

Article

Lowering the Dietary Crude Protein Content Whilst Maintaining Standardized Ileal Digestible Lysine to Crude Protein Ratios Improves Growth and Reduces Diarrhoea in Weaner Pigs

James E. Langley ^{1,*} , Kate J. Plush ² , Surinder S. Chauhan ¹ , John R. Pluske ¹, Sally Tritton ², Frank R. Dunshea ^{1,3}  and Jeremy J. Cottrell ¹ 

¹ School of Agriculture, Forestry and Eco Systems, Faculty of Science, The University of Melbourne, Parkville, VIC 3052, Australia; jpluske@unimelb.edu.au (J.R.P.)

² SunPork Group, Eagle Farm, QLD 4009, Australia

³ Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

* Correspondence: langleyj@student.unimelb.edu.au

Abstract

The use of lower crude protein (CP) diets immediately after weaning has long been associated with reduced post-weaning diarrhoea (PWD). However, failing to maintain an appropriate standardized ileal digestible lysine ratio (SID: Lys) may outweigh the benefits in improving PWD outcomes. In this experiment, 560 weaners were fed either a control diet (CON; 20.6% crude protein 1.34% standardized ileal digestible lysine, SID Lys: CP = 0.06), LH: a low crude protein, higher SID Lys: CP ratio diet (19.0% CP, 1.34% SID Lys, SID Lys: CP = 0.07), or LL: a low crude protein control SID Lys: CP ratio diet (18.7% CP, 1.1% SID Lys, SID Lys: CP = 0.05) for 1 week after weaning. Pigs were scored for the presence or absence of diarrhoea for 12 days after weaning. Blood samples were taken on days 5, 12 and 28 for inflammatory marker analyses and plasma creatinine analyses. Relative to the CON diet, there was a 42% reduction in the diarrhoea index in pigs fed the LH diet and a 63% reduction in those fed the LL diet ($p < 0.05$), but LH was significantly higher than LL. There tended to be a greater average daily gain in the LH diet in the first week post-wean ($p = 0.054$). In summary, lower crude protein diets, irrespective of Lys: CP ratio, were found to be effective at reducing PWD. Moreover, increasing the SID Lys: CP ratio by reducing the CP content of diets from 20.6 to 19% appeared to improve post-weaning growth.

Keywords: diarrhoea; growth; lysine; pig; protein; weaning



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1. Introduction

The weaning period is a stressful time for pigs and often results in a post-weaning growth check and in some circumstances, elevated mortality [1]. Factors such as changes in environment and diet, litter mixing, and low immunity all contribute to this malaise. A major aspect that contributes to this is the change in diet, from sow's milk to a solid cereal-based diet [2], and this can cause the proliferation of enterotoxigenic bacteria such as *Escherichia coli* (ETEC), a major pathogen associated with post-weaning diarrhoea (PWD) [3,4]. ETEC is highly transmissible, and once established on a farm, is difficult to eradicate with multiple environmental reservoirs, allowing infections to spread rapidly through immunologically naïve weaners [5]. Post-weaning diarrhoea is frequently observed in the first weeks following weaning, while the gut adapts from sow's milk to solid food, resulting in a short period of greatly reduced growth [6].

Reducing the dietary crude protein (CP) intake has proven effective in reducing the severity of PWD during the most at-risk phase of 5–12 days after weaning [7]. Therefore, lower crude protein diets offer an alternative to high levels of dietary zinc oxide in post-weaning diets as an alternative strategy to control PWD [8]. The consequence of low CP diets could therefore be a reduction in post-weaning growth, due to insufficient dietary lysine, protein, nitrogen or other essential amino acids [9]. Part of the mechanism by which low CP diets reduce PWD is by improvements in the microbiome, with reduced ETEC being observed [10]. The onset of PWD is thought to be associated with increased amounts of systemic inflammation in the body, a factor that has been shown to lead to reduction in growth rates [11], muscle wasting, ketosis and potentially the onset of failure to thrive syndrome in pigs [12]. Lower CP diets can maintain growth provided adequate concentrations of lysine (Lys) and other essential amino acids are maintained [13,14]. The current optimum ratio of standardized ileal digestible (SID) Lys: CP for post-weaned pigs is thought to be 0.06 [15].

