Australian Government
Department of Agriculture, Fisheries and Forestry

AUSTRALIAN Pork

Ensuring that healthy and robust piglets all thrive after weaning to improve herd feed conversion efficiency

Final Report
APL Project 2022/0003

June 2026

SunPork Group
Kate Plush, Robert Hewitt, Sally Tritton, Darryl D'Souza
6 Eagleview Place
Eagle Farm, QLD 4009

The University of Melbourne
Jeremy Cottrell, John Pluske, James Langley
Grattan Street
Parkville, VIC 3010

Acknowledgements

This project is supported by funding from Australian Pork Limited, industry levy payers and the Commonwealth.

The experiments in this project form part of the PhD thesis of James Langley, "Investigating the effects of iron injection strategies and dietary protein levels on post-weaning performance in pigs".
https://hdl.handle.net/11343/368921

Executive Summary

Background

Weaning is the most stressful period in a pig's life, combining a change of diet and environment, litter mixing and separation from the sow. Encouraging feed intake immediately after weaning is critical to a successful transition and to herd feed conversion efficiency. This project targeted two common ailments that limit a piglet's ability to grow to its genetic potential: iron-deficiency anaemia and post-weaning diarrhoea (PWD). It was hypothesised that (i) a second iron injection, given at seven days of age or at weaning, would raise haemoglobin (Hb) and growth after weaning, and (ii) a lower crude protein (CP) diet with an increased standardised ileal digestible (SID) lysine-to-CP ratio would maintain growth while reducing PWD.

Approach

Three experiments were conducted. Experiment 1 compared a second iron-dextran injection given either seven days after farrowing or at weaning (~21 days). Experiment 2 tested low-CP diets fed for the first week after weaning on the occurrence of PWD. Experiment 3 combined the best treatments in a 1,600-pig, two-by-two design (iron injection at weaning × diet) to determine which strategy improved herd feed conversion efficiency through to slaughter.

Key Findings

Iron timing (Experiment 1): A second injection at seven days of age raised Hb for ~18 days after weaning but did not increase growth. An injection at weaning did not lift Hb but tended to improve average daily gain (ADG) in the second week ($p < 0.10$), so the weaning injection was carried forward.

Low-protein diets (Experiment 2): A 19% CP diet at an SID Lys:CP ratio of 0.071 significantly reduced PWD and tended to increase first-week ADG ($p < 0.10$). A lower-ratio diet (0.059) reduced PWD most but gave no growth benefit. Both reduced-protein diets almost halved removals, with no measurable inflammation.

Combined trial (Experiment 3): At a high-health, recently depopulated site, a second iron injection at weaning raised Hb at every sampling point and significantly improved ADG in the first two weeks. Here the higher-protein diet grew pigs better than the low-protein diet, the opposite of Experiment 2, reflecting the older, healthier, lower-challenge pigs.

Lifetime performance: Early gains in Hb and growth faded by the end of finishing; pigs given a single injection finished with a slightly heavier carcase, with no difference in backfat or days to slaughter.

Implications & Recommendations

Both strategies improve part of post-weaning health, but their benefit depends strongly on pig age, growth rate and disease challenge; each works best where the post-weaning challenge is greatest. As sows are bred for larger litters and faster growth, anaemia is likely to become more common, so better pen-side detection is needed. Haemoglobin (e.g. HemoCue) alone is insufficient because it lags true iron status and misses iron held outside Hb; agreed porcine reference values for ferritin and other markers should be developed. A second iron injection is best given at weaning and targeted to the largest, fastest-growing pigs most at risk, recognising the benefit is mainly short-term. Low-protein diets remain a useful antibiotic-free tool to reduce PWD and are best deployed in younger, higher-challenge herds. The combination of treatments is an effective tool to improve Herd FCR.

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1. Background to Research

Encouraging and maximising feed intake quickly in the transition period is critical for establishing herd feed conversion (HFC) in the wean-finish period (Pluske et al. 2002). Overseas studies (USA, CA, EU) have identified that iron deficiency at weaning is common (25% of piglets; Perri 2015), and have linked low iron status to inappetence (Knight and Dilger 2018), reduced body weight (Loh et al. 2001) and peri-weaning failure to thrive syndrome (PFTS) (Perri 2015). Domestic studies are lacking, but pilot investigations in QLD and SA (PIC genetics) have reinforced overseas findings, with 58% of piglets at weaning being iron-deficient (<11 g/dL), and 10% classified as anaemic (<9 g/dL). A single iron injection after birth providing 100-200 mg elemental iron is routinely applied, but providing a further injection at weaning can prevent iron deficiency (Chevalier et al. 2021) potentially reversing inappetence in the transition phase. Post-weaning diarrhoea (PWD) continues to impact herds, resulting in poorer feed conversion efficiency (Heo et al. 2008, Heo et al. 2010). Feeding a lower crude protein (CP) diet with essential amino acids, relative to standardised ileal digestible lysine (SID Lys), reduces PWD (Heo et al. 2008, Pluske et al. 2018), and is a strategy to improve gut health, increase intake and improve feed efficiency. However, studies report reduced growth and poorer feed conversion efficiency after weaning when fed a lower CP diet (Wensley et al. 2021), suggesting there is a possible misalignment between the optimum SID Lys: CP ratio to maximise production whilst simultaneously reducing PWD. Reducing the incidence of pigs that fail to thrive during the transition period by ensuring iron status and a healthier gut structure and function after weaning should be a key driver to establish pigs for superior post-weaning performance, then impact positively on FCR and HFC.

Mortality within nursery systems range between 3 and 5%. Anecdotally at least two-thirds of this mortality is attributed to anorexia or inadequate feed intake following weaning (> 65% of mortality at one nursery site investigated is associated with hospital pen euthanasia for failure to thrive). Clearly this anorexia represents animal welfare concerns and increased medication and labour costs but will also significantly worsen FCR during the weaner phase, and with fewer pigs reaching saleable weights, thereby reduced HFC. Peri-weaning failure to thrive syndrome is a condition in which large, healthy weaner pigs become anorexic and by 2-3 weeks post-wean starve or require euthanasia. It severely reduces the well-being of a significant proportion of nursery pigs and impairs performance. Its incidence has only been detailed in the USA and is highly variable between batches, ranging from 1-20% (Perri, 2015). At weaning, these pigs are of average to above average weight but become lethargic and anorexic by seven days after weaning. A distinctive oral behavioural pattern is observed that includes licking, champing and chewing, with affected piglets' having a low-hanging head position. To date, interventions to diagnose or treat the syndrome have been unsuccessful. Maximising feed intake immediately after weaning should logically alleviate this condition, and so this proposal is targeted to this approach. In these series of experiments, we tested a number of interventions aimed at improving health, encouraging feeding immediately after weaning, reducing the number of piglets that fail to thrive in the nursery, to assist in improving herd FCR whilst also reducing antimicrobial use.

2. Objectives of the Research Project

Better understand impacts of lactation factors (i.e. sow parity, litter size, weight and growth etc) on feeding behaviours and failure to thrive.

Determine the effectiveness of a split/second Fe dose at weaning on anaemia, Fe deficiency and cognitive function (feeding behaviour) after weaning.

Demonstrate that gastrointestinal tract health can be improved by feeding an optimised SID Lys: CP diet linked to lower protein diets.

Ultimately, reduce the morbidity and mortality associated with piglets that fail to thrive after weaning, thereby improving FCR and HFC.

3. Introductory Technical Information

Cutting the crude protein (CP) content in weaner diets lowers the severity of PWD, and the effect is strongest during the riskiest period, days 5 to 12 after weaning (Wang et al., 2018). This makes low-protein diets a potentially useful substitute for the high doses of zinc oxide normally used to control PWD (Tang et al., 2024). The trade-off is that cutting protein too far can slow growth, because the pigs no longer receive enough lysine, protein, nitrogen, or other essential amino acids (Aymerich et al., 2022). Low-protein diets curb PWD partly by reshaping the gut microbiome, which lowers ETEC numbers (Miller et al., 2024). The PWD itself is linked to higher systemic inflammation, which slows growth (Hennig-Pauka et al., 2019) and can lead to muscle wasting, ketosis, and even failure-to-thrive syndrome (Perri et al., 2016). Pigs can still grow well on low-protein diets, as long as the diet supplies enough lysine (Lys) and other essential amino acids (Wellock et al., 2006; Millet et al., 2017). The best standardised ileal digestible (SID) Lys:CP ratio for weaned pigs is thought to be 0.064 (or 6.4%) (Millet et al., 2018).

Pig farmers routinely inject newborn piglets with 100 to 200 mg of iron soon after birth, because piglets are born with very little iron of their own (Venn et al., 1947). How much iron a piglet needs while suckling depends mainly on how fast it grows during that time (Venn et al., 1947; Kamphues et al., 1992; Egeli & Framstad, 1998; Van Gorp et al., 2012). Today's pigs come from genetics that produce bigger litters and faster-growing piglets. As a result, recent studies show that one standard injection at birth is no longer enough to keep a piglet's iron topped up all the way through suckling (Bhattarai & Nielsen, 2015a; Perri et al., 2016). The fastest-growing piglets run short of iron first, which can cause problems after weaning (Jolliff & Mahan, 2011).

Chevalier et al. (2021) backed this up. Piglets given 200 mg of iron at birth reached their highest Hb concentration at 17 days of age, then dropped off by weaning at 22 days. Piglets given only 100 mg of iron peaked even earlier, at 11 days, before falling. This fits with earlier work showing that many piglets have low Hb (usually below 11 g/dL) at weaning (Thorn, 2010; Bhattarai & Nielsen, 2015b; Perri et al., 2016). This matters because piglets that wean with healthy Hb tend to grow better afterwards (Bhattarai & Nielsen, 2015b; Fredericks et al., 2018; Chevalier et al., 2023). Olsen (2020) showed the benefit clearly: a second injection 4 to 6 days after the first raised the share of piglets with healthy Hb at weaning from 20% to 89% and added 454 g of body weight by 42 days after weaning. Taken together, this evidence points to a likely benefit from a second iron injection before weaning—but it is still unclear whether every type of farm would see the same result.

