ESCAPING THE DAILY GRIND – COARSER GROUND DIETS FOR IMPROVED FETAL GROWTH

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Final Report prepared for the Australasian Pork Research Institute Limited (APRIL)

By

Jessica Zemitis, Sally Tritton, Darryl D'Souza and Kate Plush

SunPork Group
Eagle Farm, QLD

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Executive Summary

Producing high numbers of piglets that are of good quality and survive through to weaning is a key driver for breeder units and so farm productivity. Pig diets are processed into fine particle sizes to increase digestibility. This is especially important for the growing pig where feed conversion ratio drives profitability, and in lactating sows where high dietary energy is required for milk production. However, there is a paucity of information on the impact of particle size in gestating sow diets. A coarser ground diet will enhance hind gut fermentation, a process which involves the production of butyrate. In rats, increased dietary butyrate fed during early gestation has been shown to improve fetal growth because of enhanced implantation spacing. The aim of these experiments was to determine if a coarser ground diet fed to sows throughout gestation improved fetal growth, reduced variation in piglet weight at birth, and ultimately increased peri-natal piglet survival, if butyrate is the mechanism that drives any performance improvement and whether the inclusion of an exogenous carbohydrate enzyme improved these traits further.

The pilot investigation involved a total of 324 sows allocated by parity to one of two treatments at mating; Control, in which the gestation diet (13.0 MJ DE/kg 0.42 g SID lysine/MJ DE**)** was prepared using a 2mm disk (sieve test; 31% > 1 mm) and Coarse using a 3mm disk (sieve test; 43% > 1 mm). Sows were fed their respective diets at 2.1kg per day if in ideal body condition, or 2.4kg per day if they were assessed to be thin (P2 backfat < 16 mm) via electronic sow feeder (Osborne Industries Inc., Osborne KS USA). At approximately day 110, sows were relocated to farrowing accommodation. Measures included pregnancy rate, farrowing rate, litter size and birth weight traits (n = 80 per treatment) as well as any piglet death that occurred prior to fostering. Data were analysed in SPSS (v26 IBM, Amarnok USA). Pregnancy rate at ~ day 30 was increased in the Coarse treatment (89.8% versus Fine 85.7%; P = 0.028), but no difference in farrowing rate could be established (Coarse 89.8% versus Fine 81.3%; P = 0.207). There was no difference in total pigs born, or pigs born alive, but born dead was reduced by 0.3 pigs per litter (P = 0.031) and pre-foster mortality tended to be reduced by 0.3 pigs per litter (P = 0.065) in the Coarse compared to Fine treatment. No significant difference in any of the birth weight traits were identified.

Experiment 1 involved a total of 607 sows allocated by parity to one of three treatments at mating; Control, in which the gestation diet (13.1 MJ DE/kg 0.55 g SID lysine/MJ DE**)** was prepared using a 2mm disk (sieve test; 30% > 1 mm), Coarse using a 3mm disk (sieve test; 65% > 1 mm) and Control + butyrate (13.1 MJ DE/kg, 0.55 SID Lys g/kg) + exogenous butyrate source (Tributyrin 0.2% inclusion; ButyPearl Kemin, Killara NSW) with 30% particles > 1 mm). Sows were fed their respective diets at 2.1kg per day if in ideal body condition, or 2.4kg per day if they were assessed to be thin (P2 backfat < 16 mm) via electronic sow feeder (Osborne Industries Inc., Osborne KS USA). At approximately day 110, sows were relocated to farrowing accommodation. Sow body condition was monitored on a subset of sows with body weight and P2 backfat measured on day 1, 60 and 110 of gestation. At farrowing, performance data were recorded and individual piglet birth weights (including live and dead animals) of a subset of sows obtained. On days 30 and 90, blood and faecal samples were taken from a subset of sows for progesterone (blood only) and short chain fatty acid concentrations (SCFA; blood and faeces). Data were analysed in SPSS. Japanese Encephalitis Virus (JEV) was confirmed at the experiment site two months into the experiment. On d 90, propionic and butyric acid concentrations were highest in the Coarse treatment in the faecal samples and there was a tendency for the Coarse treatment to have the highest serum butyric acid concentrations. There was no difference in the reproductive performance of the three treatments.

Experiment 2 involved a total of 598 sows allocated by parity to one of four treatments at mating arranged in a 2 x 2 factorial design; Control: fed standard gestation diet (13.0 MJ DE/kg, 0.55 SID Lys g/kg) with 40% particles > 1mm, Control + enzyme: fed Control gestation diet + 0.2 kg/t mixed enzyme (xylanase, beta-glucanase, cellulase and xyloglucanase; Ronozome MultiGrain, DSM Nutritional Products, Wagga Wagga NSW) with 40% particles > 1mm, Coarse: standard gestation diet (13.0 MJ DE/kg, 0.55 SID Lys g/kg) with 65% particles > 1mm, Coarse + enzyme: fed Coarse gestation diet + 0.2 kg/t mixed enzyme. Sow body condition was monitored with caliper and P2 backfat measurements, and scratch score allocated (0= absent, 1= present). Farrowing performance data was recorded and a subset of litters were weighed at birth. Data were analysed in SPSS according to the 2 x 2 factorial design. There were no significant interactive effects between the two main treatments (particle size and enzyme). Sows from the Coarse treatment recorded a higher caliper at d 60 of gestation and this tended to be higher at d 110 of gestation than the Control treatment. + Enzyme sows recorded a higher P2 backfat at d 1 and d 110. There was no significant impact of either treatment on the incidence of sows with scratches at any stage of gestation. There was no significant effect of particle size or enzyme inclusion on pregnancy or farrowing rate. Sows from the + Enzyme treatment had higher total pigs born and pigs born alive than those from the - Enzyme treatment (1.1 pigs and 0.8 pigs respectively). There was no significant impact of the Coarse treatment on litter size traits. Total litter weight tended to be higher, and coefficient of variation lower, in sows fed a Coarse compared to a Control diet. The number of piglets born under 1.1 kg was reduced by 0.5 pigs per litter in the Coarse sows. There was no significant impact of enzyme treatment on weight characteristics.

In conclusion, increased particle size increased SCFA production in the large intestine, as measured in the faeces and tended to increase serum butyrate in late gestation, in the absence of viral infection, a Coarse diet reduced the incidence of low birth weight piglets having positive impacts on perinatal piglet survival and exogenous enzyme inclusion in gestating sow diets improved litter size.

