0.78	0.92	0.97	0.98
K	-0.62	-0.78	1.00	-0.59	-0.72	-0.73
AUC	0.82	0.92	-0.59	1.00	0.95	0.98
Exp240	0.77	0.97	-0.72	0.95	1.00	0.98
Pre240	0.80	0.98	-0.73	0.98	0.98	1.00

######
Do
######

	Df	F.con	Pr
(Intercept)	1	858.9000	0.0000
grain	2	10.4800	0.0110
temp	2	1.9790	0.2187
time	1	1.5460	0.2601
grain:temp	4	0.1783	0.9415
grain:time	2	1.7940	0.2451
temp:time	2	0.1487	0.8649
grain:temp:time	4	1.0160	0.4685

#######
Doo
#######

	Df	F.con	Pr
(Intercept)	1	569.100	0.0000
grain	2	38.590	0.0004
temp	2	2.796	0.1387
time	1	2.348	0.1764
grain:temp	4	3.256	0.0958
grain:time	2	1.963	0.2105
temp:time	2	0.370	0.7055
grain:temp:time	4	2.687	0.1345

#####
K
#######

	Df	F.con	Pr
(Intercept)	1	228.7000	0.0000
grain	2	9.7480	0.0188
temp	2	1.2790	0.3560
time	1	0.4355	0.5338
grain:temp	4	1.0900	0.4515
grain:time	2	0.9710	0.4312
temp:time	2	1.8420	0.2378
grain:temp:time	4	1.2820	0.3881

''''''''''''
AUC
''''''''''''

	Df	F.con	Pr
(Intercept)	1	2.071e+03	0.0000
grain	2	2.575e+01	0.0011
temp	2	2.202e+00	0.1918
time	1	2.539e+00	0.1622
grain:temp	4	1.312e+00	0.3643
grain:time	2	1.909e+00	0.2282
temp:time	2	9.953e-02	0.9067
grain:temp:time	4	1.749e+00	0.2573

''''''''''''''''
Exp240
''''''''''''''''

	Df	F.con	Pr
(Intercept)	1	1137.0000	0.0000
grain	2	30.7100	0.0007
temp	2	1.7530	0.2514
time	1	2.5640	0.1604
grain:temp	4	2.7570	0.1288
grain:time	2	1.1160	0.3873
temp:time	2	0.1025	0.9041
grain:temp:time	4	3.0870	0.1056

''''''''''''''''
Pre240
''''''''''''''''

	Df	F.con	Pr
(Intercept)	1	1.621e+03	0.0000
grain	2	3.749e+01	0.0004
temp	2	2.902e+00	0.1313
time	1	3.836e+00	0.0979
grain:temp	4	2.424e+00	0.1594
grain:time	2	2.298e+00	0.1816
temp:time	2	1.984e-02	0.9804
grain:temp:time	4	2.631e+00	0.1393

END

APPENDIX IX

STATISTICAL REPORT ON DIFFERENTIAL SCANNING CALORIMETRY OF THE MASH AND PELLETS

Statistics for the Australian Pork Industry Technical Report Series

PS037 - Differential scanning calorimetry (DSC) studies on wheat mash and pellets

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February 21, 2013

Contents

1 Introduction

This report has been compiled for Dr Peter Sopade. The protocol for this experiment has already been described in the document titled PS037 - Differential scanning calorimetry (DSC) studies on mash and pellets from wheat. A number of traits are considered in this report. They are listed in Table 1.1.

Table 1.1: Traits analysed in this report, their unit of measurement, and abbreviation.

Trait (unit of measurement)	Abbreviation
Enthalpy or heat of gelatinisation (J per g dry starch)	enthalpy
Onset temperature of gelatinisation (°C)	onset
Peak temperature of gelatinisation (°C)	peak
End temperature of gelatinisation (°C)	end

The aim of this experiment is to investigate the effects of type, grain, conditioning temperature, and conditioning time on each of the traits listed above. Two of these traits, onset temperature of gelatinisation and peak temperature of gelatinisation are reasonably well correlated (Table 1.2).

Table 1.2: Raw correlations between the 4 traits analysed in this report.

	onset	peak	end	enthalpy
onset	1.000	0.752	0.306	-0.232
peak	0.752	1.000	0.577	-0.286
end	0.306	0.577	1.000	0.274
enthalpy	-0.232	-0.286	0.274	1.000

2 Experimental design

In this report the term "diet" is synonymous with "batch". A total of 24 batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "time" (7, 14) where the bracketed text contains the levels of each factor. Batch number and treatment are provided in Table 2.1.

Two duplicates of the 24 pelleted batches and 2 duplicates of mash samples derived from the 24 batches were analysed in the laboratory for gelatinisation characteristics. Therefore, the experiment comprises a total of 96 lab samples. An experimental design based on 4 runs of 24 samples with runs 1-2 and runs 3-4 comprising a blocking factor, referred to as dblock, with each containing one mash and pellet duplicate of every batch listed in Table 2.1. A design was generated in the R (R Development Core Team, 2011) statistical computing environment using the R package `od` (Butler, 2011).

