Investigation and refinement of the utilisation of supplementary milk in sow-suckled litters, and the effect on creep intake with reference to lifetime performance, pre-weaning behaviour and gut development

Sarah Icely

B.Sc. (Hons), M.Res. Pig Production

Department of Agriculture and Environment, Harper Adams University, Newport,
Shropshire, TF10 8NB, UK

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Publications

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Abstract

Increasing sow prolificacy has necessitated strategies for rearing supernumerary piglets. Providing supplementary milk (SM) allows litters to remain intact, and can rear at least one extra piglet compared to the sow's functional teats. This project employed a novel recording system comprising an antenna and electronic ID (EID) to record individual piglet visits to the SM bowl, and so identify which piglets were utilising SM. Duration of visits was a good estimation of consumption, and piglets without a teat had the highest consumption throughout suckling, but were lighter at weaning, probably due to the low fat content of SM. Heavy birthweight piglets had higher SM consumption in the final week before weaning. When creep was provided, duration of visits was also a good indicator of consumption. Providing creep resulted in a higher pre-weaning supplementary feeding dry matter intake (DMI) than achieved using solely SM. The lightest birthweight piglets had the lowest creep consumption. Non-suckling piglets had the highest SM and creep consumption. Piglets with high pre-weaning creep consumption were lighter at weaning, but had lower FCR and higher ADG for the first week post-weaning. After d15 post-weaning, SM consumption had no effect on ADG, but creep consumption had a positive effect on ADG to slaughter. The glycine, serine, threonine metabolism pathway was enriched by creep intake, but there was no effect of SM on metabolic profile. Withdrawing SM provision one week before weaning increased creep intake. There was no difference in duodenal morphology between low and high creep consumers, but pigs fed a high specification (lysine) diet had higher villus height:crypt depth at d9 post-weaning. Diet quality (digestibility) had no effect on duodenal morphology. The dietary regime with the lowest cost/kg gain to slaughter was high specification, high quality for pigs with low creep consumption, but high specification, low quality for pigs with high creep consumption.

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1. Introduction

This project aimed to investigate the effect of provision of pre-weaning supplementary nutrition to piglets in large litter sizes on pre-weaning performance, and determine the effects of level of consumption on post-weaning performance and physiology. Although pre-weaning provision of creep feed has been an accepted management technique to prepare piglets for weaning for many years, the use of supplementary milk (SM) has become more popular.

1.1. Background

A significant challenge to the UK pig industry is rearing litters from highly prolific sows. Over the last 10 years for UK indoor herds, average litter size has risen from 12.16-14.56 pigs born/litter, with units in the top 10 % increasing from 13.51-15.92 piglets born/litter in this period (AHDB, 2024). This presents an opportunity for improved financial performance through a higher number of pigs sold/sow per year, but there are also management challenges.

Large litter sizes present a challenge to minimising pre-weaning mortality on two fronts: the effect of birth litter size on piglet birthweight, and the effect of rearing litter size (post-fostering) on sows' milk availability. Data from Harper Adams University (2018-2023) with prolific sows (average 17.0 total born) demonstrated that as total piglets/litter born increased from 10 to 20 piglets, average birthweight reduced from 1.55-1.25 kg. The number of light birthweight pigs (<0.90 kg) also increased from 0.6-3.3 piglets (5.4-17.4 % of piglets) per litter. This reinforces an earlier report of Quiniou *et al.* (2002), who found increasing litter size from 11-16 piglets reduced average birthweight from 1.59-1.26 kg, with an increase in light birthweight (<1.0 kg) piglets from 7-23 %. In both cases, light birthweight piglets also had higher mortality to weaning than their heavier littermates (Table 1).

Table 1: Piglet survival to weaning according to birthweight.

	Survival to weaning (%)	
Birthweight (kg)	Quiniou *et al*. (2002)	HAU 2018-2023
< 0.60	15	11
0.61-0.80	48	40
0.81-1.00	71	72
1.01-1.20	85	93
1.21-1.40	89	96
1.41-1.60	92	93
1.61-1.80	95	94
1.81-2.00	95	94
2.01-2.20	98	91
2.21-2.40	96	92
> 2.40	97	94

Sows now often farrow more pigs than they have functional teats, which traditionally would be fostered to a sow with a smaller litter size. However, once the majority of sows farrow supernumerary piglets, potential for fostering is limited, particularly in a batch farrowing system. These systems are popular due to the health benefits of having an all-in, all-out operation, but shunt-fostering is not possible and utilisation of nurse sows risks transfer of disease between batches, thereby eliminating the health advantage of the batch system. Even when farrowing weekly, using nurse sows and shunt-fostering are labour-intensive and disruptive to the litters involved, and may require additional accommodation. There may also be welfare implications, as Sørensen *et al.* (2016) found that nurse sows had higher risk of leg and udder lesions, and the foster litters were dirtier, with a tendency for a higher incidence of lameness. Without management intervention, supernumerary piglets are at risk of starvation: Kobek-Kjeldager *et al.* (2020a) reported that 56 % of pre-weaning mortality in litters of 17 piglets was due to starvation, and Gourley *et al.* (2020) found that pre-weaning mortality was positively associated with the number of piglets/functional teat. The process of starvation begins at birth, with competition for colostrum being higher in larger litters (Gourley *et al.*, 2020), but colostrum management was outside the scope of this project, and SM is not intended as a colostrum replacement.

The provision of supplementary milk (SM) is a method to support the sow in rearing large litter sizes by reducing the starvation of viable piglets. When SM is provided to all litters *ad libitum* (utilising a bowl in each crate connected to a milk supply) from birth, it can increase numbers weaned/sow compared to where SM is not available (Stewart *et al.,* 2010; Kobek-Kjeldager *et al.*, 2020a). When SM was provided from seven days post-farrowing, there was no difference in the number of piglets weaned compared to no provision, demonstrating the importance of early provision, with the average age at starvation of 7.1 days, compared to average age for all other causes of mortality of 0.97 days (Stewart *et al.,* 2010). Work by Douglas *et al.* (2014) suggested that supplementary milk may benefit low birthweight piglets in particular. They found that pigs of low birthweight (defined by the authors as <1.25 kg) in mixed-weight litters with access to supplementary milk had more uniform weights to 143 days old than those without access. Previous research has solely investigated differences between litters provided with SM or not, with no consideration of the variation in consumption both between- and within-litter. By characterising which piglets are high consumers of SM, and what effects level of consumption has on performance and physiology, management strategies for large litters may be optimised.

Feeding high quality creep diets is generally accepted as a method to improve adaptation to weaning, with most researchers concluding that familiarity with solid feed resulted in higher ADFI and consequently higher ADG (Bruininx *et al.*, 2002a; Sulabo *et al.*, 2010a; Muns and Magowan, 2018). Kuller *et al.* (2007) demonstrated *in vitro* improvement in nutrient absorption due to higher creep intake, but a reduction in FCR has not been consistently demonstrated. This is potentially due to the inaccuracy of previously available measuring techniques; as with SM, creep has been assessed on a provision/no provision basis, or quantification of individual intake has been limited to markers dying the faeces of consumers. These marker dyes may be subject to dilution by milk/water intake (Byrgesen *et al.* 2020) and so are of limited accuracy. Individual creep intake is likely to be highly variable, and so determining individual intake using a more accurate method may reveal physiological adaptations associated with pre-weaning creep intake. This could inform nutritional strategies to improve post-weaning performance, with targeted strategies based upon level of creep consumption.

There is little published work on combined supplementary milk and creep feed strategies to optimise pre-weaning gut development and subsequent performance of individual piglets. In 2009 Weng *et al.* reported that providing creep feed and water to artificially-reared pigs (on a dummy-sow) reduced milk consumption compared to pigs with access to milk alone. The timing of this reduction depended on the pre-set frequency of suckling, but was from as early as 8 days old when suckling was limited to 6-hour intervals, resulting in pigs being heavier when weaning at 29 days old occurred. This indicates that it may be unnecessary to provide supplementary milk for the entire duration of lactation if creep and water are also provided in naturally-suckled litters. Allowing all pigs access to supplementary milk for the entire suckling period results in high utilisation of an expensive product (milk powder costs >£ 2000/t).

1.2. Knowledge gaps

Previous research has been conducted either comparing sow rearing *vs* artificial rearing or the farrowing pen supplemented with milk from birth on a litter basis. Neither of these scenarios has been able to distinguish between different levels of intra-litter SM consumption within the natural litter environment, and so the effect of SM on individual performance and physiology is unknown. Individual identification of piglets' consumption levels could further improve understanding of the direct physiological effects of SM consumption. Most published studies investigating the provision of SM have used litter sizes <12 pigs/litter. However, as detailed previously, there has been a large increase in litter size born in the modern prolific breeds, so this project will focus upon large litter sizes from sows currently averaging born alive of >16 piglets, >14 weaned/sow. Large litters are likely to have higher variation in birthweight and higher proportion of light-birthweight (<0.90 kg) piglets, which have a greater risk of mortality (Quiniou *et al*., 2002, Quesnel *et al*., 2008), and may benefit most from SM provision (Douglas *et al.*, 2014). This was the focus of Study 1.

To date, quantification of individual pre-weaning creep intake has solely been achieved through the utilisation of in-feed markers to colour the faeces of consumers, which is potentially inaccurate due to dilution (Byrgesen *et al.*, 2020), and limits classification to eater/non-eater. By recording visits to the creep feeder using EID, quantification of intake with higher precision can be achieved, allowing more targeted physiological investigations to be performed. There is also a knowledge gap concerning the effects of supplementary milk provision on creep intake, and the combined effects of both on post-weaning performance and physiology. This was investigated in Study 2 using performance recording and metabolomic analysis of blood plasma.

Due to the difficulty experienced in quantifying individual creep consumption, there is little research concerning optimising post-weaning diets for pigs with differing levels of pre-weaning creep consumption. If pigs with high creep consumption are better adapted to weaning it may be possible to reduce the cost of the post-weaning feed regime by reducing diet quality. Given the current interest in reducing dietary CP levels for weaners to mitigate against post-weaning diarrhoea in the wake of the zinc oxide ban, CP level is also of interest. Study 3 utilised performance recording and analysis of duodenal histomorphology to investigate the effect of pre-weaning creep consumption on optimising post-weaning nutrition.

1.3. Hypotheses

- Provision of SM enables sows to rear supernumerary piglets
- Level of SM and creep consumption will vary according to competition, birthweight, and suckling position
- Piglets with higher creep consumption will be better adapted physiologically to weaning
- The optimal post-weaning feeding regime will vary depending on pre-weaning SM and creep consumption

2. Literature review

2.1. Litter size

Due to genetic selection sow prolificacy has improved, resulting in increasing numbers of piglets born per litter. The most recent figures from AHDB for UK indoor sows (period ending 31/03/23; AHDB, 2024) show that total piglets born averaged 14.56 per litter, with the top 10 % of units achieving 15.92 piglets born per litter. Ten years prior to this, average piglets born/litter was 12.16, with the top 10 % achieving 13.51 piglets born/litter. This progress has improved financial potential, but for highly prolific sows (top 10 % of records) there are management implications, with two aspects of litter size to consider: birth litter size and rearing litter size. The effects of birth litter size are due to growth and development of the foetus, and rearing litter size due to a combination of birthweight and competition for milk.

2.1.1. Effect of litter size on weight

2.1.1.1. Foetal growth

Litter size *in utero* is negatively correlated with average foetal weight from d56 of gestation (Lyderik *et al.*, 2023). This is related to nutrient supply to the foetuses: average placenta weight is unaffected by litter size (Smit *et al.*, 2015 and Vernunft *et al.*, 2018), but uterine blood flow (Père and Etienne, 2000) and glucose uptake per foetus are lower in larger litters, resulting in lower average birthweight (Père and Etienne, 2018). Piglets with lower birthweight will also have lower organ weights (Town *et al.*, 2004; Pardo *et al.*, 2013), and may have been subjected to the phenomenon of “brain-sparing”, whereby the brain develops at a normal rate at the expense of other organs such as the liver, leaving the light birthweight piglet under-developed physiologically (Town *et al.*, 2004). Muscle fibre number may also be adversely affected by high litter size; muscle fibre number is determined by d50 of gestation (Tse *et al*., 2008), and crowding *in utero* reduces muscle fibre number (Town *et al*., 2004; Tse *et al*., 2008; Bérard *et al*., 2010) due to competition for nutrients (Pardo *et al.*, 2013), with light birthweight piglets particularly affected (Rehfeldt and Kuhn, 2006; Bérard *et al*., 2010). Lower muscle fibre number may limit the potential for growth of light birthweight piglets (Rehfeldt et al., 2008).

2.1.1.2. Birthweight

Negative associations between litter size and birthweight have been consistently reported (e.g. Milligan *et al.*, 2002; Beaulieu *et al.*, 2014; Yang *et al.,* 2023), agreeing with observations *in utero*. An increase in within-litter variation of birthweight with increasing litter size has also been reported (Milligan *et al.*, 2002; Quiniou *et al.*, 2002; Quesnel *et al.*, 2008). Milligan *et al.* (2002) and Quiniou *et al.* (2002) both observed an increase in the proportion of light birthweight piglets as litter size increased, defining light birthweight as 300 g lighter than the litter mean (Milligan *et al.*, 2002) or <1kg (Quiniou *et al.*, 2002). Quesnel *et al.* (2008) reported average piglet birthweight reduced from 1.88-1.38 kg as litter size increased from ≤9 to ≥16 piglets/litter, and a concomitant increase in CV of birthweight from 15-24 %, caused by an increase in the proportion of both light- and heavy-birthweight piglets in relation to the mean. More recently Riddersholm *et al.* (2021), with litter size ranging from ≤14 to ≥29 piglets born/litter observed that average birthweight decreased by 19.5 g (gilts) or 21.7 g (sows, parity 2-9) for each piglet born. The proportion of light birthweight piglets (<1 kg birthweight) increased as litter size increased, with a decrease in the proportion of heavier birthweight piglets (>1.6 kg birthweight). They concluded that the negative effect of litter size on piglet birthweight was due to reduced uterine blood flow (UBF) per foetus.

2.1.1.3. Effect of birthweight on performance

Quiniou *et al.* (2002) reported a strong correlation of birthweight to weaning weight (r =0.57), as ADG pre-weaning increased with birthweight. They concluded this was due to the heavier piglets being more able to stimulate and drain the teat at suckling, as has been reported by Drake *et al*. (2008) and Huting *et al.* (2018). Quiniou *et al*. (2002) found the relationship between birthweight and weaning weight was not linear, with an increase in birthweight from 1.0-1.1 kg resulting in 400 g increase in weaning weight, but the increase in weaning weight was halved when birthweight increased from 2.0-2.1 kg. The non-linear effect was also reported by Fix *et al*. (2010b). In the study of Quiniou *et al*. (2002) this resulted in the lighter birthweight piglets having a greater relative bodyweight gain to weaning, with a seven-fold increase for 0.7 kg piglets compared to a four-fold increase for 2.0 kg piglets. In this way, some of the lighter birthweight piglets were able to reduce the weight deficit with the heavier ones, and even overtake them. Surek *et al*. (2014) reported that the lightest group of piglets had a higher relative gain than the heaviest group (144 g/kg birthweight vs 111 g/kg birthweight respectively). The effect of birthweight on performance also depends on the within-litter variation in birthweight. Douglas *et al.* (2014a) found that light birthweight piglets in uniform litters with other light birthweight piglets had higher ADG (271 g/d) to weaning than light birthweight piglets in mixed-weight (50 % each light and heavy) litters (252 g/d). This was also reported by Huting *et al.* (2017), and in addition they found that heavy birthweight pigs had lower ADG to weaning in uniform litters than in mixed-weight litters. Vande Pol *et al.* (2021a) included medium weight piglets, and reported similar results to those of Huting *et al.* (2017), with the addition that medium weight piglets had highest ADG in litters with light littermates. Huting *et al.* (2017) concluded that light birthweight piglets benefitted from reduced competition for milk in uniform litters, but sows milk yield was limiting for litters of heavy birthweight pigs. Vande Pol *et al.* (2021a) calculated that the fostering regime that would give most benefit to most piglets was mixed light, medium and heavy litters, due to the low proportion of light birthweight (0.5-1.0 kg) piglets. When considering performance of piglets with regards to birthweight, it is therefore important to consider the composition of the litter in which they are reared. Piglets are also able to increase or decrease their relative weight classes by the time of weaning, dependant on competition for milk (Huting *et al.*, 2018).

A further complication to the effect of birthweight is for those piglets classed as intrauterine growth restricted (IUGR). Paredes *et al.* (2012) defined these as having a birthweight >2.5 standard deviations (SD) below the mean weight, and exhibit little chance of catching up as development *in utero* is restricted. Lynegaard *et al.* (2020) reported a >2 kg lower weaning weight at 24 d for IUGR (0.75 kg birthweight) compared to normal piglets (1.38 kg birthweight). They also found that the characteristic brain-sparing during gestation was still evident at weaning, with IUGR piglets having higher brain:heart and brain:liver weight ratio. Piglets suffering from IUGR have disproportionately large heads, and Huting *et al.* (2018) found that the ratio of birthweight:cranial circumference was positively correlated with ADG. Recently Lynegaard *et al.* (2020), have suggested that IUGR piglets that survive follow the same growth curve as their normal littermates, with the same ADG once they reach the same weight. This was evidenced by a similarity in IGF1 levels between IUGR and normal piglets, and although IUGR piglets had lighter muscles, relative to body weight there was no difference at weaning compared to normal piglets.

Generally, post-weaning ADG has been shown to improve with increasing birthweight, resulting in a large difference in weight at slaughter, or age at slaughter if weight is constant (for example Quiniou *et al.*, 2002, Fix *et al.,* 2010b, Beaulieu *et al.,* 2014). Table 2 shows the difference in ADG from birth to slaughter from six studies comparing light and heavy birthweight pigs.

Table 2: Difference in ADG from birth to slaughter of pigs, dependant on birthweight recorded in six studies.

Author	Average birthweight for comparison (kg)	Difference in ADG birth-slaughter (g/d)	Difference in ADG birth-slaughter (%)
Rehfeldt and Kuhn (2006)	0.94 vs 1.80	49.7	8.6
Bérard *et al*. (2008)	1.41 vs 1.96	51.0	8.4
Rehfeldt *et al*. (2008)	1.08 vs 1.67	29.5	5.0
Alvarenga *et al*. (2013)	1.09 vs 1.93	46.1	7.0
Beaulieu *et al*. (2014)	1.04 vs 1.93	43.3	5.8
Douglas *et al*. (2014a)	1.14 vs 1.80	52.7	10.6

The difference in ADG may not be linear over time, with Dwyer *et al* (1993) finding no effect of birthweight after pigs reached 25 kg, and this was reinforced by the findings of Lynegaard *et al*. (2020). The effect of birthweight on post-weaning ADG may be related to the aforementioned effect of uterine crowding and light birthweight on muscle fibre number (Dwyer *et al.,* 1993; Rehfeldt and Kuhn, 2006; Bérard *et al.*, 2008).

2.1.1.4. Effect of birthweight on mortality

It is widely accepted that the highest piglet mortality is experienced pre-weaning rather than post-weaning, with the UK average most recently reported as 13.2 % of born alive (AHDB, 2024). An increase in mortality as birthweight reduces has been commonly observed (for example Quiniou *et al.*, 2002, Fix *et al.*, 2010a, Kobek-Kjeldager *et al*., 2020a), but it is not a linear relationship. Figure 1 shows the results of Quiniou *et al.* (2002) in comparison with data from the Harper Adams University pig herd, from 2018-2023 (re-stocked with hyperprolific sows in 2018), with a remarkable similarity despite the large difference in time.

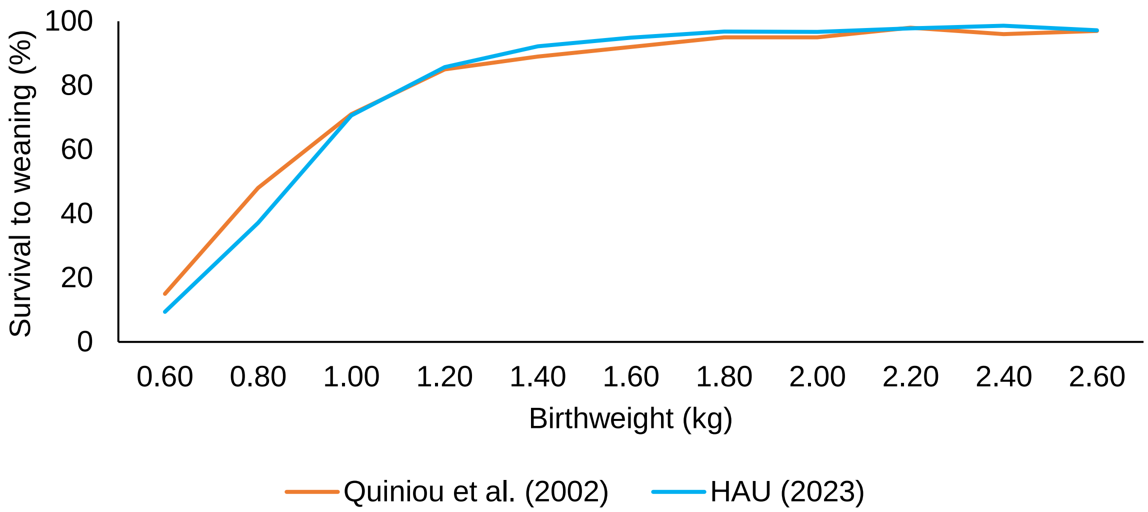

Figure 1: Survival of piglets to weaning, by birthweight (from Quiniou *et al*., 2002 and Harper Adams University, 2023).

In both cases, the flattening of the curve begins at approximately 1.20 kg birthweight, with piglets above this weight having >80 % survival to weaning. This is largely in agreement with Feldpausch *et al.* (2019), who reported a point of inflection of 1.11 kg, when combining pre-weaning mortality in European and American datasets, and Paredes *et al.* (2012), who found pigs >2 standard deviations (SD) lighter than the mean (<0.65 kg birthweight for HAU) had in excess of 80 % mortality to weaning. Feldpausch *et al.* (2019) found that piglets below their threshold of 1.11 kg had 5.9 times greater risk of death than those above the threshold, with a 34.4 % mortality rate. They concluded this was due to poor viability and locomotion, increased risk of hypothermia, and less competitive suckling resulting in lower colostrum intake. This agrees with Huting *et al.* (2018), who found that the highest pre-weaning mortality was seen in the lightest 25 % of piglets by birthweight, and the majority of deaths were recorded as being due to low piglet viability or starvation. Sufficient colostrum intake is the most important factor for survival, and this has been shown by Theil *et al.* (2014) and Declerck *et al*. (2017) to be positively associated with birthweight. As commented by Quiniou *et al*. (2002), the definition of a LBW piglet is important; Douglas *et al.* (2014a) reported no difference in mortality of LBW pigs in uniform or mixed-weight litters. They concluded it may be due to their definition of light birthweight, as they included any pig ≤ 1.25 kg birthweight, whereas other studies have had a lower threshold (for example: 1 kg; Quiniou *et al.*, 2002, Fix *et al.,* 2010a). This is reinforced in Figure 1 above, as a proportion of the lightweight piglets from Douglas *et al.* (2014a) would have been heavier than the weight at which birthweight has little effect on mortality. Their lowest average weight of LBW pigs was 1.11 kg, which is the threshold suggested by Feldpausch *et al.* (2019), at which point mortality reduces dramatically as birthweight increases.

For those LBW pigs that survive to weaning, there is evidence of higher mortality in the nursery period (to approximately 25-40 kg liveweight, dependant on management). In the study of Fix *et al.* (2010a), mortality increased post-weaning as birthweight reduced. There was no effect of birthweight on mortality in the finishing phase, but not all pigs were available for the study and only 18 % of ≤1 kg birthweight pigs survived to finisher placement (Fix *et al.,* 2010a). Huting *et al*. (2018) also reported that the lowest 25 % of pigs by weaning weight had higher mortality, and these were most likely to be the lightest at birth, due to the strong correlation between birth and weaning weight. Alvarenga *et al.* (2013) concluded that long-term effects on piglet mortality were most likely due to poor immunity and impaired digestive function, which stem from low colostrum intake and sub-optimal development *in utero*.

2.1.2. Effect of rearing litter size on pre-weaning performance

The effect of rearing litter size on pre-weaning performance is due to competition for resources.

2.1.2.1. Sow milk production

As reviewed by Zhang *et al.* (2018), the composition of sow's milk and colostrum with regards to fat, lactose and protein has remained largely consistent since the analysis performed by Klobasa *et al*. in 1987. The highest protein level is found immediately post-parturition, as the whey fraction contains the immunoglobulins of colostrum (Klobasa *et al.*, 1987; Figure 2).

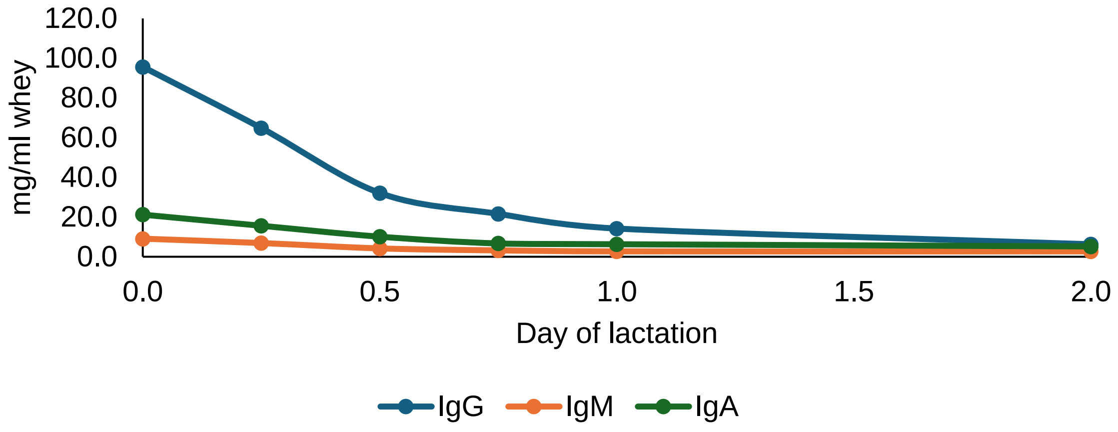

Figure 2: Reduction in immunoglobulin content of sow milk during the first two days of lactation, as measured by Klobasa et al. (1987).

Intake of 200 g/kg bodyweight of colostrum (Theil *et al.*, 2014) within the first 6-12 hours of life is critical as there is a dramatic reduction in both immunoglobulin levels and efficiency of absorption after this point, with the piglet's gut becoming impermeable to large molecules by

48 hours after birth (Holland, 1990). Colostral immunoglobulins are sourced from the sow's serum and will reflect diseases to which she has been exposed, with IgG being the most common immunoglobulin found in colostrum, as this is highest in serum (Gaskins and Kelley, 1995). It is well-established that the risk of pre-weaning mortality increases as colostrum consumption decreases (Declerck *et al.*, 2016; Gourley *et al.*, 2020). Colostrum yield is not related to litter size, but is very variable (reviewed by Quesnel *et al.*, 2012). The lack of effect of litter size on colostrum yield results in colostrum supply per piglet being lower in large litters, with Declerck *et al.* (2017) quantifying this as 9 g reduction in colostrum intake for every extra piglet born. Coupled with the findings of Tuchscherer *et al.* (2000) who reported a longer interval to first suckling as litter size increased, it is apparent that being born into a large litter presents an early challenge to the piglet. In litters where the number of piglets born exceeds the number of functional teats on the sow, split-suckling improves acquisition of colostrum-derived passive immunity (Baxter *et al.*, 2013). This ensures that all piglets get an opportunity to suckle before colostrum quality decreases.

During the first two days of lactation, as milk composition changes from colostrum to mature milk, protein content reduces and lactose and fat increase slightly (Klobasa *et al*.,1987; Figure 3).

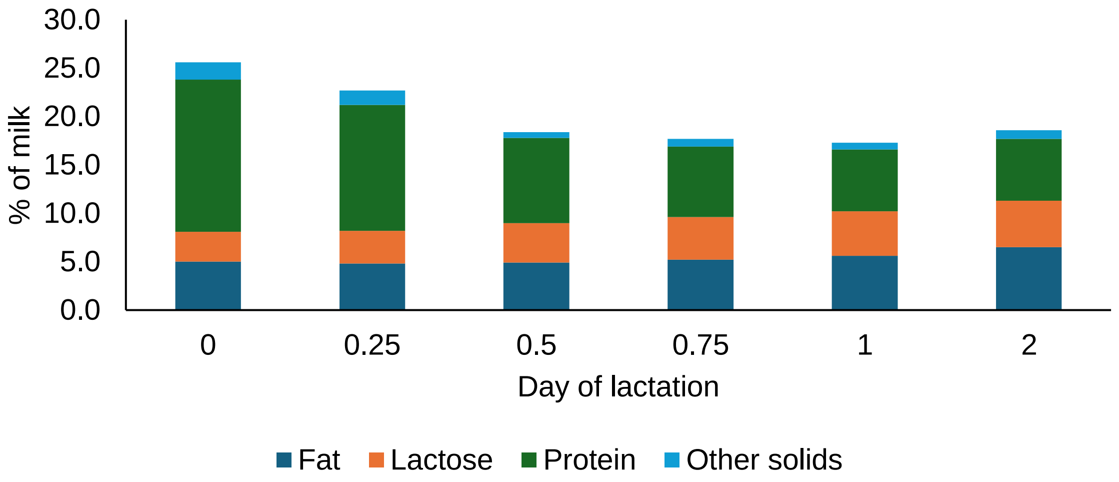

Figure 3: Change in composition of sow milk during the first two days of lactation (From Klobasa et al, 1987).

After d2, milk composition remains consistent throughout lactation, with values quoted by Zhang *et al.* (2018) of 7.5 % fat, 5 % lactose, 5 % protein. Manipulation of sow milk composition through the diet is possible: fat content, and fatty acid composition of the milk, may be modified by sow lactation diet (Rosero *et al.*, 2015), and protein content may be increased by additional dietary amino acids (Rezaei *et al.*, 2022) but a review of this is beyond the scope of this project.

The contribution to the gross energy of milk from each fraction is 65 % fat, 14 % lactose, 22 % protein. This leads to a low protein:energy ratio of 9.2-10.4 g/MJ GE (8.04-9.09 g SID protein/MJ ME; milk protein digestibility of 92 % reported by Mavromichalis *et al.* 2001a, and ME=GE*0.95 reported by Le Dividich and Sève, 2001) and so milk favours fat deposition over lean growth. Subcutaneous fat acts as thermal insulation and an energy store (Pluske *et al.,* 1995). It also results in the energy metabolism of the piglet being predominantly fat-based rather than carbohydrate-based pre-weaning (Metzler-Zebeli *et al.*, 2023), with glycerol converted to glycerol-3-phopsphate, which can enter gluconeogenesis (Shah *et al.,* 2022), and fatty acids converted to acetyl CoA before entering the citric acid cycle to be metabolised.

There are several other biologically important compounds found in sows' milk, including oligosaccharides (OS), growth factors and leucocytes (Zhang *et al.,* 2018). The OS found in porcine milk have protective and prebiotic functions, binding to pathogenic bacteria to prevent them binding to the intestinal epithelium, and acting as a fermentable energy source for beneficial bacteria of the intestinal microbiome (Tao *et al.,* 2010). The OS profile of porcine milk has some similarities with bovine milk, but is more similar to human milk, albeit with fewer isolated OS. Oligosaccharide profile changed as lactation progressed, with fucosylated-OS increasing, and sialylated-OS decreasing as a proportion of the total, which has not been reported in bovine milk (Mudd *et al*., 2016; Salcedo *et al.,* 2016). The reduction in sialylated-OS was also reported by Jahan *et al.* (2016), but they were unsure whether it was due to a reduction in secretory capacity, a dilution effect due to higher milk yield and lower DM, or a response for lower requirement from the piglet as their own sialic acid synthesis-capacity increases. IGF-1 stimulates intestinal development, increasing enterocyte proliferation, crypt depth and villus height (de Greeff *et al*., 2016) by activating the mechanistic target of rapamycin (mTOR) pathway (Yao *et al.*, 2008, 2011). Leucocytes in sow milk may assist in regulating the immune response (Moeser *et* al., 2017; Zhang *et al.* 2018). Antimicrobial proteins are also present in milk, with some genes coding for secretion of these being higher in mature milk than colostrum (Keel *et al.*, 2021), indicating an ongoing importance of sow's milk to the piglet's immune response. Antibodies present in sows' milk have a protective role against pathogenic bacteria in the intestinal lumen Kobek-Kjeldager *et al.* (2020b). Exosomes in milk may have a role in growth and immunity as they contain microRNAs (miRNA) that are thought to contribute to immunity and development. The exosomes protect their contents from enzymes and allow for uptake into cells by endocytosis (Zempleni *et al.,* 2017) Concentration of miRNA inside exosomes is higher than in milk, indicating they are an important delivery system. Humans are able to absorb miRNA from bovine milk, but there is still an argument as to whether miRNA is biologically active across species (Zempleni *et al*., 2017).

Availability of sow's milk to piglets is the main limiting factor in pre-weaning piglet growth (Auldist *et al.*, 1998), and depends upon both milk yield and the piglet's ability to access a functional teat. A milk yield of 14 kg/d is estimated to result in litter weight gain of 3.5 kg/d, requires the sow to consume 92.5 MJ NE/day (Whittemore *et al.*, 2003). Milk yield is determined by the amount of lactose synthesis within the Golgi apparatus of the epithelial tissue of the mammary gland (Boyd and Kensinger, 1998; Sadovnikova *et al.*, 2021), as the hydroxyl groups of lactose form hydrogen-bonds with water molecules (Sadovnikova *et al.*, 2021), resulting in a typical lactose content of 4.9 % (Boyd and Kensinger, 1998). The blood supply to the mammary gland directly provides 70 % of glucose required for lactose synthesis, with the remainder formed from gluconeogenesis of glycerol, lactate and surplus amino acids within the mammary gland (Boyd and Kensinger, 1998). Milk protein synthesis and mammary gland growth demand 90 % of absorbed amino acids, and 50 % of fatty acids are supplied by the blood (ingested and catabolised from body lipid), with the remainder synthesised in the mammary gland (except for linoleic acid which cannot be synthesised).

The efficiency of utilisation of ingested nutrients into milk production has been estimated between 78 % (Theil *et al.*, 2004) and 89 % (Boyd and Kensinger, 1998), and efficiency of catabolism of body lipid estimated as 85 % (Whittemore *et al.,* 2003) to 90 % (Theil *et al.*, 2004), with each kg of catabolised lipid contributing 33.4 MJ NE to the energy balance (Whittemore *et al.,* 2003). With the large demand from the mammary gland for ingested nutrients, sows typically lose weight and backfat during lactation (Feyera *et al.,* 2020). Strathe *et al.* (2017) reported a negative correlation between total born in the subsequent litter and sow weight and backfat loss, and a positive correlation between sow ADFI and litter ADG, and so although sow body tissue mobilisation contributes to milk yield, it is advantageous to maximise sow feed intake (Feyera *et al.,* 2020).

As milk yield is dependant on glucose, the sow's dietary energy intake is critical (Theil *et al.*, 2004). Feyera *et al.* (2020) observed greater sow feed intake and milk yield, and lower sow weight loss when sows were fed a high energy diet (13.8 MJ ME/kg) compared to low energy diet (12.1 MJ ME/kg). Body fat mobilisation was greatest in sows fed a low energy diet, and so although the efficiency of ingested energy:milk yield was greatest in the low energy sows, when tissue mobilisation was included, the high energy diet had higher energy efficiency of milk production from combined ingested and mobilised tissue energy source.

Dietary protein intake has a lesser effect on milk yield, but influences milk protein content (Hansen *et al.*, 2012*)*. Sinclair *et al.* (1999) reported no difference in milk yield between sows fed a diet containing 181 g/kg CP (9 g/kg lysine) and 140 g/kg CP (6 g/kg lysine), however the sows fed the higher CP diet had lower body weight loss, and higher milk protein content. More recently, Hojgaard *et al.* (2019) demonstrated that reducing standardised ileal digestible (SID) CP content of a sow lactation diet from 153-116 g/kg, but maintaining the level of lysine, methionine, cysteine, threonine and tryptophan with the use of crystalline amino acids had no effect on litter weight, average piglet weight, sow milk yield and sow weight and backfat loss. Protein content of sow milk was unaffected when SID CP level reduced from 153-126 g/kg (~5.20 %), but suffered a considerable reduction at 116 g/kg dietary SID CP (4.88 % milk protein content). Plasma urea N increased as CP level increased, which the authors concluded demonstrated a negative relationship between N-use efficiency and dietary CP content. By maintaining amino acid intake through the use of crystalline amino acids, the effect of reducing dietary SID CP on performance was eliminated in the range of 126-153 g/kg SID CP.