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1. Introduction

Pre-weaning mortality is one of the largest areas of reproductive loss/wastage on sow breeder farms, with the biggest risk factor being low birthweight (Baxter *et al.* 2008). Previous work has found, on average, four piglets per litter are born weighing less than 1.1 kg, and demonstrated that the survivability of those piglets is ~65 %, whilst those heavier than this cut-off have a 90% survival rate (Plush *et al.* 2019). With the modern sow becoming more prolific and litter sizes increasing, the frequency of lightweight piglets is anticipated to only increase.

A relatively underexplored field of study to modify birthweight relates to manipulation of the form, e.g., particle size, and type, e.g., pellet versus meal, the use of feed additives, of the diet fed to sows. The impacts of increasing particle size on grower pigs were recently summarised by Kiarie and Mills (2019). These included a slower gastric passage rate, decreased pH in the stomach, and increased flow of undigested starch into the hindgut promoting short-chain fatty acid (SCFA) production. Butyrate is preferred energy source for colonocytes, and so increasing the particle size has been suggested as a means of improving gastrointestinal health in weaner pigs with the transition away from antibiotic use. However, it may also have a role in improving pregnancy outcomes in sows.

The links between reproductive health and butyrate in livestock are only recently being reported. In beef cattle, the modulation of butyrate production has been suggested as a means of reducing the incidence of retained placenta (Boro et al, 2014). When fed to sows, sodium butyrate increased colostrum quality and litter performance, and reduced the wean to service interval (Chen et al, 2019). Whilst reported benefits around parturition are becoming more frequent, there is little information on the impacts of butyrate production during gestation in livestock. However, in rats, dietary supplementation with sodium butyrate increased embryonic implantation number, but perhaps more interestingly, improved the spacing of the embryos along the uterus (Ye et al, 2019). With implantation spacing being a critical determinant for foetal growth, this strategy shows great promise to reduce the incidence of low birth weight piglets.

The aim of this experiment was to determine the effect of increased particle size in gestating sow diets on litter birth weight characteristics. We hypothesised that when fed during gestation, a coarser milled diet would increase hindgut fermentation in the sow resulting in greater butyrate production, and reduced incidence of low birth weight piglets.

2. Methodology

Animals and management

These experiments were approved by the PIRSA Animal Ethics Committee #11-19, #23-20 and #10-21, and conducted on a 7,500 sow commercial breeder unit in South Australia. All sows (Camborough 42, PIC AU, Grong Grong NSW) were mated at first standing heat after weaning using post-cervical insemination twice, 24 h apart, and then relocated to a naturally ventilated gestation shed. This gestation shed contained 12 identical pens (maximum 55 sows per pen), on partially slatted, concrete flooring with three lying bays. Two experimental diets could be fed in each pen using electronic sow feeders (ESF), at a daily allowance of 2.2 kg from day 1 until d 110 of gestation. Treatment pairs were rotated across pens so that all possible combinations were represented. Sows were allocated to each pen using previous performance (litter size farrowed and weaned) and current parity. Approximately 5 days prior to estimated parturition, sows were relocated into naturally ventilated farrowing sheds, with each shed containing between 200 and 240 farrowing crates (1.8m x 2.4m; Stockyard Industries, Bendigo VIC) on plastic slatted flooring and heated via a lamp. Once housed in a farrowing crate, all experimental sows were fed a standard transition diet (Wijesiriwardana *et al*. 2021) at an allowance of 2 kg twice daily to farrowing.

Treatment and measurements

Pilot Experiment: We hypothesised that the incidence of low birth weight piglets would be reduced when a Coarse diet containing a higher proportion of larger particles was fed to sows from mating until farrowing compared to those fed a Control diet.

To determine the influence of disk mill setting on particle size, sieve analyses were conducted post grind on test samples milled at 2 mm and 3 mm settings (Figure 1). Increasing the disk mill setting from 2 mm to 3 mm increased particles > 1 mm in the feed from 30.6% to 42.6%, largely driven by an increase in particles > 2.5 mm. An image of the two test samples is presented in Figure 2.

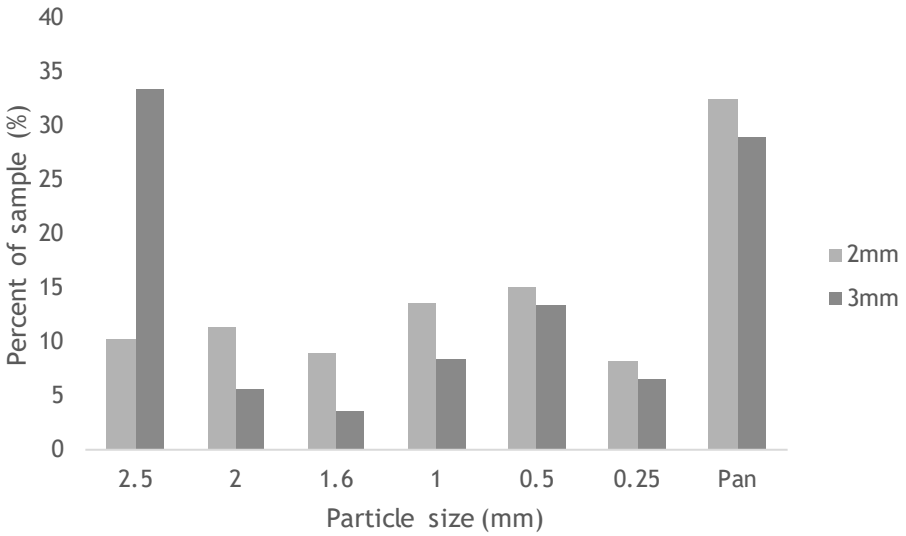

Figure 1. Percent of sample at varying particle size for gestation diets milled using a 2 mm versus 3 mm disk setting.

Figure 2. Feed samples from the two diets with the Coarse treatment (40% particles > 1 mm) on the left and Control treatment (30% particles > 1mm) on the right-hand side.

Subsequently, in spring of 2020, a total of 324 sows (parity 4.1 ± 0.05) were allocated to one of two treatments:

- Control (n = 164): fed a standard gestation diet (13.0 MJ DE/kg, 0.52 SID Lys g/kg) with 30% particles > 1mm and average micron size of 980 D_{gw}.
- Coarse (n = 160): fed a standard gestation diet (13.0 MJ DE/kg, 0.52 SID Lys g/kg) with 40% particles > 1mm and average micron size of 1530 D_{gw}.