This experiment examines the feeding strategy of lower CP diets in the Australian context. The Australian industry has a closed genetic herd for biosecurity reasons, creating a smaller genetic pool, whilst resulting in similar growth rates [16]. This work is required because Australian swine diets are heavily cereal grain-based, compared to corn-based in North America [17,18]. Therefore, optimizing the use of low crude protein diets could help reduce the incidence of enteric disease during weaning, and optimize gut health and function without additional use of antimicrobials [19]. Therefore, this experiment had two aims. Firstly, to quantify if reducing dietary crude protein reduces PWD even when fed for a short period of 7 days. Secondly, to quantify if increasing SID Lys: CP ratio from 0.06 to 0.07 increases post-weaning growth without increasing PWD occurrence in Australian weaner pigs.

2. Materials and Methods

This experiment was approved by the CHM Animal Ethics Committee (PP163/22) and all animal procedures were conducted in accordance with the Australian code for the care and use of animals for scientific purposes [20]. The experiment was conducted in 2023 on a commercial research piggery in Queensland, Australia.

This experiment comprised 39 pens (13 pens per treatment) of 14 mixed sex pigs divided into 3 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 \pm SD, PIC genetics) days of age into the nursery in 4 batches. Experimental diets (Table 1) were imposed for the first 7 days from weaning. Following this, all pigs were abruptly fed the CON diet for the remainder of the nursery period (3 weeks). Feed was provided ad libitum through a circular gravity feeder in each pen, and water was provided through 1 nipple drinker and 1 bowl drinker located at the back of each pen. For the duration of the experimental period, standard farm management occurred, including daily checking of pigs for health. The experimental nursery was an enclosed building, and pens were 1 m \times 2.8 m in area, giving an allowance of 0.2 m² per pig. A suspended bar heater was provided at the opposite end of the pen to the drinkers. The shed climate was controlled by 2 variable-speed roof-mounted dampened exhaust fans.

Table 1. Composition (%) and NIR spectroscopy analysis of treatment diets fed for the first 7 days after weaning. Lysine to CP ratios were confirmed by amino acid analysis (amino acid panel) (Symbio, Eight Mile Plains, QLD, Australia). CON 20.6% protein 0.06 SID lysine: CP LH 19.1% protein 0.07 SID lysine: CP, LL 18.7% protein 0.05 SID lysine: CP.

Feedstuff %	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 (oil removed)	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
Hilyses	2.00	2.00	2.00
Bioplus 2B 400 premix	0.08	0.08	0.08
Canola oil	2.55	3.20	3.00
Salt	0.20	0.20	0.20
ZINCO Plus	0.10	0.10	0.10
Betaine	0.10	0.10	0.10
DL Methionine	0.18	0.27	0.13
Lysine HCL	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
ROVABIO ADVANCE P 10%	0.05	0.05	0.05
ACTIVO	0.02	0.02	0.02
FORMI	0.40	0.40	0.40
LUCTAROM sweet apple flavour	0.03	0.03	0.03
Starter premix	0.02	0.02	0.02
Composition			
Crude Protein %	20.6	19.03	18.7
DE MJ/kg	14.8	14.8	14.8
SID lysine %	1.34	1.34	1.10
SID lysine %/MJ DE	0.09	0.09	0.07
SID lysine %/CP%	0.06	0.07	0.05

Starter premix provides per ton of complete diet: 10 MIU vitamin A, 2 MIU vitamin D3, 80 mg vitamin E, 3 gm vitamin K, 3 gm vitamin B1, 4 mg vitamin B2, 3 mg vitamin B6, 20 mg vitamin B12, 20 mg calcium pantothenate, 25 mg niacin, 100 mg biotin, 0.5 gm folic acid, 70 mg Fe (as S₀₄), 100 mg Zn (as S₀₄), 40 mg Mn (as oxide), 20 mg Cu (as S₀₄), 0.3 mg Se (as selenite), 0.2 mg Se (organic), 0.2 mg Cr (Picolinate), 0.75 mg Co (as picolinate), 1.5 mg I (as KI).