Feyera *et al.* (2020) reported that efficiency of piglet growth was higher in litters where sows were fed a high energy (13.8 MJ ME/kg) and SID lysine (10 g/kg) level compared to low energy (12.1 MJ ME/kg) and SID lysine (4.0 g/kg) level. Piglets from high energy/high lysine sows required 3.72 g milk for every 1 g of growth, whereas piglets from low energy/low lysine sows required 4.02 g milk for 1 g growth, indicating that milk composition (higher protein and lactose, lower fat) was optimal for piglet growth when sows were fed high energy/high lysine diets. The authors concluded that higher protein intake increases casein production by the sow, thereby increasing milk protein content. Increased casein secretory capacity was also demonstrated by Miao *et al.* (2019), when sows were supplemented with an additional 0.12 % tryptophan. Milk fat content is less important than protein content for piglet pre-weaning growth (Zhe *et al.*, 2023), but may also be influenced by the fat content of the diet. A meta-analysis conducted by Wang L *et al.* (2022), revealed that increasing sows' dietary fat content mainly resulted in higher milk fat, but it appears that fat source is an important factor. Zhe *et al.* (2023) reported that an additional 8 % of fat as coconut oil or sunflower oil increased milk fat output, but not when the supplement was fish oil or octanoic acid plus fish oil. Theil *et al.* (2004) utilised animal fat to increase dietary fat content (31 g/kg DM *vs* 113 g/kg DM), but this had no effect on milk fat content (averaged 37.4 % milk DM).

Typically, sow milk yield peaks at around week 3 of lactation (Hughes and Varley, 1980; Hansen *et al*., 2012; Feyera *et al.,* 2020), resulting in an undersupply of nutrients to the piglets if they are weaned later than this, as their demand increases throughout the suckling period (Feyera *et al.,* 2020). Figure 4 depicts sow milk yield during lactation from the studies of Vadmand *et al.* (2015), and Feyera *et al.* (2020), who both observed peak milk yield in week 3.

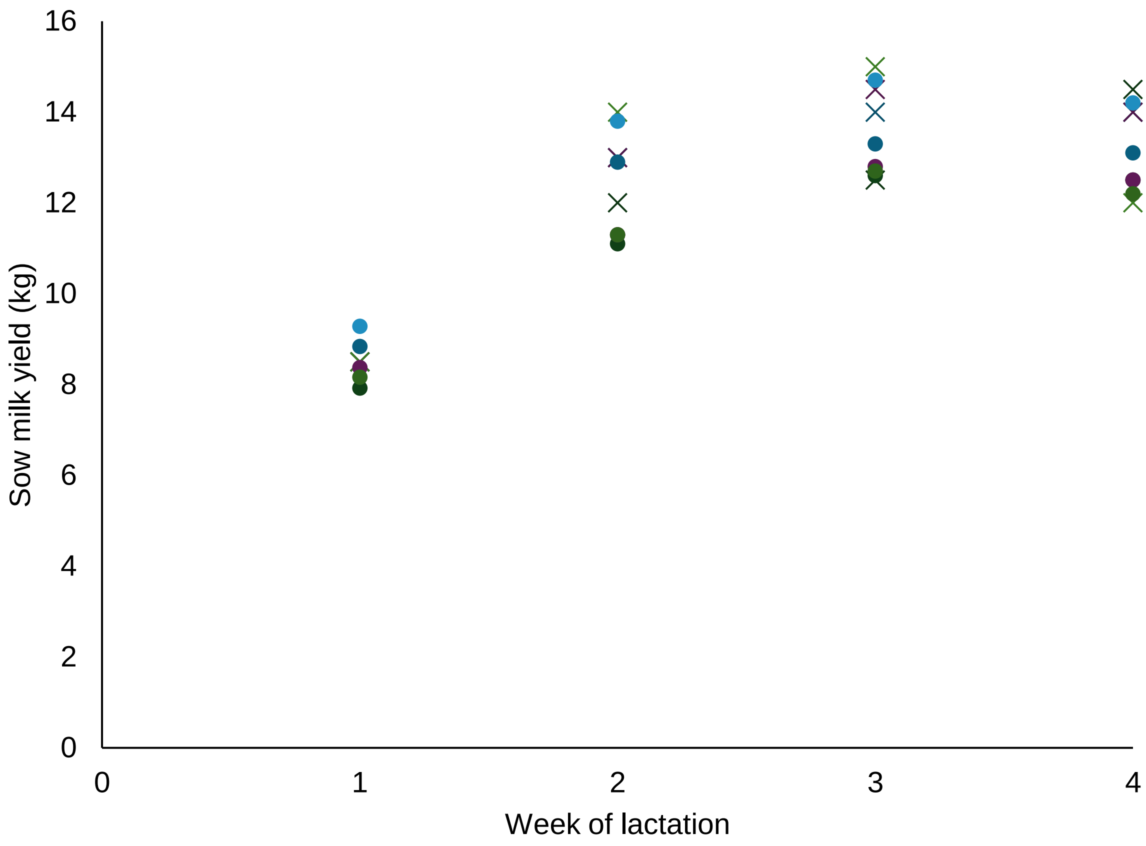

Figure 4: Sow milk yield throughout lactation, from the studies of Vadmand et al. (2015; crosses) and Feyera et al. (2020; circles).

Once the sow's maximum secretory capacity has been attained, assuming that feed intake remains constant, there will be no difference in body tissue mobilisation, regardless of litter size or competition increasing demand (Surek *et al.*, 2014). Litter size is positively associated with milk yield, as more functional teats are utilised, maximising secretory capacity (Vadmand *et al.,* 2015), but once the number of piglets exceeds the number of functional teats there is a greater risk of starvation (Andersen *et al.*, 2011; Kobek-Kjeldager *et al.*, 2020a; Vande Pol *et al.,* 2021b). With modern genotypes, the number of piglets born alive typically averages 14-17, whereas sows commonly have 13-15 functional teats and so having excess piglets is increasingly common (Vande Pol *et al.,* 2021b). Even when there are sufficient functional teats, there is a positive relationship between litter size and competition as more dominant piglets may monopolise multiple teats. Piglets without a teat, or that lose control of their teat, will die within 2-3 days, either through starvation or by being laid on as they spend more time in close proximity to the udder between suckling bouts, and are less active (Andersen *et al.*, 2011). Van de Pol *et al.* (2021) manipulated litter size in relation to functional teats, and observed that for sows that were fostered to have more piglets than functional teats, by d9 the higher mortality (17.9 % compared to 11.5 % for control litters where piglets=teats) resulted in there being no excess piglets. Among the piglets that survived to weaning, piglets in litters where there were fewer piglets than functional teats had higher weaning weight at all birthweights than those in control litters, or litters where there were initially excess piglets. This agrees with the earlier findings of Ocepek *et al.* (2017), who found that sows were unable to produce sufficient milk for the demands of piglets as litter size increased from 10-17 piglets, and this was indicated by longer intervals between suckling bouts. This demonstrates the need for nutritional supplementation of large litters to attain maximal pre-weaning growth, and minimise mortality

2.1.2.2. Suckling behaviour

Larger litter sizes may have adverse effects on suckling behaviour. Ocepek *et al.* (2017) reported that at d 10 of lactation the interval between successful sucklings increased as litter size increased (from 40 minutes with 10 piglets to 55 minutes with 17 piglets), even though piglets engaged in udder massage in an attempt to initiate further milk ejection. This is contradictory to earlier work by Valros *et al*. (2002), who reported no effect of litter size, and Auldist *et al.* (1998), who reported shorter suckling intervals in early lactation when litter size increased from 6-14 piglets. However, Auldist *et al*. (1998) did not consider successful and unsuccessful sucklings separately, and so it is possible that they over-estimated suckling (De Passillé and Rushen, 1989), and Valros *et al.* (2002) analysed the whole lactation, and so there may be litter age-based differences. Ocepek *et al.* (2017) found an increase in unsuccessful sucklings (milk ejection didn’t occur) as litter size increased, concluding that this may be due to sow discomfort due to increased aggression of piglets. A similar finding was made by Valros *et al.* (2002), as sow-terminated nursings increased through lactation, potentially due to discomfort of larger piglets suckling and thus increased physicality. However, Andersen *et al.* (2011) reported no effect of litter size on the proportion of failed nursings. Ocepek *et al.* (2017) also reported an increase in the number of piglets missing milk letdown as litter size increased, even though fostering was performed to ensure there were sufficient functional teats for the number of piglets, and a similar effect was observed by Andersen *et al.* (2011), although they did not account for functional teats. The higher mortality due to starvation observed by Andersen *et al.* (2011) resulted in the number of piglets weaned being unrelated to the number of piglets born.

Within-litter variation in weight also affects suckling behaviour, with Huting *et al.* (2017) reporting that piglets in uniform litters had a lower teat consistency (i.e. returning to the same teat pair each suckling) than piglets in mixed-weight litters. They also observed that weaning weight decreased from anterior to posterior teats, for both light and heavy birthweight piglets, agreeing with earlier work by Nielsen *et al.* (2001).

2.1.2.3. Piglet performance

Beaulieu *et al.* (2014) compared performance of different litter sizes to weaning with small (3-10 piglets), medium (11-13 piglets), large (14-19 piglets) litters. There were no differences in average weaning weight (6.81 kg, 6.45 kg, and 6.49 kg respectively) between small, medium and large litters, and so total litter weight weaned was highest in large litters. A similarity in weaning weight between pigs born in litters of ≤10 and ≥14 was reported by Bérard *et al.* (2008), but this was probably due to fostering occurring, reducing the difference in reared litter sizes. Commonly, there is a negative association between average weaning weight and rearing litter size, as reported by Douglas *et al*. (2013), who found that piglets from larger litters had lower ADG to weaning, and Kobek-Kjeldager *et al.* (2020a) who reported lighter mean weaning weight with larger litters, both indicating the limiting effect of the sow's milk yield on piglet growth. Feeding levels of the sows in Beaulieu *et al*. (2014) were not reported, and a lower feed allocation for sows with lower litter size could have reduced milk yield and so explain the lack of difference in average weaning weight between the three treatments.

2.1.2.4. Piglet mortality

Piglet mortality increases as litter size increases (van Rens *et al.,* 2005, Beaulieu *et al.,* 2014; Sanz-Fernández *et al.*, 2024), but this is probably not independent of piglet birthweight. As discussed previously, larger litter sizes have lower average birthweight and more light birthweight piglets, and lighter birthweight piglets are at greater risk of mortality. Larger litters typically have a longer duration of farrowing, with Feyera *et al.* (2018) calculating that farrowing duration increased by 12.6 minutes per extra piglet born. This results in the later piglets born having a lower chance of colostrum intake as quality decreases over time, and colostrum yield is independent of litter size (Quesnel *et al.*, 2012). Low colostrum intake is an accepted cause of high pre-weaning mortality, with Gourley *et al.* (2020) reporting that an intake of <302 g/piglet of colostrum resulted in only 52.1 % of piglets surviving to weaning. With a colostrum intake of 302-408 g/piglet, survival increased to 83.3 %, 91.6 % of piglets survived with a colostrum intake between 408-509 g/piglet, and 93.7 % survived when colostrum intake exceeded 509 g/piglet. These requirements were higher than previously reported due to the method of calculation, with prior methods under-estimating colostrum intake (Gourley *et al.*, 2020). Colostrum intake is correlated with birthweight, with Theil *et al*. (2014) estimating 200 g colostrum/kg birthweight, and so the intakes reported by Gourley *et al.* (2020) will have been influenced by birthweight.

Piglets are more at risk of starvation in larger litters due to insufficient teats and/or milk production of the sow (Andersen *et al.*, 2011). Despite high levels of mortality overall, Gourley *et al.* (2020) found that survival to weaning was higher in litters where there were <0.81 piglets/teat (76.8 % piglets survived), compared to 0.81-1.13 piglets/teat (71.1 % survived) and >1.13 piglets/teat (63.3 % survived). Piglet:teat ratio was negatively correlated with colostrum consumption. Kobek-Kjeldager *et al*. (2020a) also found that having insufficient teats/piglet resulted in higher mortality in litters of 17 piglets compared to 14 piglets (mortality 17.7 % *vs* 10.8 % respectively), and that 56 % of mortality in litters of 17 piglets was due to starvation, whereas this was 39 % in litters of 14 piglets. The number of functional teats of the sow did not differ between the two litter sizes, and number of piglets reared did not exceed the number of functional teats.

The increase in risk of starvation associated with large litter sizes has prompted interest in piglet pre-weaning nutrition.

2.2. Piglet pre-weaning supplementary nutrition

With modern hyperprolific sows the number of piglets born in a group often exceeds the number of functional teats in that group, presenting limited opportunity for fostering of excess piglets (Kobek-Kjeldager *et al.* 2020a). In these instances, additional support of the sow is required to fully realise the potential of larger litter sizes, and limit mortality through starvation. Due to large litter sizes having increased proportions of LBW piglets, and these being most at risk of mortality, it may be LBW piglets that benefit most from this additional support. Most milk replacers are based on cow milk which has higher lactose and lower fat and protein than sow milk (Pieper *et al*., 2016a) as shown in Table 3 from studies with published figures for protein, fat, and lactose content of the milk replacer.

Table 3: Comparison of milk replacer for artificial rearing/supplementation of piglets, with sow milk at d 14 of lactation.

	DM (g/kg)	Crude protein (g/kg DM)	Crude fat (g/kg DM)	Lactose (g/kg DM)
Sow milk¥	182	280	352	324
Weng *et al*. (2009)	290	270	262	380
Huygelen *et al*. (2012)*†	127	220	181	441
Miller *et al*. (2012)*	127	248	272	403
Theil and Jørgensen (2016)	127	235	257	327
Pieper *et al*. (2016a)*	127	226	200	460
Kobek-Kjeldager *et al.* (2020b)	143	234	163	436

¥At d 14 of lactation, from Klobasa *et al.* (1987). *DM unspecified by authors, but equal mixing rate to Thiel and Jørgensen (2016) of 150 g/l. †Calculated from reported as-fed specification.

In contrast to the ruminant sector where milk replacers are commonly used, there is a lack of research into sow milk replacers. The high fat content of sows' milk has so far been unable to be incorporated into piglet milk replacer products, due to the powder needing to be water soluble. An as-fed fat level of 6 % has been achieved in commercially-available lamb milk replacers (McCoard *et al.*, 2021), close to the 6.4 % fat content of sow milk reported by Klobasa *et al.* (1987), and so it seems likely that if provision of SM to piglets becomes more popular, feed manufacturers will be able to incorporate the processes such as adding emulsifiers that allow for a higher degree of fat solubility in water.The low fat content of sow milk replacer results in artificially-reared (AR) piglets getting a lower proportion of their energy from fat than suckled piglets do (Theil and Jørgensen, 2016). The performance of piglets with access to milk replacer may therefore differ compared to those solely receiving sow milk. Gut development may also be affected as sow milk contains hormones and growth factors that stimulate gut development and maturation in the suckling piglet (Darragh and Moughan, 1998), as mentioned previously. There are two commonly utilised methods of supplying milk replacer to piglets: artificial rearing (AR), and supplementary milk (SM) provision to a suckling litter.

The contribution of creep feed should also be considered, as this is an accepted method of preparing pigs for weaning (Sulabo *et al.*, 2010a; Kuller *et al.*, 2007).

2.2.1. Artificial rearing

A thorough review of AR is beyond the scope of this project, focussed as it is on SM provision to suckling litters, but it is of value to briefly review the reasoning for a preference of SM over AR.

Artificial rearing involves removing piglets from their sow after they have had the opportunity to suckle colostrum, and feeding milk replacer and creep until weaning. A whole litter may be removed, for the sow to become a nurse sow, or excess piglets from each litter may be removed. Performance compared to suckled piglets is variable, with Vergauwen *et al* (2017) reporting artificially-reared LBW (0.75-0.90 kg birthweight) and NBW (within 1 SD of mean) piglets to be heavier at d 19 than similar-birthweight suckled piglets. They found the AR piglets to have increased intestinal surface area at d19 and concluded that this may have improved their ability to absorb nutrients and therefore increased growth. Schmitt *et al.* (2019) found that AR piglets had lower ADG from d 0-weaning by 30 g/d. The authors concluded this reduction in growth rate was a direct result of the change in environment, diet and feed delivery, the stress of which caused an increase in diarrhoea. The reduction in ADG was despite higher frequency of visits to the milk bowl compared to suckling bouts of suckled piglets, and this was concluded to be due to feeding being interrupted by belly-nosing in the AR piglets, and a higher rate of displacement by other piglets at the milk cup. Other negative behaviours such as ear and tail manipulation were also increased in AR piglets. Rzezniczek *et al.* (2015) reported similar negative behaviours from AR piglets, and an increase in aggression over time.

Although AR allows excess piglets to be reared, performance is variable, and health and welfare may be compromised. There is also a requirement for additional space for the rearing pen, and extra time taken to move piglets.

2.2.2. Supplementary milk

Providing SM to litters *ad libitum* from birth allows litters to remain intact within the farrowing pen, right through to weaning with no fostering post 48 hours of birth. This therefore removes some of the negative behaviour and welfare impacts associated with AR.

2.2.2.1. Method of delivery

There are several SM delivery systems, ranging from simple manual filling of a trough or feeder with a small reservoir for each litter, to automatic systems where milk replacer is added manually to a tank and the SM is mixed for a whole day and continuously pumped around the system to a feeder in each pen, to the most modern systems where SM is mixed by the machine on demand, with cleaning cycles included. There is a negative association between cost of the system and the amount of labour required daily to provide SM to litters. Maintaining adequate supply of SM to litters is difficult when utilising manual feeding; Christensen and Huber (2021) capped SM provision to 3 L/litter per day due to the high labour input associated with filling the small reservoir on each feeder, and concluded that this may have limited any beneficial results, particularly when considering sow weight and P2 loss. Correa *et al*. (2023) also showed lower pre-weaning mortality in litters where there were more piglets than functional teats when SM was used, but only when it was fed *ad libitum* through an automatic system rather than manually added to a feeder. An objective of this project is to quantify the amount of SM utilised throughout the suckling period, and the variation in consumption between litters and piglets.

2.2.2.2. Mortality

Provision of SM has been shown to improve the number of pigs weaned per sow. Stewart *et al.* (2010) reported an increase in numbers weaned from 10.05 to 10.71 when SM was available from birth, with the increase in multiparous litters being greater than in litters reared by gilts (1.1 *vs* 0.4 extra pigs/litter respectively). Having SM available from seven days old did not reduce mortality or increase numbers weaned significantly. In 2002, Wolter *et al.* reported a tendency towards a higher number of piglets weaned/litter (11.4 *vs* 10.9) and lower mortality (5.2 % *vs* 9.4 %) when SM was available from 3 days post-farrowing, compared to when there was none provided. Both these experiments were conducted at a time when sows typically had 10-12 functional teats, rather than the 14-16 found in modern hyperprolific sows. Park *et al.* (2014) also showed a lower pre-weaning mortality from 10.3-5.6 % when SM was available, but this was not analysed statistically. None of these studies reported reasons for mortality, but Stewart *et al.* (2010) concluded that the reduction in mortality was due to additional nutritional support provided by SM, implying that starvation was reduced, and that this was required from birth to maximise effectiveness. This has been reinforced recently by the work of Kobek-Kjeldager *et al.* (2020a), who found that starvation was greatly reduced in litters of 17 piglets that had access to SM compared to those with no access, with a smaller reduction in litters of 14 piglets (Figure 5).

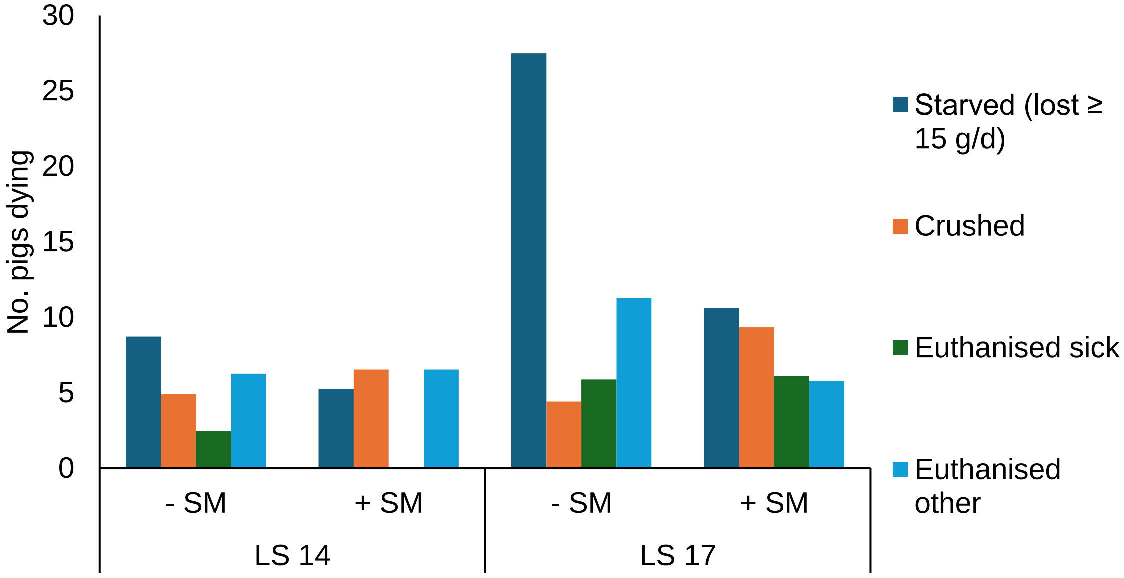

Figure 5: The effect of SM (+/-) and litter size (LS 14 =14 piglets, LS 17 =17 piglets) on cause of mortality (calculated from Kobek-Kjeldager et al., 2020a).

None of the litters of 14 piglets had more piglets than the sow had functional teats, but for the litters of 17 piglets, no sow had more than 16 teats. There was no analysis based upon litter size in relation to functional teat number. Correa *et al.* (2023) compared two different formulations and only one was effective at reducing mortality, demonstrating the variation in effectiveness of differing specifications. The milk replacer that reduced mortality had higher fat content, meaning that it was closer in composition to sow's milk. It also may have been more palatable, as the sweeteners were different, but SM intake was not measured (Correa *et al.*, 2023). The effect of SM on mortality does appear to be inconsistent, with several authors (Azain *et al.,* 1996; Miller *et al*., 2012; Pustal *et al*., 2015) reporting no effect of SM on mortality. All of these studies provided SM within 3d of birth, and so the lack of effect is unlikely to be due to delayed introduction. It could be due to limited litter size (and so piglets were at a lower risk of starvation), as litters in the study of Miller *et al*. (2012) only consisted of 10 piglets/sow, and Azain *et al.* (1996) utilised litters of 10-11 piglets, lower than the litters of Stewart *et al.* (2010, average 11.4 post-fostering), Wolter *et al.* (2002, average 12 post-fostering) and Kobek-Kjeldager *et al.* (2020a, 14 or 17 piglets), who all noted lower mortality in litters that were offered SM. Miller *et al.* (2012) concluded that pre-weaning survival is unlikely to be improved by supplemental milk as most mortality occurs before 3d of life, when piglets consume minimal SM. This may be the case with low litter sizes such as were utilised in the study of Miller *et al*. (2012), when the burden on the sow in later lactation is not so great, and there are likely to be sufficient functional teats for all piglets. Recently, it has been recognised that absolute number of piglets/litter is not as important as the number of piglets compared to functional teats of the sow (Kobek-Kjeldager *et al.*, 2020a). Number of piglets/litter post-fostering was not reported by Pustal *et al.* (2015), but in both treatments (with/without SM), number of piglets did not exceed the number of functional teats, and so an effect of supplementation reducing starvation would not be expected.

2.2.2.3. Performance

There is increasing evidence that SM provision improves survival of piglets in large litters, particularly where there are more piglets than functional teats (supernumerary piglets), but the effects of SM on performance are more variable. Wolter *et al.* (2002) reported that litters receiving SM were heavier at weaning (6.6 *vs* 5.7 kg), with the effect most apparent in litters of LBW piglets (average 1.3 kg birthweight); LBW supplemented piglets had similar weaning weights to HBW (average 1.8 kg) unsupplemented piglets. HBW litters also consumed more SM than LBW litters (14.28 *vs* 9.63 kg powder per litter over 3 weeks), attributed by Wolter *et al.* (2002) to HBW piglets consuming more milk per feed than LBW piglets. In agreement with Wolter *et al.* (2002), Azain *et al.* (1996), Dunshea *et al.* (1997) and Miller *et al.* (2012) reported higher average weaning weight when litters had access to SM, suggesting that sow milk production was insufficient to meet the requirements of the litter. However, Pustal *et al.* (2015) reported higher litter weaning weight due to a greater number of piglets weaned/litter with access to SM, but average piglet weight was similar between treatments. The higher litter weight in this study cannot be solely attributed to SM, as the litters were also offered creep feed from 7 d old, with those that received SM having a higher creep intake than litters without SM. Correa *et al.* (2023) and Luo *et al.* (2022) also found that average piglet weaning weight was unaffected by provision of SM. Christensen and Huber (2021) reported that piglets supplied with SM were heavier at weaning than those offered powdered milk replacer, commercial creep or no supplementary feeding, with a higher supplementary DM intake, and higher proportion of piglets consuming supplementary feeding from introduction at d9 to weaning on d21.

Kobek-Kjeldager *et al.* (2020a) found that the effect of SM provision on piglet growth depended upon litter size, with SM having no effect on litter weight or average piglet weight weaned with 14 piglets/litter. When litter size was 17, litters without SM access had the lowest average piglet weights, and lowest litter weaning weight despite weaning over one pig/litter more than the litters of 14 piglets, indicating that the sow’s milk supply was limiting piglet growth. The litters of 17 piglets with SM had the highest litter weaning weight (due to higher number weaned), and a similar average piglet weaning weight to litters of 14 with SM. They concluded that the lack of effect of SM on weaning weight with 14 piglets indicated that piglets would rather suckle the sow if teats were available. However, they did not measure SM intake per litter and so it is not possible to verify this. The effect of SM provision on growth is also influenced by milk production of the sow. Several studies have compared the effect of SM on performance between hot and cold seasons, and found that as sow feed intake is limited during periods of high ambient temperature (above 25 °C), litter SM consumption increased (Azain *et al.*, 1996; Miller *et al.*, 2012), resulting in higher weaning weights for piglets with access to SM (Azain *et al.*, 1996; Miller *et al.*, 2012; Park *et al.*, 2014). However, Correa *et al.* (2023) found no effect of SM provision on growth of piglets during a study when ambient temperature exceeded 26 °C. They concluded this may be due to SM being withdrawn one week prior to weaning.

In contrast to the effect of SM on mortality, where SM needs to be available from birth, a relatively short period of supplementation may be sufficient to notice an increase in weaning weight, with van Oostrum *et al.* (2016) finding that providing SM for five days prior to weaning resulted in higher weaning weight by 0.2 kg, which increased to 0.8-1.0 kg by four weeks post-weaning, and a lower FCR. Wang *et al.* (2005) also investigated the effect of SM later in lactation, assessing the effect of SM access from 22 d old (13 days pre-weaning), and found that piglets were 1.3 kg heavier at weaning where SM was available. Both these studies provided SM at the point where sows milk yield is thought to be limiting to piglets' growth.

Post-weaning ADG when SM ceases at weaning seems to be unaffected by SM consumption pre-weaning, with Wolter *et al.* (2002), Miller *et al.* (2012) and van Oostrum *et al.* (2016) reporting no effect of pre-weaning SM provision on ADG post-weaning, despite differences in weaning weight. Douglas *et al.* (2014a) found that although provision of SM from d1-28 of age (weaning) had no effect on ADG or weight at any point to slaughter (143 d), LBW piglets (< 1.25 kg birthweight) in mixed-weight litters with access to SM had more uniform weights to 143 days old than those without access (an interaction of supplementation and birthweight in mixed weight litters). In this way SM improved uniformity of light birthweight pigs at 143 d old. A source of variation was removed from post-weaning study, with piglets <4 kg weaning weight not being eligible. This was only five piglets, and four were from unsupplemented litters, which would have increased the CV of weight for unsupplemented pigs further. Park *et al.* (2014) also removed very small piglets at weaning (unspecified weight or number of piglets), but concluded that the 54 g/d higher ADG recorded by piglets who had access to SM pre-weaning was due to the very small piglets being discarded. It is therefore important to consider the performance of all piglets when assessing lifetime performance as the lightest at weaning are likely to be lightest at slaughter, requiring extra time to finish, or reducing margin (Fix *et al.,* 2010b, Beaulieu *et al.,* 2014).

An indirect effect of SM provision is any impact on the sow, but effects are inconsistent. Stewart *et al.* (2010) found that sow weight loss was reduced by 6.7 kg when SM was available to the litter from birth, but other researchers (Dunshea *et al.*, 1997; Wolter *et al.,* 2002; Pustal *et al.*, 2015) have reported no difference in sow weight or P2 loss at weaning.

2.2.2.4. Physiology

There is a small amount of research regarding the effect of providing SM to suckling piglets on their physiology. Wang *et al.* (2005) reported no effect of providing SM to suckling piglets on activity of lactase, sucrase or maltase four days pre-weaning or three, seven- or 17-days post-weaning. de Greeff *et al.* (2016) found that suckling piglets supplemented with nutrient-dense milk replacer (200 g/kg CP and 200 g/kg fat) had heavier small intestine, and tended to have higher expression of the gene for IGF-1 in the jejunal mucosa. Higher expression of IGF-1 indicating higher capacity for growth was also reported by De Vos *et al.* (2013) in AR compared to sow-suckled piglets, and they concluded this was due to higher protein intake.

Kobek-Kjeldager *et al.* (2021a) reported no effect of SM provision on piglet body composition (water, fat protein, ash) at 28 days old. When they analysed composition according to nutrition source based on direct observation, they found that piglets with low intake (missing more than one suckling bout and having lower than median SM visits) had higher water and ash content and lower fat content at 28 days old, but there was no difference in body composition between piglets that always suckled the sow, suckled and used the milk bowl, or had high usage of SM combined with missing more than one suckling bout. They concluded that the low fat retention from piglets with low suckling success combined with low SM usage was due to low fat intake and lower *de novo* synthesis, due to lower energy intake. They also speculated that increasing SM usage combined with reduced suckling success would lead to piglets having similar body composition as those that were artificially reared, with Theil and Jørgensen (2016) reporting lower body fat percentage in AR piglets due to the lower fat content of milk replacer compared to sows' milk. This makes it relevant to consider differences in physiology of AR and sow-suckled piglets.

Several studies have assessed the effect of artificial rearing on piglet physiology, but some may have been affected by differing levels of feed intake (De Vos *et al.*, 2013; Amdi *et al.*, 2022). van den Borne *et al.* (2007) found that piglets fed milk replacer had a higher pre-prandial volume of pancreatic secretion than those suckling the sow, but there was no difference in post-prandial secretion volume, or the change in volume. Protein or trypsin level of pancreatic secretion were unaffected by diet, but varied significantly between pigs and were positively correlated with ADG, and negatively correlated with FCR. Cholecystokinin (CCK) levels were unaffected by diet. They concluded that the lack of effect of diet on pancreatic protein and trypsin secretion reflected that bioactive peptides in sows' milk do not suppress pancreatic enzyme secretion, and that the higher volume of pancreatic secretion may be due to the lack of antimicrobial factors (lysozyme, lactoperoxidase, lactoferrin) in the milk replacer compared to sows' milk. Amdi *et al.* (2022) reported that enzyme activity differed between the AR and sow-suckled piglets: of the carbohydrases, sucrase activity was higher throughout the small intestine, lactase activity was higher in the medial and distal regions, and maltase activity was higher in the distal small intestine in sow-reared piglets compared to AR. For the peptidases, aminopeptidase A was higher throughout the small intestine, aminopeptidase N was higher in the distal region, and dipeptidyl peptidase IV was higher in the distal region in sow-reared compared to AR piglets. The authors concluded that higher enzyme activity indicated increased maturation of the gut, and was probably due to higher DMI as the AR piglets were restrict-fed to avoid diarrhoea.

Amdi *et al.* (2022) found no difference in intestinal morphology at 24 or 25 days old between AR and sow-suckled piglets, in contrast to Huygelen *et al*. (2012) who reported shorter villi in AR piglets at d10. This difference may have been an effect of time of sampling, as Vergauwen *et al.* (2017) observed that villus atrophy occurred immediately post-separation from the sow, but that villus height recovered by 19 days of age, and absorptive surface area was then higher in AR piglets. They also demonstrated that there was a redox imbalance during the initial period post-separation with a higher glutathione disulphide:glutathione (GSSG:GSH) ratio in AR piglets. The tight junction proteins claudin and occludin have been shown to be differentially expressed between AR and sow-suckled piglets, with Vergauwen *et al.* (2017) showing both to be higher in AR pigs, and Huygelen *et al*. (2012) reporting an increase in occludin levels in AR piglets, both of which indicate a higher barrier function of the intestine.

All these studies regarding the effect of SM on mortality, performance and physiology lack individual (or even litter) quantification of SM intake, and merely analyse provision or none, with intake likely to be highly variable within- and between litters. The proportion of piglets consuming SM, and the level of consumption of individual piglets is yet to be assessed and characterised. Assessing consumption at the individual piglet level using electronic ID (EID) may improve understanding of the effect of SM provision on performance and physiology. There is a lack of research on the effect of SM on creep consumption, and the combined effect of both on piglet performance.

2.2.3. Creep feeding

2.2.3.1. Creep feed composition

Pre-weaning creep diets are typically high quality (often defined as high complexity due to the greater variety of ingredients utilised compared to grower/finisher diets which are raw cereal and soya-based). They comprise a high proportion of highly digestible ingredients such as milk products (whey, lactose, skimmed milk powder), fishmeal and processed (finely ground or cooked) cereals, to mirror the initial post-weaning diets (Muns and Magowan, 2018; Byrgesen *et al.*, 2021). Creep diets are high in DE and CP (16.5 MJ DE/kg and 22.5 % CP in Muns and Magowan, 2018; 16.4 MJ DE/kg and 19.1 % CP in Byrgesen *et al.*, 2021). Such nutritional density is required due to the small quantities consumed. Digestibility of creep in the pre-weaning piglet is not assessed due to the majority of the piglets' nutritional intake being provided by the sow, but digestibility of high-quality diets immediately post-weaning is typically higher than that of low-quality diets. Digestibility of CP in casein-based weaner pig diets was consistently higher than soyabean meal-based diets, particularly in the first 14 days post-weaning (casein CP digestibility 0.50 at day 7 and 0.79 day 14; soyabean meal CP digestibility 0.15 at day 7 and 0.51 at day 14), in a study by Engelsmann *et al.* (2022). They did not report on performance parameters. Kim *et al.* (2002) reported that apparent digestibility of DM, N and gross energy at day 9 post-weaning was highest in complex diets (included lactose, spray-dried plasma and blood meal, spray dried wheat gluten, higher surface area due to smaller particle size) compared to simple diets (higher corn content). Pigs fed the complex diets had higher ADG and ADFI from weaning to day 10 and higher ADG and FCR overall from weaning to day 24.

Pajor *et al.* (2002) reported that piglets fed a high complexity diet had 52 % higher feed intake pre-weaning than piglets fed a simple diet, and that this effect continued for 20 days post-weaning. Heo *et al.* (2018) also reported greater feed intake pre-weaning in piglets fed a complex creep compared to simpler weaner diet, or sow feed, and this resulted in greater ADG for the final week pre-weaning for piglets fed creep. Pajor *et al.* (2002) found that piglets fed the high complexity diet also lost less weight immediately post-weaning, although whether this was due to the pre-weaning creep diet or post-weaning starter feed was unable to be determined. In contrast, Collins *et al.* (2013) reported that feed disappearance per litter was greater from d19-29 when piglets were offered a simple diet consisting of wheat, peas and soyabean meal, athough there was no difference in the proportions of piglets classified as good-, moderate-, small- or non-eaters. There was no difference in weaning weight, but pigs offered the simple diet pre-weaning also had greater ADFI, ADG and gain:feed for the first five days post-weaning. The authors offered no explanation for the improved post-weaning performance of pigs fed the simple diet pre-weaning, but the nutrient composition was slightly different, with higher CP, fat, and fibre for the simple diet. Badaras *et al.* (2022) reported greater pre-weaning weight gain in piglets fed a creep diet with additional sugar beet pulp, compared to the basal creep diet, and they attributed this to the greater fibre content acting as a prebiotic, altering the gut microbiome. Fibre is fermented into short-chain fatty acids (SCFAs) by microbes, providing an additional energy source, and reducing pH to favour beneficial bacteria such as *Lactobacillus sp.* (Badaras *et al*., 2022). Heo *et al.* (2018) found that piglets offered weaner feed pre-weaning had greater ADFI and ADG from weaning-d14 post-weaning than those offered creep feed, and concluded this may have been due to the longer duodenal villi observed in the piglets fed the weaner diet.

These varying effects on intake and performance regarding creep composition reinforce the importance of consistency throughout the experimental program.

2.2.3.2. Performance

It is now recognised that to assess the effect of creep consumption on performance and physiological adaptation, consumption should be assessed at the individual piglet level (e.g. Sulabo *et al.*, 2010a; Huting *et al*., 2017; Muns and Magowan, 2018), and this typically involves the use of indigestible markers to colour the faeces (e.g. chromic oxide, indigo carmine), and observation of defecation. Most studies have found that higher creep consumption doesn’t improve ADG pre-weaning, which Sulabo *et al.* (2010a) attributed to the relatively low levels of consumption. High creep consumers (compared to low consumers) have been reported to have lower weaning weights (Huting *et al*., 2017; Middelkoop *et al.*, 2019). This may be due to the high consumers having suffered a restriction in sows milk intake by fostering protocol (Huting *et al*., 2017), and sow feeding regime (Middelkoop *et al.*, 2019). Middelkoop *et al.* (2019) considered that energy intake from sows’ milk was the most important stimulus of creep consumption, and Collins *et al.* (2013) concluded that piglets classified as non-eaters had similar pre-weaning ADG to eaters because their sow milk intact was sufficient.