Outside the change in particle size, the diets were identical (Table 1).

Table 1. Raw ingredients for the gestation diet fed to sows from d 1 to d 110 in the Pilot Experiment.

Ingredient	%
Barley	67.5
Wheat	14.2
Millrun	8.6
Meat meal	2.2
Vegetable oil	1.3
Limestone	1.3
Monocalcium phosphate	0.5
Betaine	0.4
Breeder Premix	4.0

Sows were weighed and P2 backfat measured at day 1, 40, 80 and 110 of gestation. At between 28-35 days of gestation, sows were scanned for pregnancy using ultrasonography, with non-pregnant animals removed from the experiment. At farrowing, performance data were recorded (total pigs born, pigs born alive, pigs stillborn, mummies, assistance, pre-foster mortality) and individual piglet birth weights (including live and dead animals) of a subset of sows obtained (n = 81 Coarse, and 76 Control litters).

Experiment 1: We hypothesised that the increased particle size in the Coarse treatment resulted in higher butyrate production in the hindgut was transferred into the peripheral blood system and altered foetal growth. We also hypothesised that the addition of a coated butyrate product to the Control treatment would achieve similar results in faecal and blood analyses and animal performance to the Coarse treatment.

In summer 2021, after 330 newly mated sows were allocated to treatment, sieve analyses identified that the experimental diets were inconsistent with the pilot experiment. Both the Coarse and Control diets had upwards of 60% of particles > 1 mm. As a result, the experiment was terminated until the issue could be identified and rectified. After replacing old, worn parts at the mill, a sieve test was conducted once more with the Control diets achieving 35% particles > 1 mm, and Coarse diets 65% > 1 mm (Figure 3).

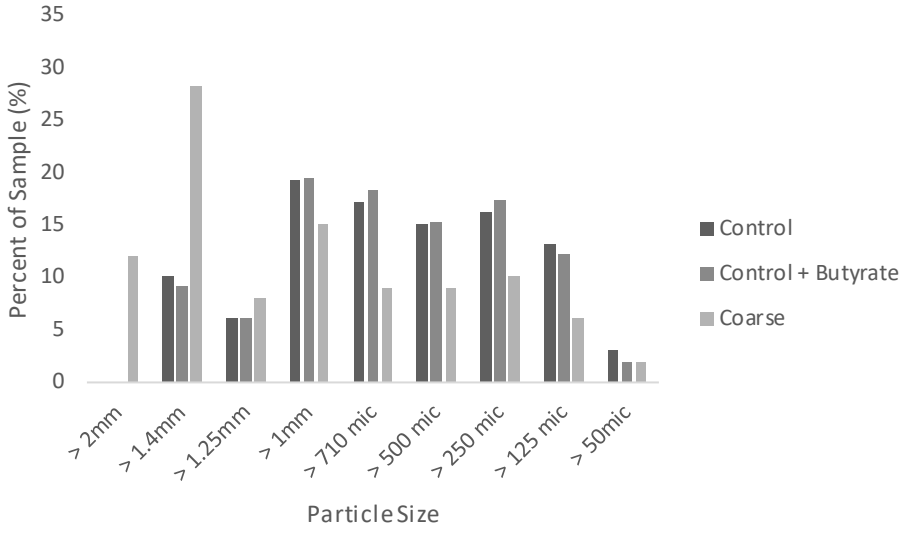

Figure 3. Average percent of sample at varying particle size for gestation diets for Experiment 1 milled using a 2 mm versus 3 mm disk setting.

In autumn 2022, a total of 607 freshly mated sows (parity 3.1 ± 0.05) were moved into a gestation house and allocated to one of three treatments:

- Control: fed standard gestation diet (13.1 MJ DE/kg, 0.55 SID Lys g/kg) with 30% particles > 1 mm and 579 D_{gw} micron (n = 197).
- Coarse: current feed formulation (13.1 MJ DE/kg, 0.55 SID Lys g/kg) with 65% particles > 1 mm 65% and 1032 D_{gw} micron (n = 209).

- Control + butyrate: current feed formulation (13.1 MJ DE/kg, 0.55 SID Lys g/kg) + exogenous butyrate source (Tributyrin 0.2% inclusion; ButyPearl Kemin, Killara NSW) with 30% particles > 1 mm and 576 D_{gw} micron (n = 201).

Diets were identical in formulation, with the only adjustment being the change in particle size and the 0.2% butyrate inclusion for the third treatment (Table 2).

Table 2. Raw ingredients for the gestation diet fed to sows from d 1 to d 110 in Experiment 1.

Ingredient	%
Barley	73.3
Wheat	15.2
Canola meal	4.0
Meat meal	4.0
Poultry tallow	1.0
Limestone	0.9
Monocalcium phosphate	0.3
Betaine	0.2
Lysine HCl	0.2
Salt	0.2
Sodium Bicarbonate	0.5
Breeder Premix	0.2

Two months after the experiment commenced, Japanese Encephalitis Virus (JEV) was confirmed at the experimental farm. Given already experienced delays, a decision was made to continue with the experiment. Sows were scanned for pregnancy using ultrasonography, with non-pregnant animals removed. Sow body condition was monitored on a subset of sows with body weight and P2 backfat measured on day 1, 60 and 110 of gestation (n= 81, 64, 56 per treatment, respectively). At farrowing, performance data were recorded (total pigs born, pigs born alive, pigs stillborn, mummies, assistance, pre-foster mortality) and individual piglet birth weights (including live and dead animals) of a subset of sows obtained (n=80 Control, n=98 Coarse, and n=88 Control + butyrate litters).

On days 30 and 90, blood and faecal samples were taken from a subset of sows (n=29, 29, 17 per treatment, respectively) for progesterone (blood only) and SCFA concentrations (blood and faeces). Blood was spun at 3,000 *g* for 10 minutes, with both resultant plasma/serum and faecal samples stored at -20°C until analyses. Only sows that had successful faecal and serum sampling on d 30 and 90 were sent for analysis.

Plasma progesterone analyses were conducted by The University of Adelaide's Research Assay Facility, Robinson Research Institute, using a radioimmunoassay kit

(#IM1188, Beckman Coulter/Immunotch, Prague CZR). Lowest limit of detection was 0.11 ng/mL, with mean intra-assay CV 5.5%, and inter-assay CV 4.0%.