4. Research Methodology

4.1 Experiment 1: Determining the best time to administer a second iron injection to pigs

Animals and housing

The experiment was conducted at a commercial research piggery in Queensland, Australia and consisted of 33 pens of 14 pigs per pen, with litters assigned a treatment at piglet processing (day 2 of life). Piglets were weaned at 20.2 ± 0.07 days into the experimental nursery in four replicates, separated by sex, balanced for weight and housed with a spacing allowance of 0.20 m^2/pig on fully slatted flooring. All pigs were fed the same standard starter diet (Table 1) with tested iron levels of 180 mg/kg of feed for the duration of the nursery period. Feed was provided by a circular gravity feeder in the center of each pen, and water was available through one bowl and one nipple drinker in each pen. The standard on-farm medication regime was followed during the nursery phase of the experiment. This comprised a water acidifier (1% Selko, Trouw Nutrition, Amersfoort, Netherlands) for all pigs from entry to day seven after weaning. On days 12-14, a preventative antibiotic was administered via water (Oxytetracycline 25 mg/L). On day 25, piglets received two vaccinations, for prevention of Mycoplasma hyopneumoniae infection (M-Pac, MSD animal health, USA) and Leptospirosis (Eryvac, Zoetis, Australia).

Treatments

Litters were randomly assigned to a treatment group from 141 sows in parities 2 to 6 at piglet processing. On day two, pigs were given an individual ear tag with a unique number used for identification during the experiment. During this time, all pigs received the first iron dextran injection using a 21G needle into the muscle directly behind the ear (1 mL Ferron 200 + B12 that included 40 µg/mL vitamin B12 as cyanocobalamin; Ferron, Elanco, IN, USA). During this time, piglets also had tails docked and were administered an oral coccidiostat (Baycox Coccidiostat, Elanco Australia, Macquarie Park, New South Wales, Australia). At seven days of age, pigs in the day 7 group were given an additional dose of Ferron 200 + B12. At weaning, all pigs were then moved to the nursery facility, where the weaning treatment group was administered the additional Ferron 200 + B12.

Measurements

Nursery performance

Pigs were weighed weekly from entry until four weeks after weaning, with individual data summed to pen growth for each period. The amount of feed offered and residuals were measured every week and assumed to be pen feed intake. Pen growth rate and feed intake data were used to calculate the gain: feed ratio on a pen basis. Any pig requiring medical intervention was recorded (cause and treatment) and removed to a hospital pen (removals). A pig was only removed if it was deemed unable to survive without treatment.

Behavioural analysis

Pigs were recorded using video cameras suspended above pens (Swann 4-channel wireless Wi-Fi NVR package, Melbourne, Australia) from 0700h to 0900h on days four to seven after weaning. Behaviours were recorded against the following ethogram (Table 2) using the scan sampling technique with a sampling interval of 30 seconds. Scan sampling is known to be an ideal way to assess the behaviour, posture and activity levels of pigs, with closer sampling periods providing more accurate data (Kobek-Kjeldager et al., 2024). For every sample, each pig within the pens was scored for both posture and activity.

Table 1 Dietary formulation (%) as fed, composition from formulation and analysed total diet iron content of weaner diet fed in experiment 1.

Raw materials, %	
Wheat	51.6
Biscuit meal	5.00
Soybean meal	2.00
Soybean isolate	4.95
Full-fat soybean	8.00
Blood meal	2.00
Meat meal	2.95
Fish meal	5.00
Whey powder	12.5
Prebiotic	2.00
Probiotic - Bacillus	0.03
Canola oil	1.95
Monodicalcium phosphate	0.25
Salt	0.20
Microencapsulated zinc oxide	0.10
Betaine	0.10
DL-methionine	0.16
Lysine	0.40
L-threonine	0.11
L-tryptophan	0.03
L-isoleucine	0.01
Carbohydrase	0.05
Phytase	0.01
Phytogenic additive	0.02
Organic acid blend	0.40
Flavour	0.03
Vitamin/mineral premix[1]	0.20
Composition	
Digestible energy (DE), MJ/kg	14.9
SID lysine, %	1.34
Crude protein (CP), %	22.6
SID lysine: DE	0.090
SID lysine: CP	0.059
Iron, mg/kg	180

[1]Premix provides per ton of complete diet, Vitamin A 10 miu, D3 2 miu, E 80 g, K 3 g, B1 3 g, B2 4 g, B6 3 g, B12 20 g, Pantothenate 20 g, Niacin 25 g, Biotin 10 g, Folic 0.5 g, Fe 70 g, Zn 100 g, Mn 40 g, Cu 20 g, Se 0.5 g, Cr 0.2 g, Co 0.75 g, I 1.5 g.

Blood collection

On days one and 28 of weaning, four focal pigs per pen were identified and had a blood sample collected via jugular venipuncture using a 21G needle into EDTA vacutainers. The timing of sampling occurred two hours after administration of iron dextran and was standardised across days. Following collection, samples were placed in a centrifuge and spun for ten minutes at 1,000g, with plasma extracted and stored at -20 °C until analyses.

Whole blood measurements

On day 0, 4, 7, 11, 14, 18, 25 and 28 after weaning, the same four pigs were sampled for Hb (HB201+ blood analyser, Haemocue AB, Sweden) and BHB concentrations (Freestyle Optimum Neo Blood Ketone Meter, Abbott Industries, Chicago, United States) from each pen (n=44 per treatment). This was done by collecting a small amount of blood from the ear using a fine needle directly into a cuvette and following the supplier's instructions. The haemocue Hb201+ was validated for use as an accurate point-of-care meter for Hb quantification (Kutter et al., 2012). The BHB meter was validated for use in cattle (Sailer et al., 2018), as BHB is an indicator of ketosis and wasting in pigs in the post-weaning period (Perri et al., 2016)

ELISA analyses

Plasma collected was analysed using ELISA for the concentrations of haptoglobin (ABClonal, Boston, Massachusetts, United States), ferritin (Cloud Clone, Houston, Texas, United States) and HAMP (Cloud Clone). The spike recoveries for haptoglobin, ferritin and HAMP were 104%, 90% and 117% respectively, and the intra-assay variability was 8%, 4% and 6%, respectively.

Statistics

Pig production data were analysed by analysis of variance with treatment as a fixed effect and pen as a blocking factor using GenStat, version 23 (VSNi, Hemel Hempstead, United Kingdom). The pig behavioural data did not meet normality assumptions and were analysed in SPSS (IBM Corp. Released 2023. IBM SPSS Statistics for Windows, Version 29.0.2.0 Armonk, NY: IBM Corp) using negative binomial regression with a repeated measure of day. As not all pens contained the same number of pigs (removals), the number of pigs in each pen was included as a covariate. The model included the fixed terms of day, treatment and the interaction between day and treatment. The ELISA results did not meet normality tests using any transformation. Therefore, analysis of variance was used to generate means, separated using non-parametric Kruskal-Wallis testing with treatment as the fixed effect in SPSS. Statistical significance was accepted with a p-value of < 0.05, and a trend was p-value $<$ 0.10.

4.2 Experiment 2: Investigating the use of low crude protein diets to reduce post-weaning diarrhoea

This experiment comprised 39 pens (13 pens per treatment) of 14 single sex pigs divided into three experimental groups. The experiment was a randomized block design with sex and pen as blocking factors. Pigs were weaned at 27.8 ± 1.64 (Mean ± SD, PIC genetics) days of age into the nursery in four batches. Experimental diets (Table 2) were imposed for the first seven days from weaning. Following this, all pigs were abruptly fed the CON diet for the remainder of the nursery period (three weeks). Feed was provided *ad libitum* through a circular gravity feeder in each pen, and water was provided through one nipple drinker and one bowl drinker located at the back of each pen.

Pens were allocated to one of three diets:
(1) control (CON); 20.6% CP, 1.34% SID Lys, SID Lys: CP ratio 0.065,
(2) low CP: high SID Lys: CP ratio (LH); 19.0 CP, 1.34% SID Lys, SID Lys: CP ratio 0.074,
(3) low CP: low SID Lys: CP ratio (LL); 18.7% CP, 1.1% SID Lys, SID Lys: CP ratio 0.059.

Diets were provided as a hydrated mash for 5 days after weaning to encourage feed intake and as a pellet for the duration of the trial via a penguin feeder in each pen (Rotecna, Lleida, Spain). Diets were analysed using near-infrared spectroscopy analysis (NIR) calibrated for the calculation of protein, moisture and fat in feed (Table 2), wet chemistry was used to determine energy content, and an amino acid panel was to ensure the formulation of SID Lys was achieved in the diets. Additional amino acids were also supplemented in diets to meet the requirements of weaner pigs but were not analysed in an amino acid panel. All diets included 0.1% zinc oxide at below anti-microbial levels (400 ppm) (ZINCO Plus; Jefo, Quebec, Canada). A water acidifier (Selko Amersfoort, Netherlands, 1% in water) was included for all pigs for days one to seven after weaning. After consultation with on-farm veterinarians, a water acidifier was deemed unable to be removed due to the risk of an uncontrollable level of PWD. In the first week after weaning, no preventive antibiotics were administered in feed or the water supply; then a preventative antibiotic (Oxytetracycline, 25 mg/L,) was included in the water supply on days 12-14 to reduce the incidence of any prolonged scouring, as PWD is most commonly seen 5-12 days after weaning significant scouring after this period would likely indicate more significant concerns to animal health and was therefore controlled with the in water antibiotic. Dietary ingredients and nutritional information are shown in Table 2.

Routine husbandry practices were undertaken for the duration of the experiment. Weekly feed and water intake was monitored by recording the weekly feed delivery, refusals and water meter readings. Pigs were weighed individually on days 0, 7, 14, 21 and 28 and monitored daily for any signs of ill health or injury. Any medications administered or removals were recorded as they occurred. Pigs removed were deemed to be unable to continue in the production system and were therefore removed from the experiment and placed in a hospital pen for closer monitoring and further treatment as per standard farm practice.

Four pigs were randomly selected from each pen for blood sampling on days 5, 12 and 28, and the same four pigs were sampled at each time point (n= 52 per treatment). A blood sample was collected via jugular venipuncture into an EDTA vacutainer, labelled, then stored on ice until plasma extraction. Blood samples were immediately chilled after collection and then centrifuged for ten minutes at 1,000g before the plasma was extracted and frozen at -20 °C until analysis. When the blood sample was collected, beta-hydroxybutyrate (BHB) concentrations were recorded using a handheld precision Xtra BHB and glucose meter (Abbott diabetes care, Abingdon, UK) for an indicator of ketosis that has previously been validated for use in pigs (Perri, O'Sullivan et al. 2016).