Greater creep intake pre-weaning has a positive effect on post-weaning performance, with most researchers reporting higher ADFI immediately post-weaning. Muns and Magowan (2018) compared post-weaning performance of piglets classified as eaters or moderate eaters with those not offered creep. The eaters/moderate eaters had higher ADFI in the first week post-weaning (205 g/d *vs* 174 g/d), but this did not translate to any significant improvement in ADG, although ADG was 15 g/d higher for creep consumers. They concluded that this was due to the increase in ADFI being too small, but that higher ADFI indicated better welfare due to diet familiarity. This was also concluded by Bruininx *et al.*

(2002a), as eaters had a greater proportion of visits to the feeder that resulted in consumption, rather than exploration, than non-eaters. Huting *et al*. (2017) found that non-consumers were heaviest at weaning, but that high consumers had higher ADG post-weaning, and so by 61 days of age piglet weights were similar. Feed intake was not recorded in this study, and so it is unknown whether the higher ADG was because of higher ADFI or lower FCR, and the piglets were not weighed more frequently to determine when the high consumers caught up with the non-consumers. Sulabo *et al*. (2010a) also found that non-eaters were heavier at weaning than eaters, but by day 3 post-weaning there was no difference in weights, due to higher ADFI and ADG (but no difference in FCR) of the eaters. Average daily gain and ADFI tended to be higher in the eaters to d7 and d14 respectively, but average piglet weights were similar until d28 post-weaning when the study finished. Bruininx *et al.* (2002a) used individual feeding recording post-weaning, and reported that piglets classified as eaters of creep pre-weaning recommenced feeding more rapidly post-weaning, with 50 % of piglets resuming eating within four hours for eaters. For non-eaters this threshold was reached at 6.7 hours post-weaning, and 6.9 hours for piglets that were not offered creep feed. Reduced latency to feeding was also reported by Kobek-Kjeldager *et al.* (2021b) in piglets with access to supplemental feed pre-weaning. For the study of Bruininx *et al.* (2002a) this lead to higher ADFI and ADG in the first eight days post-weaning for eaters than non-eaters, or piglets not offered creep feed, and there was also an improvement in gain:feed (g:f) associated with consuming creep. Most studies have failed to detect any difference in feed efficiency, and the result of Bruininx *et al.* (2002a) is probably due to the improved precision of using individual feed recording stations. There is *in vitro* evidence for an improvement in physiological adaptation to weaning associated with creep intake: Kuller *et al*. (2007) found that absorption of electrolyte solution at four days post-weaning in segments of piglet intestine was highest in those classified as creep eaters compared to non-eaters pre-weaning. As yet few *in vivo* studies have been able to demonstrate an improved FCR (only Bruininx *et al*., 2002a), or identify changes in piglet physiology to account for this. A disadvantage of using coloured markers is that they are relatively imprecise, and the colour may be diluted by high liquid intake, such that they are indiscernible, potentially resulting in mis-classification (Bruininx *et al*., 2002a; Byrgesen *et al.*, 2021). Byrgesen *et al.* (2021) reported that in one litter there were no piglets classified as eaters according to faecal colour, but that 392 g of feed had disappeared from the feeders. It is also possible that a very small amount of feed intake will colour the faeces, but this is insufficient to affect growth (Collins *et al.*, 2013). A more accurate method of quantifying individual level of pre-weaning creep consumption using EID may therefore elucidate more conclusive results.

2.2.3.3. Improving creep intake

The timing of creep introduction should be considered, as this may affect the proportion of eaters:non-eaters. Sulabo *et al.* (2010a) observed that litters where creep introduction was delayed to two days prior to weaning consumed a similar amount of creep in those two days (350 g for 11 pigs) as litters where creep was introduced six days prior to weaning. Both had higher consumption in the final two days than litters where creep was introduced 13 days prior to weaning (256 g for 11 pigs), but also had a higher proportion of non-eaters (~30 % compared to 19.8 %) compared to litters with the earliest creep introduction. The litters with two days of creep feeding also had the lowest overall intake, approximately half of that of the six- and 13- day fed litters. Although creep consumption is low early in the suckling period (Sulabo *et al.,* 2010a; Middelkoop *et al.*, 2019), earlier provision appears to encourage more piglets to consume creep, and familiarity with the diet is probably an advantage when sows milk becomes limiting in later lactation (Collins *et al.*, 2013). To maximise the proportion of piglets consuming creep, and overall creep intake, it appears that creep introduction is required at least 13 days prior to weaning.

Creep feed presentation can affect intake, with Wattanakul *et al.* (2005) utilising video observations, determining that litters where creep was offered in a tray had higher visits and duration of feeding per hour than litters where creep was offered through a hopper, on day 14, 21 and 25 of lactation. This translated to a higher litter creep intake of approximately 230 g/d for the tray-fed piglets compared to 85 g/d for the hopper-fed piglets. In common with the previously discussed studies, there was no effect of the higher creep intake on average piglet weaning weight. This study did not continue post-weaning. The higher creep intake was attributed to the tray allowing for social feeding and imitation, and rooting behaviour, but wastage may have been greater due to the more open design.Occasionally the piglet's access to the hopper feeder was restricted due to another piglet using/blocking it, which didn't occur with the tray. Both had 30 cm length of feeding space, but the tray allowed the piglets better access as it was more open.

The mechanism by which creep-feeding may be beneficial to post-weaning adaptation is not yet determined, and is likely to include physiological development and familiarity with solid feed. At weaning, the piglet's diet changes from a lipid and lactose-based energy supply to a starch-based energy supply, requiring a significant and rapid development of the digestive processes, including changes in enzyme production (for example: Kelly *et al.*, 1991; Montagne *et al.*, 2007; Martin *et al*., 2012) and absorptive capacity (for example Spreeuwenberg *et al*., 2001; Montagne *et al.*, 2007; Muns and Magowan, 2018).

2.3. Weaning

Weaning is a stressful time for the pig, there is the nutritional stress of a change in composition and form of the diet, and social stress from leaving the sow and potentially being mixed with unfamiliar pigs. This comes at a time when passive immunity from the sow is reducing, and the development of the GI tract is incomplete (Moeser *et al*., 2017). At times of acute stress, corticotrophin-releasing hormone (CRH) is secreted from the hypothalmus and inhibits the action of appetite stimulants neuropeptide Y and agouti-related peptide neurons (Sominsky and Spencer, 2014). The purpose of this is to direct behaviour away from sourcing food and prioritise the fight-or-flight response (Jiang *et al.*, 2020). Corticotropin-releasing hormone also stimulates ACTH secretion and thus increases cortisol in blood and saliva. Cortisol acts on the liver to increase glucose production from glycogen, to allow the organism to respond to threats (Jiang *et al*., 2020). The stress response of piglets to weaning is rapid, with raised cortisol levels being recorded two hours post-weaning (Yu *et al.*, 2018). Raised cortisol levels are transient, with Kick *et al*. (2012) finding cortisol levels reverted to pre-weaning levels by six days post-weaning, regardless of whether piglets were weaned at 14, 21 or 28 days old. Clinical signs observed are well-documented and include loss of appetite, reduced ADG (e.g. Cranwell 1985; Bruininx *et al.*, 2002a; Jiang *et al.*, 2020), and post-weaning diarrhoea. ((Jiang *et al.*, 2020; Meng *et al.*, 2020).

2.3.1. Reduced energy intake

Reduction in energy intake in the newly-weaned piglet is two-fold: poor feed intake due to stress and unfamiliarity with solid feed, and physiology not suitably adapted for a cereal-based diet. The mean latency of commencing eating post-weaning was reported as 10.7 hours, ranging from 0-76 hours, by Bruininx *et al.* (2002a). Metabolically, the piglet moves from fat being the dominant energy source, and lactose being the main carbohydrate, to most of the energy coming from starch. This necessitates a change in digestive enzymes (Kelly *et al.*, 1991; Makkink *et al.*, 1994; Martin *et al.*, 2012).

2.3.1.1. Intestinal morphology

Changes in small intestinal morphology have been widely observed post-weaning, with a transient reduction in villus height due to atrophy, crypt hyperplasia and lower villus height:crypt depth ratio reducing the absorptive capacity of the intestine (for example Spreeuwenberg *et* al., 2001; Montagne *et al.*, 2007; Muns and Magowan, 2018). Montagne *et al.* (2007) modelled the effects of weaning by starving 21-day old piglets for 48 hours, then recommenced feeding with a cereal-based diet through a stomach tube, to reduce variability in intake. This was a more extreme fasting period than would be observed voluntarily with Bruininx *et al.* (2002a) reporting 50 % of piglets recommenced eating seven hours post-weaning. By employing serial slaughter on days 0, 2, 5, 8 and 15, Montagne *et al.* (2007) assessed the effects of weaning through what they defined as the acute phase (d0-5) and adaptive phase (d5-15) on intestinal structure and function. They found the length of the intestine was unaffected by age, and this was also observed by Adeola and King (2006) during the two-week period post-weaning. Due to changes in the mucosal layer, Montagne *et al.* (2007) reported that intestinal weight was lighter on d2 and regained weaning weight on d5 post-weaning. However, villus length in the jejunum was lower on d2 and did not recover by d15 and crypts were deeper on d5 and thereafter. This lead to a lower villus height:crypt depth ratio post-weaning, which reduces the absorptive capability of the intestine. Even when there is diet familiarity and no induced starvation period, voluntary feed intake is lowered immediately post-weaning, with Spreeuwenberg *et al.* (2001) reporting that piglets removed from the sow, but offered a milk replacer diet consumed 43 % of that offered on the first day post-weaning, increasing to 83 % on day two and 96 and 94 % on days three and four respectively. This demonstrated the effect of stress on appetite, and lead to the piglets having a weight loss of 77 g/d over the four-day period. Proximal and medial small intestine histomorphology was also adversely affected by reduced feed intake, with villus height being lowest on d2 (317 and 253 µm for proximal and mid-small intestine respectively), and villus height:crypt depth lowest on d2 and d4. There were no difference in these parameters in the distal small intestine. This was also observed by Montagne *et al.* (2007), who concluded that this was because the enterocytes in the proximal and mid-small intestine (duodenum and jejunum) obtain nutrition from the luminal contents, whereas those in the distal small intestine (ileum) obtain most nutrients from the mesenteric artery, and so are less exposed to the effects of post-weaning anorexia.

2.3.1.2. Digestive enzymes

A thorough review of digestive enzymes is beyond the scope of this project, but the basic changes associated with weaning are presented here. The change in diet requires an adaptation in the enzymes secreted, but there is a lag between weaning and optimal secretion of enzymes suitable for hydrolysis of cereal-based diets, with Kelly *et al*. (1991) reporting changes in enzyme activity from three days post-weaning in piglets weaned at 14 days of age. This lag coincides with the period where feed intake is lowest. Enzyme secretion is initiated by the hormone cholecystokinin (CCK) due to presence of chyme in the duodenum, and so when feed intake is low, enzyme secretion is low, although production of pancreatic enzymes may be maintained and stored until required (Montagne *et al.*, 2007).

Pre-weaning carbohydrate digestion focusses on lactose and so lactase is the predominant carbohydrase. Post-weaning, there may be milk powder included in the diet (Kelly *et al.*, 1991; Makkink *et al*., 1994) and so lactase will still be present at a lower level (Kelly *et al.*, 1991; Martin *et al*., 2012), but the main carbohydrate source will be cereal-based. Carbohydrases that have been extensively studied are amylase, sucrase and maltase, with Kelly *et al.* (1991) concluding that amylase and maltase were most important post-weaning, as they showed the greatest difference in activity compared to that observed in suckling piglets of the same age. Indeed, other researchers have shown that sucrase activity does not increase rapidly post-weaning, with Adeola and King (2006) and Martin *et al*. (2012) observing sucrase did not increase in activity until 28 days post-weaning. Maltase was also observed by Martin *et al*. (2012) to increase by eight days post-weaning.

Generally, post-weaning diets will be lower in fat than sow’s milk, and so lipase activity is reduced post-weaning (Jensen *et al.*, 1997; Montagne *et al.*, 2007).

There is also a change in the proteases, with chymotrypsin being the predominant protease pre-weaning, and trypsin most common post-weaning (Makkink *et al.* 1994). This has been shown to be diet- and intake dependant, as Makkink *et al*. (1994) found that weaned piglets designated non-eaters (consumed <10 g/d) had higher chymotrypsin:trypsin ratio. Protein source also affected chymotrypsin and trypsin secretion on day three and day six post-weaning; piglets fed fishmeal or soyabean meal had lower trypsin on day 3 than piglets fed skimmed milk powder or soyabean protein concentrate, and this persisted to day 6 for the fishmeal piglets. The authors concluded that lower enzyme activity in the fishmeal-fed piglets may be due to slower gastric emptying, as these piglets had a higher level of protein hydrolysed in the stomach.

2.4. Conclusion

The sows’ ability to rear her litter has not kept pace with the rate of genetic improvement in litter size. Milk yield is insufficient, particularly during the fourth week of lactation, and where there are supernumerary piglets compared to functional teats. Providing SM pre-weaning has been shown to be beneficial in reducing mortality, particularly in large litters, and may improve weaning weights of piglets, but currently there is little data on the effect of SM at the individual piglet level; which piglets are utilising SM, and what the effect is on lifetime performance. To replicate the commercial environment all live pigs should continue to be studied post-weaning, as light weight pigs at weaning lengthen the finishing period or have to be sold light, and so are the ones with most potential to improve. Previous research characterising piglets as eaters or non-eaters of creep has identified that post-weaning performance is likely to be improved with higher creep intake, but whether this is a behavioural effect or physiological is unknown. Improved precision of classification should allow this to be determined. The effect of the combination of SM and creep pre-weaning on piglet performance pre- and post-weaning is also poorly understood. Therefore, there is a need to investigate consumption patterns of SM and creep, determine the effect of level of SM and creep consumption on pre- and post-weaning performance, and determine optimal nutrition based on pre-weaning level of consumption.

This project will utilise novel technology to characterise piglets by their SM and creep intake. Continuous accurate recording of individual consumption will be achieved through the use of EID and an antenna system to record every visit to the SM bowl or creep feeder. The first study will concentrate on SM consumption to characterise this without confounding with creep introduction. An important part of this is calibration of the recording system with weighed SM consumption. Creep will be introduced from study 2.

2.5. Hypotheses

- Provision of SM enables sows to rear supernumerary piglets
- Level of SM and creep consumption will vary according to competition, birthweight, and suckling position
- Piglets with higher creep consumption will be better adapted physiologically to weaning
- The optimal post-weaning feeding regime will vary depending on pre-weaning SM and creep consumption

3. General materials and methods

This project utilised the Harper Adams University 230-sow commercial pig unit, operating under nationally-recognised Red Tractor Assurance standards, membership number E3444 NSF. The site is also licenced under the Animals (Scientific Procedures) Act (ASPA) 1986 legislation, establishment licence number X01FC5D6A.

3.1. Animals

All studies were approved by the Harper Adams University ethics committee, project number 0287-201805-PGMPHD, individual study numbers are included in the respective materials and methods sections. Where regulated procedures were employed (Study 2), these were in accordance with the Project Licence PDE402EC7 Nutrition and Management for Sustainable Weaner Production, and were approved by the Harper Adams University AWERB committee. Details are included in Chapter 5.

Litters from multi-parous sows were utilised. Sows were Large White x Landrace (JSR TN70), with the JSR 900 (pilot and Study 1) and Topigs Tempo (subsequent studies) as terminal sires. The herd was porcine reproductive and respiratory disease (PRRS) and enzootic pneumonia (EP) free, but positive for Glassers disease and porcine circovirus (PCV2). Sows were vaccinated against *E. Coli* (Porcilis Porcoli DF; MSD, UK) to provide piglets with passive immunity three weeks prior to farrowing. Piglets were vaccinated at weaning against PCV and EP (MHyosphere PCV ID; Hipra, UK). In study 3, piglets were also vaccinated 14 days prior to weaning and at weaning against Glassers disease (Suvaxyn Respifend HPS; Zoetis, UK). This conferred sufficient protection in Batch 1, but in-water medication (Amoxinsol, Vetoquinol UK Ltd) was required in Batch 2 for five days when pigs were 13 weeks of age.

Batch farrowing was practised; each batch comprised up to 32 sows, and litters were selected with minimal variation in farrowing date. Power analysis was conducted to determine the level of replication required. Standard commercial practice of tooth reduction (Dremel, UK), iron injection (Ferroferon 200 mg/ml: Iron4u, Denmark) and iodine spray to the naval of the piglet were performed within 24 hours of birth. Boars were left intact. Piglets were given an electronic ID (EID) tag at this point, inserted into the left ear (MS quick tag, MS Schippers, Hapert, Netherlands). Functional teats of the sow were counted at farrowing by manual expression of colostrum; any litter whereby the number of piglets born alive exceeded the number of functional teats were split-suckled: the heaviest piglets (half of the litter) were removed for two hours in the morning and afternoon until fostering occurred. Fostering was performed within 48 hours of birth, and ensured that supernumerary piglets compared to functional teats were spread evenly across the batch. Farrowing performance for the room utilised for the duration of the project (July 2019-November 2022) is displayed in Table 4, and the percent of farrowings for each parity is displayed in Figure 6.

Table 4: Farrowing performance data for the duration of the project

Parameter	Mean	s.d
Total number of litters	*818*	
Litters/batch	28.2	3.18
Sow parity	3.36	0.72
Total born	17.2	1.01
Born alive	16.3	0.95
Piglet birth weight (kg)	1.40	0.07
Number weaned	13.7	0.74
Piglet weaning weight (kg)	8.58	0.38
Litter weaning age (d)	27.0	0.75

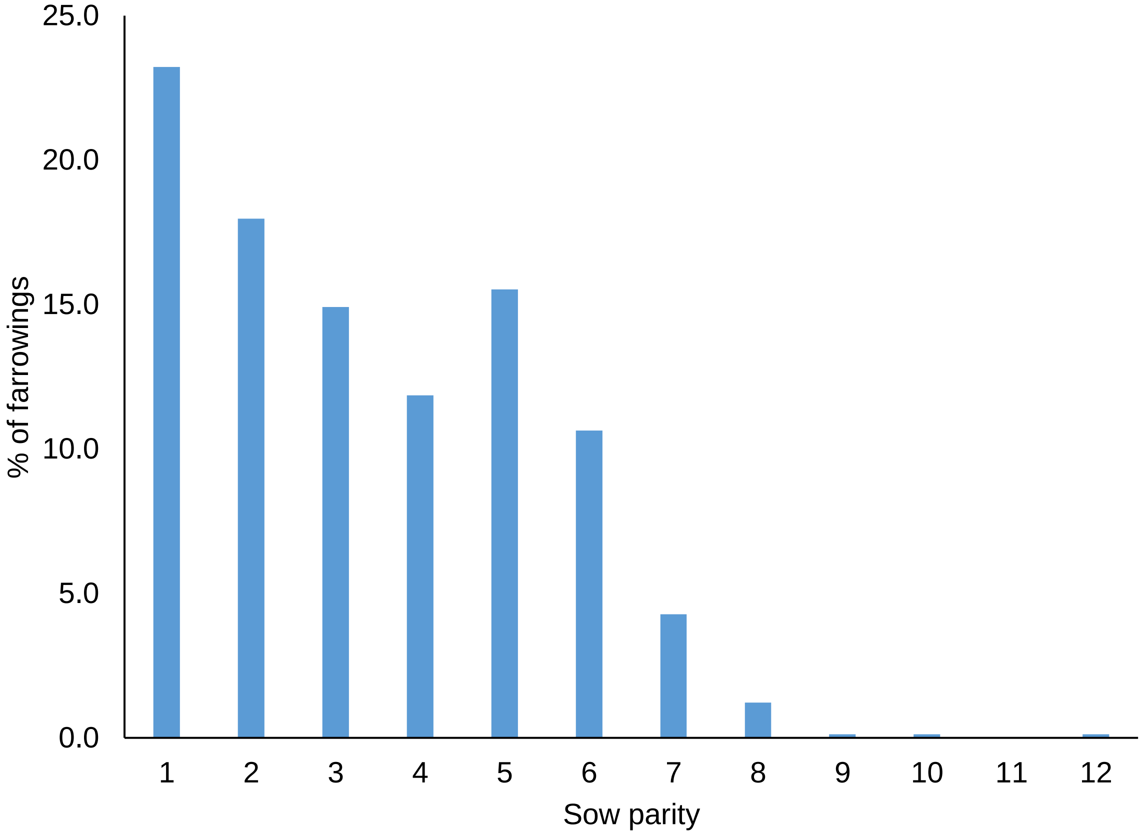

Figure 6: Percent of farrowings for each parity for the duration of the project.

3.2. Accommodation

3.2.1. Pre-weaning

Conventional fully-slatted, fixed farrowing crates were used throughout. The total pen area was 4.3 m^2. Sows entered the farrowing room five days prior to the expected earliest farrowing date of the batch. Enrichment was provided in the form of a hessian sack and a chain with plastic chew-toy (Porcichew: Nutrapet, UK) secured to the crate. Water was provided *ad libitum* through a nipple in the sow's feed trough, and a further water nipple at piglet height on the outside of the crate. Each pen contained a supplementary milk (SM) bowl connected to a single tank that fed the entire building. Temperature and ventilation were controlled by Skov (Skov, Denmark) system, with temperature set on a curve commencing at 22 °C for the first 10 days, then reducing over seven days to 18 °C and remaining at this set-point until weaning. Daily minimum and maximum temperatures were recorded. Lighting was controlled by a timer, with the light period from 06:00-21:00.

3.2.2. Post-weaning

Nursery accommodation was fully-slatted, with pigs housed in pens of five (0.49 m^2/pig) or six (0.41 m^2/pig) pigs. Each pen had a three-space hopper and two *ad libitum* water nipples. Feed was manually added to hoppers. Enrichment was provided through a compressed straw block in a dispenser and a rubber chew-toy (Easyfix Luna 86: MS Schippers UK Ltd). Temperature and ventilation were controlled by Skov system, with temperature set on a curve commencing at 28 °C at weaning and reducing to 20 °C by day 54 post-weaning (exit to finishing accommodation). Daily minimum and maximum temperatures were recorded. Lights were manually operated, with the light period from 08:00-16:00 and no night-light.

Commercial finishing accommodation was fully-slatted, with pigs housed in 17.5m^2 pens of 24 pigs (0.73m^2/pig). Each pen had a two-space feeder and three *ad libitum* water nipples. Feed was delivered by the Roxell Multifast system (Roxell, Belgium): each trough contained a sensor to enable refilling on demand. The amount of feed delivered to each pen was recorded by the Roxell computer. Enrichment was provided through a block of wood in a dispenser and rubber chew-toys (easyfix Luna 117 and Astro 200: MS Schippers UK Ltd). Temperature and ventilation were controlled by Skov system, with temperature set on a curve commencing at 21 °C and reducing to 19 °C over a four-week period. Lights were manually operated, with the light period from 08:00-16:00 and no night-light.

Pig performance testing (PPT; Nedap, Netherlands) stations were located in a fully-slatted building in four 9.8m^2 pens of 14 pigs each. The PPT station comprised a feed trough (feed delivered by Roxell Multifast system) combined with a weighing platform. At each visit, the pigs EID tag was read and the amount of feed consumed, weight of the pig and duration of visit was recorded. Enrichment was provided through a block of wood on a chain and rubber chew-toys (easyfix Luna 117 and Astro 200: MS Schippers UK Ltd). Temperature and ventilation were controlled by a Stienen controller (Stienen BE, Netherlands), with temperature set at 20 °C throughout. Lights were manually operated, with the light period from 08:00-16:00 and no night-light.

3.3. Nutrition

3.3.1. Pre-weaning

Sows were fed a single pelleted lactation diet from farrowing room entry to weaning (specification in Table 5). Sows were fed to appetite twice per day; feed allocation was 4 kg/d from entry to farrowing, and increased by 1 kg/d thereafter until reaching the maximum allocation of 16 kg/d, which was maintained to weaning. Supplementary milk was provided *ad libitum* to all litters from birth until one day prior to weaning (Faramate: Volac UK, specification in Table 5). This was mixed at a rate of 150 g powder/L milk. Where creep was offered through a single-space feeder (Studies 2 and 3), this was introduced at 14 days prior to weaning (wn-14) and followed a two-stage regime, changing at seven days prior to weaning (wn-7; Table 5).

3.3.2. Post-weaning

In Studies 1 and 2, there were no post-weaning dietary treatments, and so a single post-weaning feeding regime was employed, with feed quantity allocated on a per pig basis until the grower diet was introduced. Sufficient feed was added based on the number of pigs in the pen, with the subsequent diet added once the initial feed was virtually consumed, to ensure the trough was never empty (Table 6). Dietary treatments and regime for Study 3 are detailed in that chapter. All feed was provided *ad libitum.*

Table 5: Pre-weaning diet specifications and regimes (fresh weight basis)

	Sow lactation diet	Milk replacer powder	First creep	Second creep
Constituent	Entry-weaning	From birth	Wn-14 to wn-7	Wn-7 to weaning
Crude protein (g/kg)	203	220	240	220
Crude fibre (g/kg)	35	0.0	26	20
Crude oil and fats (g/kg)	58	140	130	85
Crude ash (g/kg)	53	75	70	60
Calculated DE (MJ/kg)*	16.0	17.2	17.3	16.5
Lysine (g/kg)	12	20	16	17
Methionine (g/kg)	3.2	n/a	3	7
Ca (g/kg)	7.0	9.0	6	7.5
Na (g/kg)	2.0	5.0	3.5	2.8
P (g/kg)	4.7	7.0	6	7.5
Vitamin A (iu/kg)	10000	25000	12500	12500
Vitamin D3 (iu/kg)	1875	10000	2000	2000
Vitamin E (iu/kg)	125	500	95	300

Wn-14: 14 days prior to weaning. Wn-7: seven days prior to weaning.* Gross energy calculated using method of Weiss and Tebbe, 2019. Converted to from Mcal/kg to MJ/kg using multiplication factor of 1 cal:4.184J. Digestible energy approximated as GE*0.84 (Noblet *et al.* 2022)

Table 6: Post-weaning diet specifications and regimes

	1st stage	2nd stage	Link	Grower	Finisher
Constituent	1 kg/pig	2 kg/pig	5 kg/pig	To 61 d p-w	To slaughter
Crude protein (g)	220	215	215	192	173
Crude fibre (g)	20	20	25	43	58
Crude oil and fats (g)	85	65	60	38	40
Crude ash (g)	60	50	55	53	53
Calculated DE (MJ/kg)*	16.5	16.3	16.1	15.6	15.6
Lysine (g)	17	15.5	15	13	11
Methionine (g)	7	6	6	3	2.8
Ca (g)	7.5	7	7	7	7
Na (g)	2.8	2	2	2.3	2.6
P (g)	7.5	6.5	6.7	4.8	5
Vitamin A (iu)	12500	12500	12500	9500	6500
Vitamin D3 (iu)	2000	2000	2000	1850	1650
Vitamin E (iu)	300	200	200	75	35

p-w: post-weaning. * Gross energy calculated using method of Weiss and Tebbe, 2019. Converted to from Mcal/kg to MJ/kg using multiplication factor of 1 cal:4.184J. Digestible energy approximated as GE*0.84 (Noblet *et al.* 2022)

3.4. Measurements

3.4.1. Weighing

Sows were weighed to +/- 1 kg (Eziweigh 7: Datamars, UK) and P2 backfat measured (Dravet BF-8) at entry to the farrowing room and at weaning. All liveborn piglets were tagged within 24 hours of birth with an EID tag (MS Schippers), weighed, and sex was recorded. Any piglets that were fostered or died were weighed at the point of removal from the litter. All piglets were weighed weekly during the suckling period, and at weaning. Post-weaning weighing regimes are detailed in each chapter. At birth, piglets were weighed to +/- 20 g using the Bosche TWI weighing platform (Bosche, Germany). Subsequent piglet weighings utilised the XRS2 stick reader (Datamars, UK) to read the EID tag and send the EID to the Eziweigh 7 (Datamars, UK) weigh head. The EID and weight were recorded on the weigh head and downloaded at the end of the session. Until six weeks of age piglets were picked up and placed in a basket weigher (Pharmweigh UK) to +/- 50 g (up to 20 kg) or +/- 100 g (≥ 20 kg). After this point, a run-through weigher was utilised (Pharmweigh, UK) to +/- 100 g.

3.4.2. Feed

Feed intake during lactation was recorded for each sow. Intake was recorded volumetrically and the volume:weight calibration was performed by weighing 10 scoops of feed (Bosche TWI weighing platform) and taking an average.

Where consumption of SM was recorded on a litter basis, each morning the remaining milk was tipped out, weighed (Bosche TWI weighing platform) and discarded. The dispenser was scrubbed and rinsed with warm water, and the quantity required was mixed as detailed above, weighed and tipped into the dispenser. If further milk was required later in the day it was mixed, weighed and added to the dispenser. The same procedure was followed with the main mixing tank where consumption of SM was recorded on a batch basis.

Creep consumption was recorded on a litter basis. Small amounts of creep were added to the hopper to ensure freshness, and recorded volumetrically. The volume:weight calibration was performed by weighing 10 scoops of feed (Bosche TWI weighing platform) and taking an average. Creep remaining was weighed at the end of each period.

Post-weaning feed intake was recorded by weight on a pen basis for each period in the nursery (Bosche TWI weighing platform) and commercial finishing accommodation (Roxell Multifast), with refusals recorded at each weighing session. Feed intake was recorded individually per visit for pigs recorded through the PPT stations.

3.4.3. Observation of suckling

Suckling position was assessed on d 4, 14, and 25 by observation of at least two successful sucklings/litter on each day. Successful was defined as occurring when all piglets were awake and milk letdown occurred. Suckling positions were: the anterior two teat pairs (A), the posterior three pairs (P), and centre teat pairs (C) with every sow having at least two pairs of teats in section C (Figure 7). Piglets not observed suckling were recorded as NONE.

Figure 7: Piglet suckling positions. A: anterior; C: centre; P: posterior; NONE: not observed suckling.

Due to inconsistent suckling positions at d4 as teat order was not established (also reported at d2 by Huting *et al.*, 2017), and difficulties in identifying piglets at d24 as the piglets were too large to identify without disrupting suckling (Figure 8), observations at d14 were utilised for analysis.

Figure 8: Suckling on d24.

3.4.4. PigTrack ® system

3.4.4.1. Development

The PigTrack ® system (Asserva, France) was utilised to record individual piglet SM and creep consumption. This system utilises and EID tag inserted at birth to identify piglets visiting the SM and creep feeders. A pilot study was conducted to finalise the design and validate the accuracy of recording. The PigTrack ® system was an existing recording system utilised in pig finishing buildings to monitor water intake, but had not been used in a farrowing room before. The system needed to be portable to allow for power-washing and disinfection between batches, and the antenna for reading EID tags needed to be protected from the pigs. The housing was designed with adjustable width such that a single pig could access the SM bowl. In litters where there was high competition for SM, it was still possible for piglets to push the occupant out, and when this recurred rapidly the PigTrack system was unable to accurately record, so data from these litters was discarded. The housing was metal-clad to prevent reading of tags from piglets external to the unit. To determine the maximum width the shoulder width of a pig weighing 12 kg was measured. The set-up is shown in Figure 9. A floor maze was added after the first prototype to prevent piglets lying in the unit. This was a narrow strip of stainless steel 2cm high that ran down the centre of the unit, from the bowl to the entrance. A plastic pipe was added to the unit to prevent the antenna cable being chewed by piglets.

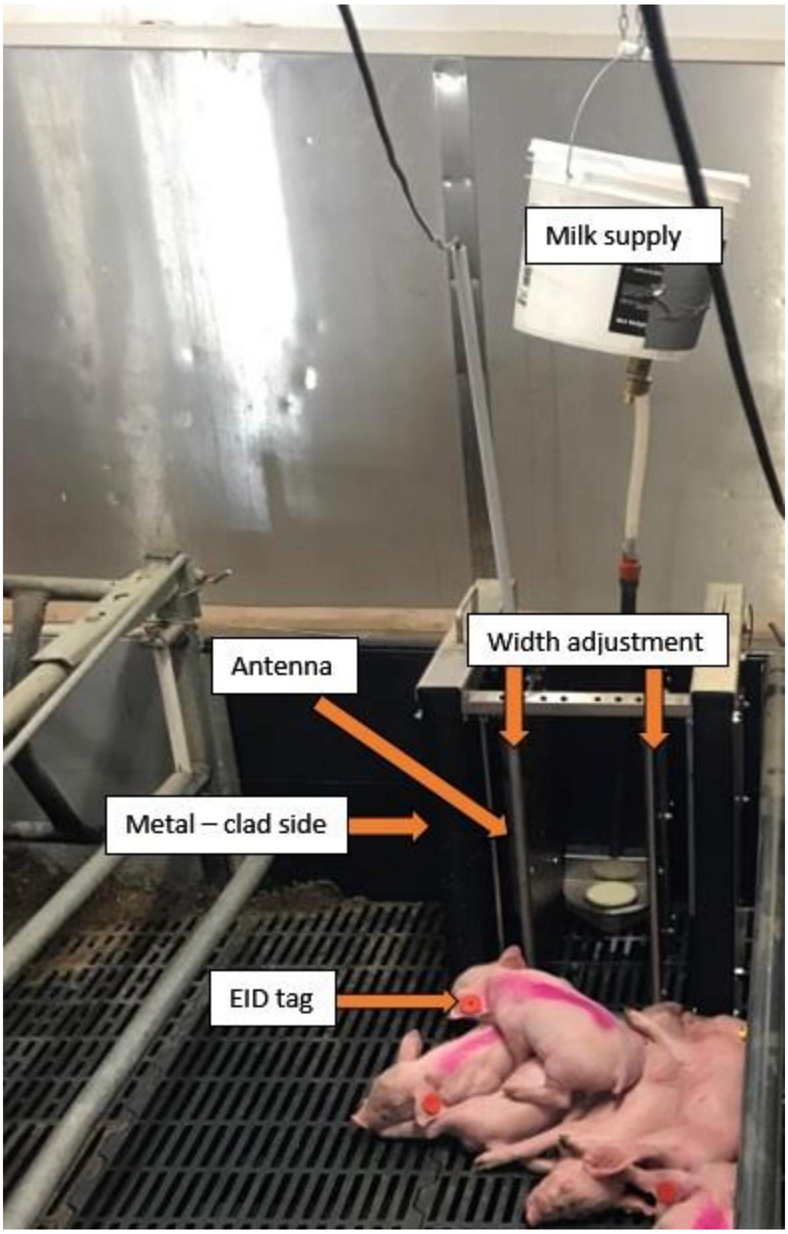

Figure 9: Set-up of PigTrack ® system around the supplementary milk bowl.

The antennae were wired into junction boxes (four antennae per box), which transmitted wirelessly to the receiver connected to the PC (Figure 10). A daily csv file containing the EID, date, time, and duration of each visit was generated.

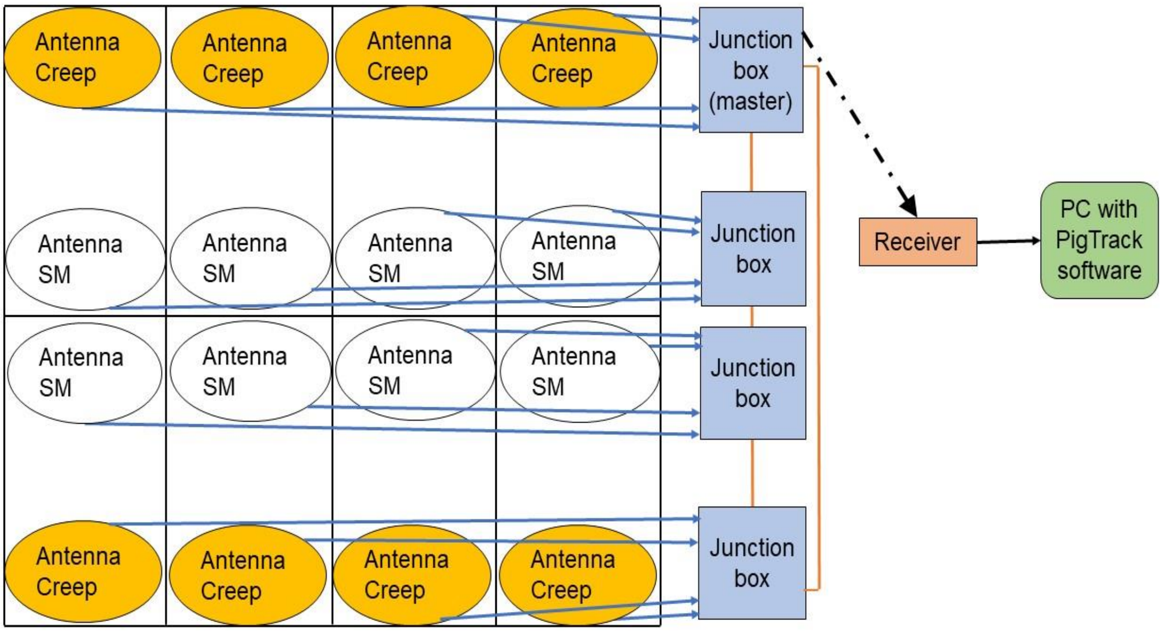

Figure 10: Schematic of PigTrack® system set up. Solid line denotes wired connection, dashed line denotes wireless transmission.

The functioning of the antennae could be checked in real-time in the junction box. Due to poor initial reading of EID tags a new board had to be added to the junction boxes, that allowed the sensitivity of the antenna to be adjusted. Sensitivity had to be adjusted each time an element was added to the system (extra antennae, junction box, connecting keyboard or mouse to PC), and so for each study all junction boxes were turned on and antennae connected to prevent needing to adjust sensitivity each time. Sensitivity was adjusted by changing the position of clips on the antenna board, with 32 permutations per box, and the positioning of the clips in each junction box was different. Once sensitivity was set, the optimum set-up within each junction box was maintained between studies.