Serum samples were analysed for SCFA by the Australian Wine Research Institute. The quantification of acetate, propionate and butyrate was performed using a derivatization technique with 3-Nitrophenylidreazine (3-NPH) and analysed by LC-MS/MS ESI (-ve) ionisation. A 50 µL aliquot of sample was added to 400 µL of extraction solvent (50-50 acetonitrile/Milli-Q water). A 5 µL aliquot of 4-methyl valeric acid was added to the mixture and vortexed for 10 seconds. The samples were placed in the freezer at -20 °C for 30 minutes to allow for protein precipitation. Samples were centrifuged at 4°C at 14,000 rpm for 10 minutes. The supernatant was stored at 0°C until derivatisation.

Carbon labelled 13C acetate, 13C propionate and 13C butyrate were added to extracts as internal standards (IS) to compensate for matrix effects and instrument variation across the analytical batch. Calibration curves were acquired in the range 0.1 – 1000 µM. Calibrants were prepared in 50% acetonitrile/Milli-Q water. At least 7 calibration points were used for each analyte. Plasma samples were spiked with known concentrations of acetate, propionate and butyrate to assess the recovery of the target analytes. A standard mix of acetate, propionate and butyrate at two concentration levels of 100 µM and 250 µM was added to plasma samples showing a %CV (Coefficient of Variation) below 20%. The recovery range was 93% - 119% across all analytes. Samples were maintained at approx. 0°C throughout the derivatisation process to minimise the evaporation of the analytes.

The following steps were performed for derivatisation of samples and calibrants:

1. The 3-NPH (20 uL, 200 mM) was added to N-(3-dimethylaminopropyl)-N' -ethyl carbodiimide (EDC) HCl (20 µL, 120mM);
2. The sample/calibrant (40 µL) was directly added to the derivatising agent and vortexed;
3. The sample/calibrant was heated for 30 min at 40°C;
4. Quinic acid (20 µL, 200 mM) was added to the mixture;
5. The sample/calibrant was heated for 30 min at 40°C;
6. The 13C-NPH labelled SCFA mixed standard (20uL, 100 µM acetate, 50 µM propionate and 25 µM butyrate) was added to the mixture;
7. An aliquot of 1.98 mL of 10% acetonitrile in MilliQ-water was added and the mixture was vortexed;
8. The samples and calibrants were analysed by LC-MS/MS ESI (-ve) mode. The separation of sample constituents was performed using Agilent 1290 Infinity liquid chromatography system coupled with AB Sciex 4500 QQQ (LCMS). Sample was acquired in Multiple Reaction Monitoring (MRM) in negative ionization mode. Data processing was carried out using SCIEX OS (1.7.0.36606) software.

Faecal samples were sent for SCFA and lactate analyses to the Wagga Wagga Agricultural Institute at the NSW Department of Primary Industries. A sub-sample of faeces was mixed (1:4) with a weak acid solution (1% ortho-phosphoric acid + 1% formic acid) containing 184 ppm 4-methylvaleric internal standard. Following vortexing, samples were centrifuged at 10,000 x *g* for 20 mins and the supernatant transferred to a glass gas chromatography (GC) vial for analysis. The SCFA

concentrations were determined by capillary GC using an Agilent 6890 gas chromatograph with an autosampler and autoinjector as described previously (Packer et al., 2011). The method employed a wide bore capillary column (BP21, 12 m x 0.53 mm internal diameter (i.d.), 0.5 um film thickness, SGE International, Ringwood Victoria, P/N 054473) with a retention gap kit (2 m x 0.53 mm i.d. guard column, SGE International, P/N RGK2).

For GC analysis, the carrier gas was helium with a total flow rate of 48.0 mL min^{-1}, a split ratio of 5:1 and a column flow of 7.84 mL min^{-1}. The inlet temperature was 155° C, inlet pressure was 19 kPa and injection volume was 1 µL. The oven temperature was set at 80° C for 2 min, increased 6° C per min to 122° C, increased 12° C per min to 144° C, increased 40° C per min to 180°C, held for 2 min and then increased 40° C per min up to 220° C and maintained to give a total run time of approximately 20 min. The flame ionisation detector temperature was set at 200° C with the following gas flow rates; hydrogen = 35 mL min^{-1}, instrument air = 350 mL min^{-1} and nitrogen make-up gas = 25 mL min^{-1}.

Sample SCFA peaks were identified by comparing their retention times with those of a standard mixture of genuine SCFAs (Sigma Aldrich) and quantified using Chemstations Version C.01 and Microsoft Excel using 4-methlyvaleric acid as the internal standard. All results were calculated as ppm and converted to mmol kg $faeces^{-1}$ for subsequent analyses.

The D- and L-lactic acid concentrations were determined by enzymatic analysis (Megazyme K-DLATE) using a microplate spectrophotometer. Prior to analysis, 1,000 uL of faeces:acid solution was added to a 1.5 mL Eppendorf tube and 200 uL of a colour removing reagent (polyvinylpolypyrrolidone, PVPP) was added. Following mixing and centrifugation at 10,000 x *g* for 5 mins, a 40 uL sub-sample was added to a 96 well microplate and mixed with 150 uL of the Megazyme enzyme solution and read on a microplate reader at 340 nm. The concentration (mmol/L) was determined by reference to a 6-point standard curve using purified D- or L-lactic acid.

Experiment 2: We hypothesised that in the absence of Japanese Encephalitis Virus, and with a greater sample size, the observed increase in SCFA production in sows fed a Coarse diet would have a reduced incidence of low birth weight piglets. We also hypothesised that the addition of a mixed non-starch polysaccharide enzyme would further enhance in-utero *piglet growth and be reflected in higher birthweights*

In spring 2022, 598 newly mated sows (parity 3.0 ± 0.05) were moved into the gestation house and allocated to one of four treatments:

- Control: fed standard gestation diet (13.0 MJ DE/kg, 0.55 SID Lys g/kg) with 40% particles > 1mm and 585 D_{gw} micron (n = 151).
- Control + enzyme: fed Control gestation diet + 0.2 kg/t mixed enzyme (xylanase, beta-glucanase, cellulase and xyloglucanase; Ronozome MultiGrain, DSM Nutritional Products, Wagga Wagga NSW) with 40% particles > 1mm (n = 135).

- Coarse: standard gestation diet (13.0 MJ DE/kg, 0.55 SID Lys g/kg) with 65% particles > 1mm and 954 D_{gw} micron (n = 173).
- Coarse + enzyme: fed Coarse gestation diet + 0.2 kg/t mixed enzyme (xylanase, beta-glucanase, cellulase and xyloglucanase; Ronozome MultiGrain, DSM Nutritional Products, Wagga Wagga NSW) with 65% particles > 1mm (n = 139).