Table 2 Dietary formulation (%) as fed, composition from formulation of treatment diets fed for the first seven days after weaning. Lysine to CP ratios were confirmed by amino acid analysis (amino acid panel) (Symbio, Queensland). CON 20.6% protein 0.065 SID lysine: CP LH 19.1% protein 0.071 SID lysine: CP, LL 18.7% protein 0.059 SID lysine: CP.

Raw materials, %	**CON**	**LH**	**LL**
Barley	4.00	8.25	4.00
Maize	10.0	53.5	19.5
Wheat	45.5	0.00	42.2
Soybean meal	9.50	2.28	3.00
Blood meal	2.20	2.50	1.50
Fish meal	9.75	10.6	10.0
Whey powder	12.5	15.0	12.9
Prebiotic	2.00	2.00	2.00
Probiotic - Bacillus	0.08	0.08	0.08
Canola oil	2.55	3.20	3.00
Salt	0.20	0.20	0.20
Microencapsulated zinc oxide	0.10	0.10	0.10
Betaine	0.10	0.10	0.10
DL-methionine	0.18	0.27	0.13
Lysine	0.44	0.60	0.43
L-threonine	0.12	0.19	0.10
L-tryptophan	0.06	0.11	0.06
L-isoleucine	0.08	0.20	0.07
L-valine	0.00	0.11	0.01
Carbohydrase	0.05	0.05	0.05
Phytogenic additive	0.02	0.02	0.02
Organic acid blend	0.40	0.40	0.40
Flavour	0.03	0.03	0.03
Vitamin/mineral premix[1]	0.02	0.02	0.02
Composition			
Crude protein (CP), %	20.6	19.03	18.7
Digestible energy (DE), MJ/kg	14.8	14.8	14.8
SID lysine, %	1.34	1.34	1.10
SID lysine: DE	0.091	0.091	0.074
SID lysine: CP	0.065	0.071	0.059

[1]Premix provides per ton of complete diet, Vitamin A 10 miu, D3 2 miu, E 80 g, K 3 g, B1 3 g, B2 4 g, B6 3 g, B12 20 g, Pantothenate 20 g, Niacin 25 g, Biotin 10 g, Folic 0.5 g, Fe 70 g, Zn 100 g, Mn 40 g, Cu 20 g, Se 0.5 g, Cr 0.2 g, Co 0.75 g, I 1.5 g.

Enzyme-linked immunosorbent assays (ELISA) were used to measure the concentrations of Pig-MAP (Major acute phase protein; Acuvet, Zaragoza, Spain) and C-reactive protein (C-RP; ABCAM, Cambridge, United Kingdom). The spike recoveries for Pig-MAP and C-RP were 90 and 92%, respectively, and the intraassay variations were 6 and 4%, respectively.

The diarrhoea index (DI) was adapted from the method described by (Heo, Opapeju et al. 2013) because there were insufficient cases of severe diarrhoea in this study, possibly due to the addition of the water acidifier. Pigs were observed from day 1-12 daily after weaning and scored based on the presence (one) or absence (zero) of PWD in the pen. The DI was then calculated based on the following eq:

Equation 1: Diarrhoea index (DI) formula used for pigs fed diets varying in protein content and SID Lys.

$$DI = \frac{(Number\ of\ occurances\ of\ diarrhoea\ in\ pen \times number\ of\ pigs\ in\ pen)}{Number\ of\ days\ observed} \times 100$$

Statistical analysis was undertaken using GenStat (v. 23, VSNi, Hemel Hempstead, United Kingdom) for DI and growth data and IBM SPSS (IBM Corp. Released 2023. IBM SPSS Statistics for Windows, Version 29.0.2.0 Armonk, NY: IBM Corp) for all other analyses. GraphPad Prism (10.22) was used to create graphics (La Jolla, California, United States). In total four batches in pigs were used in the experiment, weaned into the nursery in weekly replicates. Treatments were randomly allocated across gender and batches. Growth data was tested for normality and was found to be normally distributed. As feed intake could not be measured on an individual animal basis, the pen feed intake was used at the statistical unit and data is presented as average feed intake for the pen. Pen was used as a blocking factor in the model. The data was then divided by the number of pigs in a pen to obtain average growth for each animal in the pen. A REML analysis was used for growth data analysis. For ELISA results, normal distributions could not be achieved through any transformation, therefore, means were generated using a one-way analysis of variance and separated using non-parametric Kruskal-Wallis tests. The BHB concentrations were analysed using negative binomial regression using day as the repeated measure. Individual animals were used as the statistical unit for ELISA and BHB analysis. A p-value of < 0.05 was considered significant, and a p-value < 0.10 was considered a trend, in the interpretation of the data. Trends were defined as changes in a parameter in the same direction over time.

4.3 Experiment 3: Comparison of iron injections and low crude protein diets

The experiment commenced on a 7,500-sow breeder and nursery unit in South Australia from February to April 2025. The site had recently undergone a whole farm depopulation where all pigs were removed from the site, which was then deeply cleaned to remove residual pathogens and environmental reservoirs before being repopulated with animals from other sites. Therefore, it was assumed that the disease challenge for pigs at weaning would be reduced compared to pre-depopulation, as has been shown in the literature previously (Dee, Joo et al. 1996).

Sows and their litters (n = 150 sows and litters) were housed in farrowing crates within naturally ventilated sheds. Each farrowing crate was on plastic slatted flooring and contained one sow feeder, a sow and a piglet nipple drinker, and a heated creep area with a solid mat. On day three, farrowing information (sow parity, litter size) was collected, and piglets were weighed, individually identified, allocated to treatment, and processed. Processing involved tail docking using cauterisation, intramuscular injection with iron dextran (Ferron 200 + B12; Elanco, Macquarie Park, New South Wales, Australia) providing 200 mg of iron dextran, and oral gavage of coccidiostat (Baycox Coccidiocide for piglets and cattle, Elanco, Macquarie Park, New South Wales, Australia) and the administration of an individual ear tag for identification. Sows were fed a standard lactation diet *ad libitum*, and from day 15 after farrowing, piglets were fed a creep feed (Table 3) twice daily from a round bowl.

The day before weaning (27.5 ± 0.08 days), pigs were vaccinated (Circoflex, Boehringer Ingelheim, North Ryde, New South Wales, Australia) and transported from the farrowing shed to the nursery (~2 km). The nursery comprised four large pens, each containing approximately 400 piglets (0.17 m^2/pig) on slatted tribar flooring (two-thirds) with a concrete sleeping and feeding area (one-third). Water access was provided via a trough drinker with 25 openings and feed access via round feeders (Rotecna, Lleida, Spain) and via heavy-duty cast-iron feeders (Stockyard Industries, Clifton, Queensland, Australia). Lighting followed a 16h light and 8h dark pattern. There were no in-feed or water medications for pigs in this experiment. A water acidifier was included for the duration of the experiment (Selko, Amersfoort, The Netherlands, 1 % in water). Pig health was checked twice daily at approximately 0730h and 1500h.

At processing, litters were randomly assigned one of the following four treatments arranged in a two-by-two factorial treatment structure with a randomised design with the respective factors being dietary protein content and number of iron injections:
(1) CON single; iron injection on day 2 of life and a high protein diet (HP 22.1 % crude protein) fed after weaning for seven days,
(2) CON double: Iron injections at day 2 of life and the day before weaning and a HP diet,
(3) LP single: Iron injection at day 2 of life and a low protein diet (LP 18.5 % crude protein),
(4) LP double: Iron injections at day 2 of life and the day before weaning and a LP diet.

Iron injections at day 2 and the day before weaning were identical: 1 mL intramuscular injection of iron dextran (Ferron 200 + B_{12}) providing 200 mg of iron dextran. At weaning, piglets were sorted into four mixed sex pens, representing each of the experimental treatments. Experimental diets were fed for seven days after weaning, after which all pigs were transferred to the post-experiment diet (Table 4) for the following week of the experiment.

Table 3 Ingredients and formulated composition of lactation and creep diets fed to all sows and all piglets pre-weaning.

Raw materials, %	**Lactation diet**	**Creep feed**
Barley	21.4	
Maize		37.7
Wheat	38.2	21.1
Wheat - flour		3.00
Groats		3.00
Millrun	7.00	
Field peas	12.6	
Canola meal	5.91	
Soybean meal		3.35
Full-fat soybean	0.50	2.00
Blood meal	2.00	2.20
Meat meal	4.70	2.70
Fish meal		6.00
Whey powder		10.0
Prebiotic	0.50	2.00
Probiotic - Saccharomyces	0.01	
Probiotic - Bacillus	0.03	0.04
Salmon oil	0.43	
Vegetable oil blend	4.50	
Pork tallow	0.10	3.50
Dextrose		1.00
Limestone fine	0.36	
Salt	0.50	0.15
Copper(I) oxide		0.07
Microencapsulated zinc oxide	0.04	0.10
Vitamin C	0.01	
Betaine	0.50	0.10
DL Methionine	0.07	0.26
Lysine	0.20	0.62
L-Threonine	0.10	0.21
L-Tryptophan	0.03	0.10
L-Isoleucine	0.03	0.19
L-Valine	0.03	0.12
Carbohydrase		0.02
Phytase	0.02	0.02
Protease	0.02	
Phytogenic additive	0.01	0.02
Organic acid blend		0.30
Flavour		0.02
Food dye		0.01
Vitamin/mineral premix	0.20	0.20
Composition		
Crude protein (CP), %	18.2	18.5
Digestible energy (DE), MJ/kg	14.2	14.7
SID lysine, %	0.88	1.25
SID lysine: DE	0.062	0.085
SID lysine: CP	0.048	0.067

Each day of the experimental period, each group of pigs was visually examined for any health concerns by an experienced stockperson. If any pigs were found to be diseased or injured, this was documented before pigs were treated with the appropriate medication or euthanised. Pigs that were treated were removed from the experiment to a hospital pen, where appropriate medications were provided continuously until pigs recovered, died of natural causes or were euthanised. Staff who performed the animal husbandry during the experiment were deemed competent in the Certificate III in Pork Production, specifically modules AHCPRK311, AHCLSK301 and AHCLSK309 all relating to pig health, which define decisions and protocol for the treatment and removal of sick pigs. Stockpersons were certified for the euthanasia of sick and injured pigs as they had been certified in euthanising pigs (AHCLSK307). The most common diseases and reasons for removals were ill-thrift, paleness and physical injury (e.g., a broken leg). Pigs that died suddenly were documented and then removed from the experiment.