2 Experimental design

Table 2.1: List of batch numbers and treatments. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Batch (Diet) No.	Grain	Temp.	Time
1	1894	80	7
2	1894	60	7
3	1896_SPROUT	80	14
4	1896_SPROUT	70	14
5	1896_SPROUT	70	7
6	1896_SPROUT	80	7
7	1895	70	14
8	1895	60	14
9	1895	70	7
10	1895	60	14
11	1894	80	14
12	1894	60	7
13	1894	70	14
14	1894	70	7
15	1895	80	7
16	1895	80	14
17	1896_SPROUT	60	7
18	1896_SPROUT	80	14
19	1894	70	14
20	1894	60	14
21	1895	80	7
22	1895	60	7
23	1896_SPROUT	60	14
24	1896_SPROUT	70	7

3 Statistical Methods

For each trait linear mixed model technology was used to fit a statistical model to the data. The linear mixed model 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. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). Unlike ANOVA, the linear mixed model has the ability to accommodate missing values in the response and explanatory variables and can accommodate a wide range of variance models. The R package `asreml` (Butler et al., 2007) was used to fit all models.

To meet the aim of the experiment the following model, using the symbolic notation described by Wilkinson and Rogers (1973), was fitted to each trait,

$$\begin{aligned} \text{trait} \sim\ & 1 + \text{type*grain*temp*time} \\ & + \mathbf{dblock} + \mathbf{batch} + \mathbf{pelletday} + \mathbf{pelletorder} + \mathbf{pelletsession} \\ & + \mathbf{labday} + \mathbf{laborder} + \mathbf{units} \end{aligned} \tag{3.1}$$

where 1 is the overall mean, type, grain, temp, and time represent the treatment factors type (pellet or mash), grain, conditioning temperature, and conditioning time. Terms in bold font are fitted as random effects. For each random term there is an associated variance component. The term units represents random error and is not explicitly fitted in the R call to `asreml`.

The traits peak temperature of gelatinisation and end temperature of gelatinisation had non-treatment variation that could be attributed to the pelleting process in some way, such as variation between pelleting days, variation between pelleting orders, variation between pelleting sessions, and variation between pelleting batches. All traits had non-treatment variation that could be atrributed to variation between the days samples were measured in the laboratory.

3 Statistical Methods

Two outliers for the trait onset temperature of gelatinisation were removed. Wald type F-statistics for each trait are presented in Section 4. For each trait predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level have been provided in *.csv* files.

4 Results

Wald type F-statistics and associated p-values for the terms fitted as fixed effects in model (3.1) for all traits considered are presented in Table 4.1. The four-way interaction of type, grain, conditioning temperature, and conditioning time was statistically significant for the traits enthalpy or heat of gelatinisation and onset temperature of gelatinisation. The two-way interaction of type and conditioning temperature and the main effect of grain was significant for peak temperature of gelatinisation. The main effect of grain and type was significant for end temperature of gelatinisation.

Table 4.1: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the models for all traits considered in this report.

		enthalpy		onset		peak		end	
Term	Df	F-statistic	p-value	F-statistic	p-value	F-statistic	p-value	F-statistic	p-value
type	1	31.20	<0.001	4.32	0.051	3.79	0.066	38.98	<0.001
grain	2	30.90	<0.001	13.38	<0.001	36.86	<0.001	10.36	0.001
temp	2	0.84	0.448	0.40	0.676	0.94	0.406	0.35	0.707
time	1	3.37	0.081	0.09	0.766	0.01	0.941	0.00	0.977
type:grain	2	0.68	0.518	2.23	0.134	1.24	0.312	0.66	0.529
type:temp	2	2.39	0.117	1.30	0.295	3.72	0.042	0.23	0.799
grain:temp	4	0.80	0.542	0.69	0.610	0.86	0.505	1.51	0.238
type:time	1	1.24	0.279	2.09	0.164	3.65	0.070	0.66	0.427
grain:time	2	0.91	0.420	2.11	0.147	1.43	0.262	1.48	0.251
temp:time	2	2.77	0.087	1.23	0.313	2.38	0.118	0.54	0.594
type:grain:temp	4	4.62	0.008	0.75	0.570	0.86	0.503	2.14	0.113
type:grain:time	2	2.52	0.106	2.27	0.129	1.35	0.283	0.11	0.897
type:temp:time	2	1.56	0.234	0.35	0.712	1.13	0.342	1.69	0.211
grain:temp:time	4	4.23	0.012	1.00	0.432	0.50	0.736	0.57	0.689
type:grain:temp:time	4	6.04	0.002	3.56	0.033	2.02	0.131	1.43	0.262

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APPENDIX X

STATISTICAL REPORT ON *IN VITRO* PROTEIN DIGESTION OF THE PELLET DIETS

Statistical Consulting Report

PS036: in-vitro protein digestibility (IVPD)

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Table of Contents

1 Introduction

This report has been compiled for Dr Peter Sopade, a senior research fellow with the University of Queensland. The protocol for this experiment has already been described in the document titled *In-vitro protein digestibility of pellets from wheat using the pH-drop method*. The trait considered in this report is ***in-vitro* protein digestibility (IVPD)** which is a function of the change in pH of a sample in a 10 minute period. There are two aims to this experiment. These are stated in the next section after first considering the experimental design.