Data recording was validated by cross-referencing downloaded visits with video recordings (pilot study). This revealed that many visits were not being recorded initially, which Asserva proposed was caused by electrical interference due to the large amount of metal in the farrowing room (crates and pre-fabricated walls). To solve this problem, units were earthed, and the junction boxes within a room were wired together and earthed, with a single Master transmitting to the receiver. This required an upgraded booster transmitter and receiver, and these improvements enabled all visits of a duration where consumption was likely to have occurred to be recorded

During the pilot study, pigs were weighed weekly and daily SM consumption per litter was recorded by suspending a bucket above the bowl and weighing SM in and out. This data was used with the data from Study 1 to produce the model relating duration of visits and piglet weight recorded by PigTrack ® to physical data.

3.4.4.2. Operation

The sensitivity of the antenna was set so that it read the tag of the piglet once it reached the SM bowl or creep feeder. The entrance width of the station was increased weekly. The computer recorded the EID, date, time, and duration of visit in a daily csv file, and displayed the time since the last visit for each station on a monitor so that operation could be checked in real-time. The data was downloaded and combined at the end of each batch, and whenever necessary for selection as detailed in each chapter. The PT station was put in place on day 4 to allow the piglets to locate the SM bowl prior to it being enclosed.

3.4.4.3. Data cleaning

Raw data was cleaned to remove erroneous EID readings (tags not present in the litter), and discard records with a very short duration (indicating no SM/creep consumption), or very long duration (indicating the piglet stayed longer than consuming SM/creep). The acceptable durations were determined by analysing a cumulative frequency table and histogram of duration of visits, and rejecting those that were outside of the 1-95 % cumulative frequency range. Only days where the data file size was equal or larger to the previous day were used, as smaller files were due to missing data where connectivity had been lost.

3.5. Statistical analysis

Performance data (average weights, piglet ADG, pen ADG, FCR and ADFI) were analysed by ANOVA in Genstat (20th-23rd Editions; UK). Normality was determined by examining the residuals, and transformations applied where necessary. Where significance was determined at $P < 0.05$, a *post-hoc* Bonferroni test was applied to determine differences between treatments.

Repeated measures ANOVA was performed using antedependence modelling. This analysis determines whether observations in a time-series are dependent on the previous observations. For example: for antedependence order 1, observations are most highly correlated between adjacent timepoints, and are independent of other prior observations. For order 2, observations are influenced by the two prior observations and independent of older observations. Additional uniform correlations within-subject may be included if the variation in observations differs between subjects (Jaffrézic *et al.*, 2003).

Optimisation of the model was achieved by determining order of antedependence and validity of including additional uniform correlations. Changes in deviance and Akaike Information Criterion (AIC) were assessed at each stage. Reduction in the values of these indicators for each model change were assessed for significance on the chi-square distribution at the relevant residual degrees of freedom. A significant reduction in the deviance and AIC indicated a better-fitting model.

Where multiple regression was utilised, the maximal model was fitted initially, with a step-wise elimination of non-significant terms to obtain the minimal model. This method was used in preference to forward stepwise variable selection (ie starting with one variable and building until there were no more significant variables) to reduce the risk of type 1 statistical errors due to the number of tests performed, as outlined in Altman and Krzywinski (2017).

4. Study 1: Effect of teat supply on performance, suckling behaviour and supplementary milk consumption in sow-suckled litters.

4.1. Introduction

Supplementary milk (SM) has been shown to increase the number of piglets reared by the sow when litter size is large, but there is little research into which individual piglets benefit from SM provision, due to a lack of a viable recording system. Therefore, there is a need to investigate the effect of litter size, birthweight and suckling position on SM consumption, and the effect of level of consumption on performance pre- and post-weaning. A new recording system is available that is able to record individual electronic ID (EID) tags of piglets when they visit the SM bowl, and a pilot study was completed using four prototype PigTrack ® stations (PT stations) to assess the nature of the data that could be collected. From this, the design was refined and a further four prototypes manufactured. The pilot study revealed SM usage at the individual pig level was highly variable, but it was possible to correlate PT station data with physical SM weight consumption data. The SM data could also be utilised to divide the pigs into phenotypic usage treatments using number of visits/day or duration of visits/day.

The hypotheses for this Study were:

- Litters where there are supernumerary piglets compared to functional teats will have higher SM consumption
- Light birthweight piglets are more likely to have a poor-yielding teat and so will have higher SM consumption
- Heavy birthweight pigs will be more limited by sow's milk yield and so will have high SM consumption in late lactation
- Piglets suckling from the posterior teats will have higher SM consumption as sows's milk yield will be lower
- Pre-weaning SM consumption will have no effect on post-weaning performance

4.2. Assessment of new prototypes

This was performed during the pilot study and Study 1. The new design was shorter than the original prototype and so was less obstructive in the farrowing pen. This necessitated a thinner antenna, to avoid reading the tags of piglets outside the stations.

4.2.1. Initial adjustments

- The handle on the locking mechanism needed to be shortened further to allow side-by-side usage.
- The adjustable panel was re-drilled to allow the antenna to be fixed in close proximity to the SM bowl
- A slide was attached to secure the antenna, as there was only one fixing point on the antenna, which allowed it to rotate into the reach of the piglets.

4.2.2. Improving functionality

There were multiple issues involving antenna sensitivity, affecting both original and new prototypes, and the final design which arrived for the final batch of Study 1. These were time-consuming to resolve and delayed the commencement and completion of Study 1 considerably.

Whenever an element was added to the PT system, or the equipment changed location, the sensitivity of the antennae required adjustment, and the system restarting. The problem with changing location was thought to be due to the large amount of metalwork within and surrounding the farrowing room causing interference. Adjustment of the antennae was achieved by moving the clips in pairs on the RFID panel in the main junction box for each set of four antennae, and was simple but time-consuming. There were five pairs of pins, so the possible number of combinations was 32 per junction box. Each junction box potentially had a different configuration of clips to set the same antenna sensitivity. For the new prototypes, sensitivity had to be reduced considerably due to the stations being shorter, to avoid reading the tags of a piglet outside the station. This meant half-duplex (HDX) tags were needed for these stations, and the final design, as full-duplex (FDX) did not have a strong enough signal. The stations were set so that the antennae only read the tag of a piglet standing at the milk bowl.

Until the final design arrived, only one farrowing room was utilised in each batch, meaning that the wireless receiver could be located in the farrowing room, and the cable put through the wall to the PC in the centre passage (clean area). Once both rooms were in use, the wireless receiver was repositioned in the centre passage, but was unable to receive data from the junction boxes. To overcome this, all junction boxes within a room were wired together and the signal sent from a single booster transmitter in one of the junction boxes.

4.3. Study materials and methods

This study employed no regulated procedures under the ASPA 1986 legislation, but was approved by the Harper Adams University ethics committee, project number 0458-207905-PGMPHD.

For details of animals used, accommodation, nutrition, weighing, PT system operation, data cleaning and statistical analysis, see Chapter 3 General materials and methods.

4.3.1. Experimental design and selection

4.3.1.1. Pre-weaning

The study was performed over four batches farrowing over a six-month period, with a total of 48 litters recorded. Farrowing performance of the four batches utilised is summarised in Table 7.

Table 7: Farrowing performance of batches utilised in Study 1.

Parameter	Mean	s.d
Number of litters	*48*	
Litters/batch	12	2.55
Sow parity	3.86	0.38
Total born	16.8	0.77
Born alive	15.5	0.70
Piglet birth weight (kg)	1.40	0.05
Number weaned	13.2	0.42
Piglet weaning weight (kg)	8.64	0.19
Litter weaning age (d)	27.2	0.85

In batches 1-3, eight litters per batch were utilised and in batch 4, 24 litters were utilised as the final PT stations had arrived. Litters were selected that were closest in birth date. Experimental treatments were insufficient functional teats (IS) and sufficient functional teats (S). Sow parity and birth litter size were balanced across treatments. Functional teats were assessed by manual expression of milk during farrowing, and piglets were fostered between litters to achieve a litter size of functional teats+2 (IS), or functional teats-1 (S). Piglets were selected for fostering to maintain the weight variation in the litter. After the initial fostering to set the treatments, no additional fostering was performed. Due to early mortality, by d4 when the study began, actual teat supply *vs* number of piglets had changed. Unbalanced ANOVA was therefore performed on teat supply at d4, with IS having up to two piglets in excess of functional teats, and S having up to three piglets fewer than functional teats.

4.3.1.2. Post-weaning

Two batches (farrowing batch 1 and 3) were utilised for post-weaning study, comprising 230 piglets. Treatments were allocated based on SM consumption within batch in the final week of suckling, and only two piglets did not continue to post-weaning study (one testicular rupture and one lame). Piglets were ranked by visits/day (v/d) and split into thirds, resulting in low (L, n=75), medium (M n=75), and high (H, n=80) consumption classifications. Piglets were penned in pens of five according to these treatments, and limited to two piglets per litter in each pen. There were differences between batches in the average number of visits for L, M, H, with batch 1 being 3, 9, 25 v/d respectively and batch 3 being 10, 24, 59 v/d respectively. To reflect this, an unbalanced ANOVA was performed with new groups of L (low: batch 1L), M (medium: batch 1M and batch 3L), H (high: batch 1H and batch 3M), and V (very high: batch 3H). Individual weight and pen feed intake were recorded throughout the nursery period. On day 50 post-weaning, pigs were weighed for selection to undergo individual feed intake and weight recording through Nedap PPT stations. This was reserved for pigs from the lowest and highest third of SM users in each of the batches to ensure any post-weaning performance differences attributable to pre-weaning SM consumption could be identified. Pigs for Nedap stations were selected from the full weight-range in these groups on day 50 post-weaning and balanced for sex. Selected pigs entered the Nedap stations on d54 post-weaning and were recorded until slaughter at 20 weeks (first draw) or 22 weeks (final draw). The remainder of the batch entering commercial finishing.

4.3.2. Study-specific measurements taken

4.3.2.1. Pre-weaning

No creep feed was offered to piglets in this study.

All days of age referred to hereafter are based on a weaning age of 27 d, regardless of actual age. Piglets were weighed on day 4, 11, 18, and 27 (weaning) according to the method in Chapter 3 Birthweight groups and SM consumption groups were defined *post-hoc.* Birthweight groups were defined as group 1: <1.00 kg; 2: 1.00-1.50 kg; 3: 1.52-2.00 kg; 4: >2.00 kg. Supplementary milk consumption groups were defined as the lowest third (L), middle third (M) and highest third (H) of piglets based on average visits/d to the SM bowl in the final week of the suckling period. The final week of suckling was chosen as this was when SM usage was highest, and so any relationship with performance parameters should be easier to identify.

4.3.2.2. Post-weaning

From weaning to d54 post-weaning, pigs were housed in pens of 5 pigs, and fed according to the standard regime in Chapter 3. Feed was weighed and recorded into the pens, with refusals weighed back when pigs were weighed on d6, 19 and 54 post-weaning. In the commercial finishing pens, pigs were weighed at 20 weeks of age. The Nedap PPT stations recorded EID, feed intake, pig weight and duration of each visit.

Pigs from farrowing batches 2 and 4 did not continue to post-weaning study, but were weighed on d54 post-weaning, and at 20 weeks old.

4.3.3. Statistical analysis

All growth, suckling position and SM usage data were analysed by ANOVA as detailed in Chapter 3. Log_{10} transformation was necessary to ensure normality for visits/d (v/d) and duration/d (du/d) SM consumption data. Proportions of piglets in birthweight groups and suckling positions were analysed by Chi-square. Supplementary milk usage over lactation was analysed by regression, with grouping factor of teat supply treatment. Multiple regression models were calculated to assess the viability of using measured piglet parameters to estimate litter daily SM usage, combining the data from the pilot study and this study. The maximal model for daily litter SM consumption included total duration of visits/d (du/d), average piglet weight (calculated from previous weighing and weekly ADG), litter age, litter size, station type and a calculated term $\sum wt_i*du_i$, which was the sum of piglet weight*duration for every piglet in the litter on each day. This term was used to account for any difference in the rate of consumption of SM of piglets of differing weights.

4.4. Results

4.4.1. Pre-weaning

Three litters were discarded from all analysis: one only had six piglets by d4 due to lay-overs, and the other two sows did not lactate well, resulting in very poor performance overall and a high reliance on SM of most of the litter. The PigTrack® equipment was not able to record these litters accurately due to the amount of competition for the SM, with usage being underestimated. This was observed visually, with the recording system unable to record any EID tag when there were at least two piglets competing for the milk bowl. Competition entailed rapid changes in occupancy, and piglets climbing over each other to access the bowl.

4.4.1.1. Supplementary milk consumption

Supplementary milk consumption/litter per day increased during the suckling period, as did variation in consumption (Figure 11).

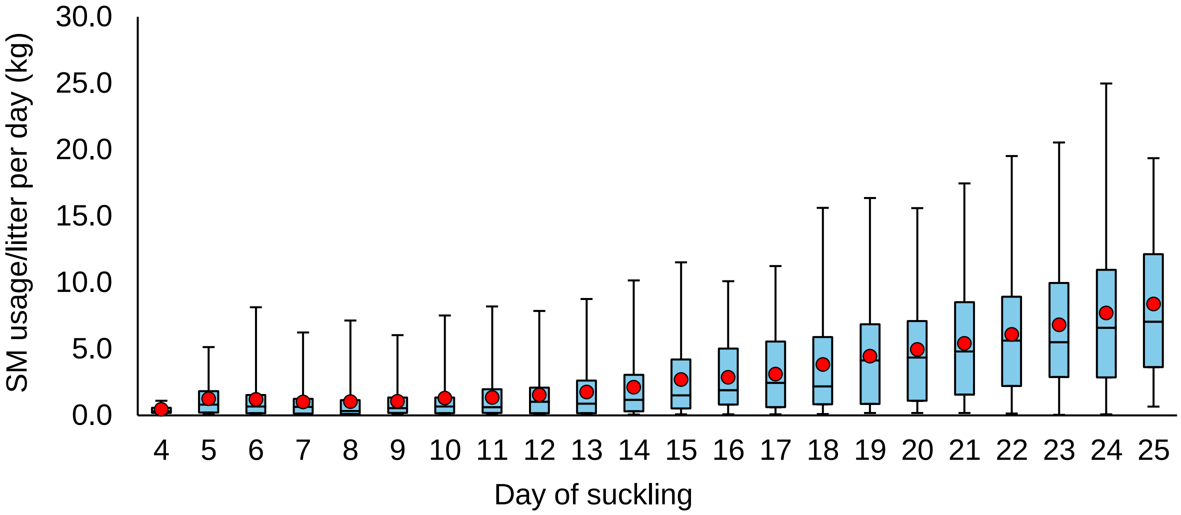

Figure 11: Supplementary milk (SM) consumption/litter per day throughout the suckling period. Red dot is the mean, horizontal line within the box is the median

4.4.1.2. Calibration of litter SM usage to PigTrack recording

Data were combined from the pilot studies and farrowing batches 1-3 of this study (Figure 12). When lactation was divided into weekly periods, litter SM consumption (kg) showed a linear relationship with litter total daily duration of visits (∑dui; s) for the periods d11-18 (B) and d18-weaning (C; *P* <0.001 for both periods, s.e 0.315 and 0.358 respectively), but not from d4-11 (A, *P* >0.05).

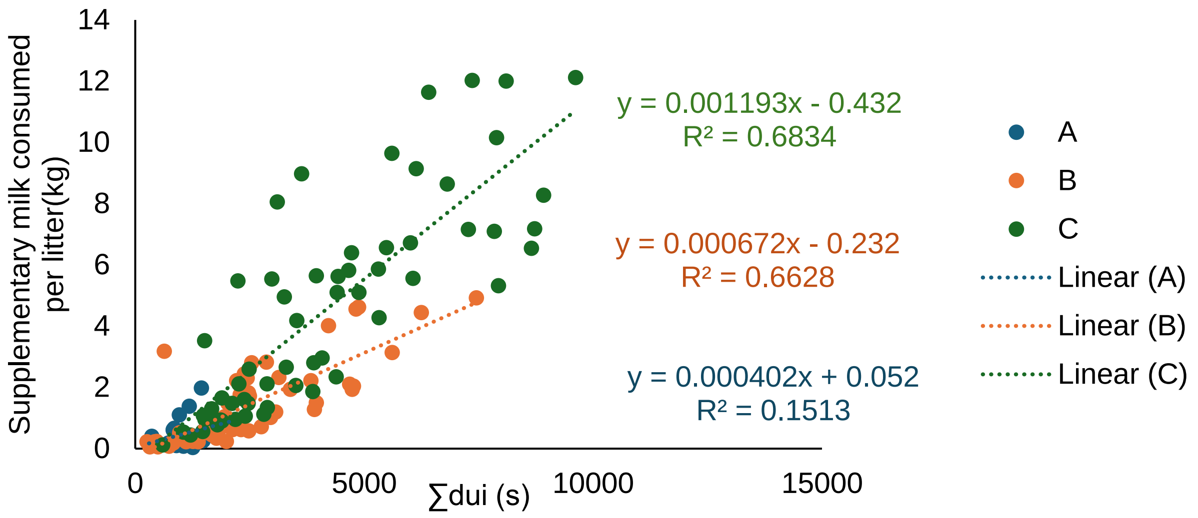

Figure 12: Relationship of supplementary milk usage with duration of visits. Period A: d4-11, B: d11-18, C: d18-weaning. ∑dui: litter total daily duration of visits.

Variance accounted for increased by including individual piglet weight with duration (∑wti*dui), as shown in Figure 13 (*P* <0.001, s.e 1.17). In this case it was not necessary to split the data into periods although for clarity these are shown on the graph in the same colours as Figure 12.

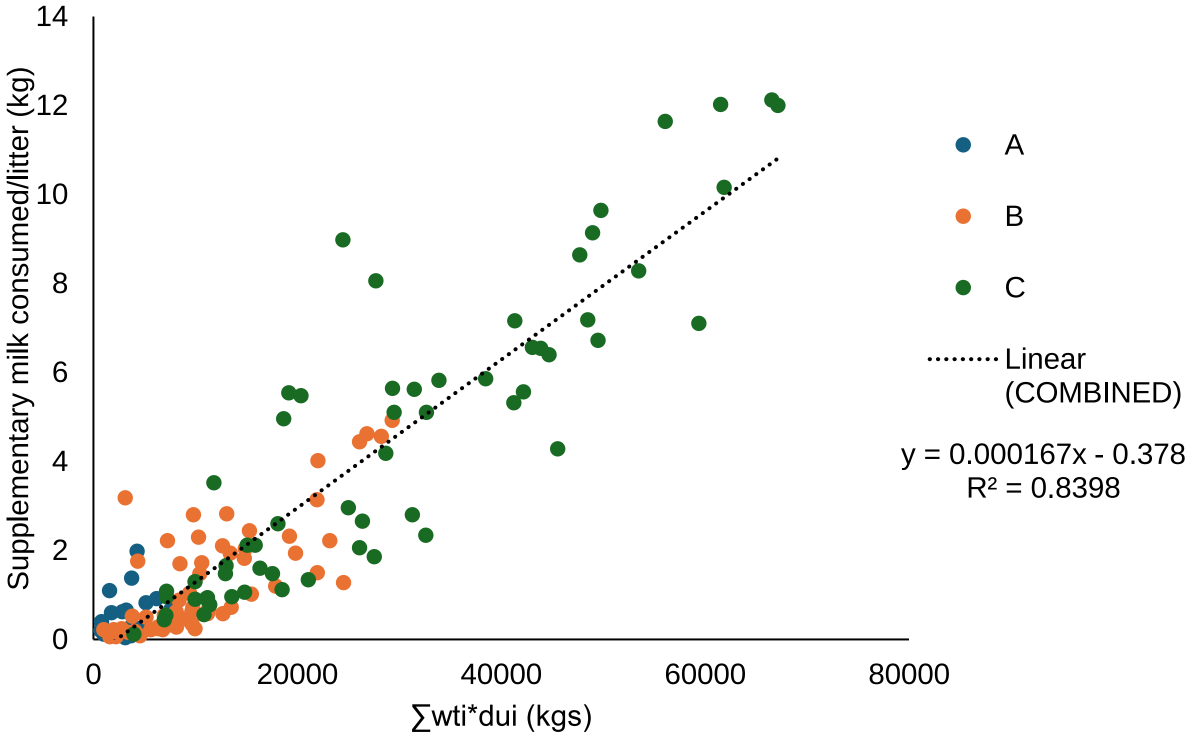

Figure 13: Relationship of supplementary milk usage with piglet weight and duration of visits ∑wti*dui: sum of piglet weight*duration for every piglet in the litter on each day. Period A: d4-11, B: d11-18, C: d18-weaning.

4.4.1.3. Effect of teat supply on litter pre-weaning performance and SM consumption

As shown in Table 8 although there was no difference in number of functional teats at farrowing (*P* =0.955), by design there were more piglets/litter in treatment IS compared to S (*P* <0.001), and this difference was maintained until weaning (*P* <0.001).

Table 8: Effect of sufficient (S) or insufficient (IS) teats/piglet at d4 post-farrowing on pre-weaning litter performance.

Performance parameters	S	IS	s.e.m	*P*-value
n	*29*	*15*		
Functional teats at farrowing	14.69	14.67	0.332	0.955
Number piglets/litter d4	13.72	15.93	0.344	<0.001
Number piglets/litter d11	13.48	15.80	0.360	<0.001
Number piglets/litter d18	13.48	15.60	0.363	<0.001
Number piglets/litter weaned d27	13.48	15.60	0.363	<0.001
Litter weight d4 (kg)	29.38	32.54	1.270	0.050
Litter weight d11 (kg)	54.33	58.83	1.950	0.081
Litter weight d18 (kg)	83.09	89.52	2.860	0.074
Litter weight weaned (d27; kg)	118.00	127.80	3.510	0.030
Total litter weight gain d4-weaning (kg)	89.00	94.94	2.812	0.091
Litter ADG d 4-weaning (kg/d)	3.76	3.90	0.154	0.459
Average piglet weight d4 (kg)	2.14	2.04	0.069	0.269
Average piglet weight d11 (kg)	4.04	3.73	0.096	0.012
Average piglet weight d18 (kg)	6.17	5.75	0.144	0.022
Average piglet weight weaning (d27; kg)	8.77	8.20	0.182	0.015
Piglet ADG d 4-weaning (g/d)	289	270	7.3	0.039
CV weight d4 (%)	22.51	23.21	1.348	0.673
CV weight d11 (%)	22.64	24.99	1.484	0.206
CV weight d18 (%)	21.12	23.98	1.504	0.150
CV weaning weight d27 (%)	19.96	22.66	1.613	0.180
Total litter weighed SM used (kg)	51.0	103.2	16.31	0.031
Weighed SM used/kg weaned (kg)	0.39	0.78	0.118	0.026
Weighed SM used/pig weaned (kg)	3.78	6.61	1.011	0.062

Litter weight was higher for IS than S due to the higher number of piglets/litter. Average piglet weight was not different at d4 (*P* =0.269), but was lower at each subsequent weighing in IS compared to S litters. As shown in Figure 14, piglets of all birthweights were lighter at weaning when they were reared in IS litters compared to S litters (*P* <0.001, s.e 1.72, overall r^2 0.265). The difference was approximately 400 g per pig. A polynomial order 2 curve produced a slightly better fit for the data points (r^2 0.298), but the inflexion point was at 2 kg birthweight where there was excessive leverage from some piglets reared in S litters that had much lighter weaning weights than would be expected.

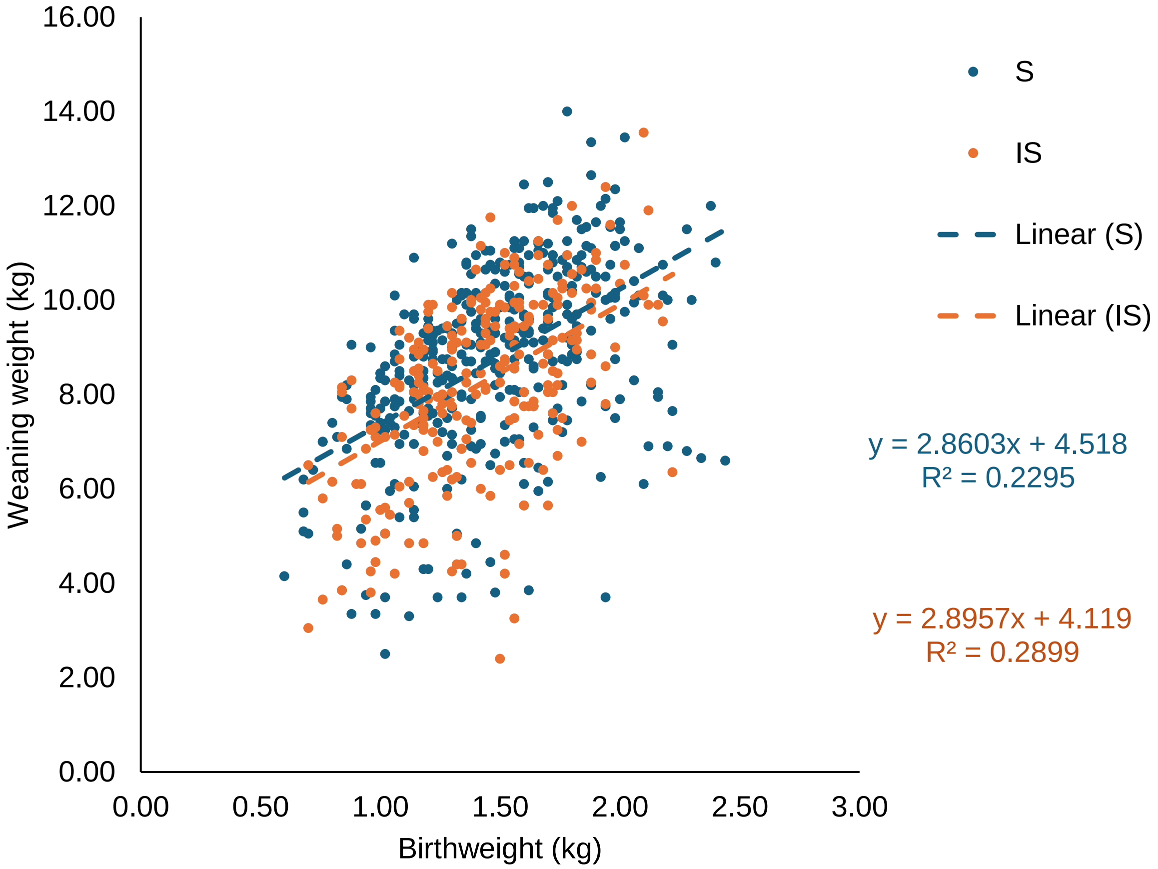

Figure 14: Relationship between birthweight and weaning weight for piglets reared in litters with sufficient (S) or insufficient (IS) functional teats.

Litters with insufficient teats had higher SM usage in total (*P* = 0.031), and per kg litter weight weaned (*P* = 0.026), with a tendency (*P* = 0.062) for higher SM used per piglet weaned. Figure 15 shows that SM consumption per litter was similar between S and IS litters until d14; thereafter IS litters had a higher rate of increase of SM consumption.

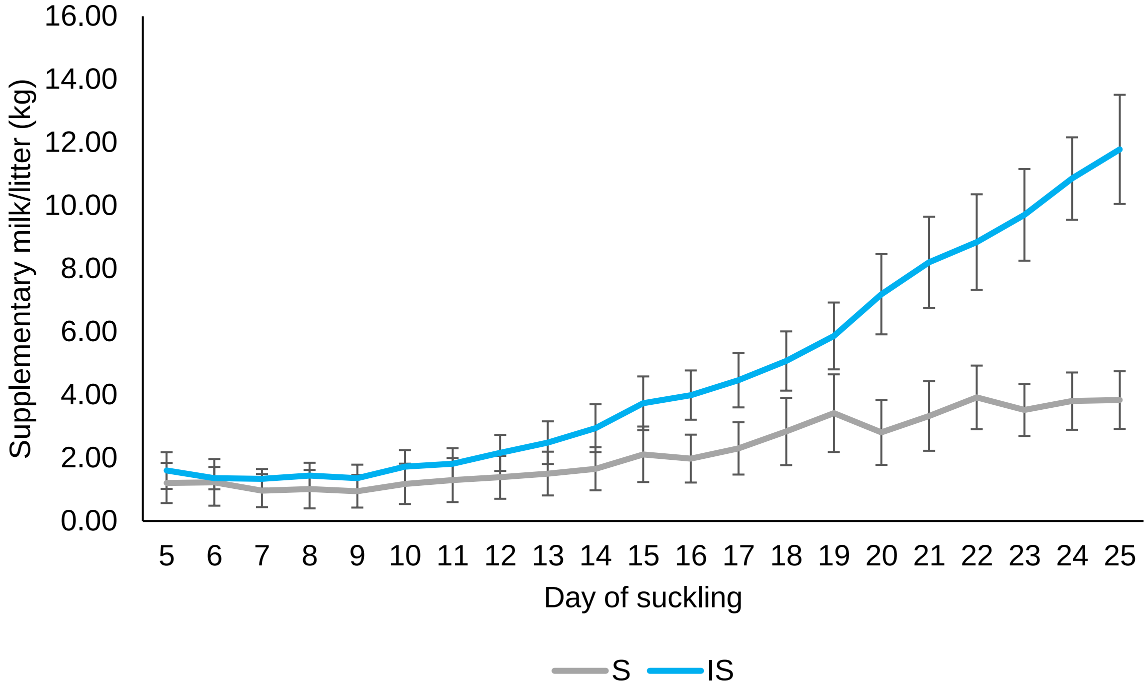

Figure 15: Effect of teat supply (sufficient: S or insufficient: IS) on litter supplementary milk consumption throughout the suckling period. Error bars denote s.e.m

In support of the weighed SM intake, litters from IS sows had higher visits/d (v/d) and duration of visits/d (du/d) to the SM bowl than litters from S sows from the period d11-weaning (*P* <0.050; Table 9).

Table 9: Effect of sufficient (S) or insufficient (IS) teats/piglet at d4 post-farrowing on pre-weaning litter supplementary milk usage behaviour.

Behaviour parameters	S	IS	S.E.M	*P*-value
n	*29*	*15*		
Average v/d $^{\alpha}$ d4-11*	60	99		0.138
Average du/v$^{\beta}$ d4-11 (s)	25	26	1.2	0.533
Average total litter du/d$^{\gamma}$ d4-11 (s)*	1456	2538		0.098
Average v/d$^{\alpha}$ d11-18*	92	158		0.003
Average du/v$^{\beta}$ d11-18 (s)	23	24	0.8	0.181
Average total litter du/d$^{\gamma}$ d11-18 (s)*	2138	3858		0.002
Average v/d$^{\alpha}$ d18-weaning*	151	310		0.003
Average du/v$^{\beta}$ d18-weaning (s)	24	25	0.8	0.284
Average total litter du/d$^{\gamma}$ d18-weaning (s)	3599	7608	1008.9	0.002

$^{\alpha}$ visits/d; $^{\beta}$ duration/visit; $^{\gamma}$ duration/d. *required log10 transformation, actual means presented without s.e.m due to non-normal distribution

Duration/visit did not vary between treatments (*P* >0.050), therefore total duration/litter per day followed the same pattern as visits/d (*P* <0.050).

Total sow feed and sow feed/d was similar between S and IS sows (*P* >0.05; Table 10), but sow feed/kg litter weight weaned and per piglet weaned was lower for IS than S (*P* <0.05). By including the weight of milk powder with sow feed, the difference in total feed input between S and IS was reduced, but IS sows still had lower feed input than S (*P* =0.031)

Table 10: Effect of sufficient (S) or insufficient (IS) teats/piglet at d4 post-farrowing on sow feed intake, weight and P2 change over lactation, and number born in the subsequent parity.

Performance parameters	S	IS	s.e.m	*P*-value
n	*29*	*15*		
Total sow feed (kg)	281.6	265.9	10.14	0.215
Sow feed/d (kg)	10.09	9.44	0.341	0.132
Sow feed/kg litter weight weaned (kg)	2.41	2.09	0.086	0.004
Sow feed/piglet weaned (kg)	21.0	17.2	0.72	<0.001
Sow feed+milk powder/kg litter weaned (kg)†	2.32	2.18	0.102	0.344
Sow feed+milk powder/piglet weaned (kg) †	21.1	18.1	0.920	0.031
Change in sow weight (kg)	-22.2	-32.0	3.91	0.046
Change in adjusted sow weight (kg)*	5.02	-0.12	4.011	0.298
Change in sow P2 (mm)	-1.82	-3.46	0.959	0.242
Total born in subsequent parity	16.24	17.46	1.092	0.405
Litter birthweight in subsequent parity (kg)	23.76	24.07	2.06	0.904
Average birthweight in subsequent parity (kg)	1.44	1.35	0.130	0.620

*Adjusted sow weight=sow weight at entry-litter weight-placenta weight. Placenta weight=litter weight/5.1 (Vernunft *et al*., 2018). † Only includes sows where SM was weighed

Although IS sows had higher weight loss between farrowing entry and weaning (*P* =0.046), this was partially accounted for by their higher litter birthweight and consequently higher placental weight (calculated as litter weight/5.1, as this was the mean placental efficiency reported by Vernunft *et al*., 2018). The change in adjusted sow weight was similar between treatments (*P* >0.05), although IS sows did lose a small amount of weight on average. Sows with insufficient teats had numerically higher P2 loss than sows with sufficient teats, but not significantly (*P* >0.05). Change in adjusted sow weight and in P2 were highly variable, as shown by relatively high s.e.m in Table 10. Of the sows that were not culled at weaning and thus continued to their subsequent service (35 of the 43 sows), 34 were served at five days post weaning and one at six days post-weaning. There was no difference between S and IS sows in the total number of pigs born, litter birthweight, or average birthweight in the subsequent parity (*P* >0.05).

Regression analyses showed no relationships between entry weight and weight change or P2 change over lactation (*P* >0.05). There was also no effect of sow weight change or P2 change over lactation with litter ADG, total number born in the subsequent parity or average piglet birthweight in the subsequent parity (*P* >0.05). Litter birthweight in the subsequent parity increased as sow weight loss decreased, independent of teat supply (*P* =0.040, $r^2$0.201, s.e 4.37), with the equation:

$$Litter\ birthweight\ (kg) = 0.1544 * change\ in\ adjusted\ sow\ weight + 23.4$$

4.4.1.4. Effect of birthweight group on pre-weaning piglet performance

Birthweight groups were defined as 1: ≤1.00 kg, 2: 1.02-1.50 kg, 3: 1.52-2.00 kg, 4: >2.00 kg.

At d18 and at weaning, piglets from birthweight groups 3 and 4 were similar in weight in S litters; however, in IS litters, piglets from birthweight group 4 were heavier than those in birthweight group 3 (*P* =0.033 and *P* =0.046 for d18 and weaning respectively; Figure 16).

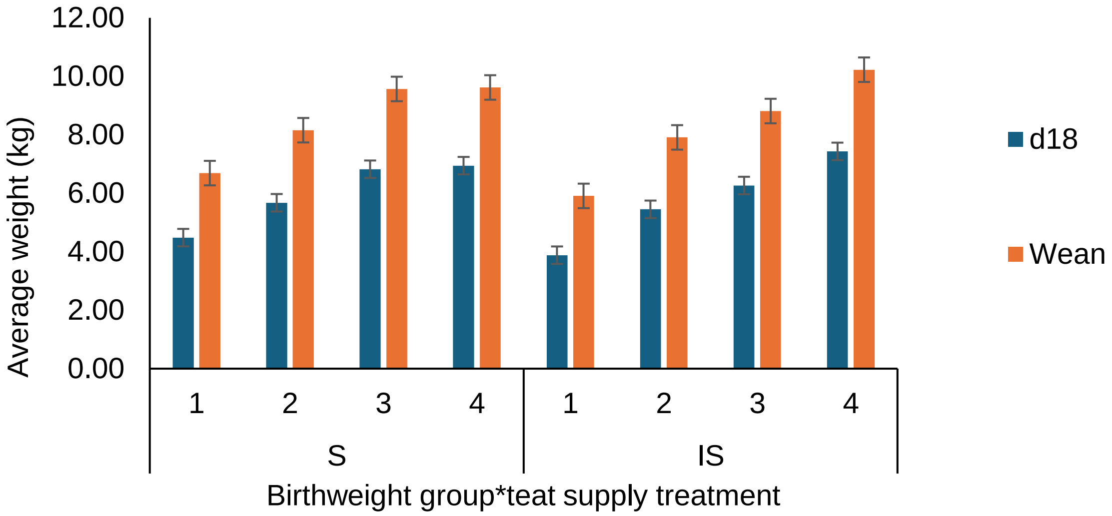

Figure 16: Birthweight group*teat supply (sufficient: S; insufficient: IS) interaction effect on piglet weight pre-weaning. Error bars represent s.e.m. Birthweight group 1: <1.00 kg; 2: 1.00-1.50 kg; 3: 1.52-2.00 kg; 4: >2.00 kg. S: sufficient teats/piglet at d 4, IS: insufficient teats/piglet at d 4.

Average daily gain from d4-11, d11-18 and overall from d4-weaning was also similar between piglets from birthweight groups 3 and 4 in S litters, but in IS litters, piglets from birthweight group 4 had higher ADG than those from birthweight group 3 (*P* <0.05; Figure 17).