Table 4 Diets fed, and calculated composition of diets fed to pigs for seven days after weaning and then post-experiment.

Raw materials, %	**Control**	**Low Protein**	**Weaner**	**Grower1**	**Grower2**	**Finisher**
Barley						40.8
Maize	34.9	44.3	55.0			
Wheat	18.7	15.0		80.0	77.8	43.4
Groats	3.00	3.00	4.00			
Canola meal					9.85	9.10
Soybean meal	9.50	3.00		10.8		
Biscuit meal			3.00			
Field peas			3.00		2.75	
Soya	3.85	1.10	5.30			
Soyabean	0.50	2.85	6.30			
Phytogenic additive			0.01			
Bloodmeal	2.20	2.20	2.50	0.90		
Meat meal	3.60	2.00	5.10	3.78	6.88	3.70
Fish meal	6.00	6.00	5.00			
Whey powder	10.0	10.5	3.50			
Prebiotic	2.00	2.00	1.00			
Pork tallow	3.10	3.55	3.50	2.60	1.65	1.00
Dextrose	1.00	1.46	1.00			
Limestone		0.15		0.80		0.91
Monocalcium phosphate		0.30				
Salt	0.14	0.15	0.20	0.22	0.22	0.20
Copper(I) oxide	0.06	0.07	0.06			
Microencapsulated zinc oxide	0.10	0.10	0.10			
Betaine	0.10	0.10	0.10			
DL Methionine	0.18	0.29	0.18	0.09	0.05	0.06
Lysine	0.34	0.66	0.43	0.45	0.45	0.45
L-Threonine	0.07	0.21	0.15	0.13	0.10	0.12
L-Tryptophan	0.06	0.10	0.06		0.02	0.02
L-Isoleucine	0.04	0.20	0.09			0.01
L-Valine		0.14				
Carbohydrase	0.02	0.02	0.02			
Phytase	0.02	0.02	0.01	0.01	0.01	0.01
Protease				0.02	0.02	0.02
Organic acid blend	0.30	0.30	0.30			
Flavour	0.02	0.02				
Food dye		0.01				
Vitamin/mineral premix	0.20	0.20	0.20	0.20	0.20	0.20
Composition						
Crude protein (CP), %	22.1	18.5	22.4	18.8	18.3	15.7
Digestible energy (DE), MJ/kg	14.8	14.8	14.8	14.2	14.0	13.5
SID lysine, %	1.30	1.30	1.30	1.02	0.94	0.84
SID lysine: DE	0.088	0.088	0.088	0.072	0.067	0.062
SID lysine: CP	0.059	0.070	0.058	0.054	0.051	0.054

Piglet weights were collected individually on days 3 and 21 of lactation, and on days 0, 7 and 14 after weaning. ADG was calculated using these values. A subset of pigs (n = 220 per treatment) was selected to have whole blood collected via the auricular vein on day 1, 8 and 15 after weaning, which was then used to quantify haemoglobin concentrations using a haemocue (Haemocue HB201+ blood analyser, Haemocue AB, Sweden) and beta-hydroxybutyrate (BHB, Precision lifestyle optimum neo blood glucose and BHB meter, Abbott industries, Chicago, Illinois, USA). The haemocue and the BHB meter, respectively, have been previously validated for use on pigs (Kutter, Mauch et al. 2012).

Due to the design of the facility, one pen was allocated to each treatment, and as treatments were applied to each individual the pigs were used as the statistical unit. All data were analysed in SPSS (Version 29.0.2.0, IBM Corp, Armonk, New York, USA). Normally distributed data (weight, growth) were analysed using a general linear model with sex (male and female), diet (CON and LP), iron (day 2 and weaning iron), and the interaction between the two treatment factors according to the two-by-two treatment structure and randomised design. Hb and BHB measures were analysed using a repeated measures analysis with diet iron injection and timepoint as factors. Incidence data (0 or 1; medication, removal and mortality) were analysed using binary regression and the same fixed effects. Pearson's correlations for all data were analysed using SPSS. After analyses, it was attained that there was a random treatment effect on day three and 21 weights post-farrowing (prior to treatment application). To account for this, the final model also included the variable of day three as a covariate to account for the differences in weaning weight. Means were separated using Tukey's LSD test, and a p-value of < 0.05 was considered significant. A p-value of < 0.10 was considered a trend.

At five weeks post-weaning, pigs were transported to the grower–finisher facility. On arrival, pigs were individually sorted into homogenous weight and sex pens and housed by treatment (n = 22 pigs/pen) in paired pens. Each pen has partial slatted flooring, one shared fenceline penguin feeder, three water points, and all pens in the shed were identical. Individual weights were collected at 9, 11, 15, and 17 weeks post-weaning. These weights were then converted to average daily gain (ADG). In the grower–finisher phase, feed intake was recorded weekly at the paired-pen level to determine average daily feed intake (ADFI) and along with growth data to calculate feed conversion ratio (FCR) for each treatment group. At 17 weeks post-weaning, a subset of 1,000 pigs were randomly selected across treatments (n = 250 per treatment) and tattooed on the right side of the neck for carcass identification at the abattoir. Pigs were marketed from 21 weeks of age according to a fixed weight target. Carcass composition data were collected and included in the final analyses.

Statistical analyses of grower-finisher data were conducted using SPSS (Version 29.0.2.0, IBM Corp., Armonk, NY, USA). Data distributions were assessed for normality prior to analysis. Normally distributed data were analysed directly; data from the grower–finisher phase were square-root transformed to achieve normality and subsequently back-transformed for presentation. Where necessary, extreme outliers with disproportionate influence on model residuals were removed. Weight, ADG, ADFI and FCR data were analysed using a general linear model including the fixed effects of sex (male, female), diet (HP, LP), iron (Single, Double), and the diet × iron interaction, consistent with the two-by-two factorial design. Removal rates were analysed using binary regression interpreted using 95% confidence intervals. Statistical significance was set at p-value < 0.05, and p-value < 0.10 was considered a trend.

5. Results

5.1 Experiment 1: Determining the best time to administer a second iron injection to pigs

The Hb levels were significantly higher in the day 7 treatment group until day 18, after which point all treatments were similar (Figure 1).

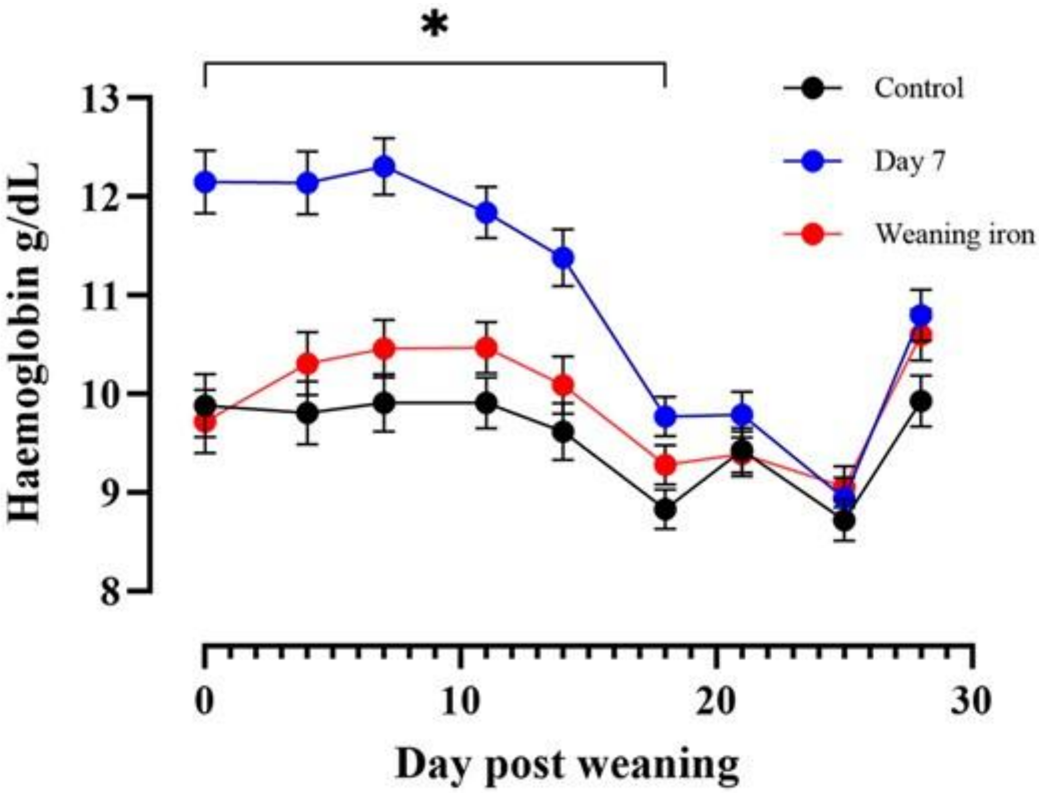

*Figure 1 Haemoglobin (Hb g/dL) concentration (mean ± SEM) for 28 days after weaning for pigs on a treatment basis given different iron treatments. The control group was given 200 mg of iron on day two after farrowing. The day 7 group was given control and 200 mg iron dextran on day seven after farrowing and weaning iron was given control and 200 mg iron dextran at weaning. Data were analysed via analysis of variance. *denotes P < 0.05 from control for day 7 group.*

Treatment had an impact on activity, weaning iron pigs were found to be inactive (sitting and lying) more frequently, followed by day 7, with the control group exhibiting the lowest frequency for this behavior (Table 5). There was no difference between treatments in eating, drinking or champing behaviors, with overall frequency of these behaviors being low (8.8, 0.91 and 0.18 % of total observations, respectively). The impact of day was more pronounced, influencing both posture (lying, standing) and activity (active, inactive and drinking). When averaged across all measurement days, pigs from the weaning iron treatment were observed to be lying more frequently, and control pigs were observed to stand more frequently, with the highest period of activity on day four after weaning. There was no significant effect of the interaction between day and treatment. There was no difference in the sick pig stance between the three treatments.

