Prepared for the CRC for an Internationally Competitive Pork Industry

# Project 2G-105

# COST EFFECTIVE FORMULATION OF VEGETABLE- PROTEIN BASED DIETS

**EXPERIMENT CODES:** AF007/AF008

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## Background:

This project addressed the following Pork CRC outputs:

**Output 1.4:** Novel methods for improving the utilisation of feed ingredients by pigs.
**Output 2.6:** Manipulation of grower pig intake.
**Output 2.12:** Improved growth and feed efficiency through manipulation of hormonal and metabolic pathways.

**Objectives:**

Exogenous phytase has been shown to effectively increase the digestibility of phosphorus and amino acids in pig and poultry diets. Unlike carbohydrase enzymes, phytate has a consistent effect across an entire diet rather than specific components of the diet, and hence nutrient values can be ascribed to phytase when formulating. Depending on the ingredient composition of the diet, addition of exogenous phytase can significantly reduce the overall diet cost and efficiency of nutrient utilisation.

Due to the nature of northern Australian cereal production, the majority of diets are based on sorghum and barley. As a consequence, northern Australian pig diets contain the highest phytic acid content in Australia, and sorghum has little to no natural phytase activity. While phytase has been shown to increase the availability of amino acids by over 5% in maize and wheat-based diets, very limited information exists on the uplift in sorghum or barley-based diets. Some work was conducted by Ravindran *et al.* (1999), reporting that added phytase increased ileal amino acid digestibility in sorghum by 6.5% in broilers. More recently phytase has been shown to significantly increase protein digestibility and growth performance in young broilers, offered sorghum based diets (Perez, per. com.)

The extent of the uplift with phytase addition varies depending on the primary protein sources and the total phytic acid content of the diet. The limited use of legumes in northern Australian production systems places heavy reliance on oilseed meals with this reliance likely to increase with moves to mono-protein feedmills (ie animal or vegetable proteins to assist compliance with ruminant feeding requirements). To effectively use phytase in diets containing sorghum and barley, it is necessary to quantify the increase in phosphorus (and other minerals), amino acid and energy digestibilities. This allows accurate diet formulation and maintenance of an ideal balance of essential amino acids and assist the nutritionist to capture the most out of diet cost savings and/or improvements in FCR and overall growth performance.

There are several other major factors, other than phytate, that limit protein and overall nutrient digestibility in sorghum and barley. In sorghum, poly-phenols (including condensed tannins) have been shown to significantly decrease protein digestibility. It has been recently reported that even small increases in condensed tannins in the modern low tannin red sorghums have been shown to significantly decrease ileal amino acids digestibility in broilers. It is postulated that both tannin and phytic acid can produce an additive negative effect on protein digestibility by hydrogen bonding and non-polar hydrophobic associations, precipitating protein out of solution and rendering it unavailable for protease hydrolysis (Duodu *et al.*, 2003). Given the optimum conditions, sorghum poly-phenols are capable of binding up to 12 times its own weight of protein. Both anti-nutritional factors have also been reported to significantly increase endogenous loss of protein in monogastrics (Cowieson *et al.*, 2004).

Barley grown in dry, semi arid conditions, tends to produce higher levels of soluble non starch polysaccharides (NSP), including high branched arabinoxylan and beta-glucanse. Moreover, the mean viscosity of barley internationally is approximately 30 cPs, whereas the average in Australia is 550 cPs, with many Western Australian barley viscosities exceeding 1000 cPs. Although viscosity is not regarded as a major problem in pigs, as it is in poultry, high levels of soluble barley NSP significantly reduce protein digestibility and can reduce feed intake (Inborr *et al*., 1991).

Figure 1. High viscosity barley (>1000 cPs) compared to low viscosity barley (6.1 cPs).

The final negative compounding effect on protein digestibility in sorghum and barley is steam pelleting above 80$^{o}$C. Cooking sorghum increases the precipitation of condensed tannin and protein. Cooking also solublises more phytate, which allows it to chelate to protein in the acidic conditions of the stomach. The steam pelleting of barley significantly increases the solubility of NSP and phytic acid in barley, decreasing the nutritive value of the cereal grain. To improve FCR (by 4 to 5%), reduce feed separation and wastage, the majority of sorghum and barley based diets are steam pelleted at or over 80$^{o}$C, by the large feed-milling groups. Much more information is required on diets processed under these conditions.

As feed costs represent the largest proportion of pig production costs, and with the greatest cost pressure against the supply of energy in diet formulations, any improvement in the measurement of available energy from diet ingredients will significantly reduce the cost of pig production in Australasia – a major priority for the Pork CRC.

Since 1996, the pork industry has been investing in the development of rapid and objective measures of feed grain quality through the Premium Grains for Livestock Program (PGLP). It has been realized that a significant amount of the differences between grains is due to differences in their digestibility and/or fermentability within the small and large intestines, mostly in relation to their carbohydrate fraction. To date, NIR calibrations have been developed for ileal and faecal DE (whole and milled, as fed and dry matter), ileal:faecal DE ratio, feed intake and a feed intake index, however, further research is required to enhance these calibrations so they can be used for the commercial trading of grains.

How to unlock sorghum's hidden potential? Relative to wheat, barley and maize, there has been very little research on the use of feed additives and enzymes to improve the feeding value of sorghum for pigs. Approximately 35% of growing pigs are fed sorghum-based diets in Australia. However, there is increasing evidence that pigs are not performing as consistently on such diets as they do on wheat-based diets. Furthermore, there is little information on the variation in voluntary feed intake and growth of pigs fed sorghum processed in different ways and in the presence of enzymes.

An important factor potentially limiting the use of sorghum by pigs is the slower rate of its starch digestion, which may be due to the type or variation in the protein surrounding the sorghum starch granule. These proteins are typically high in sulphur amino acids, and the disulphide cross-links based on cysteine residues could be limiting hydrolysis of the protein by endogenous enzymes in the pig. It is postulated that the starch granule/ protein matrix and other factors in sorghum limit the rate and extent of digestion of starch in the small intestine, and potentially increase the rate and extent of starch fermentation in the ileum and caecum. This in turn, would lower the energy available to the animal for growth. At the conclusion of this project, the amount of variation attributed to different feed processing treatments and the influence of supplementary enzymes will be quantified and this information will be used in future trials to assist in unlocking the hidden potential of sorghum-based feeds for pigs.

The objectives of this study were to:

1. improve the digestibility and pig feed efficiency on sorghum and barley based diets
2. compare diets containing either animal protein (lower phytate diets) or vegetable proteins, and in turn to increase producer and nutritionists confidence in vegetable protein only diets
3. increase data available on the increase due to exogenous phytase in amino acid, mineral and energy digestibility to allow nutritionists to safely adjust diet specifications and reduce diet costs
4. produce more information to justify the NIR calibration of phytate levels in all Australian vegetable derived diet ingredients and condensed tannin levels in sorghum.
5. improve the cost of production of pigs offered sorghum and barley based diets, steam pelleted under commercial conditions.

Two experiments were conducted at the DPI&F facility at Wacol, Queensland in February and March 2009 using surgically-prepared stock from the UQ Gatton herd. Diets using one barley variety (Gardiner) and two sorghum varieties; one containing low condensed tannins (Liberty) and the second (Bonus) were formulated to provide a moderate (0.0025ppm) and increased (0.0035) phytate content. Each ration was offered with and without 150ppm of a commercial phytase preparation (Porzyme® 8000, Feedworks, Romsey VIC 3XXX) were cold pelleted (5mm diameter) in a single batch.