2 Experimental design

In this report the term "diet" is synonymous with "pellet batch" or "batch" for short. A total of 24 batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "speed" (7, 14) where the bracketed text contains the levels of each factor. Batch number and treatment are provided in Table 2.1.

Two duplicates from each batch are analysed in the laboratory for IVPD. Therefore, the experiment comprises a total of 48 samples (2 duplicates $\times$ 24 batches). An experimental design based on 6 runs of 8 samples, where runs 1-3 and runs 4-6 comprise a blocking factor with each containing one duplicate of every batch listed in Table 2.1, was generated in the R (R Development Core Team, 2011) statistical computing environment using the R package `od` (Butler, 2011).

There are two aims of this experiment. The first is to form predictions of IVPD for each batch, and the second is to determine if the main effects and interactions of grain, temperature, and speed affect IVPD. The statistical methods used to meet these aims are discussed in the next section.

Table 2.1: List of batch numbers and treatments. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Batch (Diet) No.	Grain	Temp.	Speed
1	1894	80	7
2	1894	60	7
3	1896_SPROUT	80	14
4	1896_SPROUT	70	14
5	1896_SPROUT	70	7
6	1896_SPROUT	80	7
7	1895	70	14
8	1895	60	14
9	1895	70	7
10	1895	60	14
11	1894	80	14
12	1894	60	7
13	1894	70	14
14	1894	70	7
15	1895	80	7
16	1895	80	14
17	1896_SPROUT	60	7
18	1896_SPROUT	80	14
19	1894	70	14
20	1894	60	14
21	1895	80	7
22	1895	60	7
23	1896_SPROUT	60	14
24	1896_SPROUT	70	7

3 Statistical Methods

The two aims necessitate the fitting of two different linear mixed models to the IVPD data. The linear mixed model can be formulated in such a way that it is analagous 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. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). Unlike ANOVA, the linear mixed model has the ability to accommodate missing values in the response and explanatory variables and can accommodate a wide range of variance models. The R package `asreml` (Butler et al., 2007) was used to fit the two models presented below.

To meet the first aim, i.e., form predictions of IVPD for each batch, the following model (in symbolic notation) was fitted to the IVPD data,

$$\text{IVPD} \sim 1 + \text{batch} + + \mathbf{rblock} + \mathbf{run} + \mathbf{units} \tag{3.1}$$

where 1 is the overall mean, and batch and run have been described previously. The term "rblock" is a two level blocking factor comprising runs 1-3 and runs 4-6. The terms in bold type are fitted as random effects and a variance component is associated with each of these terms. The term "units" represents random error and is not explicitly fitted in the R call to `asreml`. The estimated variance component associated with this term is referred to as the residual variance.

To meet the second aim the following model (in symbolic notation) was fitted to the IVPD data,

$$\begin{aligned} \text{IVPD} \sim & 1 + \text{grain} + \text{temp} + \text{speed} \\ & + \text{grain:temp} + \text{grain:speed} + \text{temp:speed} \\ & + \text{grain:temp:speed} + \mathbf{rblock} + \mathbf{run} + \mathbf{units} \end{aligned} \tag{3.2}$$

where the terms 1, grain, temp, speed, rblock, run, and units have been defined previously.

An assumption of both these models is that the residuals are normally distributed and have constant variance. This assumption was satisfactorily met in both models.

4 Results

Predictions for each batch based on the model presented in (3.1) are listed in Table 4.1.

Table 4.1: IVPD predicted values and standard errors for batch.

batch	predicted.value	standard.error
1	81.14	2.069
2	79.15	2.069
3	77.84	2.058
4	78.07	2.069
5	79.00	2.069
6	74.73	2.058
7	79.09	2.069
8	82.14	2.069
9	82.80	2.069
10	82.16	2.069
11	79.55	2.069
12	75.94	2.058
13	79.83	2.058
14	79.10	2.069
15	80.47	2.069
16	82.57	2.069
17	80.21	2.069
18	78.20	2.069
19	81.88	2.058
20	79.22	2.069
21	84.87	2.069
22	81.26	2.069
23	84.78	2.058
24	80.07	2.069

Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2) are presented in Table 4.2. The two-way interaction of grain and temperature is statistically significant ($P < 0.05$) and is therefore the appropriate level at which to form predictions. These are presented in Table 4.3.

Table 4.2: Conditional F-statistics and associated P-values for the terms fitted as fixed effects in the model presented in (3.2).

Term	DF	F.con	P-value
grain	2	3.78	0.035
temp	2	0.28	0.761
speed	1	0.75	0.392
grain:temp	4	2.89	0.039
grain:speed	2	1.01	0.375
temp:speed	2	1.28	0.294
grain:temp:speed	4	0.98	0.432

Table 4.3: Predicted values and standard errors for the two-way interaction of grain and temperature based on the model presented in (3.2). The average standard error of difference (avsed) can be multiplied by 2 and 2.8 to give an approximate least significant difference at the 5% level and 1% level respectively.

grain	temp	predicted.value	standard.error
1894	60	78.49	1.418
1894	70	79.81	1.405
1894	80	80.20	1.565
1895	60	81.66	1.405
1895	70	80.80	1.565
1895	80	82.73	1.386
1896_SPROUT	60	82.33	1.561
1896_SPROUT	70	78.97	1.402
1896_SPROUT	80	76.49	1.391
avsed		1.751	

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APPENDIX XI

RIVALEA ETHIC APPROVAL 11N093C – INFLUENCE OF GRAIN AND PROCESSING CONDITIONS ON PIG GROWTH

Rivalea
AUSTRALIA

13 December 2011

Dr Cherie Collins
Rivalea (Australia) Pty Ltd
PO Box 78
COROWA NSW 2646

Dear Cherie

Please find enclosed your Animal Research Authority for the project titled:

11N093C Influence of grain and processing conditions on pig growth

This Authority is valid to 12 December 2012. Should you wish to conduct research after this date you will need to apply to the Ethics Committee for a renewal of the Animal Research Authority.