Table 5 Frequency of behaviours (120 observations each day for four days) performed by pigs given different iron doses and timings between 0700h and 0900h on days four-seven after weaning, (mean ± SEM shown). A total of 5,280 observations were recorded for treatment and 1,320 for each day were recorded. Pigs were scan sampled every 30 seconds. The control group was given 200 mg of iron on day two after farrowing. The day 7 group was given control and 200 mg iron dextran on day seven after farrowing and Weaning iron was given control and 200 mg iron dextran at weaning).

	Treatment			Day				p-value		
	Control	Day 7	Weaning	4	5	6	7	Treatment	Day	Interaction
Posture										
Lying	1092±76[a]	1190±87[a]	1378±83[b]	806±495[a]	1313±515[b]	1440±546[b]	1341±369[b]	0.032	0.001	0.99
Standing	2168±78[a]	1981±91[b]	1941±84[b]	2330±602[a]	1977±524[ab]	1852±563[ab]	1949±382[b]	0.053	0.013	0.97
Sitting	13.75±1.1	11.1±1.4	11.2±0.9	11.3±7.5	11.4±6.1	11.6±4.9	13.6±10	0.20	0.74	0.62
Sick Pig Stance	15.5±1.6	16.3±1.2	15.9±1.4	16.5±10	16.9±9.2	15.1±7.7	14.9±8.3	0.91	0.78	0.99
Activity										
Active	1637 ± 64	1466±75	1454±69	1779±522[a]	1520±419[ab]	1328±440[b]	1442±285[b]	0.06	0.002	0.98
Inactive	1109±76[a]	1208±87[b]	1389±83[c]	819±494[a]	1330±511[b]	1451±543[b]	1361±364[b]	0.047	<0.001	0.99
Eating	488±22	459±22	447±21	507±144	420±140	467±157	462±114	0.21	0.15	0.82
Drinking	45.3±3.9	54.5±3.7	44.7±5.1	50.5±18[ab]	37.1±14.7[a]	62.7±29.0[b]	41.8±37.0[ab]	0.22	<0.001	0.77
Champing	9.3±1.3	10.4±1.9	9.91±1.2	7.74±5.8	11.0±9.1	9.35±6.7	11.3±8.4	0.12	0.55	0.83

[abc]Means within treatment with different superscripts differ significantly ($P < 0.05$).

The growth rate, feed intake and production efficiency data (Table 6) were not different between treatments, although there was a tendency (P < 0.10) for improved ADG and gain: feed in week two in the weaning iron treatment group. There was a statistical difference in removals with less pigs removed from the weaning iron treatment group.

Table 6 Post-weaning feed intake and piglet growth performance (mean ± SED). The control group was given 200mg of iron on day two after farrowing. Day 7 group was given control and 200mg iron dextran on day seven after farrowing and Weaning iron was given control and 200mg iron dextran at Weaning. Data were analysed using analysis of variance with pen as the experimental unit.

	Treatment				
	Control	Day 7	Weaning	SED	p-value
Nursery (overall)					
ADFI, g/d	377	381	389	11.5	0.50
ADG, g/d	272	270	289	12.8	0.30
Gain: Feed	0.65	0.67	0.69	0.022	0.40
Weaning weight, kg	6.31	6.46	6.46	0.158	0.55
Week 1					
Weight, kg	6.60	6.79	6.74	0.164	0.49
ADFI, g/d	115	129	116	8.6	0.22
ADG, g/d	34.9	53.2	42.0	11.38	0.30
Gain: Feed	0.29	0.42	0.36	0.097	0.43
Week 2					
Weight, kg	8.31	8.52	8.61	0.201	0.30
ADFI, g/d	277	279	286	13.3	0.76
ADG, g/d	237	235	268	16.2	0.080
Gain: Feed	0.86	0.84	0.94	0.049	0.090
Week 3					
Weight, kg	10.6	10.8	11.1	0.25	0.162
ADFI, g/d	469	466	483	19.9	0.76
ADG, g/d	318	314	355	28.6	0.28
Gain: Feed	0.68	0.67	0.74	0.048	0.30
Week 4					
Weight, kg	14.1	14.3	14.5	0.33	0.51
ADFI, g/d	661	657	675	19.9	0.69
ADG, g/d	501	481	491	35.9	0.87
Gain: Feed	0.76	0.74	0.73	0.051	0.83
Number of pigs removed	5^a	6^a	0^b		< 0.001

^{ab}Means within treatment with different superscripts differ significantly (P < 0.05). ADFI, average daily feed intake; ADG, average daily gain.

An increase in HAMP was identified in the weaning iron treatment group ($p < 0.05$) when compared to the day 7 treatment group (Figure 2). There was also a significant increase in the haptoglobin concentrations of the weaning iron treatment group compared to the control group on day 28.

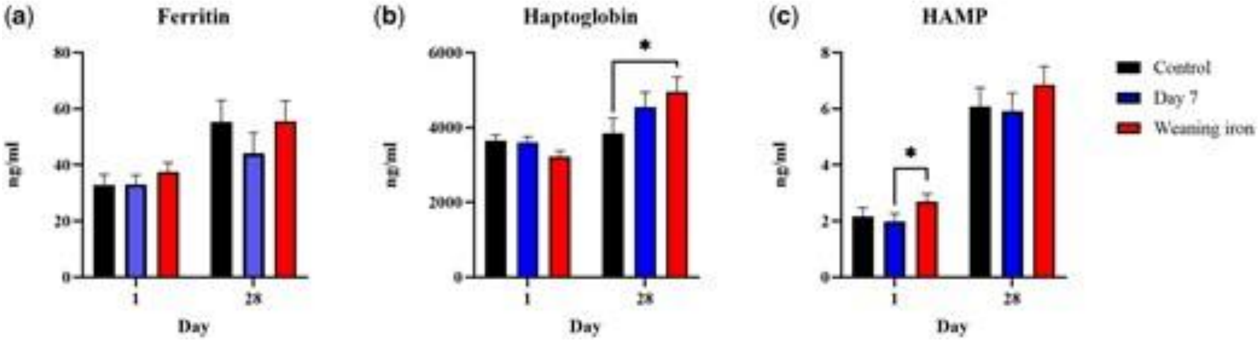

Figure 2 a) Ferritin, b) haptoglobin and c) hepcidin anti-microbial protein (HAMP) results (mean ± SED). Blood samples were collected at weaning and 28 days after via jugular venipuncture. The control group was injected 200 mg of iron dextran two days after farrowing, while the day 7 and weaning iron groups were given control and an additional 200 mg at day 7 after farrowing or at weaning. a–c indicates statistical significance ($p < 0.05$). Data was not normally distributed, analysis of variance was used to generate means, and non-parametric Kruskal-Wallis testing was used to separate means. As a normal distribution could not be achieved, no interaction p-value exists.

5.2 Experiment 2: Investigating the use of low crude protein diets to reduce post-weaning diarrhoea

There was a significant difference (P < 0.05) in both diets from CON, with diet LL having the lowest DI (30.1) and the CON diet having the highest DI (83.3). Diet LH was intermediate (48.7) and significantly different to the other two diets (Figure 3).

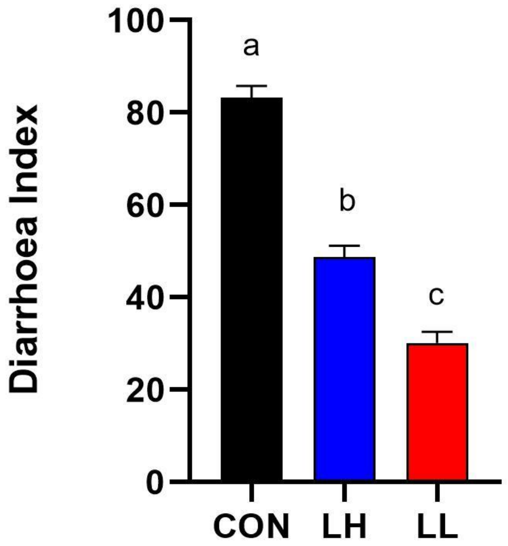

Figure 3 Diarrhoea index (mean ± SED) of weaning pigs fed diets differing in CP content and SID lysine: CP ratio. CON 20.6% protein 0.065 SID lysine: CP. LH 19.1% protein 0.071 SID lysine: CP, LL 18.7% protein 0.059 SID lysine: CP. [abc]Means with different superscripts differ significantly (P < 0.05).

There was a significant difference in plasma creatinine concentrations on day five, with pigs in the LL treatment group having a lower (P <0.05) creatine concentration compared to pigs fed the other two diets (125 versus 145 and 145 µmol/L for diets CON and LH, respectively. There was also a significant difference between days (P < 0.05) with concentrations highest on day five and decreasing on days 12 and 28 (Table 7).

Table 7 Plasma creatinine concentrations (mean ± SED) of pigs fed diets differing in CP content and SID CP: lysine ratio. CON 20.6% protein 0.065 SID lysine: CP LH 19.1% protein 0.071 SID lysine: CP, LL 18.7% protein 0.059 SID lysine: CP. The superscripts indicate statistical significance (P<0.05). Data could not be transformed to a normal distribution; therefore, means were generated using a one-way analysis of variance and separated using a non-parametric test.

	Diet				p-value		
Plasma creatinine µmol/L	CON	LH	LL	SED	Treatment	Day	Interaction
5	145[a]	145[a]	125[b]	8.9	0.042		
12	113	114	116	13.5	0.94		
28	118	114	121	10.8	0.78		
Overall	128	122	121	6.1	0.36	<0.001	0.32

[ab]Means within treatment with different superscripts differ significantly (P < 0.05).

There was no difference (P > 0.05) between diets or change over time in MAP or CRP concentrations (Figure 4a and b, respectively).