## Experimental

### Experiment 1 (AF007)

Title: Intake and live weight responses to the inclusion phytase in high and low phytate diets for weaner pigs.

> Hypothesis: Hydrolysing phytate will improve nutrient digestibility however anti-nutritional soluble NSP in barley will limit the extent of increase in amino acid, starch and mineral digestibility.

The experiment was conducted on one barley variety (Gardiner) and two sorghum varieties; one containing low condensed tannins (Liberty) and the second (Bonus) with medium levels (albeit low compared to the tannin levels in the older cultivars), to produce 12 treatments, designated as:

1. Grain type
2. Phytate level (0.25% and 0.35% Phytate Phosphorus)
3. Phytase (0 and 150 ppm)

Ten pigs, initial live weight 30.9 ± 0.38 kg, were randomly allocated to one of 12 dietary treatments (see Table 1) in an experimental design providing 5 replicates per diet. Pigs were fed test diets in successive 7 day periods and intake recorded daily. Pigs were weighed at the commencement and at the end of each period. Water was provided *ad libitum* via nipple drinkers. Faecal and ileal DE were calculated from digestibility estimates derived from reference to an indigestible acid insoluble ash (AIA) marker, Singh *et al.,* 2009. Diets included 2% Celite as the AIA marker used to determine digestibility. All values were initially calculated on an as-fed basis and then converted to a dry matter basis for statistical analysis.

**Experiment 2 (AF008)**

Title: Kinetics of digestion in grower pigs fed sorghum and barley-based diets.

> Hypothesis: Gut volume and rate of passage of material through the gastro-intestinal tract is influenced by grain type.

On completion of Experiment 1 (AF007) nine pigs (approx. 55kgs) continued to stay in the metabolic cages for a 72 hour period and were re-randomised to one of three treatment groups. The experiment was conducted in a single period with each pig used once.

Three grains were incorporated into grower rations (see Appendix 1) to include a white, low tannin variety (Diet 1, Liberty) a red, moderate tannin sorghum variety (Diet 5, Bonus) and barley (Diet 9, Gardiner). Quantities of each diet were sprayed with CrEDTA and Yb chloride solutions to provide approximately 50mg Cr and 50 mg Yb per kg feed. Known weights (c 200g) of the marked diets were then fed to 3 pigs per diet prior to their normal daily feed. Ileal samples (c. 12) were collected at intervals over the following 48 hours and faecal samples over the following 72 hours to determine a decay curve for the passage of the two markers. Feeding and management procedures through this phase of the study were as described below for ileal and faecal digestibility collection. Samples were put into uniquely labelled bottles. One sub-sample was freeze-dried and ground for chemical analysis; the other was stored frozen at the Wacol site as a reserve sample.

**Surgery**

Pigs underwent surgery approximately 6 weeks prior to commencing Experiment 1 (AF007) according to the following procedure. The pigs were fitted with simple T-piece cannulas about 150 mm anterior to the ileo-caecal valve as described by van Barneveld (1993) with the exception that skin barriers for use around stoma in human ileostomy patients (Stomahesive® System 2 with 70mm flange; Bristol-Myers Squibb, Princeton, NJ, 08543-4000 USA) will be incorporated between the flange of the cannula and the skin to promote healing of the wound and to prevent any leakage around the cannula.

**Post-surgery**

Immediately post-surgery, pigs were monitored to ensure full recovery from the administered anaesthetics. The area surrounding the cannulas was washed with Lovone® daily. Pig were housed individually in solid-sided pens (1.5 x 2m) and fed a commercial grower diet (0.65 g available lysine MJ$^{-1}$ DE; 13.5MJ DE kg$^{-1}$) Daily feeding rates will be adjusted to three times maintenance (3 x (0.5 MJ DE kg$^{-1}$ body weight$^{0.75}$)/diet DE).

Daily rations were halved and fed at 12 h intervals. Water was provided *ad libitum* via nipple drinkers. Following a 7 day recovery period, experimental diets were introduced over three days. Diets were fed for 5 days with collections of ileal digesta made on days 6 and 7. Ileal digesta was collected and immediately frozen to prevent further digestion of the sample. To facilitate collections, pigs were transferred into metabolism cages. Following collection of digesta, diets were re-allocated and the procedure repeated until all pigs had received the five diets. Following each collection, samples will be bulked, mixed, subsampled, and freeze-dried prior to chemical analysis.

While the pigs were housed in the metabolism cages for continuous collection of digesta, during this two day period, random subsamples of faeces voided were collected, bulked and stored at -20° for determination of faecal DE. At the end of each collection period, samples of digesta and faeces were thawed, subsampled, freeze-dried and ground prior to chemical analyses.

**Measurements recorded for database:**
Chemical analysis on the freeze dried ileal and faecal material, the pelleted material and the diet mash were determined as described by Singh *et al.* (2009). Phytate and phytate P were determined after the method of Selle *et al.* (1996) at a commercial laboratory (Symbio Alliance, 8 Mile Plains Qld 4XXX).

## Results & Discussion

### Experiment 1 (AF007)

The effects of grain type, phytase and phytate level on all measured parameters of ileal digestibility are shown in Tables 1 and 2.

There was a significant effect of grain type on the ileal digestibility of P, Ca, fat, DE and all the amino acids except for lysine, arginine, serine and glycine. The white sorghum produced lower P and Ca digestibility compared to the other two grains, and the red sorghum had inferior fat and ileal amino acid digestibility. The presence of phytase improved P, Ca and amino digestibility ($P<0.05$) except for cysteine, leucine and tryptophan. There was no effect of the enzyme on ileal DE ($P>0.05$). Barley and Red Sorghum were the most responsive to phytase in terms of ileal EAA digestibility, whereas the white sorghum showed little to no response, with interactions on methionine, threonine, Isoleucine, leucine, valine, histadine, arginine serine and gylcine ($P<0.05$). Phytate level only influenced ileal P digestibility ($P<0.05$), producing a negative effect on P availability. There were no significant interactions between phytate level and response to phytase.

Phytase significantly improved the faecal digestibility of P, Ca, potassium, nitrogen, threonine and fat, however it had no effect on DE (Table 3). Increasing Phytate level reduced the digestibility of phosphorus and fat ($P<0.05$), and there was a significant grain type effect, where the red sorghum produced a lower faecal digestibility of nitrogen, threonine and fat. There was an interaction between grain type and phytase on faecal P and Ca digestibility ($P<0.05$), with phytase improving Barley P and Ca by 28% and 22%, respectively, whereas phytase had a more pronounced effect on Ca (23%), compared to P (17%), in both sorghums. There three way interactions on P, Ca, nitrogen and threonine faecal digestibility. For Ca and P, the barley was the most responsive grain to phytase, however the bigger response was on the 0.25% phytate P diets, particularly for the red sorghum. The other 3 way interaction was on nitrogen and threonine, where the biggest overall response to phytase was also in barley, great in the 0.35% Phytate P diets, however the response to the phytase in the sorghum diets was more prevalent in the 0.25% Phytate P diets.