Yours sincerely

Eric Thornton
Chairperson
Animal Ethics Committee

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Rivalea
AUSTRALIA

ANIMAL RESEARCH AUTHORITY

11N093C

Issued by
Rivalea Animal Ethics Committee

Dr Cherie Collins
Rivalea (Australia) Pty Ltd
PO Box 78
COROWA NSW 2646

Is authorised to conduct the following research

11N093C **Influence of grain and processing conditions on pig growth**

Under the supervision of: Matthew Tull

at Rivalea (Australia) Pty Ltd, Redlands Road, Corowa, NSW, 2646

being animal research carried out in accordance with the Code of Practice, for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

The following conditions are enforced on this authority: NIL

This authority remains in force from 13 December 2011 to 12 December 2012 unless suspended, cancelled or surrendered.

Eric Thornton
Chairperson
Animal Ethics Committee

APPENDIX XII

STATISTICAL REPORT ON WEANER FEEDING TRIALS

Statistics for the Australian Pork Industry Technical Report Series

Rivalea weaner trial

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November 20, 2012

Contents

1 Introduction

This report has been compiled for Dr Peter Sopade (Senior Research Fellow, University of Queensland) and Dr Cherie Collins (R & I Manager, Rivalea). In previous Pork CRC experiments it was observed that conditions under which pelleted diets are manufactured can influence traits that are related to pig growth performance. This experiment has been conducted to determine if the main effects of, or interactions between, pellet conditioning temperature, pellet conditioning time, and type of wheat used affect the growth performance of weaner pigs.

2 Experimental design

There are two distinct phases in the experimental design. There is a pelleting or diet manufacturing phase and a second phase where those diets are fed to pigs in pens. The design and analysis of these types of designs in a plant breeding setting is discussed in Smith et al. (2006).

In regard to the first phase, a total of 24 diets or pellet batches were manufactured and these were the result of a partially replicated design (see Cullis et al. (2006) for a description of these designs) for 18 treatments, i.e., 6 treatments are replicated. The 18 treatments comprise the factorial of "grain" (1894, 1895, 1896_SPROUT), "temperature" (60, 70, 80), and "time" (7, 14) where the bracketed text contains the levels of each factor. Diet (batch) number and treatment, pelleting day, pelleting order, and pelleting session are provided in Table 2.1.

2 Experimental design

Table 2.1: List of diet (batch) numbers, treatments, and the days, order, and sessions in which they were manufactured. Batch numbers 10, 12, 18, 19, 21, and 24 are replicates of previous batch numbers, namely 8, 2, 3, 13, 15, and 5 respectively.

Diet No.	Temp.	Time	Grain	Pellet Day	Pellet Order	Pellet Session
1	80	7	1894	1	1	Morning
2	60	7	1894	2	1	Morning
3	80	14	1896_SPROUT	3	1	Afternoon
4	70	14	1896_SPROUT	4	1	Afternoon
5	70	7	1896_SPROUT	1	2	Morning
6	80	7	1896_SPROUT	2	2	Morning
7	70	14	1895	3	2	Afternoon
8	60	14	1895	4	2	Afternoon
9	70	7	1895	1	3	Morning
10	60	14	1895	2	3	Morning
11	80	14	1894	3	3	Afternoon
12	60	7	1894	4	3	Afternoon
13	70	14	1894	1	4	Morning
14	70	7	1894	2	4	Morning
15	80	7	1895	3	4	Afternoon
16	80	14	1895	4	4	Afternoon
17	60	7	1896_SPROUT	1	5	Morning
18	80	14	1896_SPROUT	2	5	Morning
19	70	14	1894	3	5	Afternoon
20	60	14	1894	4	5	Afternoon
21	80	7	1895	1	6	Morning
22	60	7	1895	2	6	Morning
23	60	14	1896_SPROUT	3	6	Afternoon
24	70	7	1896_SPROUT	4	6	Afternoon

2 Experimental design

The second phase of the experiment involves feeding pelleted diets to pigs in pens. The arrangement of pens in the Rivalea facility is presented in Table 2.2. The notable feature of this layout is the differing numbers of pens in columns and the differing arrangement of blocks of pens. For instance, block VI is synonymous with column 8 and contains 16 pens whereas block IV comprises columns 5 and 6 and contains a total of 56 pens. Blocks of pens are segregated by walkways.

Table 2.2: Pen layout indexed by rows and columns, and showing blocks of pens (labelled using Roman numerals).