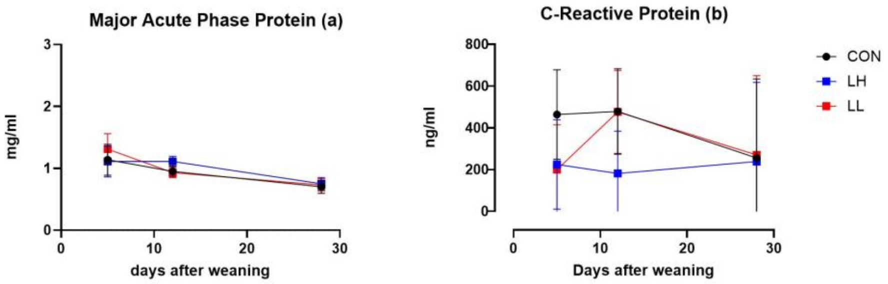

Figure 4 (a,b): (Mean ± SED) for major acute phase protein and C-reactive protein concentrations from pigs fed diets with varying SID lys: CP ratios. CON 20.6% protein 0.065 SID lysine: CP LH 19.1% protein 0.071 SID Lys: CP, LL 18.7% protein 0.059 SID lysine: CP. Data were not normally distributed; means were generated using one-way analysis of variance and separated using a non-parametric Kruskal-Wallis test.

There was no significant difference in the BHB concentrations of pigs over the nursery period (Figure 5). Moreover, no groups reached the threshold to be described as in a state of ketosis (0.2 mmol/L) as described in Perri et al. (2016). The BHB concentrations peaked on day five in all treatments after weaning and declined with increasing duration. There was a significant effect of time on BHB concentrations but no effect (P > 0.05) of treatment or an interaction effect.

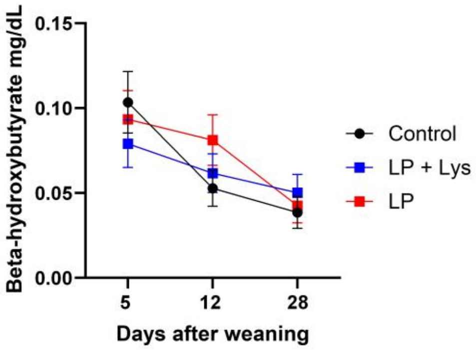

Figure 5 BHB concentrations (mean ± SED) of pigs at 5, 12 and 28 days after weaning. CON 20.6% protein 0.065 SID lysine: CP LH 19.1% protein 0.071 SID lysine: CP, LL 18.7% protein 0.059 SID lysine: CP. Data were analysed using negative binomial regression using day as the repeated measure.

There was a strong trend (P = 0.054) for pigs fed diet LH to grow faster in the first week after weaning than pigs fed CON diets (75 g/pig *versus* 50 g/pig). There was no effect of diet on weight, average daily feed intake (ADFI), or FCE during each week or when summed over the four experimental weeks. A trend for improvement (P = 0.090) was also noted in feed conversion efficiency in week two in the CON group (Table 8). Removals were almost halved by the two LP treatments relative to the CON diet; however, this was not statistically significant.

Table 8 Growth data for pigs (mean ±SED) fed diets differing in CP content and SID CP: lysine ratio from weaning to 15 weeks after weaning. CON 20.6% protein 0.065 SID lysine: CP LH 19.1% protein 0.071 SID lysine: CP, LL 18.7% protein 0.059 SID lysine: CP.

	Diet				
	CON	LH	LL	SED	p-value
Weight, kg					
Week 0	8.40	8.33	8.32	0.18	0.85
1	8.71	8.82	8.74	0.17	0.79
2	10.0	10.0	10.0	0.24	0.97
3	12.1	12.2	12.0	0.36	0.93
4	15.3	15.4	15.4	0.43	0.95
Average daily gain (ADG), g/d					
Week 1	50.0^{a}	75.0^{b}	61.0^{ab}	10.0	0.054
2	189	171	173	16.5	0.49
3	290	309	298	21.0	0.67
4	460	463	473	22.5	0.84
Average daily feed intake (ADFI), g/d					
Week 1	171	190	169	14.6	0.30
2	276	290	273	18.7	0.61
3	450	458	445	24.6	0.85
4	634	655	658	28.6	0.67
Feed: gain					
Week 1	0.32	0.41	0.36	0.053	0.28
2	0.68	0.59	0.64	0.042	0.090
3	0.64	0.68	0.68	0.036	0.55
4	0.73	0.72	0.72	0.039	0.95
Growth performance day 0-28					
ADG, g/d	251	255	251	10.5	0.94
ADFI, g/d	383	398	386	17.5	0.65
Feed: gain	0.66	0.64	0.65	0.026	0.75
Removals	13	8	7	$x^2(1)=2.15$, P=0.34	

[ab]Means within treatment with different superscripts differ significantly ($P < 0.05$).

5.3 Experiment 3: Comparison of iron injections and low crude protein diets

Diets affected weights and ADG and bodyweight at all stages of the experiment ($p < 0.05$). An effect of an additional iron injection on weight was observed when pigs were weighed at day 14 ($p < 0.05$) and increased ADG at all stages of the experiment ($p < 0.05$). There was an interaction effect of diet and iron injection on ADG during the second week of the experimental period, which was a large enough increase to improve ADG over the entire period ($p < 0.05$, Table 9).

Table 9 Weights and average daily gain (ADG) of pigs (means ± SEM) for 14 days after weaning. At farrowing, pigs were assigned to one of four treatments. CON single: High protein diet (22.1 % CP) day 2 iron injection, LP single: Low protein (18.5 % CP) diet day 2 iron injection, CON double: High protein weaning iron (D2 and weaning), or LP double: Low protein weaning iron (D2 and weaning). Data were analysed using ANOVA, and means were separated using a Bonferroni post-hoc test.

	CON		LP			p-value		
Weight, kg	Single	Double	Single	Double	*SEM*	*Diet*	*Iron*	*Interaction*
Weaning	7.92	7.84	7.63	7.62	0.073	0.003	0.79	0.45
Day 7	8.98^{a}	8.88^{a}	8.33^{b}	8.53^{ab}	0.073	<0.001	0.54	0.043
Day 14	10.7	10.9	10.1	10.3	0.09	<0.001	0.017	0.65
Average daily gain, g/d								
Day 1-7	151	158	89	122	5	<0.001	<0.001	0.18
Day 8-14	278^{a}	346^{b}	284^{a}	286^{a}	6	<0.001	<0.001	<0.001
Day 1-14	199^{a}	233^{b}	174^{c}	190^{a}	5	<0.001	<0.001	0.005

^{ab}Means within treatment with different superscripts differ significantly ($P < 0.05$).

The Hb concentrations (Figure 6a) were increased in response to a second iron injection at all stages of the experiment and the LP diet at day seven of the experiment ($p < 0.05$). An interaction effect was not observed between diet and iron on Hb. BHB (Figure 6b) was not influenced by diet or iron at weaning. On day eight of the experiment LP diets were reduced, and a second iron injection increased concentrations and an interactive effect was observed ($p < 0.05$). At day 15, diet or iron treatments individually did not affect BHB, but an interactive effect was noted with CON day 2 and LP weaning iron having higher concentrations than the other treatments ($p < 0.05$).

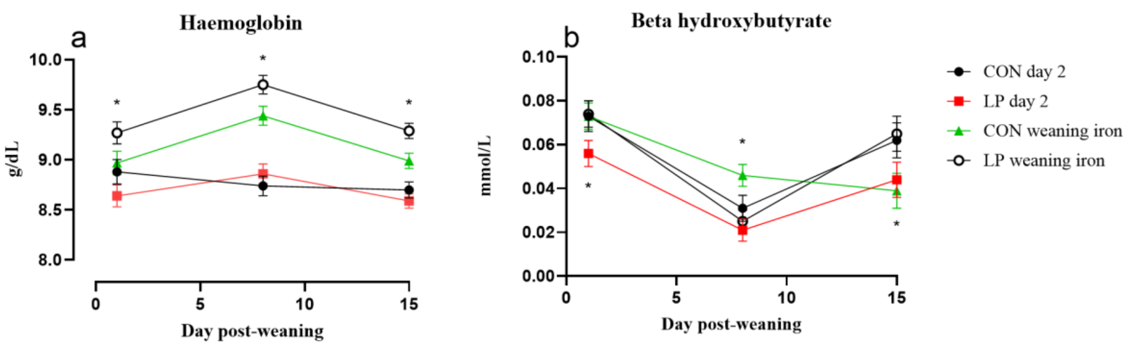

*Figure 6 Haemoglobin (Hb) concentrations (a) and Beta hydroxybutyrate (BHB) concentrations (b) means ± SED of pigs administered with either a single dose of intramuscular iron at day 2 (day 2) or day 2 and an injection the day before weaning (weaning iron; 27 days after farrowing) and fed a diet containing either high protein (22.1%) or low protein (18.5%) for seven days post-weaning. Data presented is the interaction of diet*iron.*

Pearson's correlation coefficients (Table 10) observed a negative correlation between weaning Hb and day seven and 14 weights and a weak positive correlation with ADG. The BHB concentration was negatively correlated with ADG and weight in the experiment.

*Table 10 Pearson's correlations for growth performance, haemoglobin (Hb) and beta-hydroxybutyrate (BHB). * indicates statistical significance p value < 0.05.*

	Wean weight	Wean Hb	Wean BHB	D7 weight	ADGW-7	D7 Hb	D7 BHB	D14 weight	ADG7-14	D14 Hb	D14 BHB	Nursery ADG
Wean weight	1											
Wean Hb	-0.49*	1										
Wean BHB	-0.143*	0.095*	1									
D7 weight	0.934*	-0.395*	-0.194*	1								
ADGW-7	0.043	0.107*	-0.189*	0.397*	1							
D7 Hb	-0.412*	0.588*	0.106*	-0.411*	-0.113*	1						
D7 BHB	-0.92	0.081*	0.261*	-0.115*	-0.091*	0.194*	1					
D14 weight	0.845*	-0.348	-0.184*	0.955*	0.474*	-0.380*	-0.98*	1				
ADG7-14	0.274*	-0.036	-0.113*	0.448*	0.546*	-0.146*	-0.076*	0.693*	1			
D14 Hb g/dl	-0.154*	0.135	0.016	-0.124*	0.047	0.482*	0.132*	-0.096*	-0.108*	1		
D14 BHB	-0.055	0.05	0.073	0.084*	-0.099*	0.08*	0.334*	-0.115*	-0.146*	0.159*	1	
Nursery ADG	0.173*	0.173	-0.148*	0.466*	-0.148*	-0.144*	-0.07	0.673*	0.896*	-0.046	-0.146*	1

Both diet and iron affected the incidence of pigs medicated during the nursery phase of the experiment ($p < 0.05$, Table 11), but no interaction was observed. Overall, the fewest pigs were removed in the CON weaning iron treatment group. However, there was no interaction between diet and iron injection. Mortality rates did not differ between the experimental groups at either the treatment or interaction level during the nursery phase.