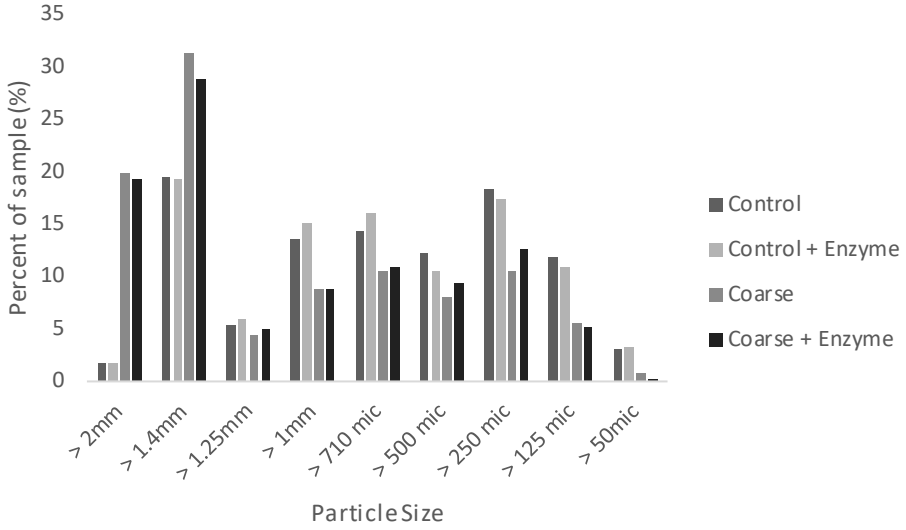

Figure 4. Average percent of sample at varying particle size for gestation diets for Experiment 2 milled using a 2 mm versus 3 mm disk setting.

Diets were identical in formulation, with the only adjustment being the change in particle size (Figure 4) and the 0.2% enzyme inclusion for the second and fourth treatments (Table 3).

Table 3. Raw ingredients for the gestation diet fed to sows from d 1 to d 110 in Experiment 2.

Ingredient	%
Barley	64.0
Wheat	15.0
Millrun	9.5
Field peas	6.8
Canola meal	5.1
Meat meal	3.3
Poultry tallow	1.0
Limestone	0.9
Monocalcium phosphate	0.2
Betaine	0.4
Lysine HCl	0.2
Salt	0.2
Sodium Bicarbonate	0.4
Breeder Premix	0.2

All sows were scanned for pregnancy confirmation at d28-35 of gestation, with non-pregnant animals removed from the experiment. Sow body condition was monitored with caliper and P2 backfat measurements, and scratch score allocated (0 = none, 1 = <5 scratches , 2 = >5 scratches) at day 1, 60 and 110 of gestation. Due to the low occurrence of scratches on the experimental animals, the score was converted to a binary trait (0= absent, 1= present). At farrowing, farrowing performance data was recorded (total pigs born, pigs born alive, pigs born dead. A subset of litters was weighed at birth for individual piglet weights (n = 101 Control, 93 = Control + enzyme, 122 = Coarse, 85 = Coarse + enzyme).

Statistical analyses

All data were analysed in SPSS (IBM, Armonk NY USA), with $P < 0.05$ deemed significant and $P < 0.10$ a trend. Continuous data (sow and piglet weights, sow P2 and caliper) were analysed using a linear mixed model, count data (parity, wean to service interval, piglet numbers) using negative binomial regression, and binary data (pregnancy and farrowing rate, incidence of sows with scratches) using logistic regression. The models included mating week as a random term, and parity and treatment as fixed effects. In Experiment 2, the 2x2 factorial design meant that particle size (Control and Coarse) and enzyme (- and +), and the interaction between these two factors, were used to examine treatment effects. Blood and faecal samples were checked for normality using Shapiro-Wilk test and transformed accordingly. Where relevant, each transformation is specified in the 'Outcomes' section, with back transformed-means presented in parentheses.

3. Outcomes

Pilot Experiment

There was no impact of treatment on sow weight or P2 backfat depth on any measurement day (Table 4). Whilst there was no difference in wean to service interval, sows from the Coarse treatment exhibited a 4.1% improvement in conception rate, but significance could not be maintained to farrowing. Sows from the Coarse treatment exhibited a reduced incidence in number of pigs born dead.

Table 4. Body condition and performance of sows fed a Control or Coarse milled gestation diet from d 1 to d 110.

		Control		Coarse		
		Mean	SEM	Mean	SEM	P value
n		164		160		
Parity		4.2	0.06	4.2	0.06	0.81
Sow condition						
Weight (kg)						
	D1	210.6	2.28	208.9	2.28	0.55
	D40	221.0	2.29	222.5	2.33	0.62
	D80	241.0	2.07	242.2	2.02	0.65
	D110	247.3	2.01	248.8	1.96	0.57
P2 fat (mm)						
	D1	18.3	0.22	18.3	0.22	0.99
	D40	19.9	0.22	19.6	0.22	0.38
	D80	20.2	0.25	20.1	0.24	0.57
	D110	20.5	0.20	20.5	0.19	0.97
Sow performance						
Wean to service interval (days)		9.2	0.73	9.5	0.74	0.78
Pregnancy rate (%)*		86	83-88	90	88-92	0.028
Farrowing rate (%)*		81	66-91	88	74-95	0.21
Total pigs born		13.7	0.28	13.6	0.26	0.77
Pigs born alive		12.6	0.26	12.9	0.25	0.47
Pigs born dead		1.0	0.13	0.7	0.10	0.031

*95% confidence intervals rather than SEM presented for binary data.

There was no significant difference in total litter weight, average piglet weight, or number of low birth weight piglets (Table 5). There was a tendency for pre-foster piglet mortality to be reduced by 0.3 pigs per litter in Coarse compared with Control sows.

Table 5. Litter weight characteristics and incidence of pre-foster piglet mortality of sows fed a Control or Coarse milled gestation diet from d 1 to d 110.