### Discussion

The present experiment provides unique data, as very little to no ileal digestibility works had been conducted in barley and sorghum based diets. The phytase influence on ileal and faecel P and Ca were highly significant, as expected, however the lower increase in faecal P digestibility in sorghum compared to barley warrants further investigation. The phytase nutrient matrices used in commercial pig formulations generally suggest an increase in P availability of 20 to 25%, with increases in Ca digestibility in the range of 15 to 20%. The present study suggests the release of P by phytase in sorghum based diet maybe over-estimated when using current industry recommendations. Increasing Phytate P level did reduce P digestibility, however the high Phytate P didn’t increase the response to the enzyme. In fact, in most incidences the response to phytase was greater in the low phytate P diets.

Ileal amino acid digestibilities were significantly increased, generally, by phytase. Again the greatest response in was in barley based diets (3.27%) and with a good response red sorghum (2.37%). The increases in essential amino acid digestibility are higher than currently suggested by phytase experts and suppliers. The present amino acid uplifts, by supplemented phytase, are on average around 2.1 to 2.2%. The amino acid digestibility of white sorghum was not influenced by phytase, only producing a 0.12% increase. Overall, the average inherent ileal amino acid digestibility of white sorghum was 4.94% more digestible than the red sorghum. The white sorghum exhibited a higher amino acid digestibility, which suggests that this variety is significantly less responsive to phytase in nitrogen availability. In fact the white sorghum was analysed to be a superior grain, compared to its red counterpart, as the white grain produced a 8.38% higher ileal DE and a 1.6% higher faecal DE. The differences between white and red sorghum have been reported in the past by Cadogan and Finn (2011), showing that the white sorghum is significantly more digestible than the red sorghum.

Phytase produced a general increase in faecal nitrogen (1.3%) and fat digestibility (2.7%), over all 3 grains, but had no significant influence on DE. The increase in nitrogen and fat digestibility would normally add up to a higher DE, however this was not observed. Several studies by Choct and Cadogan (2009) have shown that enzymes increase net energy proportionally more than ME, and it is likely that the increase in net energy by phytase was higher compared to DE in the present study. Further work should be conducted to ascertain the net energy response to phytase (and other enzyme) relative to the response in DE.

**Table 1. Effects of Grain type, phytate and phytase on the ileal digestibility coefficient of minerals, starch, fat and Digestible Energy in grower pigs fed sorghum and barley-based diets containing different levels of phytate.**

| Grain type | Phytate P (%) | Phytase (FTU/kg) | Phosphorus | Calcium | Potassium | Sodium | Starch | Fat | Digestible Energy (MJ/kg)* |
|---|---|---|---|---|---|---|---|---|---|
| **Barley** | 0.25 | 0 | 0.53 | 0.58 | 0.56 | -6.80 | 0.98 | 0.86 | 13.6 |
| **Barley** | 0.25 | 150 | 0.66 | 0.65 | 0.78 | -4.39 | 0.98 | 0.90 | 13.4 |
| **Barley** | 0.35 | 0 | 0.45 | 0.45 | 0.43 | -3.41 | 0.97 | 0.86 | 13.3 |
| **Barley** | 0.35 | 150 | 0.59 | 0.52 | 0.50 | -6.30 | 0.97 | 0.87 | 13.6 |
| **Red Sorghum** | 0.25 | 0 | 0.49 | 0.42 | 0.34 | -3.42 | 0.97 | 0.83 | 13.4 |
| **Red Sorghum** | 0.25 | 150 | 0.62 | 0.61 | 0.55 | -3.51 | 0.96 | 0.82 | 13.6 |
| **Red Sorghum** | 0.35 | 0 | 0.44 | 0.51 | 0.54 | -5.43 | 0.97 | 0.67 | 12.8 |
| **Red Sorghum** | 0.35 | 150 | 0.54 | 0.57 | 0.48 | -4.35 | 0.95 | 0.77 | 13.2 |
| **White Sorghum** | 0.25 | 0 | 0.48 | 0.38 | 0.64 | -3.85 | 0.98 | 0.82 | 13.8 |
| **White Sorghum** | 0.25 | 150 | 0.53 | 0.50 | 0.61 | -9.57 | 0.97 | 0.85 | 13.9 |
| **White Sorghum** | 0.35 | 0 | 0.43 | 0.47 | 0.52 | -8.87 | 0.98 | 0.82 | 13.8 |
| **White Sorghum** | 0.35 | 150 | 0.48 | 0.58 | 0.52 | -4.66 | 0.98 | 0.84 | 14.0 |
| | | | | | | | | | |
| **P Values** | | | | | | | | | |
| **Phytase (PS)** | | | <0.001 | <0.001 | 0.180 | NS | NS | 0.118 | 0.258 |
| **Phytate (PT)** | | | 0.002 | 0.072 | 0.067 | NS | NS | 0.140 | NS |
| **Grain type (G)** | | | <0.001 | 0.027 | NS | NS | NS | 0.003 | <0.001 |
| **PS x PT** | | | NS | NS | NS | NS | NS | NS | NS |
| **PS x G** | | | NS | NS | NS | NS | NS | NS | NS |
| **PT x G** | | | NS | <0.001 | NS | NS | NS | NS | NS |
| **PS x PT x G** | | | NS | NS | NS | <0.001 | NS | NS | NS |

**Table 2. Effects of Grain type, phytate and phytase on the ileal digestibility coefficient of amino acids in grower pigs fed sorghum and barley-based diets containing different levels of phytate.**

| Grain type | Phytate P (%) | Phytase (FTU/kg) | Lys | Meth | Cys | Thr | Iso | Trypt | Leu | Val | His | Arg | Ser | Gly |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Barley** | 0.25 | 0 | 86.6 | 89.3 | 73.5 | 70.8 | 75.9 | 76.9 | 80.6 | 75.9 | 79.0 | 80.1 | 69.5 | 62.6 |
| **Barley** | 0.25 | 150 | 88.3 | 89.5 | 76.2 | 78.5 | 81.8 | 78.2 | 84.8 | 81.6 | 85.1 | 84.5 | 78.3 | 66.0 |
| **Barley** | 0.35 | 0 | 87.8 | 88.5 | 72.7 | 76.5 | 78.8 | 76.3 | 82.2 | 80.3 | 84.4 | 83.8 | 76.1 | 67.5 |
| **Barley** | 0.35 | 150 | 88.7 | 90.0 | 75.2 | 79.7 | 81.5 | 77.1 | 84.6 | 82.8 | 86.5 | 86.8 | 79.3 | 73.8 |
| **Red Sorghum** | 0.25 | 0 | 88.6 | 86.4 | 66.9 | 75.8 | 80.4 | 75.9 | 84.4 | 80.6 | 80.5 | 79.6 | 76.3 | 59.7 |
| **Red Sorghum** | 0.25 | 150 | 91.2 | 91.4 | 70.6 | 78.2 | 82.5 | 77.9 | 85.5 | 82.2 | 81.6 | 83.7 | 78.2 | 63.2 |
| **Red Sorghum** | 0.35 | 0 | 86.0 | 87.8 | 69.1 | 72.1 | 80.2 | 77.0 | 85.0 | 77.8 | 78.2 | 79.3 | 73.2 | 55.7 |
| **Red Sorghum** | 0.35 | 150 | 88.1 | 88.0 | 67.0 | 75.8 | 80.1 | 77.6 | 82.9 | 79.8 | 81.4 | 84.0 | 76.0 | 68.9 |
| **White Sorghum** | 0.25 | 0 | 87.2 | 92.1 | 75.5 | 78.8 | 84.1 | 79.5 | 88.9 | 83.1 | 85.0 | 84.7 | 79.6 | 66.2 |
| **White Sorghum** | 0.25 | 150 | 86.4 | 90.6 | 72.1 | 77.2 | 82.9 | 81.9 | 87.4 | 82.2 | 83.0 | 81.0 | 77.3 | 62.1 |
| **White Sorghum** | 0.35 | 0 | 88.8 | 91.4 | 73.9 | 78.0 | 83.9 | 80.9 | 89.1 | 83.1 | 85.5 | 82.8 | 78.7 | 62.0 |
| **White Sorghum** | 0.35 | 150 | 89.6 | 92.8 | 75.4 | 80.2 | 84.5 | 80.9 | 88.3 | 84.5 | 84.6 | 83.1 | 77.2 | 60.6 |
| | | | | | | | | | | | | | | |
| **P Values** | | | | | | | | | | | | | | |
| **Phytase (PS)** | | | 0.017 | 0.004 | 0.212 | <0.001 | 0.006 | 0.135 | 0.131 | 0.003 | 0.002 | 0.014 | 0.008 | 0.041 |
| **Phytate (PT)** | | | NS | NS | NS | NS | NS | NS | NS | NS | 0.08 | NS | NS | NS |
| **Grain type (G)** | | | NS | 0.001 | 0.001 | 0.035 | 0.001 | 0.004 | 0.001 | 0.003 | 0.001 | 0.393 | 0.064 | 0.119 |
| **PS x PT** | | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **PS x G** | | | NS | 0.042 | 0.154 | 0.015 | 0.007 | NS | 0.001 | 0.035 | 0.001 | 0.008 | 0.001 | 0.012 |
| **PT x G** | | | 0.001 | NS | NS | 0.077 | NS | NS | NS | 0.089 | NS | NS | 0.061 | NS |
| **PS x PT x G** | | | NS | 0.001 | 0.041 | 0.086 | 0.190 | NS | NS | NS | 0.051 | NS | NS | NS |