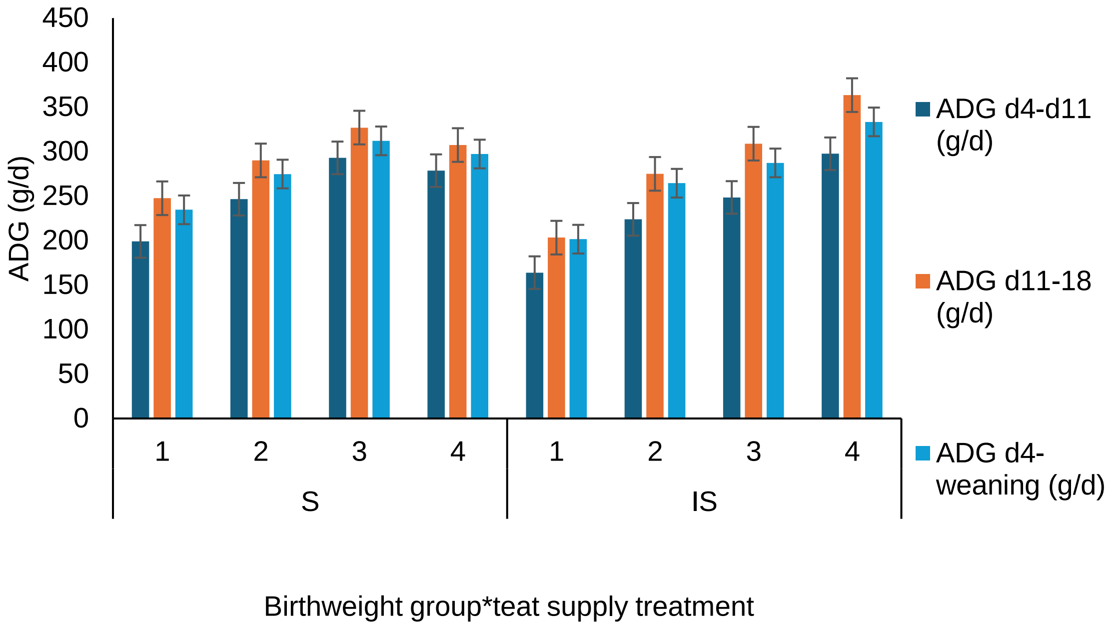

Figure 17: Birthweight group*teat supply (sufficient: S; insufficient: IS) interaction effect on piglet ADG pre-weaning. Error bars represent s.e.m. Birthweight group 1: <1.00 kg; 2: 1.00-1.50 kg; 3: 1.52-2.00 kg; 4: >2.00 kg. S: sufficient teats/piglet at d 4, IS: insufficient teats/piglet at d 4.

As there were more S litters analysed, and no difference in ADG between groups 3 and 4 in S litters, overall there was no difference in ADG between piglets from birthweight groups 3 and 4. Birthweight group1 remained lightest overall, with group 2 consistently heavier than group 1 but lighter than group 3 (*P* < 0.001;Table 11)

Table 11: Effect of birthweight group on pre-weaning piglet growth performance.

	Birthweight group					
Performance parameters	1	2	3	4	S.E.M	*P*-value
n	*70*	*248*	*251*	*68*		
Average birthweight	0.89^{a}	1.24^{b}	1.65^{c}	2.06^{d}	0.015	<0.001
Average weight d4 (kg)	1.28^{a}	1.87^{b}	2.37^{c}	2.81^{d}	0.044	<0.001
Average weight d11 (kg)	2.62^{a}	3.58^{b}	4.37^{c}	4.82^{d}	0.102	<0.001
Average weight d18 (kg)	4.25^{a}	5.59^{b}	6.60^{c}	7.14^{d}	0.158	<0.001
Average weight weaning (kg)	6.38^{a}	8.06^{b}	9.27^{c}	9.86^{c}	0.221	<0.001
ADG d4-11 (g/d)	185^{a}	238^{b}	275^{c}	286^{c}	9.7	<0.001
ADG d11-18 (g/d)	230^{a}	284^{b}	320^{c}	329^{c}	10.0	<0.001
ADG d18-weaning (g/d)	245^{a}	288^{b}	311^{c}	313^{c}	9.4	<0.001
ADG birth-weaning (g/d)	195^{a}	244^{b}	274^{c}	281^{c}	7.8	<0.001
ADG d4-weaning (g/d)	222^{a}	271^{b}	302^{c}	311^{c}	8.5	<0.001

Superscripts differing within a row denote significance at *P* <0.050. Birthweight group 1: <1.00 kg; 2: 1.00-1.50 kg; 3: 1.52-2.00 kg; 4: >2.00 kg.

4.4.1.5. Effect of birthweight group on pre-weaning piglet SM usage behaviour

There were no birthweight group*teat supply interactions in SM usage behaviour (number and duration of visits to the SM bowl) at any point (*P* >0.05). From d 18 to weaning, piglets from birthweight group 4 had higher visits/d (v/d) than those in group 1 or 2 (*P* =0.026; Table 12), with a tendency (*P* =0.060) for longer daily duration (du/d) of visit. There was no effect of birthweight group on v/d or du/d from d 4-d 11 or d 11-d 18 (P > 0.050). There was no effect of birthweight group on du/v at any point (*P* >0.05).

Table 12: Effect of birthweight group on pre-weaning piglet supplementary milk usage.

	Birthweight group					
Behaviour parameters	1	2	3	4	s.e.m	*P*-value
v/d$^{\alpha}$ d4-11*	12	7	6	5		0.485
du/v$^{\beta}$ d4-11 (s)	26	25	24	24	1.2	0.388
du/d$^{\gamma}$ d4-11 (s)*	331	182	136	122		0.468
v/d$^{\alpha}$ d11-18*	11	9	8	9		0.856
du/v$^{\beta}$ d11-18 (s)	24	23	23	22	0.6	0.198
du/d$^{\gamma}$ d11-18 (s)*	281	211	192	195		0.902
v/d$^{\alpha}$ d18-weaning*	12^{a}	14^{a}	16^{ab}	18^{b}		0.026
du/v$^{\beta}$ d18-weaning (s)	24	24	24	23	0.5	0.686
du/d$^{\gamma}$ d 18-weaning (s)*	298	334	373	424		0.060

Superscripts differing within a row denote significance at $P < 0.050$.* required log10 transformation, actual means presented without s.e.m due to non-normal distribution. Birthweight group 1: <1.00 kg; 2: 1.00-1.50 kg; 3: 1.52-2.00 kg; 4: >2.00 kg. $^{\alpha}$ visits/d; $^{\beta}$ duration/visit; $^{\gamma}$ duration/d

From d18-weaning, piglets from birthweight group 4 had higher v/d and a tendency for higher du/d than piglets from birthweight groups 1 or 2.

4.4.1.6. Effect of birthweight group on suckling position

Piglets from birthweight group 1 had the highest proportion suckling P or NONE, and piglets from birthweight group 4 had the highest proportion suckling A on d14 ($P < 0.05$; Figure 18).

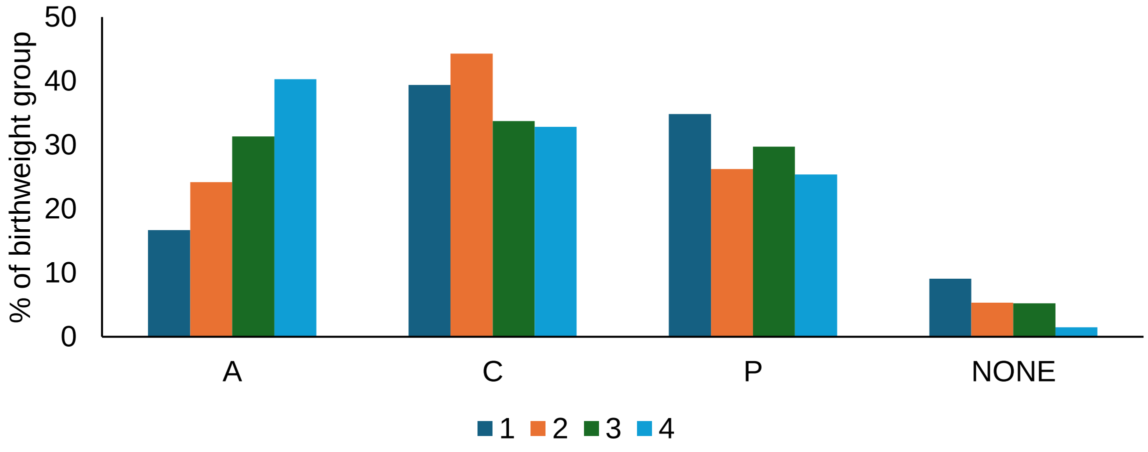

Figure 18: Proportion of piglets from each birthweight group observed suckling anterior (A), centre (C) or posterior (P) teats, or not suckling (N) at d14 of suckling. Birthweight group 1: <1.00 kg; 2: 1.00-1.50 kg; 3: 1.52-2.00 kg; 4: >2.00 kg.

4.4.1.7. Effect of suckling position on pre-weaning piglet performance

Suckling position at d14 was chosen as the most reliable for analysis, due to inconsistencies between observations on d4 as teat order had not been established, and the aforementioned high degree of competition on d25. There were no suckling position*teat supply interaction effects on performance (*P* >0.05). Of the piglets observed suckling (A, C, P), A were heaviest and had highest ADG at all points (*P* <0.001; Table 13). P were lightest and had lowest ADG, with C being lighter than A and heavier than P at most points. This was despite birthweight being similar between A, C and P (*P* > 0.050).

Table 13: Performance to weaning for piglets suckling anterior (A), centre (C), posterior (P) teats, or not suckling (N), at d14.

Performance parameters	Suckling position at udder (d14)				s.e.m	*P*-value
	A	C	P	NONE		
n	*166*	*227*	*164*	*28*		
Average birthweight	1.52^{a}	1.43^{ab}	1.42^{ab}	1.29^{b}	0.064	0.002
Average weight d4 (kg)	2.32^{a}	2.09^{b}	1.98^{bc}	1.75^{c}	0.098	<0.001
Average weight d11 (kg)	4.37^{a}	3.94^{b}	3.59^{c}	3.10^{c}	0.182	<0.001
Average weight d18 (kg)	6.70^{a}	6.02^{b}	5.46^{c}	4.69^{d}	0.263	<0.001
Average weight weaning (kg)	9.52^{a}	8.58^{b}	7.83^{c}	6.51^{d}	0.357	<0.001
ADG d4-d11 (g/d)	285^{a}	255^{b}	226^{c}	184^{c}	14.8	<0.001
ADG d11-18 (g/d)	333^{a}	298^{b}	267^{c}	228^{c}	15.4	<0.001
ADG d18-weaning (g/d)	328^{a}	299^{b}	273^{c}	214^{d}	14.3	<0.001
ADG birth-weaning (g/d)	286^{a}	256^{b}	230^{c}	186^{d}	12.0	<0.001
ADG d4-weaning (g/d)	316^{a}	284^{b}	257^{c}	208^{d}	12.9	<0.001

Superscripts within a row denote significance at *P* <0.050.

Piglets without a teat (NONE) were lighter than A at birth (*P* = 0.002) and lightest overall at d 18 and weaning (*P* < 0.001).

4.4.1.8. Effect of suckling position at d14 on pre-weaning piglet SM usage behaviour

There was no suckling position*teat supply interaction (*P* >0.05), and no effect of suckling position on du/v at any point (*P* >0.05; Table 14).

Table 14: Supplementary milk (SM) usage to weaning for piglets suckling anterior (A), centre (C), posterior (P) teats, or not suckling (N), at d14.

	Suckling position at udder (d14)					
Behaviour parameters	A	C	P	NONE	s.e.m	*P*-value
v/d $^{\alpha}$ d4-11*	4 ^{a}	6^{a}	6^{a}	19^{b}		<0.001
du/v $^{\beta}$ d4-11 (s)	24	25	24	24	1.4	0.969
du/d $^{\gamma}$ d4-11 (s)*	103^{a}	145^{a}	155^{a}	517^{b}		<0.001
v/d $^{\alpha}$ d11-18*	6^{a}	8^{a}	9^{a}	24^{b}		<0.001
du/v $^{\beta}$ d11-18 (s)	23	23	23	24	0.9	0.670
du/d $^{\gamma}$ d11-18 (s)*	145^{a}	177^{a}	206^{a}	621^{b}		<0.001
v/d $^{\alpha}$ d18-weaning*	11^{a}	12^{a}	16^{a}	36^{b}		<0.001
du/v $^{\beta}$ d18-weaning (s)	24	24	24	24	0.8	0.918
du/d $^{\gamma}$ d18-weaning (s)*	267^{a}	289^{a}	374^{a}	903^{b}		<0.001

Superscripts within a row denote significance at *P* <0.050. $^{\alpha}$ visits/d; $^{\beta}$ duration/visit; $^{\gamma}$ duration/d. * required log10 transformation, actual means presented without s.e.m due to non-normal distribution.

Between piglets suckling observed suckling (A, C, P) there was no difference in v/d or du/d (*P* >0.050), but piglets without a teat (NONE) had higher v/d and du/d at all points (*P* <0.001). Even when NONE were removed from the analysis, there was no difference in SM consumption between the suckled pigs at any point (*P* >0.05)

4.4.1.9. Classification of pre-weaning SM consumption group and effect on piglet pre-weaning performance

When SM consumption groups were defined according to v/d in the final week pre-weaning, there was separation between groups in terms of average v/d throughout the suckling period (*P* <0.001; Table 15). The difference in average v/d between the groups increased with time.

Table 15: Classification of pigs with low (L) medium (M) or high (H) SM consumption and the effect on piglet pre-weaning performance

Parameter	L	M	H	s.e.m	*P*-value
n	*215*	*215*	*214*		
v/d$^{\alpha}$ d4-11*	2^{a}	3^{b}	9^{c}		<0.001
v/d$^{\alpha}$ d11-18*	3^{a}	5^{b}	16^{c}		<0.001
v/d$^{\alpha}$ d18-weaning*	3^{a}	9^{b}	31^{c}		<0.001
Average birthweight (kg)	1.42^{a}	1.43^{a}	1.53^{b}	0.024	<0.001
Average weight d4 (kg)	2.12	2.13	2.09	0.038	0.785
Average weight d11 (kg)	4.02^{a}	4.03^{a}	3.70^{b}	0.070	0.001
Average weight d18 (kg)	6.07^{ab}	6.13^{a}	5.77^{b}	0.103	0.031
Average weight weaning (kg)	8.66^{ab}	8.77^{a}	8.20^{b}	0.138	0.009
ADG d4-d11 (g/d)	264^{a}	264^{a}	224^{b}	5.600	<0.001
ADG d11-18 (g/d)	293	301	296	5.800	0.581
ADG d18-weaning (g/d)	296	304	288	5.500	0.125
ADG birth-weaning (g/d)	259^{a}	262^{a}	241^{b}	4.600	0.002

Superscripts within a row denote significance at *P* <0.050. $^{\alpha}$ visits/d * required log10 transformation, actual means presented without s.e.m due to non-normal distribution.

Although piglets in group H were heaviest at birth (*P* <0.001), by d11 they were lighter than L or M, and were lighter than M at d18 and weaning, with L intermediate (*P* <0.05). Average daily gain was lowest for piglets in group H from d4-11 and overall from birth-weaning (*P* <0.05).

4.4.2. Post-weaning performance

4.4.2.1. Nursery performance

Due to the difference in v/d between categories in the two batches studied post-weaning, pens of piglets were re-classified as low (L), medium (M), high (H) and very high (V) SM consumption.

Although pigs in group V were lightest at weaning, with H and M heaviest, and L intermediate (*P* <0.001; Table 16), by d 54 there was no difference in weight between usage groups (*P* >0.05).

Table 16: Post-weaning performance of pigs with low (L), medium (M), high (H) or very high (V) supplementary milk usage pre-weaning, as measured by visits/day in the final week of suckling.

	L	M	H	V	s.e.m	*P*-value
n	*8*	*15*	*15*	*8*		
Average v/d final week	*3*	*9.5*	*24.5*	*59*		
Weaning weight (kg)	8.56^{a}	8.94^{b}	9.12^{b}	7.64^{c}	0.078	<0.001
Weight d6 (kg)	9.87^{a}	9.80^{a}	10.18^{a}	9.01^{b}	0.142	<0.001
Weight d19 (kg)	16.44	15.97	16.82	16.48	0.312	0.085
Weight exit d54 (kg)	45.5	45.4	45.6	45.0	0.65	0.928
ADFI wean-d6 (kg/d)	0.26	0.23	0.24	0.28	0.015	0.089
ADFI d6-d19 (kg/d)	0.64	0.59	0.62	0.63	0.023	0.211
ADFI d19-d54 (kg/d)	1.46^{a}	1.55^{ab}	1.58^{b}	1.57^{ab}	0.031	0.017
ADFI wean-d54 (kg/d)	1.12	1.17	1.20	1.20	0.024	0.093
ADG wean-d6 (g/d)	217^{ab}	173^{a}	205^{a}	275^{b}	17.7	<0.001
ADG d6-d19 (g/d)	506^{a}	443^{b}	478^{ab}	498^{ab}	17.7	0.021
ADG d19-d54 (g/d)	822	840	818	816	16.4	0.537
ADG wean-d54 (g/d)	677	668	667	680	12.8	0.805
FCR wean-d6	1.21^{ab}	1.40^{a}	1.22^{ab}	1.03^{b}	0.088	0.018
FCR d6-d19	1.27	1.33	1.30	1.28	0.055	0.836
FCR d19-d54*	1.77^{a}	1.85^{ab}	1.94^{b}	1.92^{b}		<0.001
FCR wean-d54†	1.66	1.75	1.79	1.76		0.006
CV weight wean (%)	21.19	18.03	22.57	25.65	2.722	0.146
CV weight d6 (%)	21.12	17.23	19.82	24.56	2.694	0.187
CV weight d19 (%)	20.77	17.03	16.41	20.66	2.494	0.346
CV weight d54 (%)	13.93	11.57	12.44	14.81	1.816	0.476

Differing superscripts within a row denote significance at *P* <0.05. v/d: visits to SM/day. *log10 transformation applied, actual means are presented without s.e.m due to non-normal distribution. †Kruskal-Wallis test applied to non-normally distributed data.

Pigs in group V had the highest ADG and lowest FCR during the first six days post-weaning. From weaning to d54 post-weaning, ADG was similar between the SM usage groups (*P*>0.05), but FCR was lowest for the pigs with the lowest SM usage pre-weaning (*P*=0.006). Average daily feed intake was also lowest for group L between d19 and d54 (*P*=0.017). Within-pen variation in weight was unaffected by pre-weaning SM consumption at any point (*P*>0.05).

4.4.2.2. Individual growth performance

Pigs from farrowing batches 2 and 4 were not penned by SM usage, but were weighed at weaning, d 54 post-weaning and 20 weeks old. They were included in the dataset for individual performance analysis. When multiple regression was employed to analyse individual post-weaning performance, variance accounted for was similar whether visits/day (v/d) or duration/day (du/d) were included as model terms. As du/d is a more continuous variable, models for du/d are shown in Table 17.

Table 17: Multiple regression equations for pig post-weaning weight and ADG in relation to pre-weaning SM usage (duration of visits/d: du/d) and previous weight

Parameter	Equation	r^2	s.e	*P*-value
Weight exit (d54; kg) male	0.002767*du/d+2.642*wnwt+19.39	55.9	4.64	<0.001
Weight exit (d54; kg) female	0.002767*du/d+2.642*wnwt+18.273			
ADG wean-exit (g/d) male	0.04528*du/d+30*wnwt+361.8	33.4	84.8	<0.001
ADG wean-exit (g/d) female	0.04528*du/d+30*wnwt+342			
Weight 20 weeks old (kg) male	44.01-0.00339du/d+1.3327*wtexit	64.2	6.97	<0.001
Weight 20 weeks old (kg) female	43.27+0.001011*du/d+1.2381*wtexit			
ADG exit-20 weeks (g/d) male	767.2-0.06*du/d+6*wtexit	14.6	122	<0.001
ADG exit-20 weeks (g/d) female	755.3+0.0186*du/d+4.31*wtexit			
ADG wean-20 weeks (g/d) male	401.2-0.02344*du/d+10.015*wtexit	55.1	63.9	<0.001
ADG wean-20 weeks (g/d) female	389.4+0.01467*du/d+9.286*wtexit			

du/d: duration of visits/day to supplementary milk bowl in the final week of suckling. wnwt: weaning weight. wtexit: weight at nursery exit (d54 post-weaning).

Supplementary milk consumption affected performance through to slaughter, with a positive effect from weaning to d54 post-weaning, but for male pigs, there was a negative effect of SM consumption on weight at 20 weeks, and ADG from weaning to slaughter and during the finisher phase. This negative effect of du/d was confirmed by a simple regression of ADG *vs* du/d, resulting in a negative co-efficient, and so is not a result of high estimates for other variables in the regression model. The effect of du/d for female pigs on weight at 20 weeks, and ADG to 20 weeks was not significant (*P* >0.05). Previous weight had the largest and always positive effect on subsequent performance; weaning weight had a positive effect on weight at nursery exit and ADG from weaning to exit, whereas exit weight had a greater influence over weight at 20 weeks old, and ADG from weaning to 20 weeks and exit to 20 weeks.

4.4.2.3. Individual feed recording (Nedap)

Utilising ANOVA for analysis of growth and feed data was not possible as the groupings of L and H for SM consumption represented different average v/d in each batch, therefore multiple regression was employed. In common with the pigs finished in commercial pens, variance accounted for was similar whether v/d or du/d was included in the model. There were fewer points with high leverage when du/d was included, and so equations using du/d are shown in Table 18. Start weight was a more accurate predictor of performance than weaning weight, and du/d had a negative effect on ADFI and ADG, but positive effect on FCR, confirmed by simple regression, and indicating that pigs in the finishing phase had lower feed intake, and slower, less efficient growth as pre-weaning SM consumption increased. Gilts had higher ADFI and FCR than boars, but there was no effect of sex on ADG.

Table 18: Multiple regression equations for pig post-weaning ADFI, ADG and FCR in relation to pre-weaning SM usage (duration of visits/d: du/d) and previous weight

Parameter	Equation	r^2	s.e	*P*-value
ADFI (g/d) Male	$2.062+0.01419stwt-(2.6x10^{-5}du/d)$	17.3	0.229	<0.001
ADFI (g/d) Female	$2.191+0.01419stwt-(2.6x10^{-5}du/d)$			
ADG (g/d)	$813.1+3.63stwt-0.0420du/d$	8.8	107	0.003
FCR Male	$2.394+0.00673stwt+(6.45x10^{-5}du/d)$	15.3	0.281	<0.001
FCR Female	$2.635+0.00673stwt+(6.45x10^{-5}du/d)$			

stwt: start weight at Nedap entry. du/d: duration of visits/day to supplementary milk bowl in the final week of suckling

4.5. Discussion

4.5.1. Calibration of litter SM usage and PigTrack recording

The model for litter SM usage allows this to be estimated without having to weigh SM to each litter every day, saving a lot of time, and increasing the number of litters that can be recorded in a batch. This is a unique method and allows for a greater degree of accuracy when classifying individual piglets according to usage. Alternative methods such as direct/video observations have limitations in the identification of piglets, and the time that can practically be recorded. The PigTrack system can record constantly for the entire suckling period with no observer fatigue, generating a far more complete dataset.

Due to data collection for the calibration model continuing throughout this study, it was unknown at the time of selection for post-weaning study whether duration/d or visits/d would provide the most accurate estimation of usage. Visits/d to the SM was chosen for this first study, but considering that duration/d produced a higher variation accounted for in the calibration model, in future experiments duration/d will be utilised.For the multiple regression equations linking SM consumption with post-weaning growth, duration/d was also the parameter with the highest variation accounted for, due to it being the more accurate predictor of intake. A limitation of the method employed is that the calibration of duration of visits to weighed SM consumption was performed on a litter basis, rather than individually, and so an assumption was made that duration of visits would be the most appropriate method for ranking individual piglets based on their SM consumption. It is possible that there is a higher degree of variation in the relationship between duration of visit and weight of SM consumed when considering individual piglets. Currently, technology to directly measure weight/volume of SM consumed and relate this to the individual piglet does not exist.

The importance of including piglet weight in calculations spanning the entire suckling period is assumed to be because of the effect of piglet weight on feeding rate. Light birthweight piglets typically consume a lower volume of milk per suckle (Douglas *et al.*, 2014a), and Wolter *et al.* (2002) found that SM feeding rate of heavy birthweight litters was higher than that of light birthweight litters. Kobek-Kjeldager *et al.* (2020c) concluded that lower drinking frequency on d14 and d21 compared to d7 indicated that the older (heavier) pigs had a higher consumption rate, although they did not measure duration of visits. In finishing pigs there is a positive correlation between weight and feeding rate (Hyun *et al.*, 1997).

4.5.2. Teat supply

This study demonstrated that sows were capable of rearing supernumerary piglets with the support of a SM system, in agreement with Kobek-Kjeldager *et al.* (2020a).

The IS sows reared just over two piglets/litter more than the S sows, resulting in a heavier litter weaning weight. However, piglets of all birthweights were lighter at weaning in IS litters compared to S litters, by approximately 400 g. This is consistent with previous research by, for example, Milligan *et al.* (2002), Douglas *et al.* (2013), and Kobek-Kjeldager *et al.* (2020a), whereby the nutritional demands of a larger litter size are unable to be met by the sow. However, Pustal *et al.* (2015) found that average piglet weaning weight between supplemented and unsupplemented litters did not vary, even though sows with supplemented litters reared over one pig/litter more than sows with unsupplemented litters. It is possible that this was due to the supplemented litters having the same number of piglets as functional teats and therefore SM was supporting the sow rather than rearing extra piglets.

In the current study, sow feed intake was capped at 16 kg/d, and there was no difference in intake between IS (averaged 9.4 kg/d) and S sows (averaged 10.1 kg/d). This feed intake is greatly in excess of that reported by Kobek-Kjeldager *et al.* (2020a), where sows were capped at 8.5 kg/d, and averaged ~6.4 kg/sow over lactation, and Pustal *et al.* (2015; averaged 5.3 kg/d). The average litter weaning weight of 121 kg in the current study was higher than Kobek-Kjeldager *et al.* (2020a; between approximately 90-110 kg) and Pustal *et al.* (2015; 104.9 kg), so the higher milk yield will have accounted for some of the higher feed intake.

Sow weight change in the current study was lower than that reported by both, Kobek-Kjeldager *et al.* (2020a) of around 22 kg and Pustal *et al.* (2015) of 35 kg weight loss over lactation, and so the additional feed in this study allowed the sows to maintain their bodyweight more effectively, which may have prevented a reduction in number born and litter weight in the subsequent parity in the IS sows compared to S sows. Neither Kobek-Kjeldager *et al*. (2020a) or Pustal *et al.* (2015) reported any difference in sow feed intake or weight loss from sows rearing larger litters, although they made no allowance for functional teats, and did not report subsequent performance.

Placenta weight used to calculate adjusted sow weight change is an estimate and should be treated with caution as there is high variability in this parameter (Vernunft *et al*, 2018). These considerations indicate the limitations of using sow weight pre-farrowing in weight loss calculations, although it is more convenient and easier to weigh sows on entry to the farrowing room.

A proportion of sow weight change would also have been accounted for by growth of the mammary gland. Kim *et al.* (1999a) working with gilts found each gland increased in weight from d5-28 of lactation from 381 g-582 g (53 % increase), although there was no increase between d21-28. Kim *et al.* (1999b) slaughtered sows on d21 and found that although total mammary gland weight increased, weight of individual mammary glands decreased as litter size increased, but this was mainly when increasing litter size from six to nine piglets/sow. With a litter size of 9,10 or 12 piglets, weight of each gland was similar ranging from 456, 467, 460 g respectively. Although their litter sizes were smaller than the current study, the lack of difference between their larger litter sizes suggests that an estimation of gland weight is possible by extrapolation. Kim *et al.* (1999b) also reported an overall linear relationship between litter size and total weight of mammary tissue (at d21), which seems contradictory to their results regarding individual glands, but is presumably due to non-productive (non-suckled) glands being weighed. This would allow for estimation of mammary weight at weaning, which can be subtracted from sow bodyweight. However, this is unnecessary in the current study, as S and IS sows had similar numbers of functional teats at farrowing.

Previously it has been considered that sow milk yield becomes limiting to piglet growth from around d21 of lactation (Hughes and Varley, 1980), but it can be seen in the current study that SM usage, visits and duration/d began to increase at a greater rate in IS than S from d12. This is similar to results of Miller *et al.* (2012) and Azain *et al.* (1996), who reported an increase in SM consumption from d12 in hot weather, and implies that milk yield may be insufficient for piglet's demands earlier in lactation when conditions are sub-optimal ie: when the number of piglets exceeds the number of functional teats, or sow feed intake is low.

4.5.3. Birthweight

A positive relationship between birthweight and weaning weight is well established (Quiniou *et al*., 2002; Paredes *et al.,* 2012; Douglas *et al.,* 2014b), and supported by the current study, but the effect of birthweight on pre-weaning ADG appears to be influenced by management. An increase in ADG with increasing birthweight has also been reported by Quiniou *et al.* (2002), but Surek *et al.* (2014) found no effect of birthweight group on pre-weaning ADG, and in the current study the effect was only seen between groups 1-3 (up to 2 kg birthweight). Surek *et al.* (2014) fostered litters to minimise variation in birthweight, and concluded that the lightest piglets performed well, as they were able to compete more equally for milk, but growth of the heaviest birthweight piglets was constrained by sows milk yield. It appears that if within-litter variation in birthweight is not manipulated, ADG to weaning increases with increasing birthweight, but it may not be a purely linear relationship. It is possible that the similarity in ADG between groups 3 and 4 is due to relative similarity in birthweight. The difference in average birthweight of the groups in this study was similar (0.35-0.41 kg), but at a lower birthweight the relative difference is greater, and so may have an increased effect. The birthweight group*teat supply interaction may indicate the competitive advantage of heavy birthweight piglets when competition for milk is higher, or reflect the higher SM usage observed in these piglets. The only difference in SM consumption between birthweight groups was observed from d18-weaning, indicating that sows milk yield was most limiting to the heaviest birthweight pigs at this point. In support of this, in 2002, Wolter *et al.* found that litters of heavy birthweight (1.8 kg) piglets consumed more SM than litters of light birthweight (1.3 kg) piglets, and Kobek-Kjeldager *et al.* (2020b) found that heavy birthweight piglets were more likely to consume SM. There may also be an effect of litter composition, as Douglas *et al.* (2014a) reported that light birthweight piglets (<1.25 kg) in mixed weight litters spent longer drinking SM than normal birthweight piglets (>1.75 kg), and light birthweight piglets in uniform litters, but there were no uniform litters of normal birthweight piglets with which to compare.

4.5.1. Suckling position

The effect of suckling position on performance, with weight and ADG reducing from anterior-centre-posterior suckling position throughout lactation reinforced earlier work by Nielsen *et al.* (2001) and Huting *et al.* (2017) but is contradictory to Skok *et al.* (2007), who reported no effect of suckling anterior, centre or posterior teats on piglet growth to weaning. This was despite posterior teats having a lower milk yield, and milk yield being positively correlated with growth, and it is possibly due to their being far fewer piglets observed suckling posterior teats (5 *vs* 34 anterior and 47 centre), as they were numerically 600 g lighter at weaning. The effect of suckling position on piglet weight may also be affected by within-litter weight variation. Manipulating within-litter weight variation by fostering has been shown to have contrasting results for light birthweight piglets (<1.25 kg) and heavy birthweight piglets (1.50-2.00 kg), although in all cases piglets suckling posterior teats have been lightest at weaning. Previously, Huting *et al.* (2017) observed that for light piglets in uniform litters, there was no difference in weaning weight between anterior and centre-suckled piglets, but both were heavier than posterior-suckled piglets. In mixed-weight litters, light piglets suckling anterior teats were heavier than both centre and posterior-suckled piglets. For heavy piglets in uniform litters, anterior-suckled piglets were heavier than both centre and posterior-suckled piglets, but in mixed litters there was no difference in weaning weight between anterior and centre-suckled piglets, but both were heavier than posterior-suckled piglets. What is surprising in the current study is that the posterior-suckled piglets did not increase their SM intake to compensate for the presumably lower sows milk intake. A similar observation was made by Huting *et al*. (2017) in which light birthweight piglets were expected to increase creep intake when their milk intake was restricted by competition from heavy birthweight littermates, but in fact did not consume more creep feed than light birthweight piglets in uniform litters. In the current study, there was no difference in birthweight among the suckled piglets and so the reason for the behaviour of the posterior-suckled piglets is unknown.

The proportion of piglets classified as non-sucklers (NONE; 4.8 %), was lower than the proportion of piglets classified as CUP (6-7 %) or LOW (7-13 %) by Kobek-Kjeldager *et al.* (2020b). These piglets were defined as missing more than one suckling bout and having above (CUP) or below (LOW) median usage of the SM bowl on the day of observation, and so could be considered as equivalent to NONE in the current study. The difference in proportion between the studies may be a result of litter size, as Kobek-Kjeldager *et al.* (2020b) utilised litters standardised to 14 or 17 piglets, and reported that the litters of 17 piglets had a higher proportion of piglets defined as CUP or LOW.

Piglets without a teat had the lowest ADG, and these piglets had the highest usage of SM, with the difference apparent from the first period (d4-11) of the study. Kobek-Kjeldager *et al.* (2020b) found that piglets defined as CUP had a similar ADG to those suckling the sow, or using a combination of suckling and SM, but piglets defined as LOW had the lowest ADG to d21. Kobek-Kjeldager *et al.* (2020b) observed that there was low consistency between observation days (d7, 14, 21) with regards to nutrition source, and this could be because a pig only needed to miss two suckling bouts on the observation day to be classified as CUP or LOW. These piglets suckled the sow on average 3-5 times per day (Kobek-Kjeldager *et al.*, 2020b). In the current study, a piglet was classified as NONE if it missed two consecutive suckling bouts, which would make it highly likely to genuinely be a non-suckler. This difference in methodology may explain the difference in performance results. It could also be due to mortality level: Kobek-Kjeldager *et al.* (2020b) had mortality of >10 % after d7 for piglets defined as CUP or LOW on d7, which would probably be due to starvation as the authors stated they did not train piglets to use the SM bowl, and so would remove the lightest piglets from the analysis. In the current study, mortality after d4 was 1.7 %, which demonstrates that piglets without a teat were successfully reared to weaning. Piglets without a teat to suckle could be compared with those in literature that have been artificially reared, although they have experienced a different environment, remaining with their littermates and sow. There are contrasting results where AR piglets have been compared to naturally-suckled piglets, with de Vos *et al.* (2013) reporting heavier weaning weights in AR piglets, Vergauwen *et al.* (2017) reporting heavier weight at d19, but Pieper *et al.* (2016a) found no difference in piglet weights at d14. These studies disagree with the current one, where non-suckling piglets were lighter from d4. There have been studies reporting poorer growth in AR piglets (Schmitt *et al.*, 2019 and Rzezniczek *et al.*, 2015), but these have concluded to be due to negative behaviours associated with being separated from the sow, and competition causing displacement at the milk cup. Because piglets stayed with their dam, there would not have been separation stress in the current study. There may have been competition at the milk bowl if more than one piglet was a non-suckler, but this was only the case for five litters, and so cannot explain the poor performance of the non-suckled piglets. Amdi *et al.* (2022) found that AR piglets were 2.5 kg lighter at 23 days old than sow-reared piglets, but the AR piglets were restrict-fed to avoid diarrhoea, resulting in lower DMI and much lower fat intake as the fat content of the milk replacer was approximately half that of the sows' milk. Theil and Jørgensen (2016) reported that fat retention rate (17 g/d), and corresponding energy retention (45 % of retained energy), in AR piglets was lower than in suckled piglets (21-32 g/d fat retention and 55-62 % retained energy from fat).

An important consideration when comparing these studies is colostrum intake, as piglets born into large litters are less likely to consume an optimal amount of colostrum, with the risk increasing as birthweight reduces (Declerck *et al.*, 2016). Colostrum management was not reported by Vergauwen *et al.* (2017), and piglets were selected from litters with at least 14 liveborn piglets, which is lower than the present study, and so there may have been less competition for colostrum. Pieper *et al.* (2016a) did not specify litter size, and removed piglets four hours after birth, which they assumed would provide sufficient colostrum consumption. In the current study, although split-suckling was employed, due to the high litter size it is possible that the lightest-birthweight piglets consumed less colostrum than required and there was a higher likelihood of these piglets being non-sucklers. If the only colostrum intake achieved by piglets was through split-suckling, they will have suckled the sow less frequently than their more competitive littermates. Colostrum intake has been shown to affect not only pre-weaning mortality, but also to have a positive relationship with weaning, nursery and finishing weight that is stronger in light birthweight piglets (Declerck *et al.*, 2016).

4.5.1. SM consumption

Piglets were classified by SM consumption during the final week of suckling as this was predicted to be the period where differences were most apparent, but the differences in v/d and du/d between L, M and H were evident from the first week of recording (d4-11). The effect of birthweight group on SM consumption was insignificant until the final week of suckling (and would explain the heavier birthweight of H piglets), and so being without a teat had the greatest effect on SM consumption, as this was significantly higher in non-suckled piglets from d4. The lower weaning weight of H piglets is a result of there being a higher proportion of non-sucklers, and piglets from IS litters, who were lighter on average at weaning. There is a little previous research regarding the effect of individual SM consumption on piglet performance, focussing instead on whether provision of SM is beneficial to piglets on a litter basis. These studies have been inconclusive regarding the effect of SM on performance, probably because there has been no accounting for level of consumption, merely whether SM is offered or not. A 2021 (b) study by Kobek-Kjeldager *et al.* using direct observation on selected days showed no effect of birthweight on v/d, but piglets with low suckling success the day before weaning had higher v/d to the supplemental feeding, although they didn’t classify these as non-sucklers, and the supplement was a wet creep rather than SM. They concluded the lack of effect of birthweight was due to feeder design, as multiple piglets could access it at once, leading to more social feeding.