Pens were allocated to 1 of 3 diets: (1) control (CON; 20.6% CP, 1.34% SID Lys, SID Lys: CP ratio 0.06); (2) LH: low CP: high SID Lys: CP ratio (19.0 CP, 1.34% SID Lys, SID Lys: CP ratio 0.07); (3) LL; low CP: low SID Lys: CP ratio (18.7% CP, 1.1% SID Lys, SID Lys: CP ratio 0.05). 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 1). Wet chemistry was used to determine energy content, and an amino acid panel was used 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/ton) (ZINCO Plus; Jefe, Saint-Hyacinthe, Quebec, Canada). A water acidifier (Selko, Amersfoort, The Netherlands, 1% in water) was included for all pigs for days 1–7 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 the feed or water supply; then a preventative antibiotic (Oxytetracycline, 25 mg/L, International animal health products, Huntingwood, NSW, Australia) was included in the water supply on days 12–14 to reduce the incidence of any prolonged scouring, as PWD is most seen 5–12 days after weaning. Significant scouring after this period would likely present more crucial concerns to animal health and was therefore controlled with the in-water antibiotic. These standard management practices are commonplace in the Australian industry and the treatments imposed are designed to complement this existing regime. Dietary ingredients and nutritional information are shown in Table 1.

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.

On days 5, 12 and 28, blood samples were collected. Four pigs were randomly selected from each pen for blood sampling on day 5, and the same number of pigs were sampled at each time point ($n = 52$ per treatment). A blood sample was collected via jugular venipuncture into an EDTA vacutainer, labelled, and then stored on ice until plasma extraction. Blood samples were immediately chilled after collection and then centrifuged for ten minutes at 1000 g 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 [12].

Enzyme-linked immunosorbent assays (ELISAs) were used to measure the concentrations of Pig-MAP (Major acute phase protein; Acuvet, Zaragoza, Spain) and C-reactive protein (C-RP; ABCAM, Cambridge, UK). 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 [6] because there were insufficient cases of severe diarrhoea in this study, possibly due to the addition of the water acidifier. Pigs were observed daily from day 1 to 12 after weaning and scored based on the presence (1) or absence (0) of PWD in the pen. The DI was then calculated based on the following equation:

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

$$\text{Diarrhoea Index} = \frac{(\text{Number occurrences of diarrhoea observed in pen} * \text{number of pigs in pen})}{(\text{Number of days observed}) * 100} \quad (1)$$

Statistical analysis was undertaken using GenStat (v. 23, VSN International, Hemel Hempstead, UK) for DI and growth data and IBM SPSS (IBM Corp. Released 2023. IBM SPSS Statistics for Windows, Version 29.0.2.0, IBM Corp: Armonk, NY, USA) for all other analyses. GraphPad Prism (10.22, La Jolla, CA, USA) was used to create graphics. In total, 4 batches of pigs were used in the experiment, weaned into the nursery in weekly replicates. Treatments were randomly allocated across gender and batches to a pen. Pen was included as a random factor in data analysis. Data was tested for normality using a Shapiro-Wilk test to determine the statistical method. Growth data was found to be normally distributed

and was analyzed using a REML using individual pig weights and calculated average feed intake per pig calculated from pen feed intake. Creatinine concentrations were normally distributed and were analyzed using a two-way ANOVA using treatment and day in the model with pen as a random effect. ELISA results could not be transformed to a normal distribution and were analyzed using a non-parametric Kruskal-Wallis test using treatment as the model; means were then generated using a one-way ANOVA. The BHB concentrations were analyzed using negative binomial regression using day as the repeated measure. A p -value of <0.05 was considered significant, and a p -value < 0.1 was considered a trend in the interpretation of the data. Trends were defined as changes in a parameter in the same direction over time.

3. Results

3.1. Diarrhoea Index

There was a significant difference ($p < 0.05$) in all treatments from CON, with diet LL having a significantly lower DI (30.1) than LH 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 1).