**Table 3. Effects of Grain type, phytate and phytase on the faecal digestibility coefficient of minerals, starch, fat and Digestible Energy in grower pigs fed sorghum and barley-based diets containing different levels of phytate.**

| Grain type | Phytate P (%) | Phytase (FTU/kg) | Phosphorus | Calcium | Potassium | Nitrogen | Threonine | Fat | Digestible Energy (MJ/kg) |
|---|---|---|---|---|---|---|---|---|---|
| **Barley** | 0.25 | 0 | 57.4 | 58.2 | 66.5 | 85.8 | 80.0 | 86.5 | 84.8 |
| **Barley** | 0.25 | 150 | 74.0 | 71.9 | 67.5 | 84.1 | 83.8 | 85.6 | 84.2 |
| **Barley** | 0.35 | 0 | 45.6 | 43.9 | 57.0 | 82.5 | 82.5 | 83.5 | 82.9 |
| **Barley** | 0.35 | 150 | 67.8 | 59.3 | 69.2 | 88.6 | 87.0 | 85.7 | 85.0 |
| **Red Sorghum** | 0.25 | 0 | 53.8 | 41.9 | 59.5 | 81.0 | 79.0 | 76.2 | 87.7 |
| **Red Sorghum** | 0.25 | 150 | 69.7 | 65.4 | 64.9 | 84.2 | 82.6 | 78.0 | 89.2 |
| **Red Sorghum** | 0.35 | 0 | 52.3 | 55.7 | 61.1 | 81.2 | 79.6 | 68.5 | 87.5 |
| **Red Sorghum** | 0.35 | 150 | 57.7 | 58.5 | 60.7 | 80.1 | 76.2 | 68.6 | 86.5 |
| **White Sorghum** | 0.25 | 0 | 54.9 | 41.9 | 61.9 | 84.7 | 84.3 | 76.2 | 89.2 |
| **White Sorghum** | 0.25 | 150 | 68.6 | 63.4 | 78.0 | 86.2 | 85.1 | 82.8 | 90.0 |
| **White Sorghum** | 0.35 | 0 | 52.0 | 51.5 | 56.5 | 86.1 | 84.1 | 77.0 | 88.5 |
| **White Sorghum** | 0.35 | 150 | 61.0 | 61.5 | 66.2 | 84.8 | 87.1 | 80.0 | 88.9 |
| | | | | | | | | | |
| **P Values** | | | | | | | | | |
| **Phytase (PS)** | | | <0.001 | <0.001 | <0.001 | <0.001 | 0.008 | 0.030 | NS |
| **Phytate (PT)** | | | <0.001 | 0.134 | 0.137 | 0.024 | NS | <0.001 | 0.049 |
| **Grain type (G)** | | | 0.166 | 0.053 | NS | <0.001 | <0.001 | <0.001 | NS |
| **PS x PT** | | | 0.135 | <0.001 | NS | <0.001 | NS | NS | <0.001 |
| **PS x G** | | | 0.004 | <0.001 | NS | 0.001 | 0.038 | 0.117 | NS |
| **PT x G** | | | NS | NS | NS | 0.006 | NS | <0.001 | NS |
| **PS x PT x G** | | | 0.02 | 0.002 | NS | <0.001 | 0.02 | NS | 0.119 |

**Experiment 2 (AF008)**

Intake and marker clearance parameters for grower pigs fed rations containing low (Liberty) and moderate (Bonus) tannin sorghum varieties or barley (Gardiner) are shown in Table 5. Intake and live weight are shown for descriptive purposes only and indicate that despite surgical preparation and confinement, the animals were performing as might be expected for animals of this type.

Marker excretion patterns differed markedly for ileal (see Figures 1 to 2) and faecal (Figure 3 and 4) samples. A feature of these curves is the variation observed between animals, reflecting differences in gut fill and intake. A more complete consideration of marker excretion by time is provided in Appendix 2.

Key features of the analysis of marker concentration vs time include the significant effect due to the interaction sample type and time after feeding (p-value < 0.001) for both Cr and Yb concentrations. This effect was the same for all varieties. There was also a sigficant difference between pigs and replicates, both having p-values < 0.05.

Markers appeared in ileal digesta within hours of feeding and showed clearance rates (k) from the ileum roughly three times (P<0.001) that found in faecal samples. Appearance of marker in the faeces showed numerical differences between sorghum and barley diets indicating a reduced rate of passage for both markers through the hindgut of sorghum-fed animals. The results suggest that differences in DE yield from sorghum and barley diets may be due in part to variation in the kinetics of digestion affecting the composition and quantity of material delivered to the hindgut. This finding is consistent with an increased hindgut digestion of sorghum-based feeds and provides further evidence for quantitative differences in the site as well as extent of digestion of sorghum relative to other cereal grains.