There are differences between studies in the nutrient composition of SM which could contribute to performance differences; in most studies CP is 220-250 g/kg DM, but fat content is more varied, with the current study being intermediate at 140 g/kg DM compared to 90 g/kg DM (Schmitt *et al.,* 2019), 110 g/kg DM (Vergauwen *et al.,* 2017) and 200 g/kg DM (Pieper *et al.,* 2016a). Sow's milk is typically 75 g/kg fat and 50 g/kg crude protein from 175 g/kg solids (Zhang *et al*., 2018), resulting in 430 g/kg DM fat and 286 g/kg DM CP content. Net energy content of sows milk fat as a feed source for piglets is 5.4 MJ/kg (Whittemore *et al.,* 2003), resulting in an energy content of 2.32 MJ/kg DM from fat for sow's milk (5.4 MJ/kg * 0.430 kg/kg DM). Employing the same calculation for the SM fat contents from the studies stated above, the energy from fat in milk replacer ranged from 0.486 (5.4*0.090) to 1.08 (5.4*0.200) MJ/kg DM, meaning piglets consuming SM obtain a lot less energy from fat per volume consumed. Kobek-Kjeldager *et al.* (2020b) concluded that this was the reason for piglets suckling the sow in preference to consuming SM, even when their suckling success was low. Digestibility of ingested fat is also lower in milk replacer compared to sows' milk, leading to lower body fat retention (Theil and Jørgensen, 2016; Hojgaard *et al*., 2020). There may be differences in essential amino acid and fatty acid levels, given that commercial milk replacers are formulated from cow's milk, although synthetic amino acids (particularly lysine) may be added. Yao *et al.* (2023) reported similar amino acids profiles between human and porcine milk, but cow and goat milk were only 60 % similar to human milk, in particular over-supplying methionine and phenylalanine. Digestibility of the protein in SM and sows' milk may to differ: chymosin and pepsin in the stomach of piglets has been shown to be less active against reconstituted bovine skimmed milk powder (the basis of SM) than porcine milk (Foltmann, 1992). Chymosin acts on κ-casein at the 105-106 peptide bond which in bovine milk is phenylalanine-methionine, whereas in porcine milk it is phenylalanine-isoleucine, and this difference is assumed to be the cause of the inefficiency of pig chymosin initiating proteolysis of bovine milk (Foltmann, 1992). However, Theil and Jørgensen (2016) reported that protein retention rates for AR piglets was similar to those observed in sow-reared piglets, indicating that intake of digestible protein was unaffected. A recent study by Roy *et al.* (2022) investigated the digestion of cow, sheep, and goat milk in piglets, although with no sows' milk comparison. They reported differences in fat globule and casein micelle diameter, and differences in the softness of the curds over time with sheep milk resulting in the softest curds and cow milk resulting in the hardest curds at 210 minutes post-ingestion. Consequently, the percentage of fat and protein retained in the stomach was lowest for sheep milk and highest for cows' milk after 210 minutes, with a consistent positive relationship between fat and protein loss for the milk of all species. Differences in the clotting ability of chymosin will lead to differences in rate of gastric emptying, with protein and fat retention highest where emptying is slowest. Fat retention is positively correlated with protein retention as the fat globules are encased in the clot (Roy *et al*., 2022). Thus, fat digestibility is linked to protein digestibility, and piglets with lower fat retention should have lower protein retention, although this was not observed by Theil and Jørgensen (2016), with the milk replacer having 7 % lower protein:energy ratio than sows' milk and similar protein retention levels between AR and sow-reared piglets, and they had no explanation for this.

Growth factors, prebiotics and probiotics present in sow milk will also be absent in SM (Pieper *et al.*, 2016a), and different immunoglobulins are present in sows milk compared to the bovine milk on which milk replacer is based, which may inhibit the ability of the piglet to eliminate pathogens in the intestinal lumen (Kobek-Kjeldager *et al.*, 2020b).

The research of both Vergauwen *et al.* (2017) and Pieper *et al.* (2016a) reported physiological differences between AR and suckled piglets as AR piglets had higher villus surface area, and tended to have deeper crypts at d14, but this was not observed by Amdi *et al.* (2022). De Vos *et al* (2013) observed higher IGF-1 levels in AR piglets, which could explain the increased villus height. In the study of Pieper *et al.* (2016a), there were also indications of increased gut maturity in the AR piglets, with lower lactase and higher maltase activity compared to suckled piglets. This change in enzyme activity is similar to that which occurs at weaning, but the reason for it was unknown, as there was no source of starch in the milk replacer (Pieper *et al.*, 2016a). They speculated that maturation of the gut may be hormone-dependant, as well as substrate-dependant. Amdi *et al.* (2022) found that sucrase, lactase and maltase activity were all higher in sow-reared piglets than AR piglets, but they concluded this may be due to higher DMI in the sow-reared piglets, as the AR piglet were restrict-fed.

These contradictions highlight the need for investigation of the physiology of sow-reared piglets according to their SM consumption level.

4.5.2. Nursery performance

Piglets with the highest SM usage were lightest at weaning, as this was a continuation of the pre-weaning work, with no piglet rejected on the basis of weight, and so this was due to the combination of a high proportion of non-sucklers and piglets from IS sows. It is interesting that there was no difference in exit weight, as traditionally there has been shown to be a positive relationship between weaning weight and subsequent weights (Pluske *et al.*, 2003; Magowan *et al.*, 2011; Collins *et al.*, 2017). It was in the first 19 days post-weaning that piglets from V had highest ADG, and they had the lowest FCR from weaning-d6. There are few published works whereby individual piglet SM intakes have been assessed to evaluate effects on post-weaning performance, but Kobek-Kjeldager *et al.* (2021b) used direct observation, and determined that piglets that had access to supplementary feeding (SM to d19, liquid creep to d26) recommenced eating more rapidly on the day of weaning, and that the latency to feeding showed a negative relationship with feeding bouts (visits to the feeder). Other studies have assessed the effect of provision of SM pre-weaning on post-weaning performance with contrasting results, for example Correa *et al*. (2023), and Miller *et al.* (2012) reported no effect of SM provision on post-weaning performance, but Vodolazska *et al.* (2023) and van Oostrum *et al.* (2016) found that supplemented piglets had higher ADG and ADFI in the first week post-weaning than unsupplemented piglets. Wolter *et al.* (2002) found that SM provision resulted in a higher gain:feed from weaning-14 kg bodyweight, and ADFI was higher in supplemented pigs from 25-65 kg bodyweight, but with no effects on growth rate. The composition of the supplementary feeding must also be considered, as Vodolaszka *et al*. (2023) and Kobek-Kjeldager *et al.* (2021b) used SM to d19 of suckling, and then a meal creep diet mixed with water for the final week pre-weaning, whereas Correa *et al*. (2023), Miller *et al.* (2012) and van Oostrum *et al.* (2016) provided SM to weaning. Using a liquid creep is likely to be more effective in preparing the piglet for weaning as the diet is more similar to that which they will be consuming post-weaning, and so some physiological adaptation should have occurred.

4.5.3. Finishing performance

This is the first study that has followed pigs to slaughter and assessed performance related to SM consumption pre-weaning. Wolter *et al.* (2002) recorded pigs to slaughter, but solely in groups of supplemented or unsupplemented, and reported no effects of SM on ADG. Douglas *et al.* (2014a) found that although there was no overall effect of SM provision on weight or ADG to 143 days old, low birthweight piglets that were offered SM had lower CV of weight than unsupplemented low birthweight pigs, whereas in normal birthweight pigs the effect was reversed. In the current study nursery exit weight had the greatest effect on weight at 20 weeks and ADG to 20 weeks, and it is unknown why SM du/d had a negative effect on these parameters for boars, but no effect for gilts especially considering du/d had a positive effect on weight at nursery exit and ADG from wean-exit for both sexes. From the Nedap data, ADFI decreased as pre-weaning SM consumption increased, with the resultant decrease in ADG compounded by an increase in FCR, although there was no effect of sex on ADG with these pigs. The variance accounted for in ADFI, ADG and FCR by weight and SM usage was very low in the finishing phase and so other more intangible factors such as health, environment and social hierarchy will have had a greater influence.

4.6. Conclusion

Although litter weight weaned was higher in litters with insufficient teats, average piglet weight was lower and so SM was unable to fully compensate. Usage was considerably higher in these litters due to rearing approximately one piglet extra compared to the number of functional teats of the sow, and two piglets more than the sows with sufficient teats. Piglets without a teat had the highest SM usage, but there was no effect of suckling position on usage. There was little effect of birthweight on SM usage, but the heaviest piglets at birth had the highest SM usage in the final week of suckling, presumably due to requiring additional nutrition. Post-weaning, SM intake had a beneficial effect on performance during the nursery phase, but a negative effect on finisher performance, although these were weak relationships.

4.7. Plan for study 2

The next study investigated the effects of offering creep feed in addition to SM, as Pustal *et al.* (2015) found that piglets offered SM had higher creep intakes. Metabolomic analysis of plasma was undertaken to determine the effects of SM and creep consumption on piglet physiology.

5. Study 2: Effect of supplementary milk and creep on piglet performance and plasma metabolome pre- and post-weaning

5.1. Introduction

Increasing prolificacy of sows through genetic improvement has exceeded the increase in functional teats of the sow. With hyperprolific sows the number of piglets born in a group often exceeds the number of functional teats in that group, presenting limited opportunity for fostering of excess piglets (Kobek-Kjeldager *et al.* 2020a). Supplementary milk (SM) provision as additional nutrition *in situ* for litters suckling a sow has been shown to improve survival of piglets and eliminates the need for artificial rearing and shunt-fostering (Stewart *et al*. 2010, Kobek-Kjeldager *et al*. 2020a), but the effect on growth pre- and post-weaning is less conclusive. In Study 1, in litters with insufficient teats for the number of piglets, pre-weaning mortality was no higher than in litters where there were sufficient teats, demonstrating that SM prevented starvation, but the piglets that were observed as being non-sucklers were significantly lighter at weaning than piglets with a teat to suckle. Consequently, the average piglet weight at weaning was lower in litters with insufficient teats, by 0.57 kg, indicating that SM could not fully compensate for the absence of sow milk provision. Study 1 enforced the value of SM for rearing piglets in large litters, but in order to simplify the classification of piglets, creep-feeding was omitted, and this is an important consideration for piglet physiological development, as reviewed in Chapter 2. Previous studies have relied on in-feed marker dyes or direct observation to classify piglets as eaters or non-eaters of creep, but these techniques have their flaws, with dilution of the marker dye and timing of observations being sources of inaccuracy. Employing the antenna system utilised in Study 1 should enable a more accurate quantification of creep consumption at the individual piglet level. Study 2 investigates the interaction between SM and creep consumption pre-weaning, the effect on performance post-weaning, and by analysing the plasma metabolome, compares the physiology of piglets of differing pre-weaning levels of SM and creep consumption at weaning and 29 days later.

Metabolomics is a technique that is utilised to identify and quantify differences in the metabolic profile of biological samples at a particular point in time (Zhao and Rhee, 2023). It can also be used to identify differences in biological pathways. There are several techniques, but liquid chromatography (LC) or gas chromatography (GC) coupled with mass spectrometry (MS) have been used in the majority of studies (Alseekh *et* al, 2021). The advantage of GC-MS over LC-MS analysis is that it has higher chromatographic resolution (greater ability to separate peaks), can identify a wider variety of compounds using a single column, and there are more extensive databases to identify isolated metabolites (Munjal *et* *al*., 2022). Gas chromatography-mass spectrometry does however require a greater degree of sample preparation (Munjal *et al.,* 2022). Methods vary according to target metabolites (Kiseleva *et al.*, 2022), but the underlying principles are consistent: Samples are frozen to prevent further metabolism, then a solvent (for example methanol) is added to precipitate the protein and extract metabolites. After the solvent is evaporated, the sample is reconstituted and then derivatised to allow for volatilisation of metabolites (Alseekh *et al.*, 2021). Reconstitution is achieved with the use of an aprotic solvent (cannot donate protons, for example pyridine). Derivatisation is achieved by silylation, whereby an acidic hydrogen on the metabolite is replaced by an alkylsilyl group, increasing volatility (Kiseleva *et al.*, 2022). The sample is then subjected to GC-MS analysis, using an inert carrier gas (for example helium), with the temperature increasing in stages to the desired maximum. The rate of temperature increase can be adjusted; depending on the metabolites identified, some peaks may not separate fully, and need a longer time period and thus slower rate of temperature increases. Blank quality control samples are included periodically between samples to account for contamination, and analytical standards should be run to confirm compound identification (Alseekh *et al*, 2021). Compound identification is achieved by comparison of retention times with published databases (for example National Institute of Science and Technology; NIST), and confirmed using analytical standards that were analysed concurrently with the samples. The identified metabolites and their concentrations can be analysed through specialist packages (for example MetaboAnalyst) to determine differences in both metabolites and the metabolic pathways to which they contribute.

The metabolome of pigs is usually described by analysis of faeces/intestinal contents or blood plasma. Samples collected from intestinal contents will contain metabolites pertaining to the gut microbiome and diet (Beaumont *et al.*, 2021), whereas those obtained from plasma will reflect the influence of diet, and the pig’s ability to absorb and metabolise nutrients and so more accurately indicates metabolic status (González *et al.*, 2023).

Previous research has identified differences in the piglet metabolome around weaning, and as a result of differing nutrition. Beaumont *et al.* (2021) investigated the difference in faecal metabolome between piglets at 13 days old (suckling) and 23 days old (two days post-weaning). They identified 39 metabolites (using NMR), of which 18 differed between suckling and weaned piglets. Suckled piglets had higher levels of choline (present in sow milk) and metabolites associated with the fucose degradation pathway (fucose is present in milk oligosaccharides). Weaned pigs had higher concentrations of SCFA including butyrate and propionate, which are produced from bacterial fermentation of starch. Li *et al.* (2018) identified 433 (GC-MS) metabolites that differed between colonic samples from weaned and suckling piglets. These metabolites were involved in phenylalanine metabolism, TCA cycle, glycolysis/gluconeogenesis, propanoate metabolism and nicotinate/nicotinamide metabolism.

There are differences in composition between milk replacer and sow milk: most milk replacers are based on bovine milk. Bovine milk has higher lactose and lower fat and protein than porcine milk (Pieper *et al*., 2016a), and so piglets consuming SM will get a lower proportion of energy from fat than do those that predominantly suckle their dam, which may affect performance (Theil and Jørgensen, 2016) and metabolism. Differences in pre-weaning dietary composition between sow's milk, SM and creep may be reflected in the piglet metabolome, with Rosa *et al.* (2020) finding that piglets fed human milk had higher abundance of fatty acids in the large intestinal lumen at 21 days old, whereas those fed bovine formula milk had increased carbohydrate metabolites. This was attributed by the authors as potentially indicating a difference in energy source metabolism for the different diets. There were also differences in amino acids, bile acids and cholesterol in the luminal metabolome between the diets. Xiao *et al.* (2012) reported reductions in fatty acids, cholesterol and galactose serum levels in weaned pigs compared to suckling pigs of a similar age. The effects of feeding a solid creep, and the SM*creep combination, on the piglet metabolome have yet to be determined.

Metzler-Zebeli *et al.* (2023) took a targeted metabolomics approach (focussing on lipids, carbohydrate/energy, bile acid and amino acid/nitrogen metabolism pathways) to analysis of piglet serum, every seven days from d7-28 (weaning) of life, and on d31 and d35. Piglets were offered liquid milk replacer as a creep diet from d10-d24, then a starter diet was mixed into the milk replacer for two days, before solely being offered starter diet as a creep. They reported differences in serum metabolites pre- and post-weaning, and according to whether piglets had been offered creep feed or not. They found that weaning had the greatest effect on the fatty acid profile, with most triglycerides, phospholipids and carnitine being lower post-weaning, and concluded this reflected the piglets' dietary change from lipid-rich sow milk to carbohydrate-rich starter feed. Metabolites solely detected post-weaning were benzoic acid and hippuric acid (originating from plant polyphenols), and butyrate and propionate (originating from microbial fermentation of starch). Urea metabolites were lower post-weaning, and Metzler-Zebeli *et al.* (2023) concluded this was an indication of low feed intake resulting in a reduction of arginine and ornithine (pre-cursers to urea formation). Offering creep feed also altered the metabolomic profile of piglets. Carnitine and acylcarnitine were raised compared to solely sow-suckled piglets, which they concluded was due to a higher rate of glycolysis from ingested starch. Most triglycerides were higher in creep-fed piglets, with the authors inferring that this indicated higher insulin secretion due to higher starch ingestion, thereby increasing lipid synthesis in the liver. This was combined with preferential use of glucose compared to fat as an energy source, and the effect recurred until the final blood-sampling at seven days post-weaning (Metzler-Zebeli *et al.,* 2023).

Weaning is a time of stress for the pig. Psychological stress due to separation from their sow may result in a changed metabolome due to secretion of stress hormones (glucocorticoids and catecholamines), which increase the rate of gluconeogenesis, lipolysis and deamination of protein (He *et al.,* 2011). He *et al.* (2011) also reported that the effect of weaning stress on the gut microbiota may manifest in the metabolome, specifically that decreased levels of choline they observed in pigs seven days post-weaning compared to suckling pigs of the same age may have been caused by alterations to intestinal yeast levels, but may also have been due to the removal of sows' milk as a dietary source (Beaumont *et al.*, 2021). By taking blood samples 29 days post-weaning it is hoped that psychological stress-induced disruption to the metabolome will have ceased, and that any differences in metabolomic profile observed will be due to pre-weaning SM and creep consumption. Differences in metabolomic profile have been shown to persist after cessation of treatments, with the effect of pre-weaning feeding regime of calves being identifiable after two years (Kenéz *et al*, 2018).

Management and pre-weaning supplementary feeding of litters with supernumerary piglets compared to functional teats is an important tool to improve profitability of pig production. The development of electronic identification (EID) technologies now allows quantification of duration and visits at the individual piglet level throughout the entire suckling period, potentially providing a more accurate assessment of individual consumption than previous more subjective methods, demonstrated in Study 1. The effects of weaning, SM provision and creep feeding on the piglet plasma metabolome are not well-described, and identifying differences in the metabolome according to pre-weaning nutrition could inform strategies for, and improve the efficacy of, supplementary feeding of suckling piglets. Previous studies involving cattle have indicated that effects on the metabolome are detectable for a considerable period after cessation of dietary treatments (Kénez *et al,* 2018) and for at least 14 days post-weaning (González *et al,* 2023), although it is unknown whether this is the case in pigs, and so this Study will investigate the plasma metabolome at weaning and 29 days later to determine any persistent effects of SM or creep consumption level. Metzler-Zebeli *et al* (2023) reported that effects of pre-weaning creep provision were identifiable at seven days post-weaning, but there is a lack of research into the pig plasma metabolome in general.

Hypotheses for this Study:

- Creep consumption will vary according to birthweight and suckling position

- Piglets with high creep consumption will be better physiologically adapted to consuming a carbohydrate-based diet, which will be detected in the performance measurements and plasma metabolomic profile
- Effects of SM and creep consumption on the plasma metabolome will persist post-weaning

5.2. Study materials and methods

This study was performed under the Animals (Scientific Procedures) Act 1986, Project Licence PDE402EC7 Nutrition and Management for Sustainable Weaner Production, protocol 1, points 1 (commercial housing standards) and 9 (blood sampling). Ethical approval was granted by the Harper Adams University Animal Welfare and Ethics Review Board (Protocol 02-21), project number 0311-202103-PGMPHD.

5.2.1. Pre-weaning

Two batches farrowing six weeks apart, with up to 18 sows/batch (from a total of 32 sows/batch) were used. Sows were selected that had farrowed over a period of three days in each batch. Farrowing performance of the two batches utilised is summarised in Table 19.

Table 19: Farrowing performance of batches utilised in Study 2.

Parameter	Mean	s.d
Number of litters	*62*	
Litters/batch	31.0	2.00
Sow parity	2.75	0.18
Total born	16.9	0.24
Born alive	16.0	0.40
Piglet birth weight (kg)	1.43	0.04
Number weaned	13.3	0.19
Piglet weaning weight (kg)	8.77	0.23
Litter weaning age (d)	27.4	0.21

Details of animals used, management, accommodation, piglet and sow nutrition, weighing, feed recording, and the PigTrack® system used to record SM and creep consumption can be found in Chapter 3.

Piglets had access to supplementary milk (SM; Faramate, Volac UK) *ad libitum* from birth to two days prior to weaning, provided through a bowl in the crate, constantly supplied from a single tank per batch. Creep was introduced at 14 days prior to weaning (d12), fed through a single space feeder. Weight of creep consumed per litter was recorded. Individual piglet SM and creep consumption was measured using the PigTrack ® system (Asserva, France).

5.2.1.1. Weighing

Piglets were individually weighed on d4, 11, 18, 25 (the day prior to weaning), 26 (weaning). Days were based on the batch average days post-farrowing.

5.2.1.2. Observation of suckling

On day 14, suckling was observed, using the same methodology as in Chapter 3.

5.2.2. Experimental design

This study had a split-plot design; for the whole plots, piglets were assigned a SM usage treatment by calculating a milk score prior to creep introduction, using duration of visits and ADG from d4-11:

$$Milk\ score = \frac{Total\ duration\ of\ visits\ to\ SM\ bowl}{ADG}$$

Piglets were ranked by milk score, then split in half to give the whole-plot treatments SM low (ML) and SM high (MH). As duration of visit was the most strongly correlated variable measured with weighed litter intake (from Pilot and Study 1), this parameter was used to classify consumption levels, but it was decided that a consideration of the relative importance of supplementary feeding to the piglets' overall nutritional intake was required. To achieve this, duration of visits was divided by the individual piglet's ADG for the selected period, giving a proxy FCR. A low figure therefore implied that the piglet obtained more nutrition from other sources (ie the sow), whereas a high figure implied that the piglet was more reliant upon supplementary feeding provided. This method allowed a distinction to be made between two scenarios with potentially similar supplementary feed intake durations:

1. A small piglet with consequently low appetite that mainly consumed supplementary nutrition
2. A large piglet that mostly consumed sows' milk, but satisfied its' appetite with supplementary nutrition

Durations of visit <7s were discarded, as were those >70s. Ninety-five per cent of visits fell within these boundaries.

Within each SM treatment, piglets were assigned a creep usage treatment by calculating a creep score using duration of visits and ADG from d12-26.

$$Creep\ score = \frac{Total\ duration\ of\ creep\ feeder\ visits}{ADG}$$

Pigs were ranked by creep score, to give the sub-plot treatments creep low (CL) and creep high (CH). An assumption was made that duration would also be the main defining term for creep consumption, as no creep was provided in Study 1. Creep score was calculated using the same method and reasoning as milk score, with a 95% acceptance range of 3-80s. A schematic of the design and selection is shown in Figure 19.

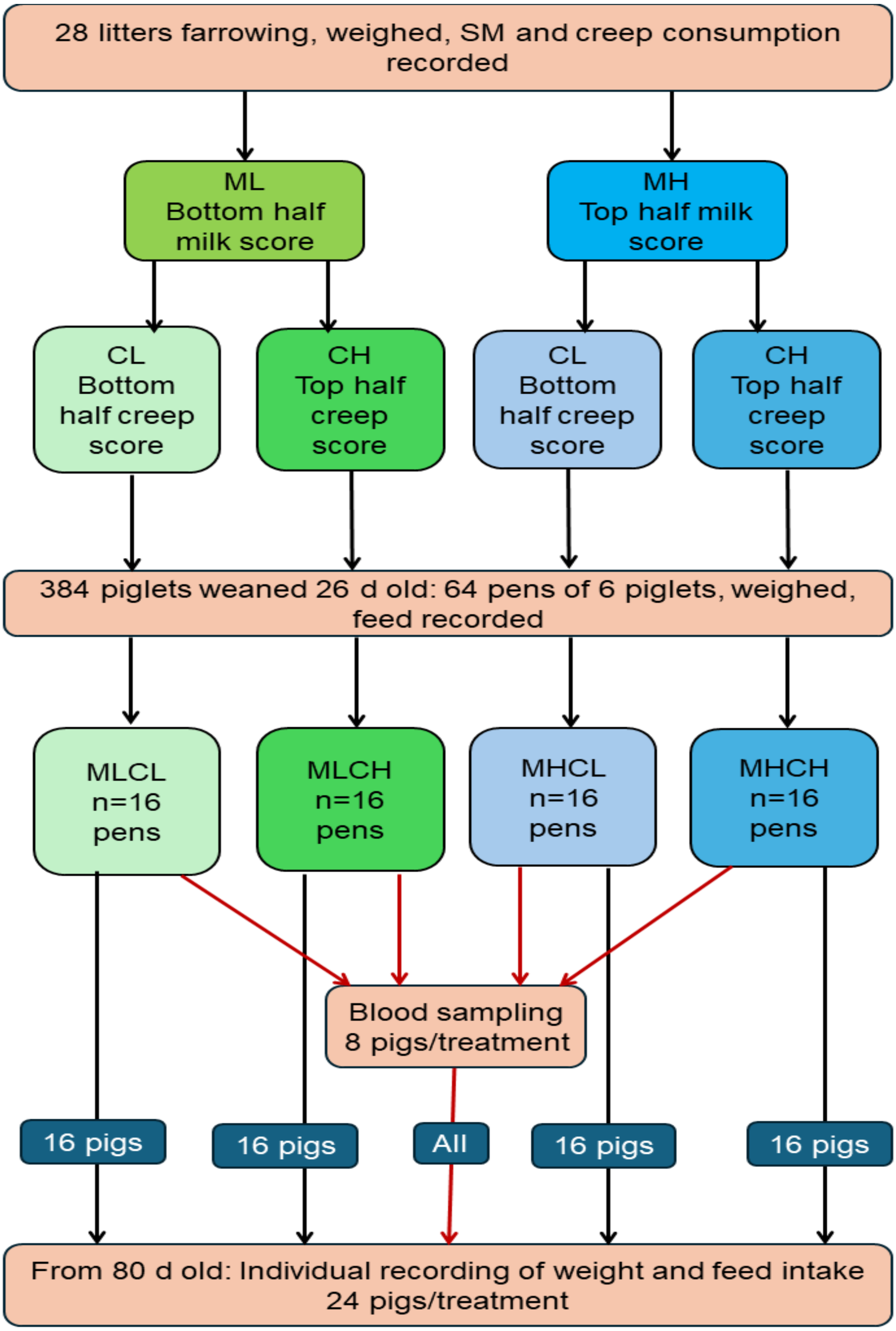

Figure 19: Experimental design and selection of study 2, comparing performance and metabolome of pigs with low (L) or high (H) pre-weaning supplementary milk (M) or creep consumption (C). Milk score = (SM duration of visits d4-11)/(ADG d4-11). Creep score = (creep duration of visits d12-26)/(ADG d12-26).

Piglets were allocated within treatment to post-weaning pens of six piglets (experimental unit was pen, 64 pens, n=16 pens/treatment), with average pen weight equalised within treatment. There were six piglets rejected from the pre-weaning selection pool that did not continue onto the post-weaning study: four were lame, one had an eye cyst, and one piglet was in excess of the number required. Pigs to be blood-sampled were distributed between pens such that there were no more than two blood-sampled pigs in any one pen to minimise any environmental/pen effects on metabolomic profile

Details of nursery and finishing accommodation, and nutrition can be found in Chapter 3.

5.2.3. Post-weaning

5.2.3.1. Weighing

Piglets were individually weighed at weaning, d7, 15, 29, and 54 post-weaning (pw), when they exited the weaner accommodation. Pen feed intake was recorded for each period. In batch 2, at d54 post-weaning 96 pigs entered Pig Performance Testing stations (PPT; Nedap, Netherlands). Pigs selected for the PPT comprised all 32 blood sampled pigs, plus a further 16 pigs/usage treatment (eight each male and female) from the entire range of weights on d50 pw. Usage treatments and sex were mixed within PPT station pens to ensure no confounding effects of environment or feeder.

5.2.3.2. Blood sampling.

In batch 2, eight piglets were selected to be blood sampled from each of the four usage treatments. Piglets were selected from those with the lowest and highest scores for low and high treatments respectively, but were also within 1 sd of the mean weight 1 day prior to weaning (d26; 7.05-9.90 kg, mean 8.63, sd 1.59). This was to ensure that the sampled piglets were sufficiently different in consumption between treatments, and that the effect of the ADG denominator for selection was minimised.

Piglets were blood-sampled from the exterior jugular vein into 10 mL heparin vacutainer tubes (Becton Dickinson Vacutainer systems, Plymouth, UK) on d26 (1 day prior to weaning) and 21 days later, by Personal Licence holders. Piglets were restrained by lying them on their back in the channel of a V-board, with one person holding the piglet’s head level (veins neither stretched nor loose), and one holding the hind legs. The person taking the blood sample manipulated the foreleg to maximise the jugular furrow at the side of the trachea, and expose the external jugular vein sampling site. The sampling site was wiped with an antiseptic wipe, and a 25 mm 20-gauge sampling needle (double-ended) screwed into the vacutainer collar, after removing the rubber cover from the needle contained within the collar. The sampling needle was inserted into the deepest part of the jugular furrow, perpendicular to the skin and approximately one third of the distance between the neck and shoulder. The labelled vacutainer was placed fully upon the needle within the vacutainer collar, and the sampling needle was pushed in to the piglet up to the hilt, then retracted slowly until blood flowed. Once blood flow was established, the needle was held in place until the tube was at least two-thirds full. All piglets were able to be blood-sampled within the recommended 5-minute time limit. The vacutainer was then removed from the needle in the collar and gently inverted and rolled to mix the blood with the heparin coating. The needle was gently removed from the piglet, and gentle pressure maintained on the sampling site using a cotton wool pad to ensure bleeding had ceased. Samples were refrigerated overnight, then centrifuged (Sigma 3-16 KL) at 1000 g for 10 mins at 4°C. The plasma supernatant was pipetted into three aliquots of 1-2 mL (dependant on yield of blood from each pig) in 2 mL screw-top vials and frozen at -20°C.

5.2.3.3. Metabolomic analysis

5.2.3.3.1. Sample preparation

Samples were prepared for GC-MS analysis according to a method adapted from Dervishi *et al*. (2017). From the defrosted plasma samples, 100 µL was pipetted into a 1.5 mL Eppendorf. An internal standard of 0.4 mg/mL ribitol in water (10 µL), and 800 µL of HPLC-grade methanol/ultra-pure water (8:1 vol/vol) was added. Methanol was identified as the most effective solvent for extraction by Jiye *et al* (2005) when analysing metabolites found in blood plasma by GC-MS. Each sample was vortexed (SciQuip Vortex VariMix) for 5 s, chilled at 4 °C for 20 minutes, and centrifuged (SciQuip Sigma 1-14) at 16000 g for 10 minutes. From the resulting supernatant, 200 µL was pipetted into a 2 mL glass GC vial with 300 µL insert, and dried at 60 °C in an oven (Philip Harris Ltd) until completely dry to remove the methanol. Dried residues were reconstituted with 40 µL of a 20mg methoxamine HCl:1 mL pyridine solution as an aprotic solvent, and incubated at room temperature for 16 hours. Use of an aprotic solvent (has no hydroxyl or amine group) ensures there is no hydrogen-bonding between reconstitution solvent and the metabolites (Parkinson, 2012). A derivatisation solution of N-methyl-N-trifluroacetamide (MSTFA) containing 1 % trimethylchlorsilate (TMCS) was added (50 µL/sample). This mixture derivatises a wide range of metabolites, and so is suitable for untargeted metabolomics approach (Parkinson, 2012). The samples were vortexed for 5 s and incubated for 1 hour in an oven (Philip Harris Ltd) at 90 °C. After incubation, samples were vortexed for 5 s, and stored at 4 °C until analysis. This method was also used to prepare four blanks containing no plasma, and ten QC samples that contained an amino acid analytical standard (Amino Acid Standard AAS18). All reagents were purchased from Sigma Aldrich UK.

5.2.3.3.2. Analysis

An untargeted approach was followed. An autosampler (G4513A; Agilent Technologies, Cheadle, UK) injected 2 µL of the derivatised samples into Agilent 7890B gas chromatograph (GC;Agilent Technologies) coupled with Agilent 5977A single quad mass selective detector (Agilent Technologies),. Methanol (HPLC-grade) was utilised as the cleaning solvent between samples to prevent contamination. The helium carrier gas flow rate was set at the manufacturer recommended rate of 1.2 mL/min. Oven temperature was initially 50 °C and increased by 5 °C per minute to 310 °C. Solvent delay was set at 6 minutes. Blank samples were injected every 10 samples. Amino acid QC samples and an alkane standard solution C_8-C_{40} (1 µL, Sigma Aldrich UK) were injected at the beginning and end of each session.

5.2.4. Statistical analysis

5.2.4.1. Performance data

In addition to analysis detailed in Chapter 3, birthweight groups were assigned *post-hoc* as group 1 (G1) ≤1.04 kg, group 2 (G2) 1.06-1.52 kg, group 3 (G3) 1.54-1.90 kg, group 4 (G4) ≥1.92 kg. These groupings followed the same distribution as in study 1 (11, 39, 39, 11 % of the batch when ranked by weight). Post-weaning pen performance data (ADG, FCR, ADFI) to d54 pw was analysed by split-plot ANOVA (Genstat 20th edition; VSN International, Hemel Hempstead, England) for each period. Finisher performance to slaughter of the individually recorded pigs was analysed by ANOVA (Genstat 20th edition). Significance between treatments was determined by Bonferroni test. Multiple regression (Genstat 20th edition) was used to determine the effects of weight, birthweight group, sex, SM duration and creep duration on post-weaning performance. The maximal model was fitted initially, with a step-wise elimination of non-significant terms to obtain the minimal model.

5.2.4.2. Metabolomic data

Peak areas for each retention time (RT) were integrated using Agilent MassHunter Quantitative Analysis B.06.00 (Agilent Technologies UK) to calculate the concentration. Compound identification was performed using AMDIS (Automated Mass spectral Deconvolution and Identification System; AMDIS.net) and comparing mass spectra to the database of the mass spectral library of the National Institute of Science and Technology (NIST), and comparing retention time to the C_8-C_{40} alkane ladder. Where compounds were identified more than once per sample, a mean concentration was calculated. Compounds with RT corresponding to the blank samples were discarded as these were remaining from derivatisation, or contamination. Peak areas (concentrations) were analysed using

MetaboAnalyst (V6.0; https://www.metaboanalyst.ca/). Initially, one-factor analysis was selected with data uploaded as concentrations in a csv file. Data were normalised by median and scaled by mean. Heat maps were obtained of relative metabolite concentrations by treatment. Principle component analysis (PCA) and t-test (two treatments) or ANOVA (three or more treatments) were performed. For the pathway analysis, to maximise pathways identified the Kyoto Encyclopedia of Genes and Genomes (KEGG) ID for each compound was obtained from the KEGG database (genome.jp./kegg/), and any that were unable to be matched were discarded from the dataset. The concentration table (csv file) was uploaded with KEGG IDs in place of compound names. Data were normalised by median and scaled by mean. Scatterplot visualisation was selected, and a reference metabolome of all the metabolites identified in this study was uploaded. This follows the recommendation of Wieder *et al.* (2021) to avoid false positives which may occur if comparisons are made with metabolites in the default reference list that were not identified in the present experiment. The pathway library utilised was the KEGG *Homo sapiens* library as this is most commonly used for pigs due to the high degree of physiological similarity, but is a limitation (Wieder *et al*., 2021; Metzler-Zebeli *et al.*, 2023). A scatterplot was produced of all pathways, and pathways were listed by *P*-value, FDR (false discovery rate; *P*-value adjusted for multiple tests) number of metabolites matched, and impact. Only pathways where more than one metabolite was identified were considered significantly altered to avoid over-estimation of impact due to changes in a single metabolite (Luise *et al.* 2020).

5.3. Results

5.3.1. Pre-weaning

5.3.1.1. Comparison of study means with study 1.

Mean performance levels between Study 1 and Study 2 were similar, as shown in Table 20.

Table 20: Comparison of performance and SM usage between Study 1 and 2.

	Study 1	Study 2
n (individual piglets at d4)	*638*	*517*
Age at weaning (d)	27.2	27.4
Average birthweight	1.45	1.38
Average weight d4 (kg)	2.10	2.07
Average weight d11 (kg)	3.92	3.93
Average weight d18 (kg)	6.00	6.26
Average weight weaning (kg)	8.54	8.68
ADG birth-d4 (g/d)	125	127
ADG d4-d11 (g/d)	252	265
ADG d11-18 (g/d)	296	288
ADG d18-weaning (g/d)	295	302
ADG birth-weaning (g/d)	253	261
ADG d4-weaning (g/d)	284	287
n (litter)	45	36
Born alive	16.7	17.4
Stills	1.1	1.1
Number of pigs weaned	14.2	14.3
Pre-weaning mortality (%)	14.8	17.8

Mortality was higher in Study 2, with a higher proportion of piglets being splayed or chilled (Figure 20).