	Block and Column							
	I	II	III		IV		V	VI
Row	1	2	3	4	5	6	7	8
1	200	149	148	93	92	37	36	1
2	199	150	147	94	91	38	35	2
3	198	151	146	95	90	39	34	3
4	197	152	145	96	89	40	33	4
5	196	153	144	97	88	41	32	5
6	195	154	143	98	87	42	31	6
7	194	155	142	99	86	43	30	7
8	193	156	141	100	85	44	29	8
9	192	157	140	101	84	45	28	9
10	191	158	139	102	83	46	27	10
11	190	159	138	103	82	47	26	11
12	189	160	137	104	81	48	25	12
13	188	161	136	105	80	49	24	13
14	187	162	135	106	79	50	23	14
15	186	163	134	107	78	51	22	15
16	185	164	133	108	77	52	21	16
17	184	165	132	109	76	53	20	
18	183	166	131	110	75	54	19	
19	182	167	130	111	74	55	18	
20	181	168	129	112	73	56	17	
21	180	169	128	113	72	57		
22	179	170	127	114	71	58		
23	178	171	126	115	70	59		
24	177	172	125	116	69	60		
25		173	124	117	68	61		
26		174	123	118	67	62		
27		175	122	119	66	63		
28		176	121	120	65	64		

The design consists of three batches of 200 pigs, from here on referred to as pig batches. In each case the 200 pigs arrive by truck, are individually weighed, and then randomly allocated to pens. The allocation of the 24 diets, i.e., pellet batches, to 192 (24×8) pigs is done after receiving

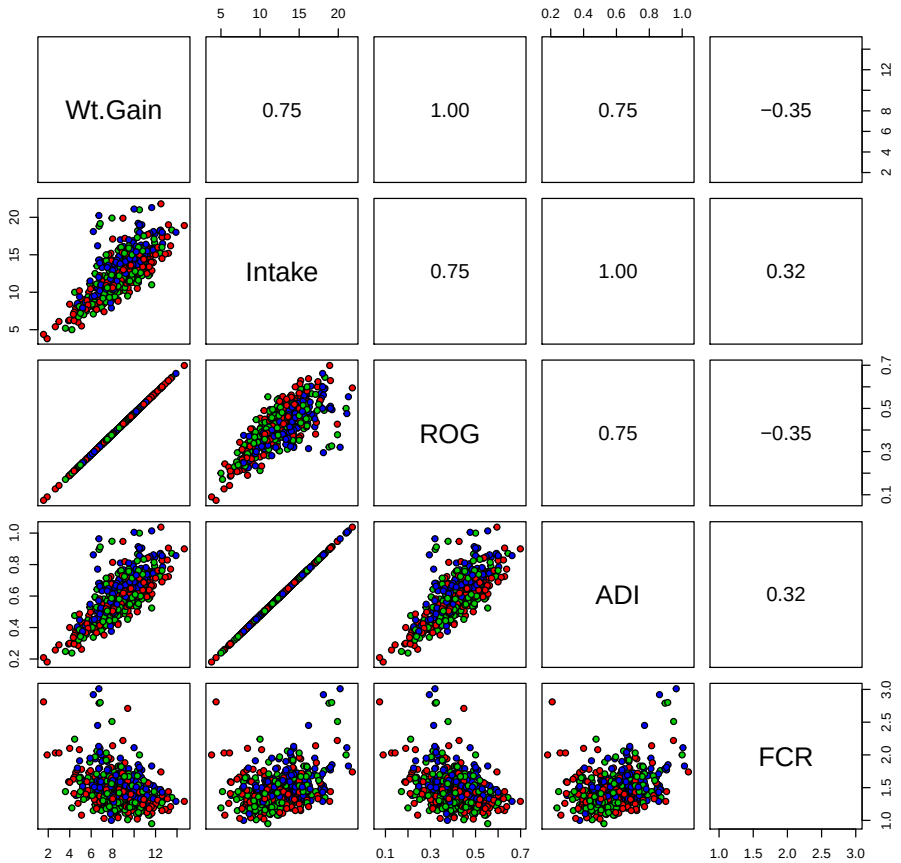
the initial start weight and pen allocation information. Eight pigs are omitted from the trial. The choice of which pigs to omit is based on initial weight, with lighter pigs typically being excluded from the trial although they remain in their allocated pen for the length of the trial on a commercial diet. In the first 5 days all pigs are fed a commercial diet. Before commencement of the trial proper pigs are again individually weighed. This measurement is referred to as start weight. Start weight and the weight gained in the first 5 days are used as covariates in the statistical analysis of all traits of interest. This is discussed further in the next section. Traits of interest are feed intake and weight gain. These are recorded every 7 days for 21 days. Other traits of interest, namely average daily intake, rate of growth, and feed conversion ratio are derived from the feed intake and weight gain traits.

The randomisation of diets to the 192 pigs in a pig batch was done in the statistical computing environment R (R Core Team, 2012) using the `od` (Butler, 2011) package. Due to unequal block size, some blocks having less than 24 pens, and 8 pens not being part of the random allocation of diets to pens, "design blocks" comprising at least 24 pens were created for the purposes of randomly allocating diets to pens. These design blocks were created in such a way as to be contiguous as possible. For example, a design block encompassing column 8 would typically only involve pens from column 7. The final design for each pig batch could be described as a randomised block design with some incomplete blocks.