	Control		Coarse		
	Mean	SEM	Mean	SEM	P value
n	81		76		
Total litter weight (kg)	18.1	0.46	17.6	0.46	0.41
Average piglet weight (kg)	1.4	0.03	1.3	0.03	0.52
Minimum weight (kg)	0.9	0.04	0.9	0.04	0.86
Maximum weight (kg)	1.8	0.03	1.9	0.03	0.40
STDEV weight	0.3	0.01	0.3	0.01	0.16
CV weights (%)	20.7	0.01	21.4	0.01	0.50
Pigs < 1.1 kg	3.6	0.42	2.9	0.36	0.15
Pre-foster piglet mortality	0.7	0.13	0.4	0.10	0.065

Experiment 1

Short chain fatty acid concentration of faeces and serum are presented in Table 6. On d 30 of gestation, faecal acetic, propionic and butyric acid concentrations were all highest in the Coarse treatment, but there were no significant differences observed in the serum. On d 90, there was no significant difference in acetic acid, but again propionic and butyric acid concentrations were highest in the Coarse treatment in the faecal samples. There was a trend for the Coarse treatment to have the highest serum butyric acid concentrations in late gestation.

There was no impact of treatment on plasma progesterone concentration, but it did rise with stage of gestation (d 30 7.4 ± 0.7 ng/mL versus d 90 10.9 ± 0.8 ng/mL; $P < 0.05$).

Sows from the Control + butyrate treatment weighed less at d1, d60 and d110, but they were not different in P2 backfat (Table 7). There was no effect of treatment on any of the sow performance measures or litter weight characteristics (Table 8).

Table 6. Short chain fatty acid and D- and L-lactate concentrations in faeces and serum on d 30 and d 90 of gestation from sows fed the experimental diets (Control, Coarse and Control + butyrate), and where transformations have occurred, back-transformed means are presented in parentheses (Experiment 1).

	Faeces (nM/kg)							Serum (uM)						
	Control		Coarse		Control + butyrate			Control		Coarse		Control + butyrate		
	Mean	SEM	Mean	SEM	Mean	SEM	P value	Mean	SEM	Mean	SEM	Mean	SEM	P value
n	16		19		12			16		19		12		
d30														
Acetic acid	78.7[a]	6.61	95.9[b]	5.68	26.4[c]	7.51	<0.001	37.9	5.20	50.4	4.47	43.4	5.90	0.19
Sqrt propionic acid	5.1[a]	0.35	6.2[b]	0.31	1.9[c]	0.41	<0.001	2.2	0.37	2.1	0.32	2.1	0.42	0.60
	(25.3)		(38.3)		(3.8)			(4.7)		(4.5)		(4.4)		
Sqrt butyric acid	2.96[a]	0.248	4.1[b]	0.21	1.1[c]	0.28	<0.001	4.4	0.30	4.3	0.26	4.3	0.35	0.94
	(8.8)		(16.7)		(1.2)			(19.5)		(18.3)		(18.2)		
D-lactate														
L-lactate	Too many samples below limit of detection													
Total lactate														
d90														
Acetic acid	82.5	5.10	80.9	4.41	87.9	7.42	0.22	76.2	10.56	82.2	9.13	66.7	15.37	0.67
Propionic acid	27.7[a]	2.71	37.5[b]	2.34	35.3[ab]	3.94	0.039	Too many samples below limit of detection						
Butyric acid	10.6[a]	1.71	17.4[b]	1.48	16.5[b]	2.49	0.020	15.4	1.41	19.7	1.22	17.2	2.05	0.082
D-lactate	0.4[a]	0.07	0.5[a]	0.07	0.8[b]	0.09	0.003							
L-lactate	0.4[a]	0.10	0.7[ab]	0.10	0.9[b]	0.13	0.021							
Total lactate	0.6[a]	0.11	0.9[ab]	0.11	1.2[b]	0.13	0.002							

Table 7. Body condition and performance of sows fed a Control, Coarse or Control + butyrate milled gestation diet from d 1 to d 110 (Experiment 1).

		Control		Coarse		Control + butyrate		
		Mean	SEM	Mean	SEM	Mean	SEM	P value
n		197		209		201		
Parity		3.1	0.05	3.0	0.05	3.1	0.06	0.33
Sow condition								
Weight (kg)								
	d1	214.9[a]	2.79	218.1[a]	2.92	202.8[b]	3.69	0.004
	d60	234.3[a]	2.76	237.2[a]	2.69	224.9[b]	3.39	0.016
	d110	244[a]	3.04	257.1[b]	3.47	239.7[a]	3.40	0.020
P2 fat (mm)								
	d1	18.5	0.12	18.5	0.13	18.3	0.16	0.41
	d60	18.9	0.11	19	0.11	19.03	0.14	0.70
	d110	19.6	0.20	19.8	0.23	19.5	0.22	0.54
Sow performance								
Wean to service interval (d)		8.5	0.96	8.5	00.96	9.7	1.19	0.39
Pregnancy rate (%)*		84	72-91	82	71-90	83	70-91	0.95
Farrowing rate (%)*		75	58-87	76	60-88	67	48-82	0.19
Total pigs born		13.2	0.33	13.5	0.34	13.2	0.45	0.76
Pigs born alive		11.9	0.31	12.2	0.33	12.1	0.43	0.76
Pigs born dead		1.3	0.15	1.3	0.16	1.4	0.20	0.95

*95% confidence interval rather than SEM presented for binary data.

Table 8. Litter weight characteristics and incidence of pre-foster piglet mortality of sows fed a Control, Coarse or Control + butyrate gestation diet from d 1 to d 110 (Experiment 1).

	Control		Coarse		Control + butyrate		
	Mean	SEM	Mean	SEM	Mean	SEM	P value
n	197		209		201		
Total litter weight (kg)	16.6	0.73	16.4	0.68	16.8	0.78	0.85
Average piglet weight (kg)	1.4	0.04	1.4	0.04	1.4	0.04	0.76
Minimum weight (kg)	0.9	0.05	0.9	0.05	0.9	0.07	0.99
Maximum weight (kg)	1.9	0.04	1.8	0.04	1.8	0.05	0.51
STDEV weight	0.3	0.01	0.3	0.01	0.3	0.02	0.26
CV weight (%)	20.6	0.01	19.4	0.01	19.1	0.01	0.34
Pigs < 1.1 kg	3.0	0.34	3.0	0.33	2.6	0.40	0.78
Pre foster piglet mortality	1.1	0.1	1.3	0.1	1.2	0.2	0.56
Piglets alive at weighing (birth)	10.9	0.4	11.1	0.3	11.3	0.4	0.71

Experiment 2

There were no significant interactive effects between the two treatments for any measurements collected and so only main effects are presented. Whilst no difference in caliper measurement was identified on d 1 of gestation, sows from the Coarse treatment recorded a higher caliper at d 60 of gestation and this tended to be higher at d 110 of gestation than the Control treatment (Table 9). No significant difference between Control and Coarse treatments was identified for P2 backfat. There was no impact of the enzyme treatment on sow body condition measured using the caliper, but + Enzyme sows recorded a higher P2 backfat at d 1 and d 110 but without any difference on d 60 of gestation. There was no significant impact of either treatment on the incidence of sows with scratches at any stage of gestation.