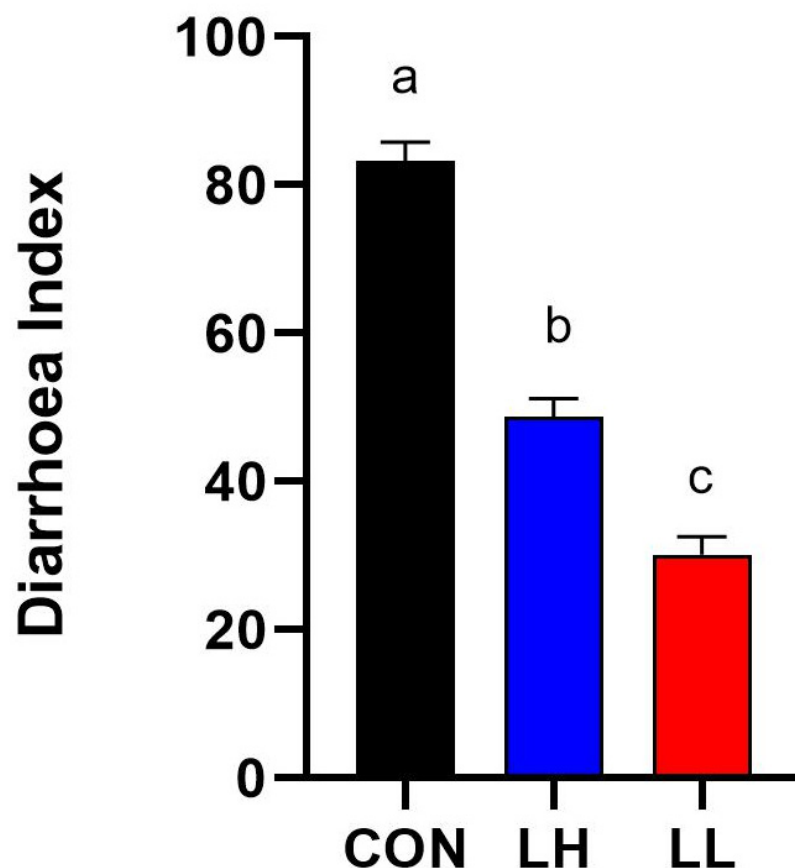


Figure 1. Diarrhoea index (mean \pm SED) of weaning pigs fed diets differing in CP content and SID lysine: CP ratio. A water acidifier—1% in water—was included during the observation period. CON 20.6% protein 0.06 SID lysine: CP. LH 19.1% protein 0.07 SID lysine: CP, LL 18.7% protein 0.05 SID lysine: CP. Data was analysed using ANOVA with pen as the statistical unit. ^{a-c} Indicates statistical significance ($p < 0.05$).

3.2. Blood and Plasma Analyses

There was a significant difference in plasma creatinine concentrations on day 5, with pigs in the LL treatment group having a lower ($p < 0.05$) creatinine concentration compared to pigs fed the other two diets (125 vs. 145 and 145 $\mu\text{mol/L}$ for diets CON and LH, respectively). There was also a significant difference between days ($p < 0.05$) with concentrations highest on day 5 and decreasing on days 12 and 28 (Table 2).

Table 2. Plasma creatinine concentrations (mean \pm SED) of pigs fed diets differing in CP content and SID CP: lysine ratio. CON 20.6% protein 0.06 SID lysine: CP LH 19.1% protein 0.07 SID lysine: CP, LL 18.7% protein 0.05 SID lysine: CP. The superscripts ^{a,b} 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.

Plasma Creatinine $\mu\text{mol/L}$	Diet			SED	<i>p</i> -Value		
	CON	LH	LL		Treatment	Day	T \times D
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

There was no difference ($p > 0.05$) between treatment groups at any of the blood sampling periods in regard to C-reactive protein (CRP) or major acute phase protein (MAP) (Figure 2).