**Table 4. Intake and marker clearance parameters for grower pigs fed rations containing low (Liberty) and moderate (Bonus) tannin sorghum varieties or barley (Gardiner).**

| | Bonus | Gardiner | Liberty | Bonus | Gardiner | Liberty | sem[1] | P value | |
|---|---|---|---|---|---|---|---|---|---|
| n | 3 | 3* | 3 | | | | | | |
| Live weight kg | 58.9 | 60.9 | 59.8 | | | | 1.02 | 0.75 | |
| Intake kg/day | 1.80 | 1.84 | 1.75 | | | | 0.02 | 0.39 | |
| **Sample** | Faeces | | | Ileum | | | | Variety | VxS[2] |
| k Cr[3] $h^{-1}$ | -0.068 | -0.073 | -0.060 | -0.210 | -0.202 | -0.147 | 0.015 | 0.35 | 0.57 |
| k Yb[4] $h^{-1}$ | -0.086 | -0.099 | -0.058 | -0.228 | -0.178 | -0.179 | 0.020 | 0.59 | 0.69 |
| pt[5] Cr h | 58 | 43 | 56 | 3.7 | 4.3 | 3.3 | 2.1 | 0.100 | 0.147 |
| pt Yb h | 58 | 42 | 56 | 4.7 | 3.0 | 4.0 | 2.1 | 0.076 | 0.148 |

[1]pooled standard error mean; [2]S, sample site; V, Variety; [3]Cr, Chromium; [4]Yb, Ytterbium; [5] pt, peak time; *no faecal data for animal 3

The rate of passage traits observed here are within the range recorded for grower pigs by Ehle *et al*., 1982 and Wilfart *et al.,* 2007 using slaughter techniques and marker techniques, respectively. Lack of access to a more sophisticated package for the calculation of marker kinetics such as that used by Wilfart *et al.,* 2007 has substantially limited the evaluation of passage rate in the present study and understated the significance of the data collected.

**Figure 1. Indicative Cr excretion patterns from the ileum for individual pigs fed diets containing Bonus (5439), Gardiner (5540) and Liberty (5442) grains.**

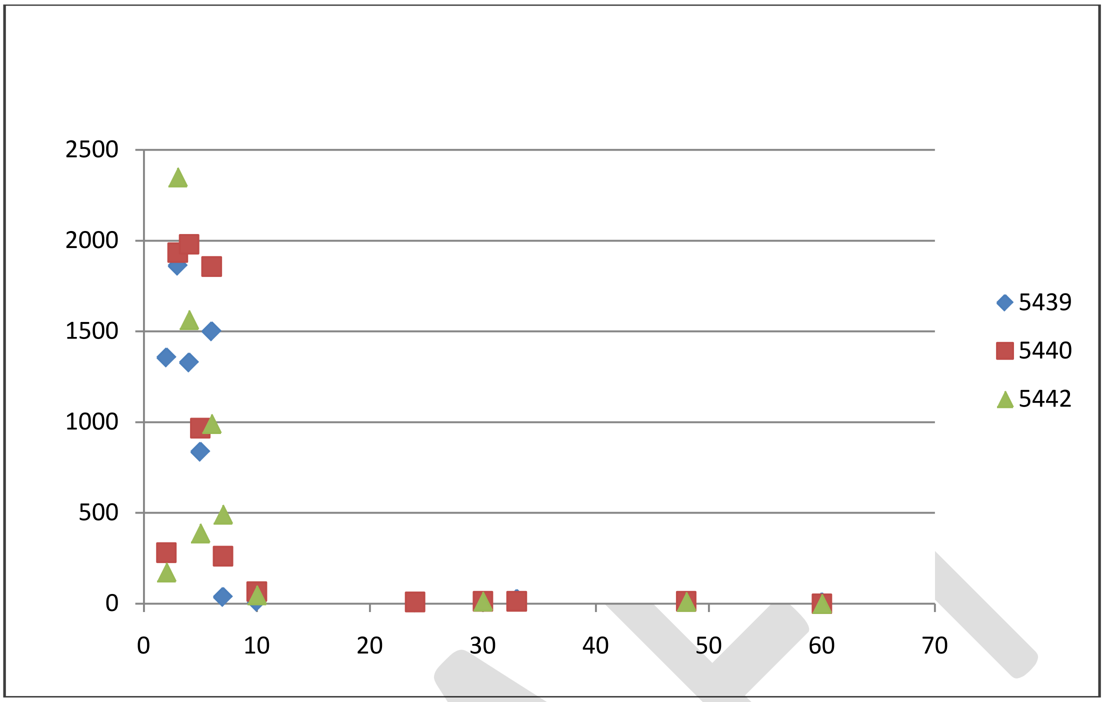


**Figure 2. Indicative Yb excretion patterns from the ileum for individual pigs fed diets containing Bonus (5439), Gardiner (5540) and Liberty (5442) grains.**

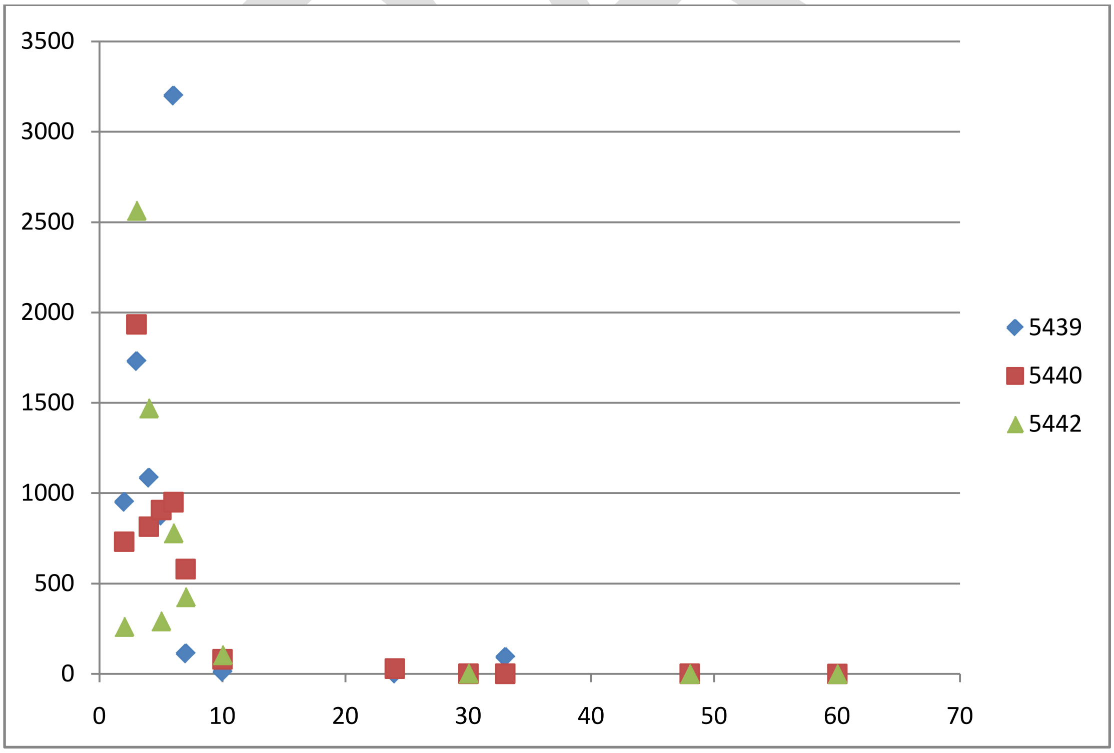


**Figure 3. Indicative Cr excretion patterns from the faeces for individual pigs fed diets containing Bonus (5439), Gardiner (5540) and Liberty (5442) grains.**