There was no significant effect of particle size or enzyme inclusion on pregnancy or farrowing rate. Sows from the + Enzyme treatment had higher total pigs born and pigs born alive than those from the - Enzyme treatment (1.1 pigs and 0.8 pigs respectively). There was no significant impact of the Coarse treatment on litter size traits.

Table 9. Body condition, incidence of sows with scratches and performance of sows fed a Control versus Coarse, or – Enzyme versus + Enzyme gestation diet from d 1 to d 110 (Experiment 2).

	Control		Coarse		- Enzyme		+ Enzyme		P value		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Grind	Enzyme	Grind x Enzyme
n	286		312		274		324				
Parity	3.1	0.05	3.0	0.05	3.1	0.06	3.0	0.05	0.78	0.28	0.68
Sow condition											
Caliper units											
d1	12.9	0.18	12.9	0.17	12.8	0.16	13	0.19	0.97	0.29	0.6
d60	13.5	0.14	13.8	0.13	13.6	0.12	13.6	0.15	0.03	0.96	0.46
d110	14.0	0.14	14.2	0.14	14.1	0.13	14.1	0.15	0.055	0.47	0.15
P2 fat (mm)											
d1	13.8	0.27	13.6	0.25	13.3	0.24	14.1	0.28	0.39	0.006	0.11
d60	15.2	0.27	15	0.26	14.9	0.24	15.3	0.29	0.65	0.13	0.53
d110	15.9	0.25	16	0.24	15.6	0.22	16.4	0.27	0.56	0.002	0.48
Scratch score > 0 (%)*											
d1	19	12-29	20	13-30	16	10-26	23	15-34	0.72	0.17	0.26
d60	7	4-10	7	5-10	8	6-12	6	4-9	0.89	0.14	0.28
d110	11	5-23	14	7-28	10	5-22	15	7-29	0.47	0.31	0.42
Sow performance											
Wean to service interval (d)	8.4	0.66	7.6	0.54	8.2	0.57	7.7	0.6	0.19	0.49	0.7
Pregnancy rate (%)*	93	85-97	90	82-95	93	86-97	90	81-95	0.4	0.37	0.9
Farrowing rate (%)*	87	79-92	85	78-91	85	78-91	87	79-92	0.66	0.7	0.67
Total pigs born	13.5	0.37	13.6	0.36	13	0.33	14.1	0.41	0.83	0.004	0.59
Pigs born alive	12.5	0.33	12.4	0.35	12.1	0.31	12.9	0.38	0.72	0.013	0.45
Pigs born dead	0.8	0.15	1	0.16	0.8	0.13	1	0.18	0.33	0.16	0.67

*95% confidence interval rather than SEM presented for binary data.

Litter weight characteristics are presented in Table 10. Total litter weight tended to be higher, and coefficient of variation lower, in sows fed a Coarse compared to a Control diet. The number of piglets born under 1.1 kg was reduced by 0.5 pigs per litter in the Coarse sows. There was no significant impact of enzyme treatment on weight characteristics (as total pigs born was included in the model as a covariate). There was a significant interaction between grind and enzyme treatments for pre-foster mortality, with no difference between Control and Coarse sows with – Enzyme (0.8 ± 0.1 versus 0.6 ± 0.1 pigs per litter), but in + Enzyme, Control sows exhibited fewer ($P < 0.05$) piglet deaths prior to fostering (0.6 ± 0.1 versus 0.9 ± 0.1 pigs per litter).

Table 10. Litter weight characteristics and incidence of pre foster piglet mortality of sows fed a Control versus Coarse, or – Enzyme versus + Enzyme gestation diet from d 1 to d 110 (Experiment 2).

	Control		Coarse		- Enzyme		+ Enzyme		P value		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Grind	Enzyme	Grind x Enzyme
n	194		207		178		223				
Total litter weight (kg)	17.7	0.27	18.1	0.26	17.9	0.25	18.0	0.29	0.083	0.75	0.45
Minimum weight (kg)	0.8	0.03	0.8	0.03	0.8	0.03	0.8	0.03	0.28	0.63	0.47
Maximum weight (kg)	1.8	0.03	1.8	0.03	1.8	0.03	1.8	0.02	0.45	0.42	0.17
Average piglet weight (kg)	1.3	0.02	1.4	0.02	1.4	0.02	1.4	0.02	0.13	0.58	0.37
STDEV weight	0.3	0.01	0.3	0.01	0.3	0.01	0.3	0.01	0.32	0.62	0.44
CV weight (%)	22.6	0.01	21.6	0.01	22.1	0.01	22.1	0.01	0.09	0.90	0.61
Pigs < 1.1 kg	3.2	0.27	2.7	0.23	2.8	0.22	3.0	0.28	0.032	0.38	0.36
Pre foster piglet mortality	0.7	0.1	0.7	0.1	0.7	0.1	0.7	0.1	0.75	0.57	0.041
Pigs alive at weighing	12.1	0.16	12.0	0.16	12.0	0.15	12.1	0.17	0.52	0.84	0.62

4. Application of Research

The aim of this project was to determine whether changing the particle size of the gestation sow diet could alter piglet birth weight characteristics as a result of altered foetal growth. Any impact seen potentially due to a change in bacterial population, fermentation patterns and so SCFA production in the large intestine, which entered the circulation as a potential energy source for the developing piglets. We were able to demonstrate in two separate experiments that sows fed a Coarse gestation diet had 1; elevated SCFA in both faeces and serum, and 2; a reduced variation in piglet birth weight, and frequency of low birth weight piglets.

Grain is ground into smaller particle sizes for monogastrics to improve feed utilization by allowing endogenous digestive enzymes greater access to specific surfaces for energy release. Because of this, there was some concern in feeding gestating sows a diet with increased particle size, and the potential consequence this would have on body condition, especially in late pregnancy. In fact, in both experiments there was evidence contrary to this, with Coarse sows not dissimilar (P2 backfat), or even showing improved condition (weight or caliper), compared to those from the Control group. This is likely due to the fact that sows have a higher hindgut digestion and energy production than younger pigs (Noblet and Shi 1993). Using corn particle sizes between 400 – 1000 um, Ma *et al*. (2021) found no changes in nutrient digestibility, which although not measured in this project, is in line with the current sow body condition results. These authors emphasised that there was a paucity of information on the impacts of particle size in gestating sow diets, highlighting the importance of the current project.