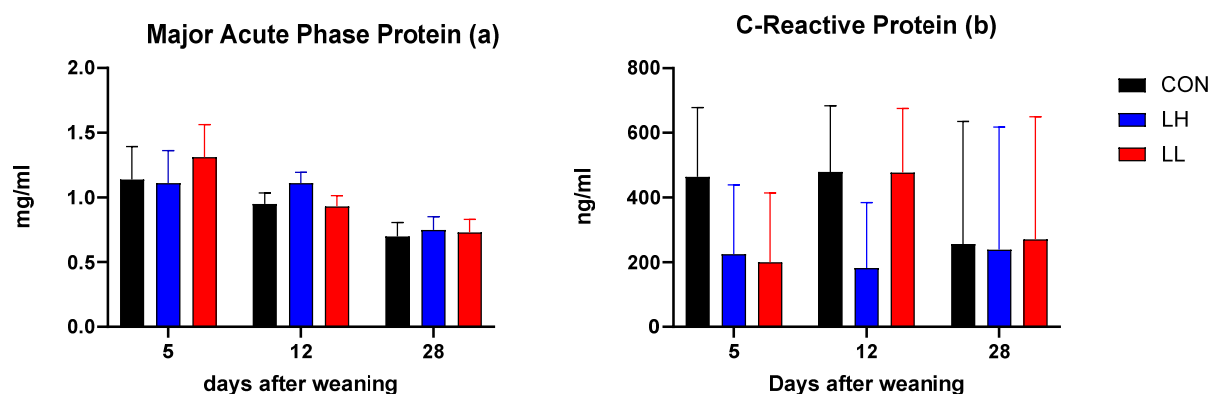


Figure 2. (a,b): (Mean \pm SED) for major acute phase protein and C-reactive protein concentrations from pigs fed diets with varying SID lys: CP ratios. CON 20.6% protein 0.06 SID lysine: CP LH 19.1% protein 0.07 SID Lys: CP, LL 18.7% protein 0.05 SID lysine: CP. Data were not normally distributed; means 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 3). 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) [12]. The BHB concentrations peaked on day 5 in all treatments after weaning and declined over time. There was a significant effect of time on BHB concentrations but no effect ($p > 0.05$) of treatment or an interaction effect.

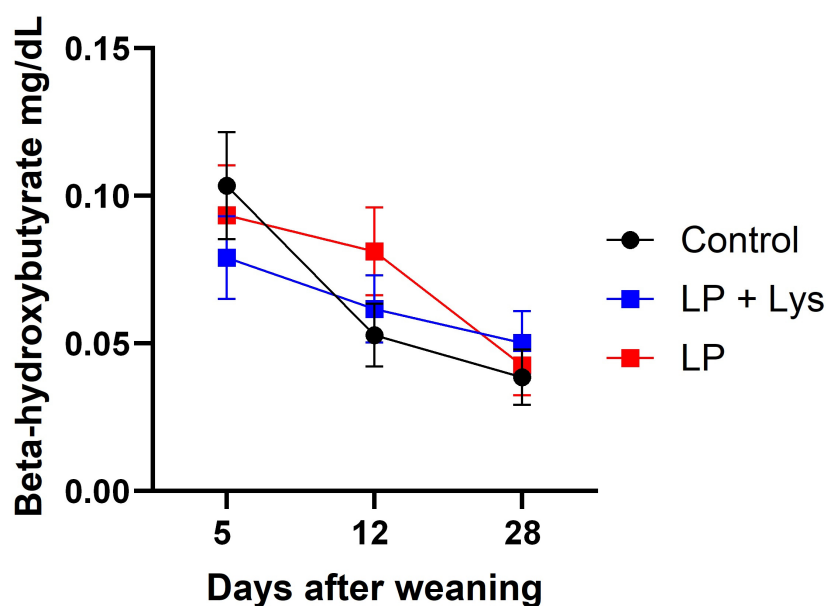


Figure 3. BHB concentrations (mean \pm SED) of pigs at 5, 12 and 28 days after weaning. CON 20.6% protein 0.06 SID lysine: CP LH 19.1% protein 0.07 SID lysine: CP, LL 18.7% protein 0.05 SID lysine: CP. Data was analyzed using negative binomial regression using day as the repeated measure.