Table 11 Deaths and medications (%) in the weaning period for pigs assigned to one of the following treatments at farrowing, CON single: High protein diet (22.1 % CP) day 2 iron injection, LP single: Low protein (18.5 % CP) diet day 2 iron injection, CON double: High protein weaning iron (D2 and weaning), or LP double: Low protein weaning iron (D2 and weaning). Diets were fed for seven days after weaning and iron injections provided 200mg iron dextran intramuscularly.

	CON		LP			p-value		
	Single	Double	Single	Double	SEM	Diet	Iron	Interaction
Medications, %	8.30	3.40	10.6	7.10	0.082	0.013	0.028	0.14
Deaths, %	1.10	1.20	1.40	0.70	0.094	0.73	0.90	0.73

No differences between treatments in weights collected at the grower site were established (Table 12). At day 91 and 105 weights tended to follow a similar trend ($P \leq 0.10$), where the HP double iron group tend to have a slightly higher weight than single iron (Day 91 +0.6 kg, Day 105 +0.5 kg).

Table 12 Weights of pigs, transformed data (back-transformed means in brackets) at 77, 91, 105, 133 and 147 days after weaning. At farrowing, pigs were assigned to one of four treatments. CON single: High protein diet (22.1 % CP) day 2 iron injection, LP single: Low protein (18.5 % CP) diet day 2 iron injection, CON double: High protein weaning iron (D2 and weaning), or LP double: Low protein weaning iron (D2 and weaning).

	CON		LP			p-value		
Weight, kg	Single	Double	Single	Double	SED	Diet	Iron	Interaction
Day 77	4.3 (18.2)	4.3 (18.4)	4.3 (18.4)	4.2 (17.8)	0.02	0.43	0.54	0.14
Day 91	5.7 (32.3)	5.7 (32.9)	5.7 (32.8)	5.6 (31.8)	0.02	0.46	0.59	0.06
Day 105	6.5 (42.8)	6.6 (43.3)	6.6 (43.3)	6.5 (41.8)	0.02	0.41	0.45	0.10
Day 133	8.3 (68.9)	8.3 (68.3)	8.3 (69.1)	8.2 (66.7)	0.03	0.38	0.08	0.26
Day 147	9.3 (85.5)	9.2 (85.0)	9.2 (84.6)	9.1 (82.8)	0.03	0.12	0.25	0.54

The ADG calculated at the grower site showed a tendency ($P = 0.052$) in the first period (Table 13). A difference of 0.03 kg/day ($P < 0.05$) was identified between the single and double iron treatments within the HP treatment only. There were no significant differences found between treatments in the rest of the period. Whilst there was no treatment effect on ADFI during any period (Table 14).

Table 13 Average daily gain (ADG) of pigs in the periods between 77, 91, 105, 133 and 147 days after weaning. At farrowing, pigs were assigned to one of four treatments. CON single: High protein diet (22.1 % CP) day 2 iron injection, LP single: Low protein (18.5 % CP) diet day 2 iron injection, CON double: High protein weaning iron (D2 and weaning), or LP double: Low protein weaning iron (D2 and weaning).

	CON		LP			p-value		
ADG, kg/d	Single	Double	Single	Double	SED	Diet	Iron	Interaction
d77-91	0.65^{a}	0.68^{b}	0.67	0.66	0.006	0.66	0.43	0.052
d91-105	0.75	0.75	0.76	0.71	0.008	0.26	0.18	0.23
d105-133	0.92	0.91	0.92	0.90	0.007	0.85	0.17	0.64
d133-147	1.19	1.20	1.11	1.16	0.010	0.030	0.22	0.41
d77-147	0.87	0.86	0.86	0.85	0.005	0.22	0.42	0.51

Table 14 Average daily feed intake (ADFI) of pigs in the periods between 77, 91, 105, 133 and 147 days after weaning. At farrowing, pigs were assigned to one of four treatments. CON single: High protein diet (22.1 % CP) day 2 iron injection, LP single: Low protein (18.5 % CP) diet day 2 iron injection, CON double: High protein weaning iron (D2 and weaning), or LP double: Low protein weaning iron (D2 and weaning).

	CON		LP			p-value		
ADFI, kg/d	Single	Double	Single	Double	SED	Diet	Iron	Interaction
d77-91	1.01	1.07	1.05	1.00	0.02	0.76	0.90	0.32
d91-105	1.58	1.71	1.59	1.56	0.04	0.43	0.60	0.34
d105-133	2.21	2.24	2.17	2.14	0.05	0.44	0.99	0.70
d133-147	2.37	2.46	2.24	2.45	0.06	0.55	0.21	0.58

The FCR did not show a significant difference through the whole period (P > 0.05), as shown in Table 15. Removal data also did not show any significant difference (P > 0.05). With regards to pig removal to a hospital pen, the HP single showed 4.0%, HP double showed 3.0%, LP Single showed 4.0%, and LP double showed 4.0%. The HP double iron group showed 1.0% less removals but this was not significantly different from the other treatments (P>0.05). The 95% confidence interval showed to be between 2.0-6.3%.

Table 15 Feed conversion ratio (FCR) of pigs in the periods between 77, 91, 105, 133 and 147 days after weaning. At farrowing, pigs were assigned to one of four treatments. CON single: High protein diet (22.1 % CP) day 2 iron injection, LP single: Low protein (18.5 % CP) diet day 2 iron injection, CON double: High protein weaning iron (D2 and weaning), or LP double: Low protein weaning iron (D2 and weaning).

	CON		LP			p-value		
FCR, kg/d	Single	Double	Single	Double	SED	Diet	Iron	Interaction
d77-91	1.6	1.7	1.6	1.7	0.04	0.83	0.46	0.97
d91-105	2.0	2.2	2.1	2.1	0.07	0.90	0.57	0.43
d105-133	2.5	2.5	2.5	2.5	0.06	0.93	0.70	0.99
d133-147	2.1	2.2	2.2	2.2	0.06	0.68	0.39	0.75

There was a significant effect of the iron treatments on carcase weight (P<0.05), with the single Fe treatment mean of 86.0 ± 0.40 kg compared to the double Fe treatment, 84.7 ± 0.41 kg (Table 16). Carcase P2 backfat depth did not show any significant difference between treatments. The days to slaughter showed no difference between treatments (P>0.05).

Table 16 Carcase characteristics of pigs assigned to one of four treatments at farrowing. CON single: High protein diet (22.1 % CP) day 2 iron injection, LP single: Low protein (18.5 % CP) diet day 2 iron injection, CON double: High protein weaning iron (D2 and weaning), or LP double: Low protein weaning iron (D2 and weaning).

	CON		LP			p-value		
	Single	Double	Single	Double	SED	Diet	Iron	Interaction
Carcase weight, kg	86.6	85.0	85.5	84.5	0.29	0.18	0.030	0.62
P2 fat depth, mm	12.4	12.0	12.3	12.1	0.10	0.94	0.11	0.85
Days to slaughter	161.4	160.2	160.0	160.2	0.44	0.44	0.58	0.41

5.4 Impact of low protein diets and double iron treatment on Herd FCR.

With the neutral impact on lifetime performance, the impact of the low protein diets and a second iron injection on HFC can be conservatively calculated by the reduction of fixed feed costs associated with the breeder herd. The ~36% reduction in post-weaning mortality observed in the LP-Double treatment compared to the control (CON-Single, Table 11), means that more pigs will be sold per year for the same amount of breeder feed consumed. A model was produced to calculate the improvement in Herd FCR, which showed a 0.01 unit improvement in Herd FCR if these experimental results were achieved in a commercial operation.

Assessment of FCR benefits from a reduction in post-wean mortality.

Assumptions:
The number of breeder spaces is limited but not grower space. Can sell extra pigs.
There is no grower herd improvement in FCR.
Improvement in Herd FCR is dependent on the allocation of sow feed across progeny.

N. sows	1000
Pigs weaned/sow/year	24.0
Pigs weaned/year	24,000
Current post-wean mortality, %	6.0%
Proportion of post-wean mortality during the weaner stage (to 9 weeks of age)	67%
Relative reduction in post-wean mortality in *+iron treatment* during the weaner stage	63.6%
Absolute reduction in post-wean mortality, %	2.5%
New post-wean mortality, %	4.5%
Extra number of pigs sold/year	611
Breeder feed/sow/year, t	1.2
Total breeder feed used/year, t	1200
Breeder feed average cost, $/t	$520.00
Reduced weight of breeder feed/pig sold, kg	**0.81**
Reduced cost of breeder feed/pig sold, $	**$0.42**
Average carcase weight/per pig, kg	84.0
Improvement in Herd FCR	**0.010**

Figure 7 Screenshot of model built to calculate the improvement in Herd FCR, from the reduced fixed breeder feed use, arising from an improvement in post-wean mortality associated with the combination of low protein weaner diets and a double iron injection program.

6. Discussion

This project investigated two common malaises of piglets in the post-weaning period, iron-deficient anaemia and post-weaning diarrhoea. It was hypothesised that a second injection of iron dextran would improve growth after weaning and it was found that administration at weaning was the most beneficial. Gastrointestinal health of piglets and PWD was hypothesised to be improved when pigs were fed a diet of reduced crude protein and an increased SID Lys: CP ratio would improve gut health and performance in pigs. This hypothesis was undetermined; this was due to the two different experimental sites that work was conducted on.

In the first experiment, a second iron injection at seven days of age raised Hb concentrations above that of pigs given only the routine day-two dose. This advantage lasted until about day 18 after weaning, after which the groups were similar. Haemoglobin responds slowly because red blood cells have a long half-life, so it reflects an animal's iron status over weeks rather than its most recent injection. A single iron dose at birth often fails to keep fast-growing piglets in good iron status up to weaning, and extra iron has been shown to improve blood measures and growth before and after weaning (Chevalier et al. 2021, Loh et al. 2001). The timing of the second injection therefore decides how long the Hb benefit lasts, not whether iron status is eventually corrected.