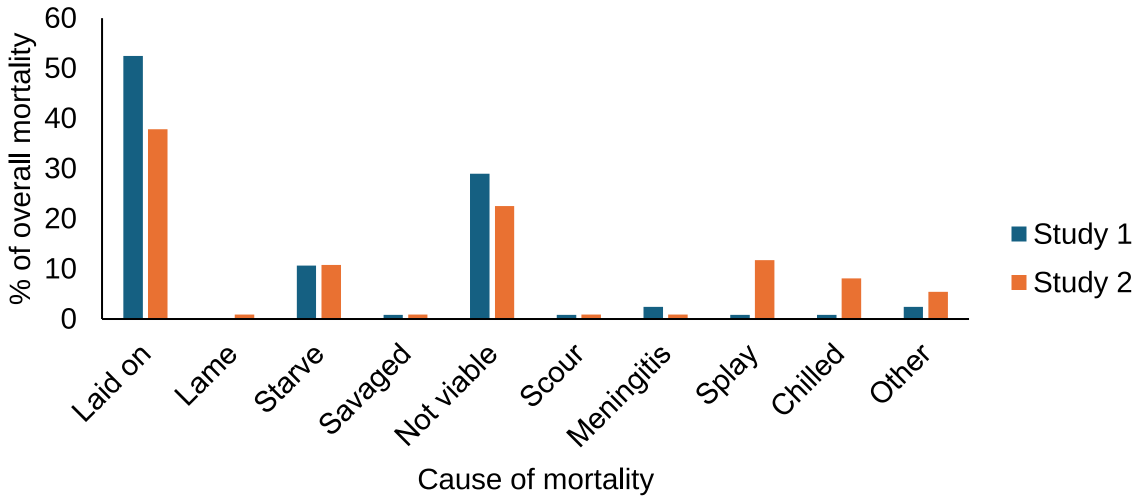

Figure 20: Causes of mortality in Studies 1 and 2.

When piglets were grouped by birthweight using the same bands as in study 1, the distributions between the studies were different (*P* <0.001), with fewer piglets in groups 1 and 4 in study 2 than in study 1 (Figure 21). To ensure that the analysis by birthweight groups represented similar proportions of piglets, birthweight group boundaries were based on the proportion of piglets being equal to that in study 1 (11, 39, 39, 11 % piglets for groups 1, 2, 3, 4 respectively). The new birthweight groups were used for analysis throughout, and were Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg.

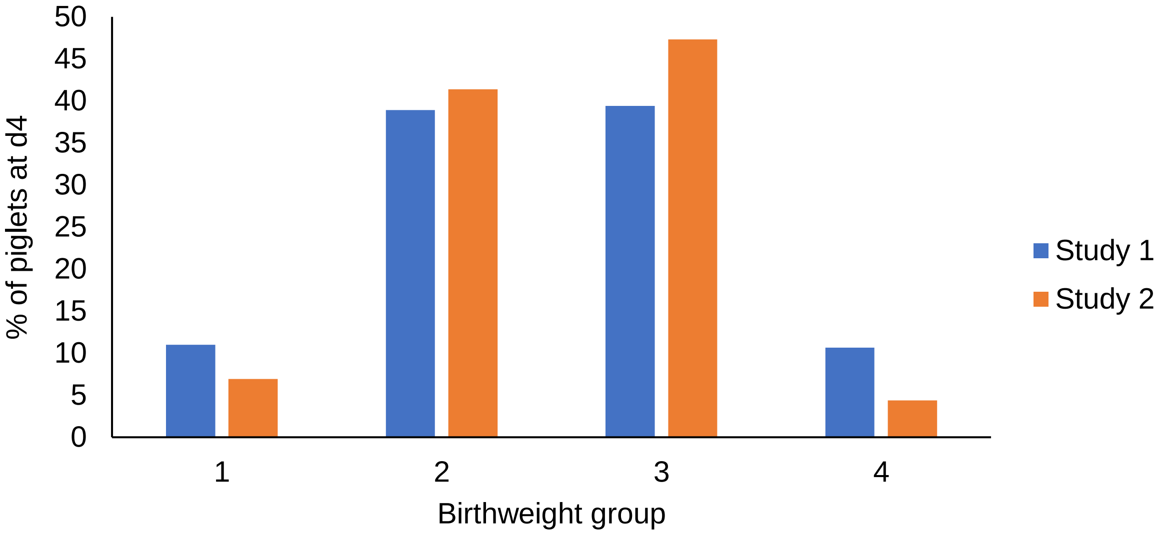

Figure 21: Proportions of piglets in each birthweight group in Study 1 and 2. Birthweight groups in ascending birthweight, with 11, 39, 39 and 11 % of piglets per batch respectively.

5.3.1.2. Consumption of SM and creep

Calibration of litter creep consumption to duration of visits and performance data was assessed using multiple regression, using the same methods as for SM in Study 1. To maximise variance accounted for, two separate models were required for the periods d11-18 and d19-weaning for creep intake. These are displayed in Table 21, along with the model for SM obtained in Study 1.

Table 21: Calibration equations linking weighed SM and creep consumption with duration of visits and pig performance.

Parameter	Equation	r2	s.e	*P*-value
SM (kg/d)	$0.000167\sum wt_i{*}du_i-0.378$	84.0	1.17	<0.001
Creep d12-18 (g)	$0.02185du_{12\text{-}18}+190.2avewt_{11}-978$	54.3	145	<0.001
Creep d19-wean (g)	$0.01571\sum wt_i{*}du_i+186.5litwtgain-59.4litwt_{19}-1265$	66.8	1012	<0.001

$\sum wt_i{*}du_i$: Sum of individual piglet weight x duration of visits for each day during the period d19-wean; $du_{12\text{-}18}$: duration of visits d12-18; $avewt_{11}$: average piglet weight d11; litwtgain: litter weight gain d19-wean; $litwt_{19}$: litter weight d19.

Using these equations, daily DM consumption was calculated to compare total supplementary nutrition intake between Study 1 and 2 (SM DM 150 g/kg, creep DM 880 g/kg). The calibration equations for SM were calculated on a daily basis, so could be used as in Table 21, but for creep they were calculated on a weekly basis, to match the frequency of weighing of litter creep intake. To estimate daily intake, the duration, $\sum wt_i{*}du_i$, and litter weight gain for each day was used, and coefficients for average weight and litter weight, and the constants, were divided by the number of days in each period. Negative intakes were discarded. For the period d12-18, using the equations under-estimated creep consumption by 23 %, but for d19-wean consumption was over-estimated by 25 % compared to weighed totals for the period. Consumption of SM was similar between studies (Figure 22), until d23 when there was a large decrease in SM consumption in study 1, the reason for which is unknown. Date for d14 from study 1 was discarded as it showed a very large drop in intake, which was assumed to be due to errors in recording.

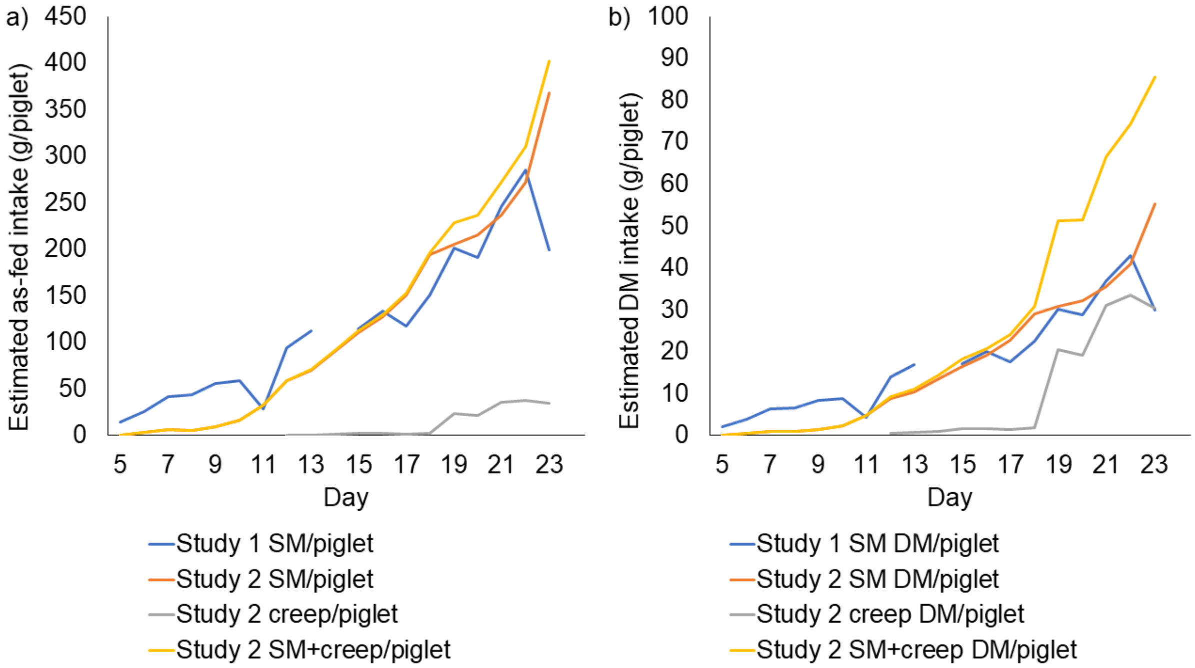

Figure 22: Comparison of piglets supplementary feeding pre-weaning between studies, as-fed (a) and DM basis (b). Creep DM 880 g/kg, SM DM 150 g/kg.

Although creep intake was low, due to the relatively high DM, total supplementary DMI was considerably higher in study 2 from d19. Creep intake for the first week after introduction was very low, but showed a large increase on d19. This may be partly due to the different equations used to calculate creep intake, and their under- and over-estimation of intake, but there was a large increase in creep consumption (weighed) for d19-weaning (0.16 kg/piglet compared to 0.013 kg/piglet for d12-18).

Diurnal variation in SM and creep intake was similar, with a morning and afternoon peak in activity, and followed that observed in study 1 (Figure 23). The pattern of SM consumption didn't vary between pre-creep and post-creep introduction periods.

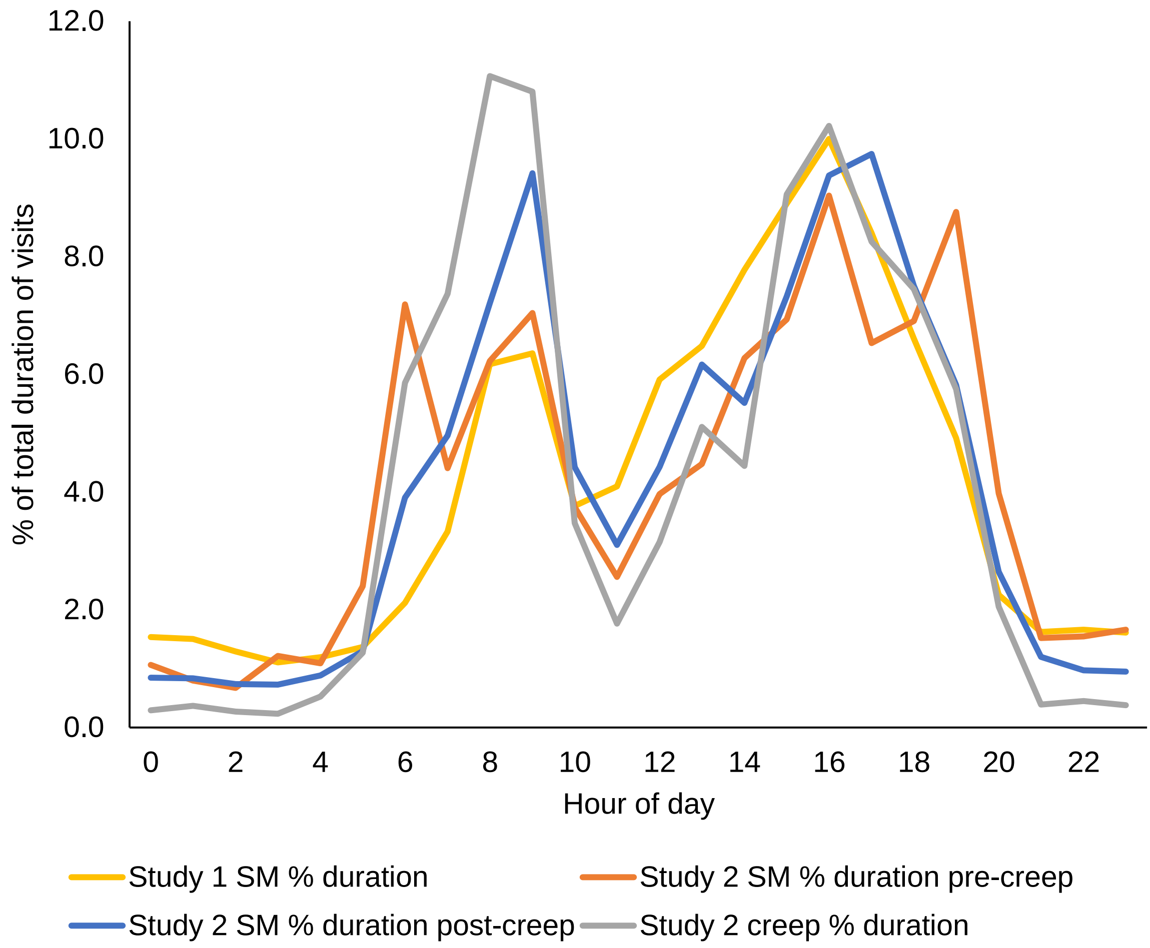

Figure 23: Diurnal variation in piglet pre-weaning SM and creep consumption. Pre-creep: up to d12; post-creep: d12-weaning.

The proportion of piglets consuming SM increased gradually from 32 % on day 5 (Figure 24). The rate of increase slowed on d11 (81 % of piglets consumed SM), and reached a maximum of 97 % on day 19. Creep consumption was high from the day of introduction with 87 % of piglets consuming creep on day 12, and also reached a maximum of 97 % of piglets consuming creep on day 19. Of the 47 piglets that didn't consume SM on every day after d19, 44 were low consumers. Of the 35 piglets that didn't consume creep on every day after d19, 30 were low creep consumers.

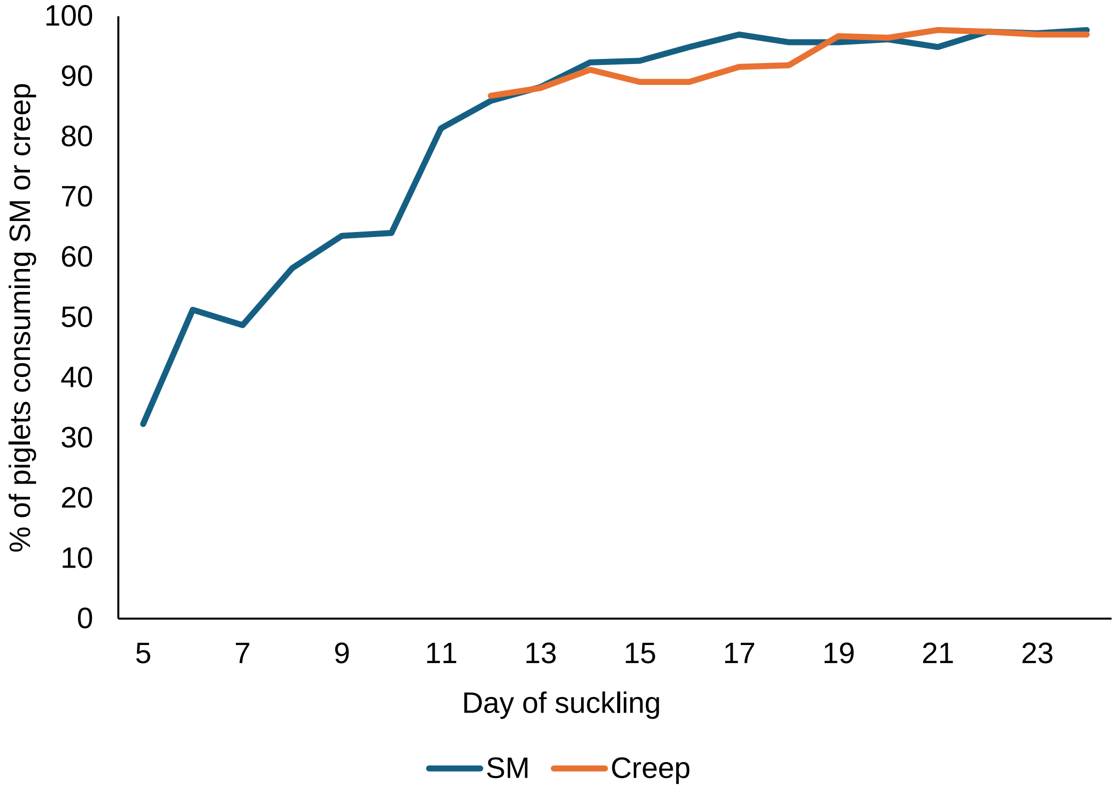

Figure 24: Percentage of piglets consuming SM or creep during the suckling period.

5.3.1.3. Effect of birthweight group

5.3.1.3.1. Performance

As shown in Table 22, the difference in weight between piglets of the lightest three birthweight groups was maintained until weaning. Birthweight group 4 were heaviest until d11; thereafter the weight of these pigs was similar to those in birthweight group 3 (*P*<0.001).

Table 22: Effect of birthweight group on pre-weaning piglet growth performance.

	1	2	3	4	s.e.m	*P*-value
n	*42*	*155*	*151*	*41*		
Average birthweight (kg)	0.93^a	1.32^b	1.69^c	2.04^d	0.018	<0.001
Average weight d4 (kg)	1.36^a	1.90^b	2.34^c	2.72^d	0.046	<0.001
Average weight d11 (kg)	2.73^a	3.72^b	4.40^c	4.84^d	0.106	<0.001
Average weight d18 (kg)	4.56^a	5.88^b	6.93^c	7.37^c	0.168	<0.001
Average weight weaning (kg)	6.53^a	8.13^b	9.57^c	10.14^c	0.227	<0.001
ADG birth-d4 (g/d)	90^a	125^b	145^c	146^c	8.0	<0.001
ADG d4-d11 (g/d)	196^a	260^b	294^c	303^c	11.0	<0.001
ADG d11-18 (g/d)	229^a	269^b	316^c	317^c	10.3	<0.001
ADG d18-weaning (g/d)	247^a	282^b	330^c	347^c	10.6	<0.001
ADG birth-weaning (g/d)	202^a	246^b	287^c	294^c	8.2	<0.001
ADG d4-weaning (g/d)	225^a	271^b	314^c	323^c	8.9	<0.001

Superscripts differing within a row denote significance at *P* <0.050. Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg.

ADG was similar for piglets in birthweight groups 3 and 4 throughout, but was lower in piglets of birthweight groups 1 and 2 at all points (*P* <0.001).

5.3.1.3.2. Consumption of SM and creep

Average number of visits/d (v/d) and duration of visits/d (du/d) to the SM were similar between birthweight groups until the period d 20-weaning, when piglets from group 4 had higher visits/d (*P* =0.031) and a trend for higher duration/d (*P* =0.052) than those in group 2, with group 1 and 3 intermediate (Table 23).

Table 23: Supplementary milk (SM) usage to weaning of piglets with differing birthweight groups.

	1	2	3	4	s.e.m	*P*-value
n	*42*	*155*	*151*	*41*		
Average v/d d4-11*	3.4	2.2	2.2	2.8		0.398
Average v/d d12-19*	8.3	7.3	7.1	9.7		0.190
Average v/d d20-weaning*	12.5^{ab}	10.1^{a}	10.7^{ab}	15.6^{b}		0.031
Average du/d d4-11 (s)*	82	52	52	62		0.471
Average du/d d12-19(s)*	184	169	162	216		0.438
Average du/d d20-weaning (s)*	293	233	252	358		0.052
Average du/v d4-11 (s)	23	22	22	21	1.0	0.439
Average du/v d12-19 (s)	23^{b}	23^{b}	22^{ab}	21^{a}	0.6	0.019
Average du/v d20-weaning (s)	23	23	23	22	0.5	0.407

Superscripts differing within a row denote significance at *P* <0.050. Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg. v/d: visits/d; du/d: duration/d; du/v: duration/visit. *Required log10 transformation, actual means presented without s.e.m due to non-normal distribution.

Figure 25 shows SM daily average du/d for each birthweight group. Excepting piglets from group 1, SM du/d was consistent and similar between groups until d11. From d11-18, both SM du/d and the rate of increase in SM du/d was similar between groups; thereafter piglets from group 4 had consistently higher SM du/d than those in group 2 and 3, with group 1 being more variable (*P* <0.001; antedependence order 1 with additional uniform correlations).

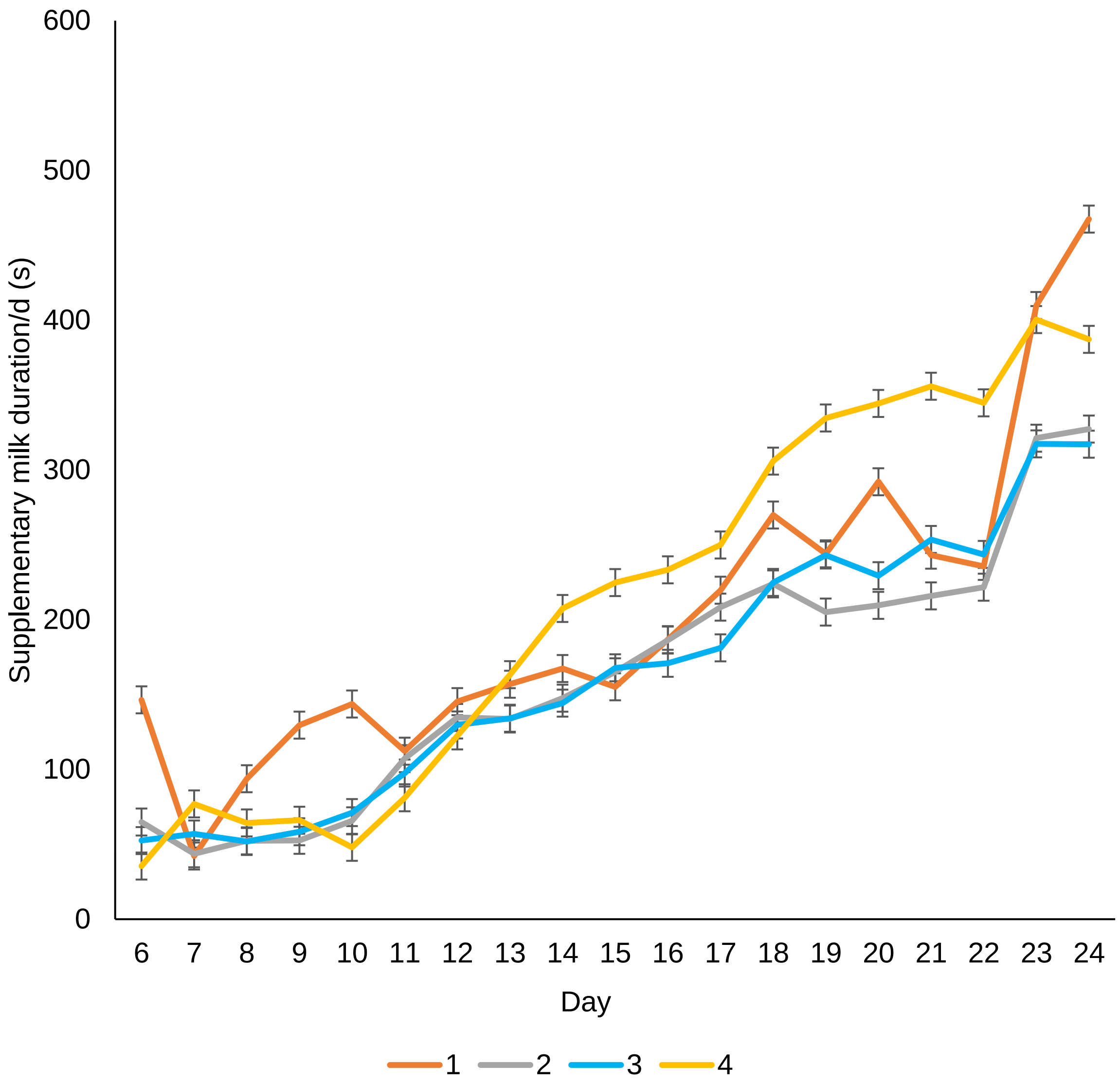

Figure 25: Average daily duration of visits to supplementary milk bowl, by birthweight group. Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg. Error bars denote pooled s.e.m

When SM du/d was calculated relative to ADG, piglets from groups 2, 3, 4 showed similar relative usage of SM (*P* <0.001; Figure 26). Piglets from birthweight group 1 had higher relative SM du/d from d18 compared to all others.

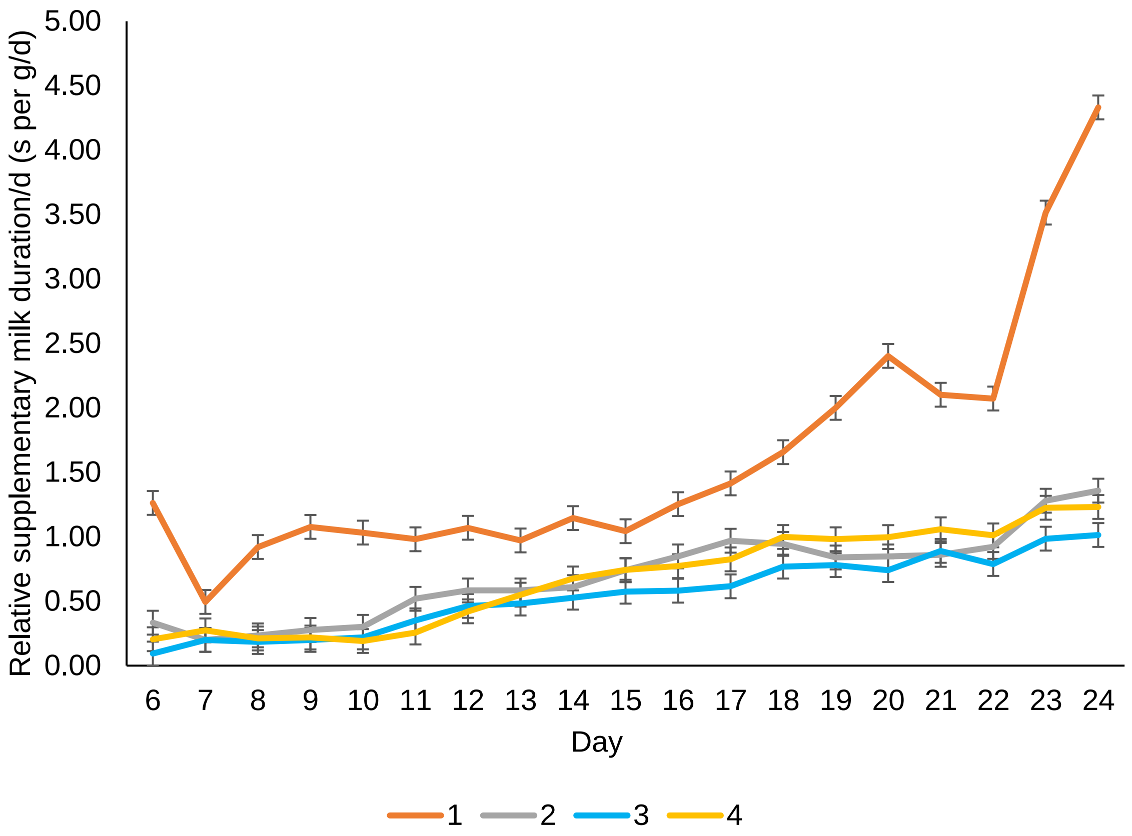

Figure 26: Supplementary milk average daily duration of visits/d relative to ADG for each birthweight group. Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg. Error bars denote pooled s.e.m

Piglets in group 1 consistently had the lowest creep usage, with group 3 having highest usage d12-19, and group 4 from d20-weaning (*P*<0.05; Table 24).

Table 24: Creep usage to weaning of piglets of differing birthweight groups.

Creep	1	2	3	4	s.e.m	*P*-value
n	*42*	*155*	*151*	*41*		
Average v/d d12-19	4.6^{a}	6.0^{ab}	6.4^{b}	5.5^{ab}	0.55	0.024
Average v/d d20-weaning	7.9^{a}	10.4^{b}	9.8^{ab}	11.1^{b}	0.82	0.024
Average du/d d12-19(s)	123^{a}	156^{bc}	165^{c}	131^{ab}	14.7	0.028
Average du/d d20-weaning (s)	214^{a}	271^{ab}	255^{ab}	297^{b}	22.0	0.040
Average du/v d12-19 (s)	26^{a}	26^{a}	25^{ab}	24^{b}	0.7	0.010
Average du/v d20-weaning (s)	27	26	26	26	0.6	0.361

Superscripts differing within a row denote significance at *P* <0.050. Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg. v/d: visits/d; du/d: duration/d; du/v: duration/visit.

Average creep duration of visits/d was similar between birthweight groups until d21. After this point, piglets from group 1 had lower du/d than those in group 4 until weaning, and lower than groups 2 and 3 excepting d23 (*P* <0.001; Figure 27)

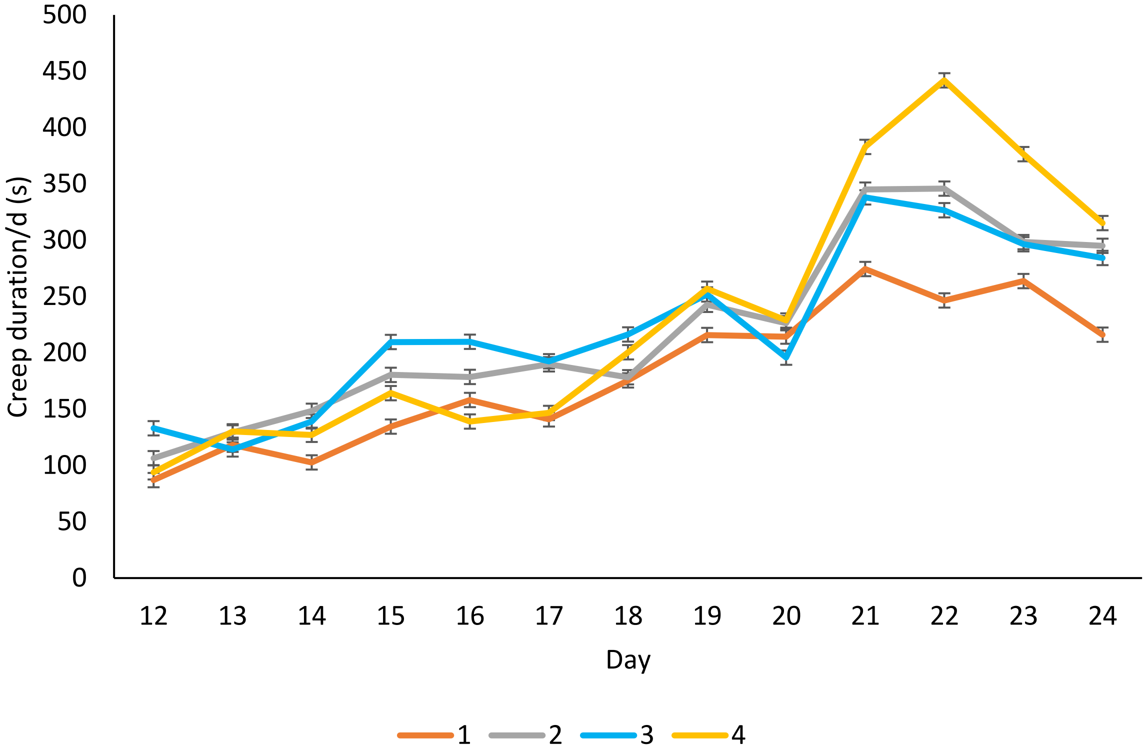

Figure 27: Average daily duration of visits to creep feeder, by birthweight group. Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg. Error bars denote pooled s.e.m

When average daily creep duration of visits was calculated relative to ADG, there was no difference in usage between the birthweight groups (*P* >0.050; Figure 28).

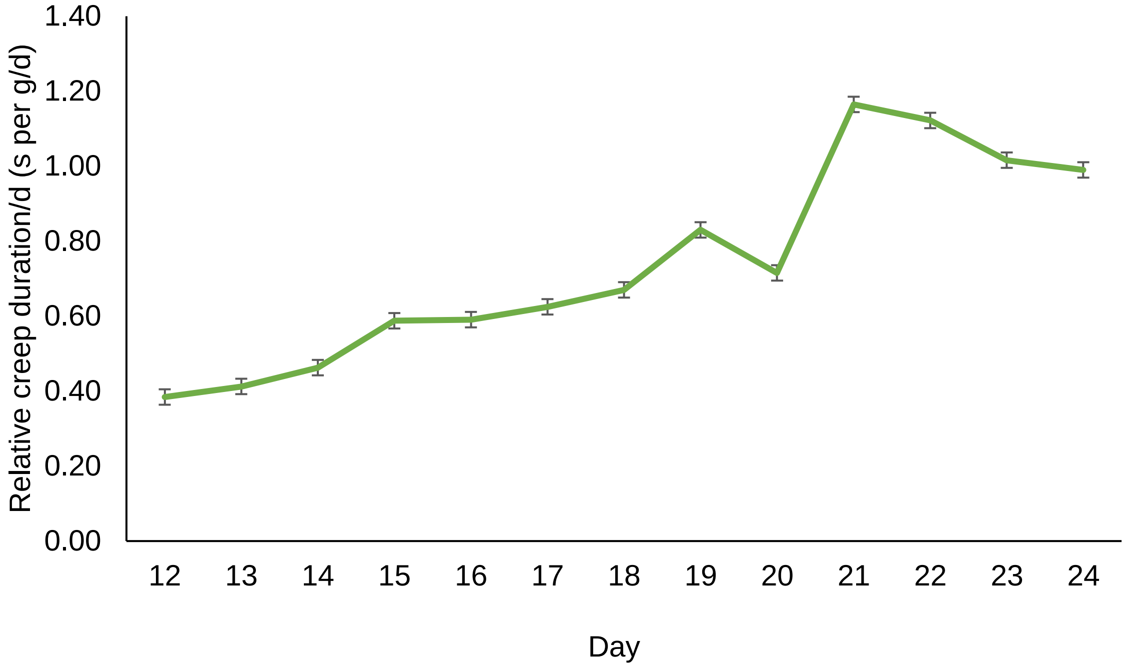

Figure 28: Change in creep average daily duration of visits, relative to ADG, from d12-24. Error bars denote pooled s.e.m

5.3.1.3.3. Effect of birthweight group on suckling position

Piglets from birthweight group 1 had the highest proportion suckling P or NONE, and piglets from birthweight group 3 and 4 had the highest proportion suckling A on d14 (*P* <0.001; Figure 29)

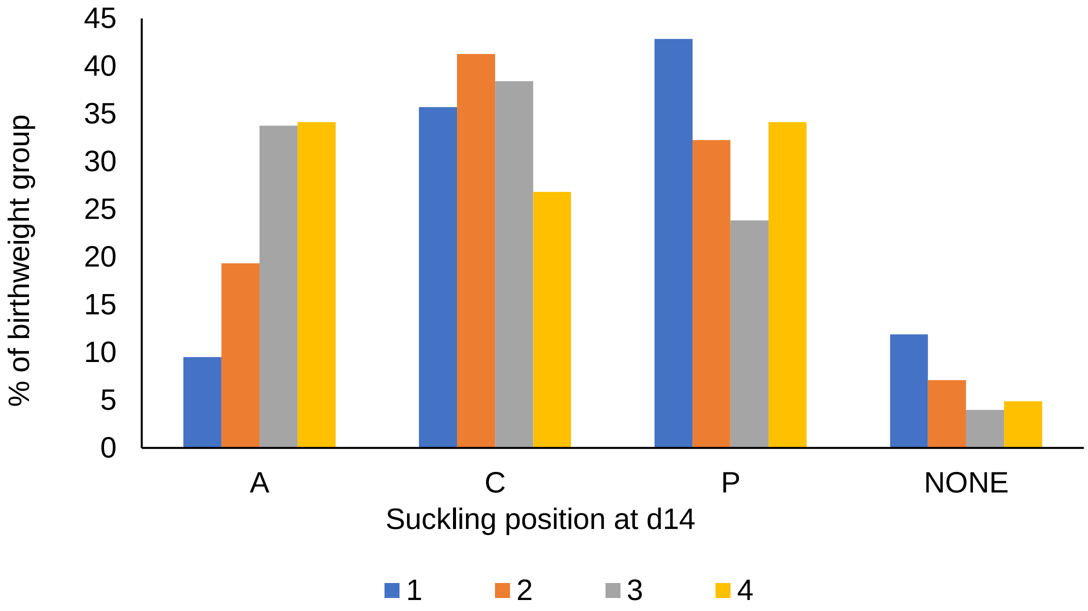

Figure 29: Effect of piglet birthweight group on suckling position at d14 of suckling. Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg

5.3.1.4. Suckling position at d14

5.3.1.4.1. Performance

Piglets suckling anterior teats were heavier throughout, but those suckling from centre or posterior teats, or not suckling were similar in weight until d11 (*P* <0.001; Table 25). From d11, piglets suckling centre teats were heavier than those suckling posterior teats, who were in turn heavier than piglets without a teat (*P* <0.001).

Table 25: Performance to weaning for piglets suckling anterior (A), centre (C), posterior (P) teats, or not suckling (N), at d14.