3.3. Growth Performance

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 vs. 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 4 experimental weeks. A trend for improvement ($p = 0.090$) was also noted in feed conversion efficiency in week two in the CON group (Table 3). Removals were almost halved by the two LP treatments relative to the CON diet; however, this was not statistically significant.

Table 3. Growth data for pigs (mean \pm SED) fed diets differing in CP content and SID CP: lysine ratio from weaning to 4 weeks after weaning. Weight data was calculated with pig as the statistical unit and average pen feed intake was used to calculate other parameters. CON 20.6% protein 0.06 SID lysine: CP LH 19.1% protein 0.07 SID lysine: CP, LL 18.7% protein 0.05 SID lysine: CP. Superscripts ^{a,b} indicate statistical significance ($p > 0.05$).

	Diet			SED	<i>p</i> -Value
	CON	LH	LL		Diet
Weight (kg/pig)					
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 (g/pig)					
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

Table 3. Cont.

	Diet			SED	<i>p</i> -Value
	CON	LH	LL		Diet
Average Daily Feed Intake (g/pig)					
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	0.0530	0.28
2	0.68	0.59	0.64	0.0420	0.090
3	0.64	0.68	0.68	0.0360	0.55
4	0.73	0.72	0.72	0.0390	0.95
Growth Performance Day 0–28					
ADG (g/d.pig)	251	255	251	10.5	0.94
ADFI (g/d.pig)	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, <i>p</i> = 0.34					

4. Discussion

The experiment confirmed results observed elsewhere that low CP diets are effective in reducing PWD when used with accompanying standard farm management practices, such as water acidifiers and in-water antibiotics, and are therefore, a valuable management strategy in the Australian pig herd [21]. Both low CP diets reduced PWD, but the LH had significantly higher PWD than the LL, indicating that the provision of additional lysine in the diet may play a role in the occurrence of PWD. In this experiment, the low CP diets were used as an intervention for 7 days post-weaning. Although there was a trend for improved ADG while experimental diets were provided, no differences in BW or other growth parameters were observed after 1 week.

Compared to the CON diet, there was a 42% reduction in the DI of the LH diet and a 63% reduction in the LL diet group compared to CON-fed pigs. The removal of the LP diets resulted in the DI returning to CON levels after day 7 of the experiment. Therefore, the first aim of this experiment was achieved: low CP diets were successful in reducing PWD. The diets used in this experiment all contained 400 ppm zinc oxide and all pigs had a water acidifier during the initial weaning period. Normal zinc oxide inclusion can range from 1000 to 3000 ppm in the immediate post-weaning period, and is used as an anti-scouring agent [17]. In 2022, the European Union's restriction on zinc oxide inclusion to 150 ppm came into effect, increasing the need for alternative strategies for managing PWD, this limit is something that is of concern to Australian producers, as regulatory bodies could soon follow the European trend for limiting zinc inclusion. As weaners adapt from exclusively sow's milk to solid food, there is a greater proportion of undigested food, malabsorption and a richer environment providing nutrients for protein fermentation [4], resulting in the proliferation of the microbiome and reduced intestinal morphology [22]. Adequate protein is essential to maximise piglet growth, but if this is beyond the absorptive capacity of the weaner pig gut then this surplus protein not only represents wastage, but will be available to stimulate microbial overgrowth and exacerbate diarrhoea [19]. Therefore, lowering the CP during the post-weaning period not only prevents wastage, but prevents microbial

proliferation and overgrowth by improving the function of the gut and not providing nutrients for overgrowth [19], with as much, if not greater effect than high levels of zinc oxide when quantified by dry matter content of the stool [23]. This approach contrasts with the use of antimicrobials, not only as it addresses one of the root causes of PWD, but it does so without the emergence of antimicrobial resistance genes, which lead to a declining efficacy of antimicrobials and antibiotics [23]. This supports what others have found overseas, that low CP diets are a useful strategy to reduce PWD, even when used without antimicrobial concentrations of zinc oxide [19,24].