3 Statistical Methods

The traits of interest are weight gain, feed intake, rate of growth, average daily intake, and feed conversion ratio. In this report we have considered these traits for the time period 0 to 21 days. The traits weight gain and rate of growth and the traits feed intake and average daily intake are almost perfectly correlated (Figure 3.1). In the interests of completeness we have analysed all 5 traits.

Figure 3.1: Pairs plot of weight gain (Wt.Gain), feed intake (Intake), rate of growth (ROG), average daily intake (ADI), and feed conversion ratio (FCR). Red, green, and blue circles correspond to grains 1894, 1895, and 1896_SPROUT respectively.



3 Statistical Methods

A linear mixed model was fitted to each trait of interest. The linear mixed model 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. The preferred method for estimating these effects is residual (or restricted) maximum likelihood (REML) (Patterson and Thompson, 1971). Unlike ANOVA, the linear mixed model has the ability to accommodate missing values in the response and explanatory variables and can accommodate a wide range of variance models. The R package `asreml` (Butler et al., 2007) was used to fit all models.

The following model (in symbolic notation) was fitted to all traits,

$$\begin{aligned} \text{Trait Response} \quad \sim \quad & 1 + \text{Grain*Temp*Time} + \mathbf{PigBatch} + \mathbf{PelletBatch} \\ & + \mathbf{PelletDay} + \mathbf{PelletOrder} + \mathbf{PelletSession} \\ & + \mathbf{at(PigBatch):Block} + \mathbf{units} \end{aligned} \tag{3.1}$$

where 1 is the overall mean and the expression Grain*Temp*Time encompasses the three main effects, three two-way interactions and the three-way interaction between the terms grain, temperature, and time. Terms in bold font are random effects. There is a variance component associated with each of the random terms **PigBatch**, **PelletBatch**, **PelletDay**, **PelletOrder**, and **PelletSession**. The "at" in the random term **at(PigBatch):Block** indicates that there is three variance components to be estimated, one for blocks in the three pig batches. The term **units** represents random error and is not explicitly fitted in the R call to `asreml`. The estimated variance component associated with this term is referred to as the residual variance. A residual maximum likelihood ratio test (REMLRT) was used to determine if a separate residual variance for each pig batch was appropriate.

The variables starting weight at the commencement of treatment allocation and weight gain in the first 5 days while on a commercial diet have been included in all fitted models as a covariate and tested for significance using Wald type F-statistics. The justification for using the weight gain in the first 5 days is that it provides a pseudo measure of pig adaptation to the new environment. These two covariates have a correlation of 0.49 for the first and third pig batch and a correlation of 0.40 for the second pig batch, which are not large enough to preclude fitting both covariates together. These two covariates were significant ($P < 0.001$) for all traits except 0 to 21 day feed conversion ratio. The coefficients associated with each of these covariates for the traits weight gain and feed intake are provided in Table 3.1.

Table 3.1: Coefficients associated with the covariates start weight, and weight gain in the first 5 days while on a commercial diet.

Covariate	weight gain coeff.	feed intake coeff.
start weight	0.5618	0.7370
weight gain (1st 5 days)	0.8927	1.2401

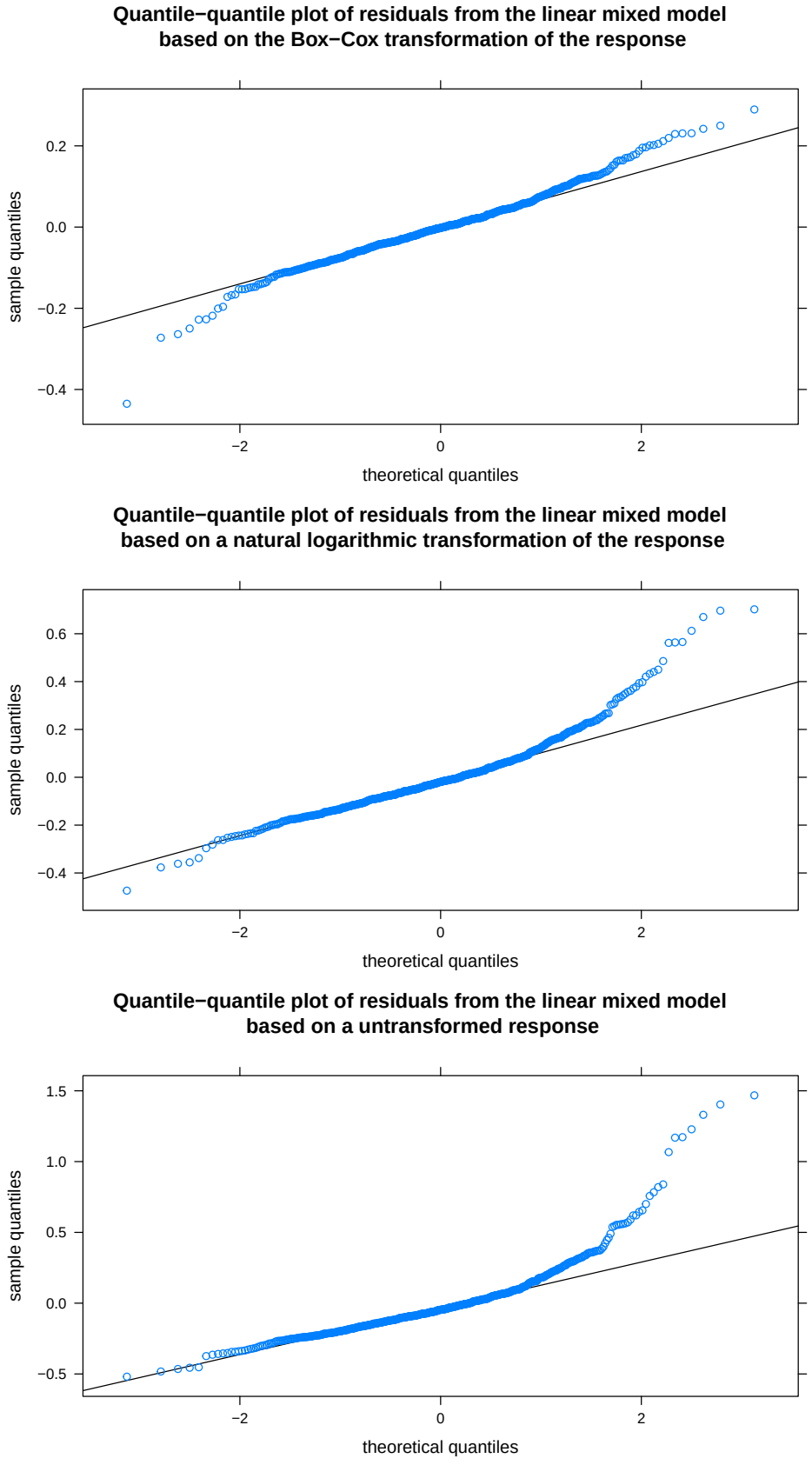
An assumption of both these models is that the residuals are normally distributed and have constant variance. This assumption was satisfactorily met for all traits except for the trait 0 to 21 days feed conversion ratio. For this trait we considered a number of transformations, including the natural logarithmic transformation, but found that the Box-Cox transformation defined as