Pigs given iron at weaning had higher HAMP concentrations than those injected at day seven, and this is informative. Hepcidin controls how the body handles iron and rises in response to both iron loading and inflammation, blocking further iron uptake and locking iron into stores (Ganz and Nemeth 2006). Haptoglobin concentrations also rose in the weaning-iron group, which points to a mild acute phase response to an injection given at the same time as the stress of weaning. A large iron dose at weaning may therefore trigger a response that limits its own benefit. This helps explain why the day-seven advantage did not last and why Hb alone is a poor measure of iron status.

Pigs given iron at weaning were less active and lay down more than control pigs. This likely reflects lower energy use rather than poor health, and it fits with the lower removal rate in this group, where fewer pigs needed hospital treatment. Growth, feed intake and efficiency did not differ between treatments. But the tendency for better daily gain and feed conversion in the second week, together with fewer removals, suggests a small production gain from correcting iron status at weaning. This agrees with reports that iron status at weaning affects later nursery performance (Perri et al. 2016a) and that iron-deficiency anaemia and its correction affect growth in young pigs (Knight and Dilger 2018).

In the second experiment, cutting dietary crude protein in the first week after weaning by 1-2 percentage points sharply reduced the diarrhoea index. The largest drop came from the diet with the lowest crude protein and the lowest SID Lys:CP ratio. Undigested protein reaching the hindgut feeds harmful bacteria such as enterotoxigenic *Escherichia coli*, so lowering protein is a proven way to reduce PWD without in-feed antibiotics (Heo et al. 2008, Heo et al. 2010, Heo et al. 2013, Pluske et al. 2002). The stepwise response across the three diets shows that both the amount of CP and its amino acid balance matter for gut health just after weaning, and it supports lower-protein diets as one of several ways to prevent PWD (Canibe et al. 2022).

Pigs on the lowest-protein diet had lower plasma creatinine, and creatinine fell over time in all groups, both in line with lower protein intake and turnover. The acute phase proteins, major acute phase protein and C-reactive protein, did not differ between treatments, so the drop in diarrhoea came without measurable inflammation. Beta-hydroxybutyrate concentrations peaked in the first week then fell, and no group reached the level that signals ketosis, which suggests the low-protein diets did not sharply cut feed intake. Beta-hydroxybutyrate is a validated marker of poor appetite and wasting in nursery pigs (Perri et al. 2016).

For production, the diet with less crude protein but the same SID Lys:CP ratio tended to grow pigs faster in the first week than the control, while the lowest-protein, lowest-ratio diet cut diarrhoea the most. Weight, feed intake and feed conversion over the four-week nursery period did not change, and both reduced-protein diets almost halved removals. The clear gain in gut health therefore came without a lasting cost to growth, as long as amino acids stayed adequate. This fits the idea that steady nutrient intake after weaning supports both health and performance (Wensley et al. 2021).

The third experiment tested both strategies together at a recently depopulated, high-health site. A second iron injection raised Hb content at every sampling point and improved average daily gain across the period, including a diet-by-iron effect in the second week large enough to lift gain overall. Here the higher-protein diet grew pigs better than the low-protein diet, the opposite of the diarrhoea benefit seen in the second experiment. The pigs' health and age best explain this difference: the depopulated site had a lower disease challenge (Dee et al. 1996), and the pigs were older. Older pigs have better gut function and immunity, so a higher-protein diet is less likely to upset them (Ming et al. 2021, Pluske et al. 2018).

The correlations add detail. Weaning Hb content was negatively related to day-seven and day-14 weights and only weakly positively related to daily gain, while BHB concentration was negatively related to both gain and weight. The link between higher BHB content and slower growth is expected, since it reflects lower feed intake and the use of body reserves. The inverse link between weaning Hb content and early weight fits the idea that larger, faster-growing piglets use up their limited iron faster, so heavier pigs can carry lower Hb at weaning despite growing well (Perri et al. 2016a).

Health effects showed up in the nursery phase: both diet and iron changed the share of pigs medicated, the higher-protein weaning-iron group had the fewest removals, and mortality did not differ. In the grower–finisher phase these early effects mostly faded, with no clear differences in weight, feed intake or feed conversion to slaughter. Pigs given a single iron injection had a slightly heavier carcase than those given a second, with no difference in backfat or days to slaughter. Against otherwise neutral lifetime performance, this suggests the value of a second iron injection lies in supporting health and growth through the risky early weeks, not in better carcase output. It also warns against assuming that early blood gains in iron carry through to finishing.

Together, the experiments show that a second iron injection at weaning and a short-term cut in dietary crude protein can each improve part of post-weaning health, but their measurable benefit depends heavily on the pigs' age, growth rate and health. Both worked best where the post-weaning challenge was greatest and faded in older, high-health pigs. This dependence on context, not a failure of the methods themselves, is why the expected benefit of lower-protein diets could not be fully settled across the different sites. It also shows the value of matching these strategies to each herd's level of risk.

7. Implications & Recommendations

The results point to a growing problem as sows are bred for larger litters. A bigger litter shares the sow's resources among more piglets, so each piglet is likely born with smaller iron stores. Pigs are also being selected to grow faster than ever (Bertolini et al. 2018), which uses iron up more quickly. Anaemia is therefore likely to become more common, and the industry needs better ways to detect it and to raise haemoglobin before weaning. The HemoCue is the preferred tool because it gives a fast Hb reading pen-side, but Hb content alone is not enough. About 40% of the body's iron sits outside Hb (Ganz and Nemeth 2006), so a normal reading can still hide low iron. Haemoglobin concentration is also slow to react: a red blood cell lives about 120 days, so the effect of an iron injection builds up gradually and shows late. Other markers matter too. Plasma ferritin, widely used in human medicine, is especially useful, but pigs lack agreed reference values for ferritin and other non-haemoglobin markers. These should be developed so that iron-deficiency anaemia can be confirmed rather than assumed.

Weaning age is increasingly debated. Older, more mature piglets handle weaning better than younger ones (Canibe et al. 2022). Larger, faster-growing pigs also use up their iron and other reserves faster than smaller pigs (Perri et al. 2016a) and cannot eat enough creep feed or milk to keep up, so they enter weaning short of iron. Weaning age also shaped these experiments. Pigs in Experiments 2 and 3 were weaned older, at about 27 days, while Experiment 1 used younger pigs more typical of commercial practice. Older pigs tend to have better gut health and stronger immunity, so they are less troubled by a high-protein diet or a heavy disease load (Ming et al. 2021). Experiment 3 used older, healthier pigs, which was probably a poor setting to test low-protein diets. The low-protein diets still worked, but the test would be sharper in younger pigs, for which weaning is a greater stress.

The timing of the second iron injection matters in practice. In Experiment 1, pigs injected at seven days held higher Hb contents until about day 18, but it was the pigs injected at weaning that had fewer removals and grew and converted feed slightly better in the second week. Experiment 3 showed the same pattern, with a second injection lifting both Hb content and growth after weaning. Giving the second injection at weaning, rather than at day seven, therefore looks the better option for getting pigs through the early weeks, when fast-growing pigs are most likely to run short of iron (Perri et al. 2016a). It should target the pigs most at risk of anaemia rather than be given to every pig.

This benefit does not last to slaughter. In Experiment 3, the early gains in Hb concentration and growth had gone by the end of finishing, and pigs given a single injection finished with a slightly heavier carcase than those given two, with no difference in backfat or days to slaughter. So, a second injection helps pigs through the risky weeks just after weaning but adds nothing to final carcase weight. Better Hb content early in life does not guarantee better finishing, and the cost and handling of an extra injection should be weighed against a benefit that is mainly short-term.

8. Intellectual Property

There has been no protectable intellectual property developed or discovered as part of this study.

9. Technical Summary

This project refined several practical techniques for diagnosing and managing the two main post-weaning ailments of piglets: iron-deficiency anaemia and post-weaning diarrhoea (PWD).

Iron-status diagnostics. Pen-side haemoglobin measurement (HemoCue) was confirmed as a fast, practical screening tool, but its limitations were established: because red blood cells live approximately 120 days, haemoglobin lags true iron status by weeks, and because around 40% of body iron sits outside haemoglobin, a normal reading can mask deficiency. Plasma ferritin, hepcidin (HAMP) and haptoglobin were trialled as complementary markers, and the work identified that the industry lacks agreed reference (cut-off) values for ferritin and other non-haemoglobin iron markers; these should be developed so that anaemia can be confirmed rather than assumed.

Iron injection protocol. A second iron-dextran injection timed at weaning (rather than at 7 days of age) was identified as the more effective protocol for carrying at-risk pigs through the early post-weaning weeks, and is best targeted to the largest, fastest-growing pigs rather than given to every animal. Early haemoglobin and growth gains did not persist to slaughter.

Dietary strategy. A short-term (first week) low-crude-protein diet (approximately 19% CP) formulated to hold the SID Lys:CP ratio at 0.071 was developed as an antibiotic-free means of reducing PWD while maintaining growth; reducing CP further (ratio 0.059) cut diarrhoea most but gave no growth benefit. Beta-hydroxybutyrate (pen-side meter), plasma creatinine and the acute-phase proteins (MAP, CRP) were applied as markers of inappetence, protein turnover and inflammation.

Key methodological learning. The measurable benefit of both strategies depends strongly on pig age, growth rate and disease challenge; both worked best under high challenge and faded in older, high-health pigs. Low-protein diets should therefore be evaluated in younger, higher-challenge pigs for a definitive test. No protectable intellectual property was generated.

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11. Publications Arising

Langley, J. E., Plush, K. J., Chauhan, S. S., Pluske, J. R., Tritton, S., Dunshea, F. R., Cottrell, J. J. (2025). Lowering the Dietary Crude Protein Content Whilst Maintaining Standardized Ileal Digestible Lysine to Crude Protein Ratios Improves Growth and Reduces Diarrhoea in Weaner Pigs. *Agriculture*, 15, 1516. https://doi.org/10.3390/agriculture15141516

Langley, J. E., Plush, K. J., Chauhan, S. S., Pluske, J. R., Dunshea, F. R., Cottrell, J. J. (2025) Additional intramuscular iron injections tended to improve post-weaning growth when administered at weaning, but not at day seven of life. *Translational Animal Science*, 9, txaf133. https://doi.org/10.1093/tas/txaf133