The immediate post-weaning period is a highly stressful time for pigs and can result in reduced caloric intake, muscle loss and ketosis [12]. It was noted that all experimental groups had positive weight gain in the first week post-weaning. Additionally, none of the pigs in the experiment had BHB levels that would indicate they were undergoing ketosis (>0.2 mmol/L, [12]), meaning that these pigs likely transitioned into the weaning period in good health despite the incidence of diarrhoea, possibly due to the average weaning age of 27 days. The high weaning age in this experiment may also be a factor when considering low zinc oxide usage, as low zinc anti-microbial strategies are effective in older weaners [25] and may be an important factor in the reductions in DI and trends for growth increases that were seen in this experiment. Regardless, there was a significant reduction in plasma creatinine in LL group during the first week after weaning. This reduction was transient, but corresponded to the peak reduction in DI, potentially reflecting a reduction in muscle catabolism due to improved health, as shown previously [26]. The significance of this observation is unclear as it did not translate to an improvement in ADG as per the LH group, possibly because lysine, or another unknown factor was limiting weight gain in the LL group. Therefore, a reduced creatinine concentration in the LL diet is likely associated with improvements in the DI of pigs after weaning but the mechanism is unclear, and this reduction did not translate to improved growth.

The optimum ratio of SID Lys: CP has previously been established [27] with the LH diet in our experiment being 0.07, as was shown to be optimal. This diet shows similar growth-promoting results achieving the second aim of the experiment. Increased SID Lys: CP ratios had a strong trend for increasing post-weaning growth. However, the LH treatment resulted in significantly more diarrhoea than the LL. The standard SID Lys: CP ratio in a lower crude protein diet is the most effective at reducing PWD occurrence (LL diet) but does not increase growth rate as seen in the LH diet. As the immediate post-weaning period is an expected period of inappetence and anorexia for pigs, body weight gain appeared to be separate from feed intake. However, the optimum SID Lys: CP ratio LH diet was the most successful at maximizing growth from a reduced feed intake in the first week after weaning, whilst still significantly reducing PWD compared to the CON diet.

5. Conclusions

In conclusion, feeding LP diets were successful for reducing PWD in pigs during the first week after weaning, irrespective of SID Lys: CP ratio when used in conjunction with a water acidifier. Furthermore, this experiment indicates that increasing SID Lys: CP ratios tended to improve post-weaning growth rates in more mature weaner pigs. This significant improvement in DI and trend for growth improvements are significant as they were observed with zinc oxide supplementation of 400 ppm, which is a non-antimicrobial concentration commonly used in Australian weaner pig diets and in the presence of a water acidifier, which is standard practice during weaning in Australia.

Author Contributions: Conceptualization and design K.J.P., J.R.P. and J.J.C.; methodology, J.J.C., K.J.P. and J.R.P.; Software J.E.L., K.J.P. and J.J.C.; Validation J.E.L., K.J.P. and J.J.C.; Formal analysis J.E.L., J.J.C. and K.J.P.; Investigation, J.E.L., S.T. and K.J.P.; Resources, K.J.P. and S.T.; data interpretation: J.J.C., K.J.P., J.E.L. and J.R.P.; draft manuscript: J.E.L. and J.J.C.; review and editing J.J.C., J.E.L., K.J.P., J.R.P. and S.S.C.; supervision F.R.D., J.J.C., J.R.P., S.S.C. and K.J.P.; funding acquisition J.J.C., K.J.P. and J.R.P. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of CHM Alliance (approval code PP163/22), 20 January 2023.

Data Availability Statement: Data is available on request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ADG	Average daily gain
BHB	Beta-Hydroxybutyrate
CP	Crude protein
C-RP	C-Reactive Protein
DE	Digestible energy
DI	Diarrhoea Index
EDTA	ethylenediaminetetraacetic acid
ETEC	Enterotoxigenic <i>Escherichia coli</i>
GIT	Gastrointestinal tract
LP	Low protein
MAP	Major acute-phase protein
PWD	Post-weaning diarrhoea
SID Lys	Standard ileal digestible lysine

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