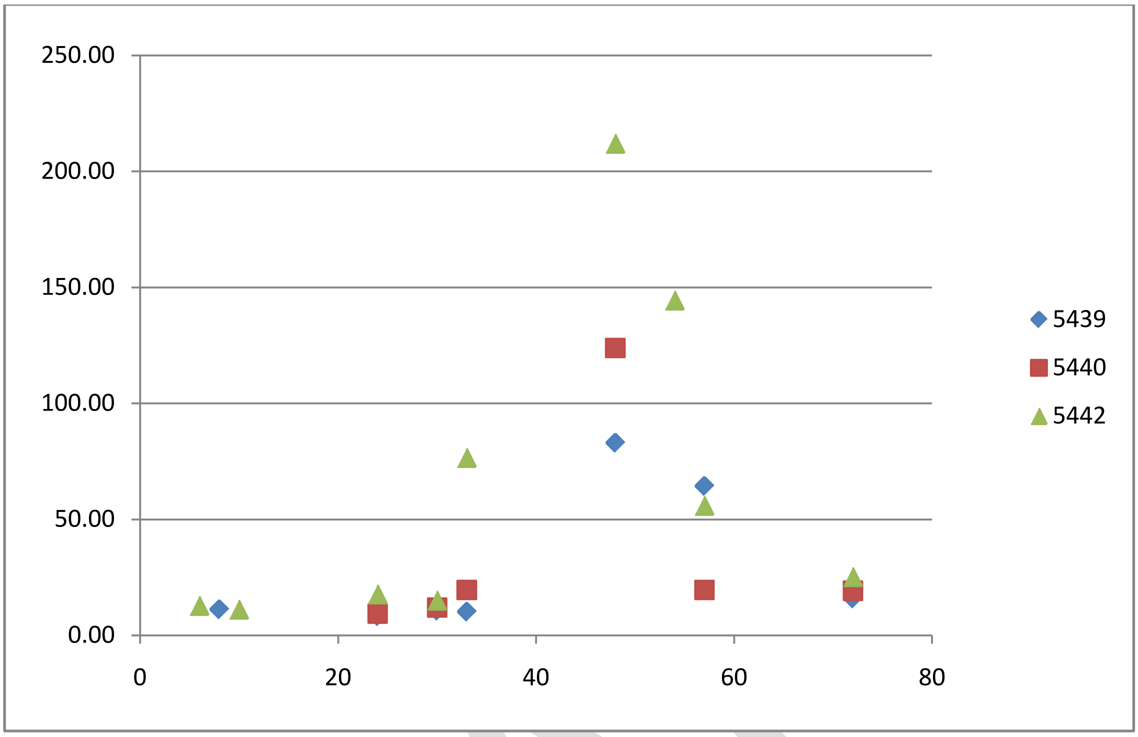
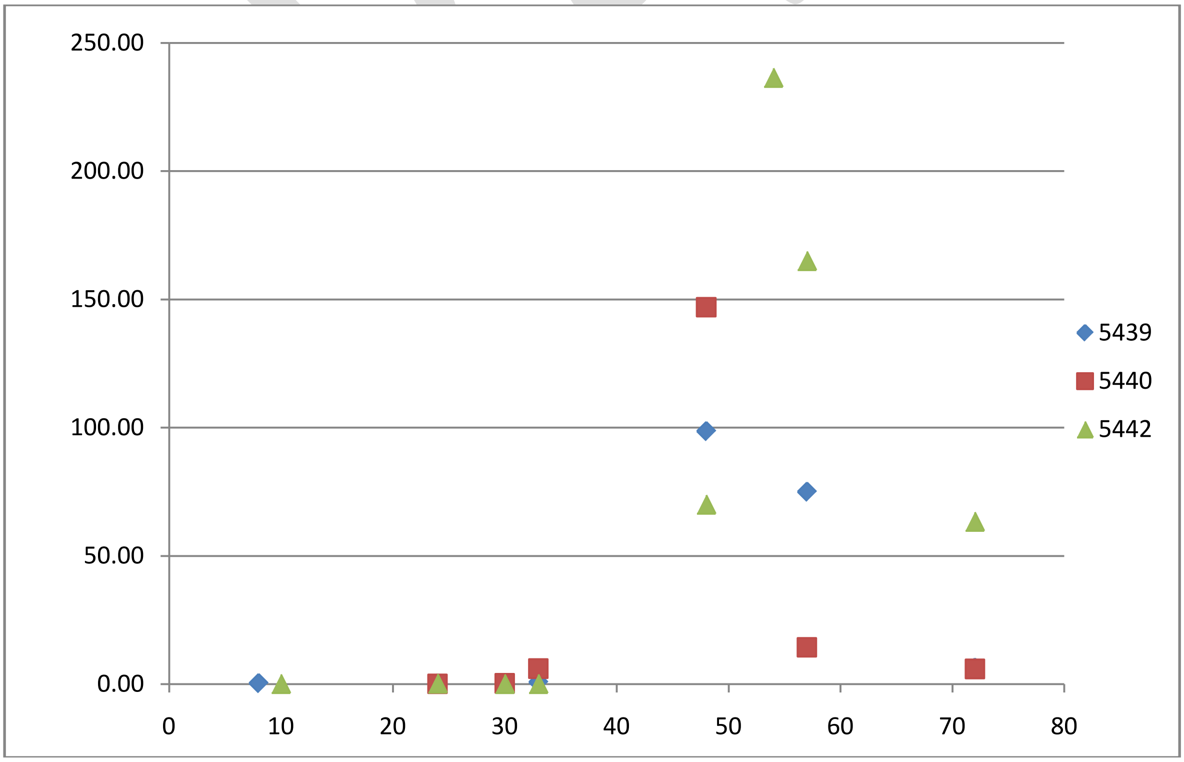


**Figure 4. Indicative Yb excretion patterns from the faeces for individual pigs fed diets containing Bonus (5439), Gardiner (5540) and Liberty (5442) grains.**



# Implications

The objectives intended for this project were substantially addressed. This study provided:

- further evidence of the value of exogenous enzymes in improving the digestibility and pig feed efficiency of sorghum and barley based diets (Objective 1)
- clear evidence of the ability of phytase to improve mineral and EAA digestibility that should enhance the confidence of both producers and nutritionists to use phytase in a wider range of applications (Objective 2). The present results confirm the current commercial recommendation for amino acid and mineral uplifts are accurate, although some more conservative uplifts may need to be applied to sorghum based diets.
- markedly increased data available on the nutrient uplift that can be obtained through the use of exogenous enzymes to allow nutritionists to safely adjust diet specifications and reduce diet costs (Objective 3) and
- more information to justify the NIR calibration of phytate levels in all Australian vegetable derived diet ingredients and condensed tannin levels in sorghum (Objective 4).

In addition, the measures of nutrient digestibility and marker kinetics are indicative of important differences in the magnitude of responses to exogenous enzymes due to grain type. This study has demonstrated the application of an alternative approach to the investigation of factors affecting nutrient yield that should be included in future feed evaluation studies. Assessment of the marker excretion curves point to retention times in the stomach of less than 4 hours with significant implications for the duration of exposure of components in the digesta to pepsin digestion and the erosion of the protein-carbohydrate matrices surrounding individual feed particles. Despite the limitations of individual experimental designs that have prevented resolution of all objectives as originally intended, this study has demonstrated that the use of marker techniques is an important method for taking into account interactions that exist between nutrients and the animal’s digestive process.