$$y^{(\lambda)} = \frac{y^{\lambda} - 1}{\lambda}$$

with $\lambda = -1.5$ was the best transformation for meeting model assumptions. We have provided three quantile-quantile plots of the residuals in Figure 3.2 which highlight the non-normality of the residuals without a transformation, the slightly better but still not satisfactory case when using the natural logarithmic transformation, and the case when using the Box-Cox transformation described above.

In regards to outliers we have taken the view that a data point would have to be obviously different to all other data points before being removed. Observation 62 was found to be significantly lower than all other data points for the traits feed intake, average daily intake, and feed conversion ratio. Observation 457 was found to be significantly higher than all other data points for the traits feed intake and average daily intake. We have provided the data file used in the analysis so that these observation numbers might be checked by the researchers if desired.

Figure 3.2: Quantile-quantile plots of untransformed, natural logarithmic transformation, and Box-Cox transformation of 0 to 21 days feed conversion ratio.



4 Results: 0 to 21 days

For the traits weight gain, feed intake, and Box-Cox transformed feed conversion ratio a table of conditional F-statistics and associated p-values for the main effects, two-way interactions, and the three-way interaction of the treatments grain, temperature, and conditioning time are provided in Table 4.1. Note that the Wald type F-statistics and associated p-values for rate of growth and average daily intake are the same as that for weight gain and feed intake respectively due to the near perfect correlation between these sets of traits.

Table 4.1: Conditional F-statistics and associated p-values for the main effects, two-way interactions, and the three-way interaction of the treatments grain, temperature, and conditioning time for the traits weight gain, feed intake, and feed conversion ratio.

		weight gain		feed intake		feed conversion ratio	
Term	Num. DF	F.con	p-value	F.con	p-value	F.con	p-value
Grain	2	1.05	0.616	14.70	<0.001	15.23	<0.001
Temp	2	11.63	<0.001	2.92	0.100	15.85	<0.001
Time	1	2.44	0.055	8.63	0.001	4.42	0.038
Grain.Temp	4	1.39	0.269	0.53	0.687	0.97	0.427
Grain.Time	2	0.55	0.642	0.77	0.399	2.00	0.141
Temp.Time	2	2.29	0.102	1.09	0.339	2.44	0.092
Grain.Temp.Time	4	2.16	0.072	1.44	0.221	1.57	0.188

4.1 Weight gain

The main effect of temperature is statistically significant ($P < 0.001$). Predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level are provided in Table 4.2.

Table 4.2: Weight gain predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level associated with the main effect of temperature.

Temp.	predicted value	standard error
60	9.11	0.142
70	8.66	0.142
80	8.32	0.142
avsed	0.182	
lsd(5%)	0.361	
lsd(1%)	0.478	

4.2 Feed intake

The main effects of grain and conditioning time are statistically significant ($P < 0.001$ and $P = 0.001$ respectively). Predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level are provided in Table 4.3 and Table 4.4.

Table 4.3: Feed intake predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level associated with the main effect of grain.

Grain	predicted value	standard error
1894	11.97	0.192
1895	12.04	0.190
1896_SPROUT	13.08	0.192
avsed	0.270	
lsd(5%)	0.536	
lsd(1%)	0.709	

Table 4.4: Feed intake predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level associated with the main effect of conditioning time.