An increase in particle size slows gastric emptying time (Kiarie and Mills 2019), which may have positive implications for sow welfare. Standard commercial feeding practices restrict the sow to below half of the *ad libitum* intake which results in increased restlessness and stereotypies (Read *et al*. 2020). Whilst 'bulking' the diet with higher fibre levels is one option to reduce hunger (Danielsen and Vestergaard 2001), the increased volume of feed acts to increase milling, freight and storage costs, as well as effluent management. To determine whether the larger particle sizes in the Coarse diets increased satiety and so reduced aggression in the ESF system imposed in the current experiment, injury scores were collected throughout gestation. The overall incidence of injuries was low, which speaks to the fact that the experimental site was experienced in the management of group housed sows, and is why scratches were converted to a binary trait (were scratches present or not). This project was designed to address the influence of particle size on birth weights characteristics, and so in all experiments, pens housed both small and larger particle size treatments. There was no treatment difference in the level of injuries quantified on the sows, but this is likely because both Control and Coarse sows were housed within the same pen. Thus, any potential hostile behaviour exhibited by sows of one treatment group was likely to impact the injury scores of both treatment groups. Future work should apply the same treatments but on a pen basis to see if there are any satiety, behavioural and consequently welfare benefits of the Coarse diets applied.

During Experiment 1, the research farm was diagnosed with Japanese Encephalitis Virus (JEV). The virus is spread by mosquitoes, with pigs acting as an amplifying host. Infection leads to a substantial increase in the risk of stillbirth, congenital defects, and postnatal mortalities (Mansfield *et al*. 2017). The data clearly demonstrated the impacts of the disease on sow reproductive performance, with the incidence of piglets born dead rising from between 0.7-1.0 in the pilot experiment and Experiment 2 when active infection was not observed, to over 1.3 pigs per litter in Experiment 1 when infection was confirmed. As JEV impacts foetal development, the lack of treatment effects on piglet birth weight characteristics observed in Experiment 1 is not surprising.

Short chain fatty acid concentrations were elevated in the faecal samples collected from sows fed the Coarse treatment in Experiment 1, in line with the hypothesis, and previous studies conducted in younger animals (Zhao *et al*. 2019). Whilst we demonstrated a tendency for increased circulating butyrate concentrations in late gestation, the exact mechanism by which this increased SCFA production may improve foetal development remains to be confirmed. The roles of SCFA are far reaching and include fuel for colonocytes (Kiarie and Mills 2019), anti-inflammatory actions (Liu 2015), hormone signaling (Jiao *et al*. 2020), and direct energy sources (Schönfeld and Wojtczak 2016), which all could have acted to potentially improve *in utero* piglet growth. Further, more targeted work to better understand the underlying physiological mechanisms is required.

Experiment 1 included a gestating sow diet containing coated butyrate to determine whether this resulted in similar alterations in piglet birth weight to a larger particle size. But from the faecal and plasma analyses, and the fact the JEV infection altered fetal development during this component of the project, no conclusions can be drawn. Previous work in rats has demonstrated that exogenous butyrate, whilst having similar positive effects on pregnancy rate and fetal number, does not result in the same antioxidant or metabolic status (Lin *et al*. 2014). Again, this highlights that further mechanistic studies should be conducted to better explain the improved reproductive performance in the presence of increased SCFA production.

The multi-component enzyme treatment was included in Experiment 2 to determine whether it further enhanced the Coarse treatment by potentially increasing direct energy release, as well as breaking down the grain into smaller fragments for bacterial fermentation almost acting as a prebiotic (Zhe *et al*. 2022), as has been previously reported (Crome *et al*. 2023). Whilst there were few interactive effects between the particle size and enzyme treatments, litter size was significantly increased by the inclusion of the enzyme. This is in contrast to previous work (Acosta *et al*. 2024). The experimental diets in the present study were fed to sows immediately after mating, whereas previous work tested enzyme inclusion a week after mating. This would suggest that the improvement in litter size when exogenous enzymes are included is driven by very early pregnancy energy release. This aligns with the period of implantation and so is a logical conclusion.

5. Conclusions

- Feeding gestating sows with larger grain particle size does not alter sow body condition
- Increased particle size increased SCFA production in the large intestine, as measured in the faeces and tended to increase serum butyrate in late gestation
- In the absence of viral infection, a Coarse diet reduced the incidence of low birth weight piglets having positive impacts on perinatal piglet survival.
- Exogenous enzyme inclusion in gestating sow diets improved litter size.

6. Limitations/Risks

The particle size distribution and eventual mean micron size of the diet differed significantly during this experiment and caused substantial delays when the desired treatment targets were not met. There is evidence of this in the discrepancies between the distribution of sieve results in the Pilot Experiment, Experiment 1 and Experiment 2. General wear and tear of the milling machinery was responsible for this drift, and so should be monitored regularly.

All experimental diets in this project were mash feed. When the particle size of the diets was changed, several alterations to the feeders had to be made to ensure correct delivery to the sows. Firstly, all the mixers in the ESFs were replaced and checked for working order to prevent bridging. Second, the feed setting had to be altered between the particle size treatments. The Coarse treatments flowed through the auger and feedline better than the Control. As the feed system settings were based on auger rotations, this meant that at the same setting of 2.2 kg per sow, sows allocated to the Coarse treatment were receiving more feed than those on the Control. As a result, the feed setting was reduced with the larger particle size treatments to ensure the weight of feed was consistent. Throughout all experiments, the ESF feed delivery systems were calibrated monthly.

During Experiment 1, JEV was confirmed at the research farm. Given that this virus has significant impacts on fetal development, we argue that any reproductive data obtained within this experiment be disregarded.

7. Recommendations

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

- Gestation sow diets should be milled to a grain particle size of ~ 1000 um to optimise piglet birth weight and survival.
- Exogenous enzyme inclusion into gestating sow diets is recommended to improve litter size.

- Further work is required to determine the exact mechanism(s) by which a Coarse diet fed to gestating sows reduced the incidence of low birth weight piglets.
- There may be other benefits to feeding larger grain particle sizes to sows (health and welfare) that were not quantified in this project.

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