# Recommendations

1. The present experiment showed that Barley responds to phytase in a very similar way as wheat, and recommend the same uplift in amino acids and minerals. The Phytase effect on mineral and amino acids digestibility in sorghum was generally lower, particularly in Phosphorus. When using phytase on red sorghum based diets, it is recommended that nutritionists use 70% of the mineral and amino acids uplift recommended for wheat base diets. More work is required on white sorghum to further assess the amino acid and mineral digestibility response to phytase.
2. Data was collected during the course of this study for the further development of NIR calibrations for Australian vegetable-derived diet ingredients and steam-pelleted feeds and is on-going as part of a related project also supported by the Pork CRC, Project 1B107. A Final Report on this project is expected by the end of 2011. It is recommended that funding to permit the further collection and consolidation of data to support the further development of NIR calibrations for feeds and feed ingredients be made available.

3. Little progress was made to directly improve the cost of production of pigs offered sorghum and barley based diets, steam pelleted under commercial conditions. In hindsight, it is unfortunate that this study was not conducted according to the protocols proposed in the original application document. Diets were cold-pressed rather than steam pelleted and logistical and staffing limitations lead to truncation of individual experimental periods in Experiment 1 and the completion of only a single measurement period in Experiment 2. In combination, these factors substantially impacted on the ability to identify statistical significance between traits and the broader application of the findings. Given there is ready access to the raw data it is strongly recommended the marker data be re-analysed and funding made available to continue the examination of site and extent of diet components under commercial conditions.

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**Appendix 1. Ration Specifications and Ingredient inclusions for sorghum and barley-based diets containing different rates of phytate and phytase.**

|  | AF080001 WHITE SORGHUM + PHYTATE 0.25% | AF080002 WHITE SORGHUM + PHYTATE 0.35% | AF080003 WHITE SORGHUM 1 + PHYZYME 150PPM | AF080004 WHITE SORGHUM 2 + PHYZYME 150PPM | AF080005 RED SORGHUM+ P HYTATE 0.25% | AF080006 RED SORGHUM+ P HYTATE 0.35% | AF080007 RED SORGHUM1 + PHYZYME 150PPM | AF080008 RED SORGHUM2 + PHYZYME 150PPM | AF080009 BARLEY + PHYTATE 0.25% | AF080010 BARLEY + PHYTATE 0.35% | AF080011 BARLEY 1 + PHYT ASE 150PPM | AF080012 BARLEY 2 + PHYT ASE 150PPM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PROTEIN | 17.8 | 17.8 | 17.9 | 17.9 | 17.8 | 17.8 | 17.9 | 17.9 | 18.6 | 18.6 | 18.6 | 18.6 |
| C_FIBRE | 2.41 | 3.05 | 2.41 | 3.04 | 2.41 | 3.05 | 2.41 | 3.04 | 6.08 | 6.37 | 6.08 | 6.37 |
| DE_PIGS_MJ | 13.6 | 13.6 | 13.6 | 13.7 | 13.6 | 13.6 | 13.6 | 13.7 | 13.6 | 13.6 | 13.6 | 13.6 |
| LYSINE | 1.06 | 1.06 | 1.26 | 1.27 | 1.06 | 1.06 | 1.26 | 1.27 | 1.16 | 1.17 | 1.37 | 1.37 |
| CALCIUM | 0.745 | 0.744 | 1.24 | 1.24 | 0.745 | 0.744 | 1.24 | 1.24 | 0.707 | 0.710 | 1.21 | 1.21 |
| COPPER | 19.9 | 19.3 | 19.9 | 19.3 | 19.9 | 19.3 | 19.9 | 19.3 | 27.5 | 26.0 | 27.4 | 25.9 |
| SELENIUM | 0.095 | 0.095 | 0.095 | 0.095 | 0.095 | 0.095 | 0.095 | 0.095 | 0.164 | 0.158 | 0.163 | 0.157 |
| ZINC | 105 | 104 | 105 | 104 | 105 | 104 | 105 | 104 | 108 | 107 | 108 | 106 |
| VIT_A | 9479 | 9479 | 9474 | 9474 | 9479 | 9479 | 9474 | 9474 | 8953 | 8953 | 8948 | 8948 |
| VIT_D3 | 1422 | 1422 | 1421 | 1421 | 1422 | 1422 | 1421 | 1421 | 1343 | 1343 | 1342 | 1342 |
| VIT_E | 42.7 | 42.7 | 42.6 | 42.6 | 42.7 | 42.7 | 42.6 | 42.6 | 40.3 | 40.3 | 40.3 | 40.3 |
| CHOLINE | 1128 | 1148 | 1127 | 1148 | 1128 | 1148 | 1127 | 1148 | 1418 | 1410 | 1417 | 1409 |
| FAT/EE | 2.97 | 3.70 | 2.97 | 3.70 | 2.97 | 3.70 | 2.97 | 3.70 | 5.34 | 6.05 | 5.34 | 6.05 |
| PHYT_PHOS | 0.245 | 0.342 | 0.244 | 0.341 | 0.245 | 0.342 | 0.244 | 0.341 | 0.241 | 0.316 | 0.241 | 0.316 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| AF PIG GROWER PREMIX | 18.0 | 18.0 | 18.0 | 18.0 | 18.0 | 18.0 | 18.0 | 18.0 | 17.0 | 17.0 | 17.0 | 17.0 |
| BARLEY FEED | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 74.0 | 68.0 | 74.0 | 68.0 |
| WHITE SORGHUM | 74.0 | 70.0 | 74.0 | 70.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SORGHUM RED FEED | 0 | 0 | 0 | 0 | 74.0 | 70.0 | 74.0 | 70.0 | 0 | 0 | 0 | 0 |
| MILLRUN 16% | 4.00 | 0 | 4.00 | 0 | 4.00 | 0 | 4.00 | 0 | 0 | 0 | 0 | 0 |
| RICE BRAN | 0 | 8.50 | 0 | 8.50 | 0 | 8.50 | 0 | 8.50 | 4.00 | 9.50 | 4.00 | 9.50 |
| VEGETABLE OIL | 0 | 0.500 | 0 | 0.500 | 0 | 0.500 | 0 | 0.500 | 3.00 | 3.50 | 3.00 | 3.50 |
| SODIUM BENTONITE | 2.00 | 1.00 | 2.00 | 1.00 | 2.00 | 1.00 | 2.00 | 1.00 | 0 | 0 | 0 | 0 |
| PHYZYME XP 5000 | 0 | 0 | 0.050 | 0.050 | 0 | 0 | 0.050 | 0.050 | 0 | 0 | 0.050 | 0.050 |
| CELITE | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
|  | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

CHARLES STURT UNIVERSITY

# AF008

# Kinetics of Digestion Sorghum & Barley

May 31, 2011

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# Kinetics

## 1.1 Model

The data is based on a designed experiment. A complete block design was used, where each replicate contained a complete set of diets -2 sorghum varieties and a barley variety. Unfortunately the design was not followed as planned -but the results were analysed according to the planned experiment.

A linear mixed model using restricted maximum likelihood were used to analyse the data using ASReml-R (Butler, Cullis, Gilmour & Gogel 2007). There are two types of responses in this report, chromium and ytterbium -measured over time and peak time for both chormium and ytterbium. Two different models are required for these responses. The model that will be used for chromium and ytterbium measured over time can symbolically be written as:

response � mean + Variety + SampleType + Variety:SampleType + TimeFromFeeding +
Variety:TimeFromFeeding + SampleType:TimeFromFeeding +
Variety:SampleType:TimeFromFeeding + Replicate + Pig

The model that will be used for the peak time for chromium and ytterbium can symbolically be written as:

response � mean + Variety + SampleType + Variety:SampleType +
Replicate + Pig

Notation: Terms fitted in the model as random are italicised; all other terms are fitted as fixed terms.