Time	predicted value	standard error
7	12.70	0.155
14	12.03	0.158
avsed	0.220	
lsd(5%)	0.436	
lsd(1%)	0.578	

4.3 Rate of growth

Rate of growth is almost perfectly correlated with weight gain, therefore the same main effect is statistically significant. Predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level are provided in Table 4.5.

Table 4.5: Rate of growth predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level associated with the main effect of temperature.

Temp.	predicted value	standard error
60	0.43	0.007
70	0.41	0.007
80	0.40	0.007
avsed	0.009	
lsd(5%)	0.018	
lsd(1%)	0.024	

4.4 Average daily intake

Average daily intake is almost perfectly correlated with feed intake, therefore the same main effects are statistically significant. Predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level are provided in Table 4.6 and Table 4.7.

Table 4.6: Average daily intake predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level associated with the main effect of grain.

Grain	predicted value	standard error
1894	0.57	0.009
1895	0.57	0.009
1896_SPROUT	0.62	0.009
avsed	0.013	
lsd(5%)	0.026	
lsd(1%)	0.034	

Table 4.7: Average daily intake predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level associated with the main effect of conditioning time.

Time	predicted value	standard error
7	0.60	0.007
14	0.57	0.007
avsed	0.010	
lsd(5%)	0.020	
lsd(1%)	0.026	

4.5 Feed conversion rate

The main effects of grain, temperature and conditioning time are statistically significant ($P < 0.001$, $P < 0.001$, and $P = 0.038$ respectively). Predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level are provided in Table 4.8, Table 4.9, and Table 4.10.

Table 4.8: Box-Cox transformed feed conversion rate predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level associated with the main effect of grain. Note that all comparisons should be made on the transformed scale. The back-transformed predicted values are provided in brackets to provide a reference to the original scale.

Grain	predicted value	standard error
1894	0.25 (1.37)	0.010
1895	0.26 (1.39)	0.010
1896_SPROUT	0.29 (1.48)	0.010
avsed	0.009	
lsd(5%)	0.018	
lsd(1%)	0.024	

Table 4.9: Box-Cox transformed feed conversion ratio predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level associated with the main effect of temperature. Note that all comparisons should be made on the transformed scale. The back-transformed predicted values are provided in brackets to provide a reference to the original scale.

Temp.	predicted value	standard error
60	0.25 (1.37)	0.010
70	0.26 (1.39)	0.010
80	0.29 (1.47)	0.010
avsed	0.009	
lsd(5%)	0.018	
lsd(1%)	0.024	

Table 4.10: Box-Cox transformed feed conversion ratio predicted values, standard errors, average standard error of difference, and least significant differences at the 5% and 1% level associated with the main effect of conditioning time. Note that all comparisons should be made on the transformed scale. The back-transformed predicted values are provided in brackets to provide a reference to the original scale.

Time	predicted value	standard error
7	0.28 (1.43)	0.009
14	0.26 (1.40)	0.009
avsed	0.008	
lsd(5%)	0.016	
lsd(1%)	0.021	

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APPENDIX XIII

MINITAB OUTPUT ON HARDNESS AND DURABILITY OF THE PELLET DIETS

HARDNESS

General Linear Model: Hardness versus temp, time, grain

Factor	Type	Levels	Values
temp	fixed	3	60, 70, 80
time	fixed	2	7, 14
grain	fixed	3	HARD, SOFT, SPROUT

Analysis of Variance for Hardness, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
temp	2	1.083	1.900	0.950	0.81	0.450
time	1	1.389	1.089	1.089	0.93	0.340
grain	2	18.398	16.033	8.017	6.84	0.002
temp*time	2	0.646	1.944	0.972	0.83	0.442
temp*grain	4	10.261	9.600	2.400	2.05	0.101
time*grain	2	1.534	1.544	0.772	0.66	0.522
temp*time*grain	4	1.355	1.355	0.339	0.29	0.884
Error	54	63.333	63.333	1.173		
Total	71	98.000				

S = 1.08298 R-Sq = 35.37% R-Sq(adj) = 15.03%

Grain main effects

Grouping Information Using Tukey Method and 95.0% Confidence

grain	N	Mean	Grouping
SOFT	24	5.111	A
HARD	24	4.917	A
SPROUT	24	3.972	B

Means that do not share a letter are significantly different.

DURABILITY

General Linear Model: Durability versus temp, time, grain

Factor	Type	Levels	Values
temp	fixed	3	60, 70, 80
time	fixed	2	7, 14
grain	fixed	3	HARD, SOFT, SPROUT

Analysis of Variance for Durability, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
temp	2	5.43	13.84	6.92	0.31	0.746
time	1	4.42	3.46	3.46	0.15	0.708
grain	2	85.83	65.03	32.51	1.45	0.307
temp*time	2	26.70	31.12	15.56	0.69	0.536
temp*grain	4	2.82	7.66	1.91	0.09	0.984
time*grain	2	20.13	21.34	10.67	0.47	0.644
temp*time*grain	4	35.89	35.89	8.97	0.40	0.803
Error	6	134.81	134.81	22.47		
Total	23	316.03				

S = 4.74008 R-Sq = 57.34% R-Sq(adj) = 0.00%