Note concerning all predicted values: The predicted values have been assigned a rank based on the Tukey family of pairwise differences with a family confidence level of 5%. Therefore, no overall standard error or difference or least significant difference will be provided.

## 1.2 Responses

The responses measured were ileal and faecal chromium and ytterbium concentration (measured over time) and ileal and faecal peak time for chromium and ytterbium.

A histogram of the response was used to identify any outliers. Both chromium and ytterbium measured in the ileum have a much larger spread than the faecal data. This could be due to the fact that these measurements were changing over time.

## 1.3 Results

### Chromium Concentration

The ANOVA table for Chromium follows:

| | Df | denDF | F.inc | Pr |
|---|---|---|---|---|
| Variety | 2 | 138.0 | 0.53 | 0.591 |
| SampleType | 1 | 138.0 | 60.27 | 0.000 |
| Variety:SampleType | 2 | 138.0 | 0.20 | 0.819 |
| TimeFromFeeding | 1 | 138.0 | 61.80 | 0.000 |
| Variety:TimeFromFeeding | 2 | 138.0 | 1.22 | 0.298 |
| SampleType:TimeFromFeeding | 1 | 138.0 | 193.40 | 0.000 |
| Variety:SampleType:TimeFromFeeding | 2 | 138.0 | 0.14 | 0.873 |

| | SampleType | TimeFromFeeding | PredictedCr | SE | BackTransformedCr |
|---|---|---|---|---|---|
| 1 | faecal | 2 | 2.41 | 0.32 | 11.08 |
| 2 | faecal | 4 | 2.46 | 0.30 | 11.67 |
| 3 | faecal | 6 | 2.51 | 0.29 | 12.28 |
| 4 | faecal | 10 | 2.61 | 0.27 | 13.61 |
| 5 | faecal | 20 | 2.87 | 0.21 | 17.60 |
| 6 | faecal | 30 | 3.12 | 0.17 | 22.75 |
| 7 | faecal | 40 | 3.38 | 0.15 | 29.41 |
| 8 | faecal | 60 | 3.90 | 0.20 | 49.17 |
| 9 | faecal | 70 | 4.15 | 0.26 | 63.56 |
| 10 | ileal | 2 | 6.56 | 0.16 | 708.69 |
| 11 | ileal | 4 | 6.32 | 0.15 | 552.92 |
| 12 | ileal | 6 | 6.07 | 0.14 | 431.38 |
| 13 | ileal | 10 | 5.57 | 0.13 | 262.58 |
| 14 | ileal | 20 | 4.33 | 0.13 | 75.91 |
| 15 | ileal | 30 | 3.09 | 0.17 | 21.94 |
| 16 | ileal | 40 | 1.85 | 0.23 | 6.34 |
| 17 | ileal | 60 | -0.63 | 0.37 | 0.53 |
| 18 | ileal | 70 | -1.88 | 0.45 | 0.15 |

**Ytterbium Concentration**

The ANOVA table for Ytterbium follows:

| | Df | denDF | F.inc | Pr |
|---|---|---|---|---|
| Variety | 2 | 6.2 | 0.79 | 0.497 |
| SampleType | 1 | 133.7 | 134.60 | 0.000 |
| Variety:SampleType | 2 | 133.6 | 1.19 | 0.309 |
| TimeFromFeeding | 1 | 133.6 | 14.12 | 0.000 |
| Variety:TimeFromFeeding | 2 | 133.5 | 2.21 | 0.114 |
| SampleType:TimeFromFeeding | 1 | 133.2 | 316.50 | 0.000 |
| Variety:SampleType:TimeFromFeeding | 2 | 133.2 | 0.73 | 0.486 |

The data was ln transformed and after transformation there was not any evidence to suggest that the assumptions associated with the residuals had been violated.
There is a significant effect due to the interaction sample type and time after feeding (p-value < 0.001). This effect ws the same for all varieties. There was also a significant difference between pigs and replicates, both having p-values < 0.05.

The predicted sample type by time after feeding follows:

| | SampleType | TimeFromFeeding | | PredictedYb | SE | BackTransformedYb |
|---|---|---|---|---|---|---|
| 1 | faecal | 2 | - | 1.59 | 0.44 | 0.20 |
| 2 | faecal | 4 | - | 1.42 | 0.43 | 0.24 |
| 3 | faecal | 6 | - | 1.25 | 0.41 | 0.29 |
| 4 | faecal | 10 | - | 0.90 | 0.38 | 0.41 |
| 5 | faecal | 20 | | -0.04 | 0.31 | 0.96 |
| 6 | faecal | 30 | | 0.83 | 0.25 | 2.28 |
| 7 | faecal | 40 | | 1.69 | 0.23 | 5.42 |
| 8 | faecal | 60 | | 3.42 | 0.29 | 30.46 |
| 9 | faecal | 70 | | 4.28 | 0.36 | 72.23 |
| 10 | ileal | 2 | | 6.89 | 0.24 | 984.76 |
| 11 | ileal | 4 | | 6.57 | 0.23 | 712.18 |
| 12 | ileal | 6 | | 6.24 | 0.22 | 515.05 |
| 13 | ileal | 10 | | 5.60 | 0.20 | 269.38 |
| 14 | ileal | 20 | | 3.98 | 0.20 | 53.29 |
| 15 | ileal | 30 | | 2.36 | 0.25 | 10.54 |
| 16 | ileal | 40 | | 0.74 | 0.33 | 2.09 |
| 17 | ileal | 60 | | -2.51 | 0.51 | 0.08 |
| 18 | ileal | 70 | | -4.13 | 0.61 | 0.02 |

## Chromium Peak Time

The predicted sample type follows.The data was ln transformed and after transformation there was not any evidence to suggest that the assumptions associated with the residuals had been violated.

The predicted sample type follows:

| SampleType | PredictedCrPeakTime | SE | Ranking | BackTransformedCrPT |
|---|---|---|---|---|
| faecal | 3.90 | 0.10 | b | 51.40 |
| ileal | 1.30 | 0.10 | a | 3.70 |

The ANOVA table for chromium peak timefollows:

| | Df | denDF | F.inc | Pr |
|---|---|---|---|---|
| Variety | 2 | 12.0 | 0.15 | 0.866 |
| SampleType | 1 | 12.0 | 576.40 | 0.000 |
| Variety:SampleType | 2 | 12.0 | 2.16 | 0.158 |

## Ytterbium Peak Time

The ANOVA table for Ytterbium peak timefollows:

| | Df | den DF | F.inc | Pr |
|---|---|---|---|---|
| Variety | 2 | 10.0 | 3.60 | 0.066 |
| SampleType | 1 | 10.0 | 513.80 | 0.000 |
| Variety:SampleType | 2 | 10.0 | 0.14 | 0.871 |

The data was ln transformed and after transformation there was not any evidence to suggest that the assumptions associated with the residuals had been violated. There is a significant effect due to the main effect of sample type (p-value < 0.001). There was also a significant difference between pigs and replicates, both having p-values < 0.05.

The predicted sample type follows:

| SampleType | PredictedYbPeakTime | SE | Ranking | BackTransformedYbPT |
|---|---|---|---|---|
| faecal | 3.90 | 0.10 | b | 50.80 |
| ileal | 1.30 | 0.10 | a | 3.70 |