	A	C	P	N	s.e.m	*P*-value
n	*99*	*148*	*118*	*24*		
Average birthweight (kg)	1.60^{a}	1.49^{b}	1.44^{b}	1.39^{b}	0.066	<0.001
Average weight d4 (kg)	2.28^{a}	2.12^{b}	1.97^{b}	1.86^{b}	0.093	<0.001
Average weight d11 (kg)	4.40^{a}	4.12^{b}	3.69^{c}	3.03^{d}	0.167	<0.001
Average weight d18 (kg)	6.94^{a}	6.51^{b}	5.83^{c}	4.69^{d}	0.249	<0.001
Average weight weaning (kg)	9.56^{a}	8.97^{b}	8.14^{c}	6.68^{d}	0.336	<0.001
ADG d4-d11 (g/d)	303^{a}	286^{a}	245^{b}	168^{c}	14.0	<0.001
ADG d11-18 (g/d)	318^{a}	298^{a}	268^{b}	207^{c}	13.7	<0.001
ADG d18-weaning (g/d)	327^{a}	308^{ab}	289^{bc}	249^{c}	14.8	<0.001
ADG birth-weaning (g/d)	289^{a}	272^{a}	243^{b}	192^{c}	11.1	<0.001
ADG d4-weaning (g/d)	316^{a}	298^{a}	268^{b}	209^{c}	12.0	<0.001

Superscripts differing within a row denote significance at *P* <0.050.

Piglets suckling anterior and centre teats had similar ADG throughout, with posterior and non-suckling being lower (*P* <0.001). Reflecting this, when birthweight was included as a covariate, there was no difference at any time point in the adjusted weights of piglets between suckling position A and C (*P* >0.05). Piglets suckling P were lighter than those suckling A or C, but heavier than non-suckling piglets from d11 to weaning (*P* <0.001).

5.3.1.4.2. Consumption of SM and creep

Piglets not suckling had highest v/d to the SM, with no difference between suckling position of the suckled pigs from d4 to weaning (*P* <0.05; Table 26).

Table 26: Supplementary milk (SM) usage to weaning for piglets suckling anterior (A), centre (C), posterior (P) teats, or not suckling (N), at d14.

	A	C	P	N	s.e.m	*P*-value
n	*99*	*148*	*118*	*24*		
Average v/d d4-11*	2.2^{ab}	1.9^{a}	2.4^{ab}	6.8^{b}		0.021
Average v/d d12-19*	7.0^{a}	6.6^{a}	7.1^{a}	18.7^{b}		<0.001
Average v/d d20-weaning*	10.7^{a}	9.7^{a}	11.3^{a}	22.1^{b}		0.003
Average du/d d4-11 (s)*	51	44	55	163		0.062
Average du/d d12-19(s)*	158^{a}	151^{a}	162^{a}	424^{b}		<0.001
Average du/d d20-weaning (s)*	250^{a}	223^{a}	262^{a}	526^{b}		0.003
Average du/v d4-11 (s)	22	22	23	22	1.3	0.964
Average du/v d12-19 (s)	22	23	22	23	0.8	0.083
Average du/v d20-weaning (s)	23	22	24	23	0.7	0.316

Superscripts differing within a row denote significance at *P* <0.050. v/d: visits/d; du/d: duration/d; du/v: duration/visit. *Required log10 transformation, actual means presented without s.e.m due to non-normal distribution

Piglets not suckling had higher du/d of SM visits from d8-weaning than suckled piglets, amongst whom suckling position had no effect on SM du/d (*P* <0.001; Figure 30; repeated measures antedependence order 1 with additional uniform correlations).

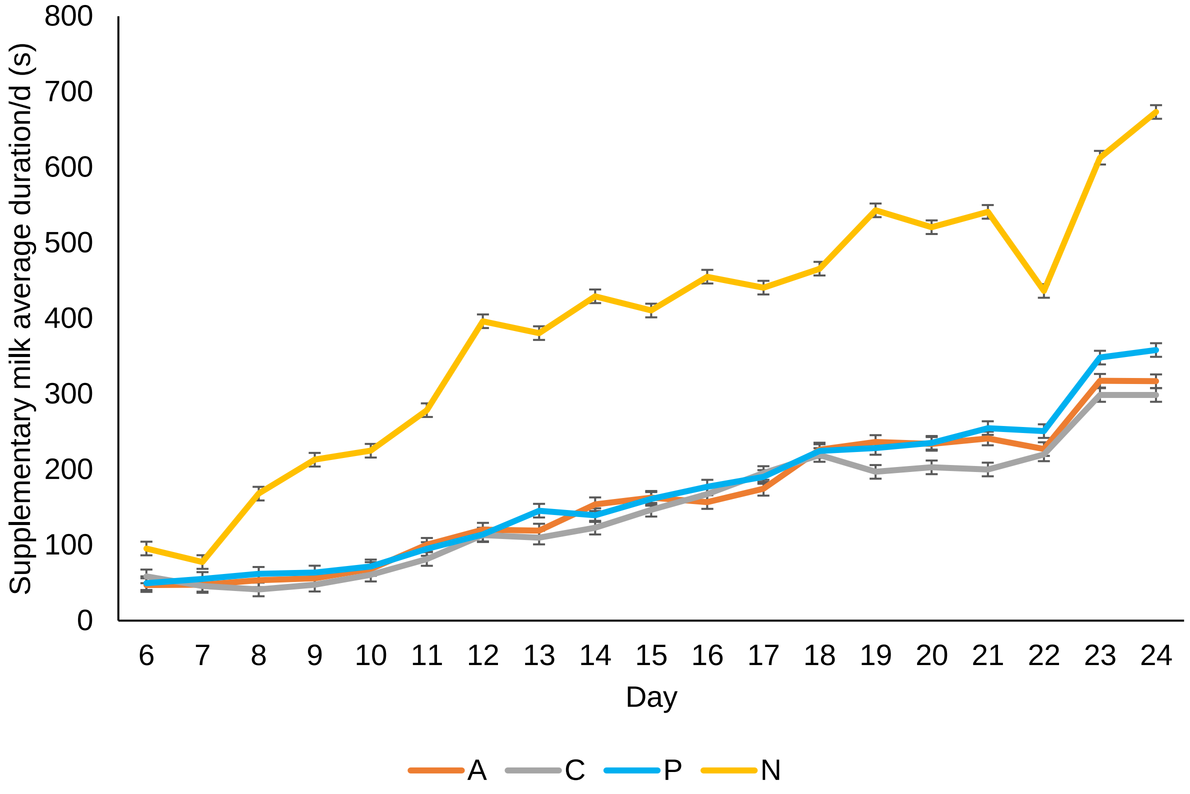

Figure 30: Effect of piglet suckling position on supplementary milk usage from d6-24. A: anterior; C: centre; P: posterior; N: none. Error bars denote pooled s.e.m

There was no evidence of a reduction in SM du/d after the introduction of creep.

From d20-weaning, piglets not suckling had the highest creep du/d (*P*=0.023), resulting from a combination of a tendency towards increased du/v (*P*=0.053) and numerically higher v/d (Table 27).

Table 27: Creep usage of piglets to weaning for piglets suckling anterior (A), centre (C), posterior (P) teats, or not suckling (N), at d14.

	A	C	P	N	s.e.m	*P*-value
n	*99*	*148*	*118*	*24*		
Average v/d d12-19	6.0	5.9	6.1	5.2	0.73	0.781
Average v/d d20-weaning	9.3	9.7	10.5	11.8	1.07	0.103
Average du/d d12-19(s)	155	152	155	144	19.4	0.953
Average du/d d20-weaning (s)	242^{a}	251^{ab}	277^{ab}	329^{b}	28.8	0.023
Average du/v d12-19 (s)	25^{a}	25^{a}	25^{a}	28^{b}	0.9	0.010
Average du/v d20-weaning (s)	26	26	26	28	0.8	0.053

Superscripts differing within a row denote significance at *P* <0.050. v/d: visits/d; du/d: duration/d; du/v: duration/visit.

Repeated measures REML antedependence order 1 with additional uniform correlations showed an increase in creep du/d from d12-weaning across all suckling positions, with a sharp increase at d21, driven by an increase in v/d rather than du/v (*P* <0.001; Figure 31). Until day 21, average du/d was unaffected by suckling position. From d21-24, piglets without a teat had the longest creep du/d, but this was similar to piglets suckling P on d21 and 23.

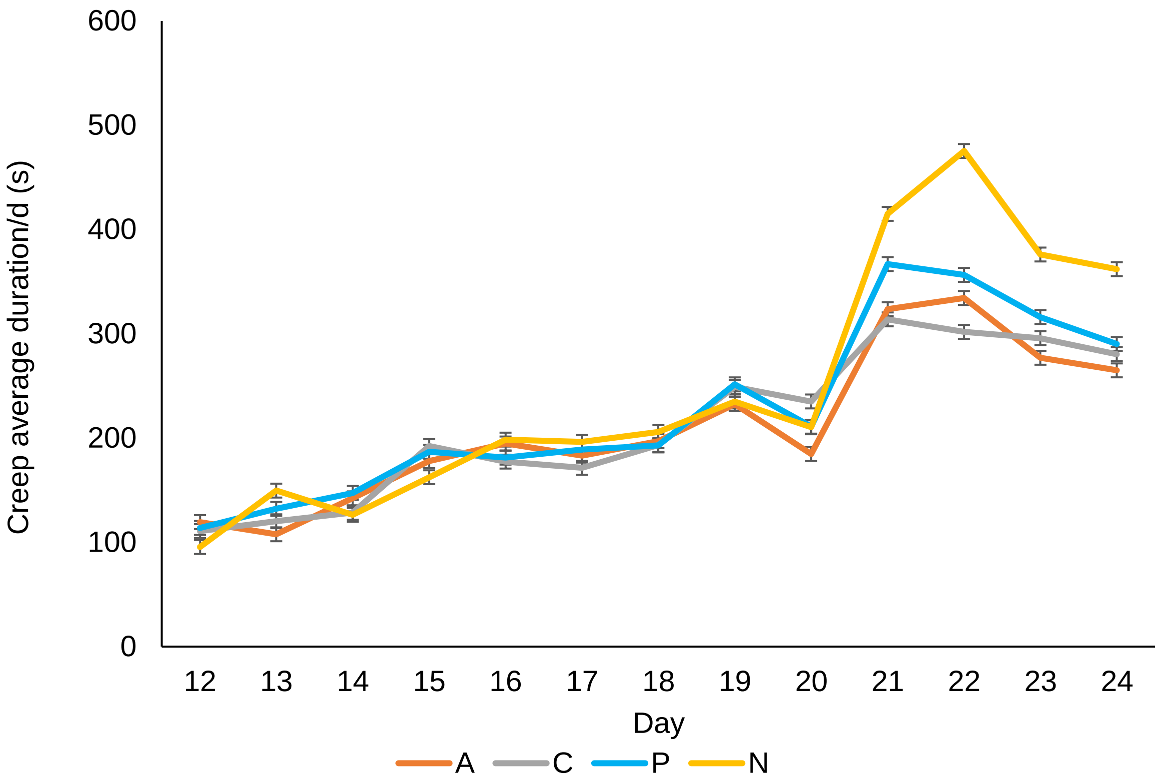

Figure 31: Effect of piglet suckling position on creep usage from d12-24. A: anterior; C: centre; P: posterior; N: none. Error bars denote pooled s.e.m

5.3.1.4.3. Consumption of SM and creep relative to weekly ADG

Supplementary milk usage (duration) relative to weekly ADG followed a similar pattern to absolute usage. Creep duration relative to weekly ADG emphasised the increased usage of creep for piglets not suckling compared to those suckling any position (*P* <0.001; Figure 32). The creep usage of piglets suckling a posterior teat was higher than those suckling anterior teats from d21-24, and centre teats on d21,22,24 using this method.

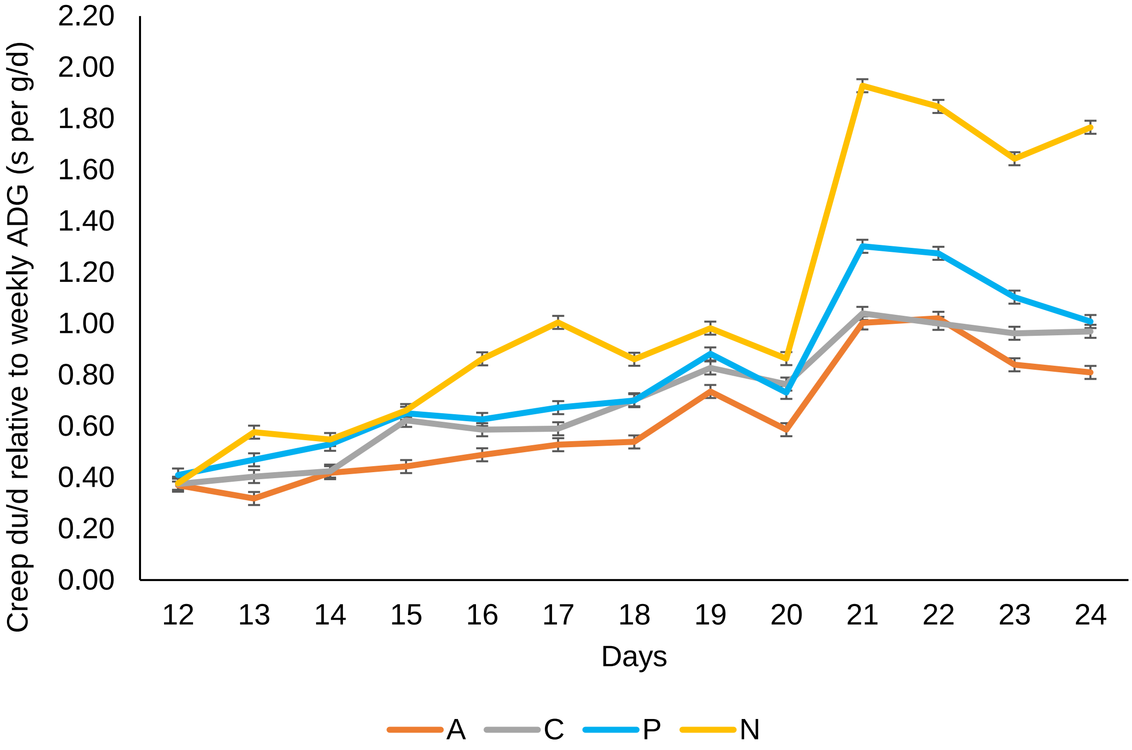

Figure 32: Effect of piglet suckling position on relative creep usage from d12-24. A: anterior; C: centre; P: posterior; N: none. Error bars denote pooled s.e.m

5.3.1.5. Duration of SM and creep visits

There was no effect of creep duration of visit on ADG from birth to weaning (*P* >0.05), however there was a negative relationship between SM duration of visit and ADG from birth to weaning, across all birthweight groups (*P* <0.001, r^2 =0.21, s.e =53.8; Table 28).

Table 28: Relationship between SM duration of visits and ADG from birth-weaning, for piglets in birthweight group 1 (≤1.04 kg), group 2 (1.06-1.52 kg), group 3 (1.54-1.90 kg), group 4 (≥1.92 kg).

Birthweight group	Equation	r^2	s.e	*P*-value
1	ADG=224.24-0.00632 x du	0.341	8.52	<0.001
2	ADG=267.70-0.00632 x du	0.126	4.96	<0.001
3	ADG=301.31-0.00632 x du	0.091	5.23	<0.001
4	ADG=318.07-0.00632 x du	0.006	8.72	<0.001

5.3.2. Post-weaning results

5.3.2.1. Level of SM and creep consumption split-plot interaction

There was no SM*creep consumption classification effect on ADG or FCR at any point (*P*>0.050; Table 29). From d7-15 and overall from weaning-d54, ADFI of pigs with low SM consumption increased when creep consumption was high, but there was no effect when SM consumption was high (*P* <0.050).

Table 29: Post-weaning performance of pigs with low (L) or high (H) supplementary milk (M) and creep (C) consumption pre-weaning.

	ML		MH			
	CL	CH	CL	CH	s.e.m	*P*-value M*C
n	*16*	*16*	*16*	*16*		
Average milk score	*476*	*444*	*5779*	*3385*		
Average creep score	*7643*	*12988*	*9441*	*14129*		
Weight at weaning (d0; kg)	9.45	8.72	8.68	8.18	0.293	0.683
Weight d7 (kg)	10.84	10.46	10.20	9.95	0.262	0.733
Weight d15 (kg)	14.24	14.02	13.89	13.65	0.259	0.957
Weight d29 (kg)	23.1	23.5	22.9	22.8	0.27	0.492
Weight exit (d54; kg)	44.3	45.9	44.5	44.7	0.51	0.402
ADFI wean-d7 (kg/d)	0.24	0.29	0.27	0.29	0.013	0.431
ADFI d7-15 (kg/d)	0.49^{a}	0.53^{b}	0.53^{b}	0.53^{b}	0.006	0.024
ADFI d15-29 (kg/d)	0.87	0.89	0.89	0.91	0.013	0.850
ADFI d29-54 (kg/d)	1.52	1.60	1.54	1.57	0.013	0.266
ADFI wean-d54 (kg/d)	1.03^{a}	1.08^{b}	1.05^{ab}	1.07^{b}	0.002	0.008
ADG wean-d7 (g/d)	198	253	218	253	12.0	0.627
ADG d7-15 (g/d)	425	445	462	462	9.4	0.518
ADG d15-29 (g/d)	613	657	621	630	6.3	0.125
ADG d29-54 (g/d)	865	905	871	881	17.4	0.565
ADG wean-d54 (g/d)	646	683	658	669	6.7	0.279
FCR wean-d7	1.20	1.14	1.24	1.15	0.017	0.564
FCR d7-d15	1.66	1.80	1.84	1.87	0.046	0.195
FCR d15-29	1.42	1.36	1.44	1.45	0.019	0.231
FCR d29-54	1.76	1.77	1.77	1.79	0.021	0.818
FCR wean-d54	1.59	1.58	1.60	1.60	0.014	0.840

5.3.2.2. Level of supplementary milk consumption

Piglets with high pre-weaning SM consumption had a tendency towards increased ADG from weaning-d7 (*P*=0.093), and increased ADFI d15-29 (*P*=0.087), but there were no other effects of pre-weaning SM consumption level on post-weaning performance (Table 30).

Table 30: Post-weaning performance of pigs with low (L) or high (H) supplementary milk (M) consumption pre-weaning.

	ML	MH	s.e.m	*P*-value
n	*32*	*32*		
Average milk score	*460*	*4582*		
Average creep score	*10316*	*11785*		
Weight at weaning (d0; kg)	9.08	8.43	0.235	0.299
Weight d7 (kg)	10.65	10.07	0.237	0.336
Weight d15 (kg)	14.13	13.77	0.206	0.432
Weight d29 (kg)	23.3	22.8	0.15	0.261
Weight exit (d54; kg)	45.1	44.6	0.22	0.343
ADFI wean-d7 (kg/d)	0.26	0.28	0.005	0.223
ADFI d7-15 (kg/d)	0.51	0.53	0.005	0.245
ADFI d15-29 (kg/d)	0.88	0.90	0.002	0.087
ADFI d29-54 (kg/d)	1.56	1.55	0.004	0.536
ADFI wean-d54 (kg/d)	1.05	1.06	0.002	0.266
ADG wean-d7 (g/d)	225	235	1.0	0.093
ADG d7-15 (g/d)	435	462	3.9	0.128
ADG d15-29 (g/d)	635	626	4.0	0.354
ADG d29-54 (g/d)	885	876	7.6	0.558
ADG wean-d54 (g/d)	664	664	2.8	0.911
FCR wean-d7	1.17	1.20	0.008	0.231
FCR d7-d15	1.73	1.85	0.042	0.286
FCR d15-29	1.39	1.44	0.014	0.220
FCR d29-54	1.76	1.78	0.016	0.538
FCR wean-d54	1.58	1.60	0.006	0.403

5.3.2.3. Level of creep consumption

Piglets with high pre-weaning creep consumption had lower FCR from weaning-d7 (*P*=0.034; Table 31), and increased ADFI overall from weaning-d54 (*P*=0.043).

Table 31: Post-weaning performance of pigs with low (L) or high (H) creep (C) consumption pre-weaning.

	CL	CH	s.e.m	P-value
n	*32*	*32*		
Average milk score	*3128*	*1915*		
Average creep score	*8542*	*13559*		
Weight at weaning (d0; kg)	9.06	8.45	0.151	0.064
Weight d7 (kg)	10.52	10.21	0.095	0.104
Weight d15 (kg)	14.06	13.83	0.128	0.290
Weight d29 (kg)	23.0	23.2	0.22	0.651
Weight exit (d54; kg)	44.4	45.3	0.47	0.290
ADFI wean-d7 (kg/d)	0.25	0.29	0.012	0.134
ADFI d7-15 (kg/d)	0.51	0.53	0.008	0.187
ADFI d15-29 (kg/d)	0.88	0.90	0.011	0.246
ADFI d29-54 (kg/d)	1.53	1.58	0.015	0.086
ADFI wean-d54 (kg/d)	1.04	1.07	0.007	0.043
ADG wean-d7 (g/d)	208	253	10.5	0.057
ADG d7-15 (g/d)	443	453	8.0	0.454
ADG d15-29 (g/d)	617	644	8.2	0.108
ADG d29-54 (g/d)	868	893	14.2	0.298
ADG wean-d54 (g/d)	652	676	7.2	0.100
FCR wean-d7	1.22	1.14	0.014	0.034
FCR d7-d15	1.75	1.83	0.028	0.118
FCR d15-29	1.43	1.40	0.017	0.363
FCR d29-54	1.76	1.78	0.011	0.411
FCR wean-d54	1.59	1.59	0.010	0.850

Piglets with high pre-weaning creep consumption tended to be lighter at weaning (*P*=0.064), but with higher ADG weaning-d7 (*P*=0.057), and increased ADFI from d29-54 (*P*=0.086).

Multiple regression analysis showed the effect of duration of creep consumption was more persistent than the effect of duration of SM consumption. There ceased to be an effect of SM consumption on ADG after d15 post-weaning, whereas creep consumption had a positive effect on ADG to slaughter (*P*<0.001; Table 32).

Table 32: The effect of birthweight group, weaning weight, SM and creep consumption on ADG of pigs to d54 post-weaning.

Period	Equation	r^2	s.e	*P*-value
Birth-wean	Cgroup$^{\alpha}$-0.007milk du	31.8	47.9	<0.001
Wean-d7	Cgroup$^{\beta}$+0.005milk du+0.016creep du	13.0	94.8	<0.001
Wean-d15	107+0.007milk du+0.013creep du+20.2wn wt	17.5	88.5	<0.001
Wean-d29	237+0.017creep du+22.8wn wt	23.9	80.6	<0.001
Wean-d54	363+0.019creep du+28.6wn wt	30.8	82.4	<0.001

$^{\alpha}$: C group 1: 230; 2: 269; 3: 309; 4:322. $^{\beta}$: C group 1: 123; 2:158; 3:175; 4:224. Birthweight group 1: ≤1.04 kg; 2: 1.06-1.52 kg; 3: 1.54-1.90 kg; 4: ≥1.92 kg. wn wt: weaning weight. milk du: duration of visits to supplementary milk bowl pre-weaning; creep du: duration of visits to creep feeder pre-weaning.

Birthweight group ceased to have an effect after d7, but weaning weight had a positive effect on ADG post-weaning from d15. There was no effect of sex on weight or ADG from weaning to d54 post-weaning (*P*>0.05).

5.3.3. Finisher performance

5.3.3.1. Three-way interaction sex*supplementary milk*creep

There was no sex*SM *creep interaction effect on lifetime performance (*P*>0.05; Table 33).

Table 33: Lifetime performance of boars (B) and gilts (G) with low (L) or high (H) supplementary milk (M) and creep (C) consumption pre-weaning.

	B				G					
	ML		MH		ML		MH			
	CL	CH	CL	CH	CL	CH	CL	CH	s.e.m	*P*-value
n	*12*	*10*	*12*	*12*	*11*	*10*	*10*	*11*		
Average milk score	*361*	*139*	*1535*	*1828*	*300*	*316*	*2077*	*3856*		
Average creep score	*4717*	*16516*	*5746*	*11204*	*4292*	*11108*	*6314*	*16928*		
Start weight (d11; kg)	56.1	56.58	57.05	54.74	54.41	57.67	55.12	54.9	2.14	0.906
Weight at first slaughter draw (d63; kg)	110.5	115.5	110.5	111.5	105.1	108.7	105.9	105.3	3.48	0.988
Slaughter weight (kg)	124.3	123.2	124.9	121	121.5	126.4	119.9	121.2	2.37	0.923
ADFI finisher (kg/d)	2.96	2.98	2.96	3.01	2.89	3.16	2.92	2.91	0.077	0.163
ADG birth-slaughter (g/d)	784	812	793	781	749	777	740	753	16.1	0.599
ADG wean-slaughter (g/d)	888	933	901	895	943	881	837	861	20.3	0.495
ADG finisher (g/d)	1109	1209	1107	1157	1048	1040	1022	1034	41.5	0.539
FCR finisher	2.78	2.61	2.77	2.77	2.94	3.16	3.12	2.94	0.131	0.105

5.3.3.2. Two-way interaction SM*creep consumption

There was no SM*creep classification interaction on lifetime performance (*P*>0.05; Table 34)

Table 34: Lifetime performance of pigs with low (L) or high (H) supplementary milk (M) and creep (C) consumption pre-weaning.

	ML		MH			
	CL	CH	CL	CH	s.e.m	*P*-value
n	*23*	*20*	*22*	*23*		
Average milk score	*332*	*228*	*1781*	*2798*		
Average creep score	*4514*	*13812*	*6004*	*13942*		
Start weight (d11; kg)	55.3	57.1	56.2	54.8	1.49	0.267
Weight at first slaughter draw (d63; kg)	107.9	112.2	108.3	108.5	2.42	0.383
Slaughter weight (kg)	122.9	124.8	122.6	121.1	1.68	0.296
ADFI finisher (kg/d)	2.93	3.07	2.94	2.96	0.055	0.255
ADG birth-slaughter (g/d)	767	795	769	767	12.0	0.208
ADG wean-slaughter (g/d)	866	907	972	879	15.1	0.251
ADG finisher (g/d)	1080	1127	1066	1098	29.2	0.794
FCR finisher	2.86	2.88	2.93	2.85	0.092	0.547

5.3.3.3. Effect of SM consumption classification

There was no effect of SM consumption classification pre-weaning on lifetime performance (*P* >0.05; Table 35).

Table 35: Lifetime performance of pigs with low (L) or high (H) supplementary milk (M) consumption pre-weaning.

	ML	MH	s.e.m	*P*-value
n	*43*	*45*		
Average milk score	*284*	*2301*		
Average creep score	*8838*	*10061*		
Start weight (d11; kg)	56.2	55.5	1.02	0.641
Weight at first slaughter draw (d63; kg)	110.0	108.4	1.65	0.519
Average slaughter day*	75.9	75.4		0.929
Slaughter weight (kg)	123.8	121.8	1.15	0.219
Age slaughter*	157	157		0.912
ADFI finisher (kg/d)	2.99	2.95	0.038	0.447
ADG birth-slaughter (g/d)	780	768	8.3	0.305
ADG wean-slaughter (g/d)	885	875	10.4	0.510
ADG finisher (g/d)	1103	1081	19.8	0.486
FCR finisher	2.87	2.89	0.063	0.784

*non-normal, Kruskal-Wallis test employed

5.3.3.4. Effect of creep consumption classification

There was no effect of creep consumption classification pre-weaning on lifetime performance (*P*>0.05; Table 36).

Table 36: Lifetime performance of pigs with low (L) or high (H) creep (C) consumption pre-weaning.

	CL	CH	s.e.m	*P*-value
n	*45*	*43*		
Average milk score	*1041*	*1603*		
Average creep score	*5242*	*13881*		
Start weight (d11; kg)	55.7	55.9	1.02	0.888
Weight at first slaughter draw (d63; kg)	108.1	110.3	1.65	0.337
Average slaughter day*	76.8	74.4		0.298
Slaughter weight (kg)	122.7	122.9	1.15	0.926
Age slaughter*	158.4	155.9		0.374
ADFI finisher (kg/d)	2.93	3.01	0.038	0.139
ADG birth-slaughter (g/d)	768	780	8.2	0.294
ADG wean-slaughter (g/d)	869	892	10.3	0.110
ADG finisher (g/d)	1072	1112	19.8	0.155
FCR finisher	2.89	2.86	0.063	0.741

* non-normal, Kruskal-Wallis test employed

5.3.3.5. Effect of birthweight group

There were insufficient replicates to analyse the three-way interaction of birthweight group*SM*creep; this was highly unbalanced due to birthweight group not being part of the post-nursery selection criteria.

Pigs from birthweight group 1 were lighter at all points, older at slaughter, and had lower ADG from birth-slaughter and weaning to slaughter ($P<0.05$; Table 37). Birthweight group had no effect on ADG, FCR or ADFI in the finishing period ($P > 0.05$).

Table 37: Effect of birthweight group on lifetime performance.

	1	2	3	4	s.e.m	*P*-value
n	*9*	*22*	*38*	*19*		
Average milk score	*1676*	*885*	*1657*	*958*		
Average creep score	*9248*	*10684*	*9422*	*9236*		
Start weight (d11; kg)	46.1^{a}	55.4^{b}	57.2^{b}	58.1^{b}	1.90	<0.001
Weight at first slaughter draw (kg)	94.2^{a}	106.8^{b}	111.9^{b}	113.4^{b}	3.23	<0.001
Average slaughter day*	89.2^{a}	79.5^{ab}	71.7^{b}	72.6^{b}		<0.001
Slaughter weight (kg)	122.0^{ab}	125.7^{b}	120.5^{a}	124.5^{ab}	2.42	0.044
Age slaughter (days)*	172^{a}	161^{ab}	153^{b}	154^{b}		<0.001
ADFI finisher (kg/d)	2.87	2.99	2.96	3.03	0.080	0.437
ADG birth-slaughter (g/d)	706^{a}	774^{b}	778^{b}	797^{b}	16.2	<0.001
ADG wean-slaughter (g/d)	802^{a}	881^{b}	885^{b}	908^{b}	21.0	<0.001
ADG finisher (g/d)	1013	1072	1106	1122	46.2	0.209
FCR finisher	3.00	2.91	2.86	2.81	0.150	0.720

Birthweight group 1 ≤1.04 kg; group 2 1.06-1.52 kg; group 3 1.54-1.90 kg; group 4 ≥1.92 kg. * non-normal, Kruskal-Wallis test employed. Superscripts denote differences at $P < 0.05$.

5.3.3.6. Multiple regression

Multiple regression revealed weaning weight and sex as important factors for ADG and FCR ($P < 0.05$; Table 38). Creep du also had a positive effect on ADG. Substituting creep score for creep du had no effect on r^2, but increased the standard error.

Table 38: Multiple regression equations for lifetime ADG and finisher FCR of boars (B) and gilts (G), and ADFI of pigs with low (CL) or high (CH) pre-weaning creep consumption

Parameter	Equation	r^2	s.e	*P*-value
ADG birth-slaughter B	49.9bwt+0.00784creep du+686.0	24.7	46.6	<0.001
ADG birth-slaughter G	49.9bwt+0.00784creep du+655.8			
ADG wean-slaughter B	12.88wn wt+0.01423creep du+741.2	25.8	58.7	<0.001
ADG wean-slaughter G	12.88wn wt+0.01423creep du+697.3			
ADG finisher B	17.72wn wt+0.02162creep du+914.5	19.8	125	<0.001
ADG finisher G	17.72wn wt+0.02162creep du+812.8			
FCR finisher B	3.104-0.0387wn wt	11.8	0.405	0.002
FCR finisher G	3.381-0.0387wn wt			
ADFI d11-slaughter CL	0.01809d11 wt+1.926	14.6	0.228	<0.001
ADFI d11-slaughter CH	0.00469d11 wt+2.749			

Creep du: total duration of creep visits d12-weaning.

5.3.4. Metabolomic analysis

In total 186 distinct peaks arose from the GC-MS analysis. After exclusion of compounds in common with the blank samples (contamination, sample preparation), and resolution of multiple peaks of a single metabolite due to drift, 67 metabolites were able to be identified confidently. There were 45 metabolites that were able to be matched with KEGG IDs for pathway analysis.

5.3.4.1. Effect of weaning

Metabolites identified and relative concentrations pre- and post-weaning are shown in Figure 33.

Figure 33: Heatmap of relative concentrations of metabolites detected in the plasma of pigs pre- (d26) and post-weaning (d55).

Post-hoc t-test identified metabolites that differed in concentration pre- and post-weaning, with the majority of the fatty acids being down-regulated and amino acids up-regulated (Table 39). Aldose sugars were all down-regulated post-weaning.

Table 39: Metabolites in pig plasma that were up-regulated (↑) or down-regulated (↓) post-weaning compared to pre-weaning, and their classification.

Metabolite	*P*-value	FDR	↑↓	Classification
N-Acetyl glucosamine	1.24x10^{-18}	8.16x10^{-17}	↑	Amino sugars
Malic acid	2.12x10^{-16}	6.99x10^{-15}	↑	Dicarboxylic acid
L-Proline	7.38x10^{-16}	1.62x10^{-14}	↑	Amino acids
Imidazol-2-amine	9.33x10^{-14}	1.54x10^{-12}	↑	Peptides
Erythrulose	1.95x10^{-11}	2.58x10^{-10}	↑	Ketoses
D-Glucose	2.36x10^{-11}	2.59x10^{-10}	↓	Aldoses
9-Hexadecenoic acid	3.52x10^{-11}	3.32x10^{-10}	↓	Fatty acids and conjugates
Arachidonic acid	5.77x10^{-11}	4.76x10^{-10}	↓	Fatty acids and conjugates
9,12-Octadecadienoic acid	5.16x10^{-10}	3.78x10^{-9}	↓	Fatty acids and conjugates
D-xylose	6.47x10^{-10}	4.27x10^{-9}	↓	Aldoses
L-Valine	1.80x10^{-9}	1.08x10^{-8}	↓	Amino acids
L-Lysine	4.69x10^{-9}	2.58x10^{-8}	↓	Amino acids
L-Isoleucine	7.86x10^{-9}	3.99x10^{-8}	↑	Amino acids
D-talose	5.08x10^{-8}	2.39x10^{-7}	↓	Aldoses
L-5-Oxoproline	1.22x10^{-7}	5.38x10^{-7}	↑	Amino acids
DL-Phenylalanine	6.08x10^{-7}	2.51x10^{-6}	↑	Amino acids
2-Ketoisocaproic acid	7.32x10^{-7}	2.84x10^{-6}	↑	Fatty acids and conjugates
D-Allofuranose	1.82x10^{-6}	6.67x10^{-6}	↑	Pentoses
Threonic acid	3.09x10^{-6}	1.07x10^{-5}	↑	Sugar acids
Lactic Acid	5.68x10^{-6}	1.87x10^{-5}	↓	Hydroxycarboxylic acids
N-Acetyl glucosamine	7.26x10^{-6}	2.26x10^{-5}	↓	Amino sugars
1,5-Anhydrohexitol	7.52x10^{-6}	2.26x10^{-5}	↓	Sugar alcohols
Erythrulose	8.25x10^{-6}	2.37x10^{-5}	↓	Ketoses
D-galactose	2.01x10^{-5}	5.54x10^{-5}	↓	Aldoses
Cholesterol	2.22x10^{-5}	5.87x10^{-5}	↓	Sterols
L-Serine	2.70x10^{-5}	6.85x10^{-5}	↑	Amino acids
Glycolic acid	3.16x10^{-4}	7.46x10^{-4}	↑	Organic acid
9-Octadecenoic acid	3.98x10^{-4}	8.76x10^{-4}	↓	Fatty acids and conjugates
2-Methoxyestradiol	4.15x10^{-4}	8.83x10^{-4}	↑	Steroids
L-Threonine	4.52x10^{-4}	9.09x10^{-4}	↑	Amino acids
Ethanol	4.55x10^{-4}	9.09x10^{-4}	↑	Organic alcohol
Methyl stearate	1.17x10^{-3}	2.27x10^{-3}	↓	Fatty acids and conjugates
D-mannose	1.57x10^{-3}	2.96x10^{-3}	↓	Aldoses
L-Hydroxyproline	3.22x10^{-3}	5.90x10^{-3}	↑	Amino acids
Eicosatetraenoic acid	0.020	0.034	↑	Fatty acids and conjugates
Myo-Inositol	0.025	0.042	↓	Sugar alcohols
D-allofuranose	0.026	0.042	↓	Ketoses

FDR: False discovery rate; *P*-value adjusted for multiple tests.

Of the amino acids that differed in plasma concentration between pre- and post-weaning, threonine showed a trend for a positive correlation with ADG from d19-weaning (*P* =0.069, R =0.348; Table 40). There was insufficient data for correlations of isoleucine and proline with ADG. Post-weaning, oxoproline showed a trend for a positive correlation with ADG from d41-55 (*P* =0.087, R =0.312). There was insufficient data for correlations of plasma lysine with ADG as lysine was not detected in sufficient number of samples.

Table 40: Correlations between ADG and plasma amino acid concentration in piglets pre-weaning and post-weaning.

	Pre-weaning ADG d19-26		Post-weaning ADG d41-55	
	R	*P*-value	R	*P*-value
Hydroxyproline	0.297	0.125	0.224	0.225
Isoleucine	*	*	0.089	0.634
Lysine	0.149	0.449	*	*
Oxoproline	0.226	0.247	0.312	0.087
Phenylalanine	0.103	0.602	-0.066	0.722
Proline	*	*	-0.227	0.220
Serine	0.123	0.533	0.269	0.143
Threonine	0.348	0.069	0.144	0.439
Valine	0.165	0.400	0.196	0.289

Comparing the amino acid profile (as a proportion of total detected in at least 50 % of samples) pre-weaning, valine was detected in higher proportion, and lysine, serine, threonine, and leucine in lower proportion in plasma than was predicted to be in sow's milk on d10 (using values from Yao *et al.*, 2023; Figure 34) or formulated in the creep. The proportion of amino acids predicted in sows' milk and formulated in the creep were similar.

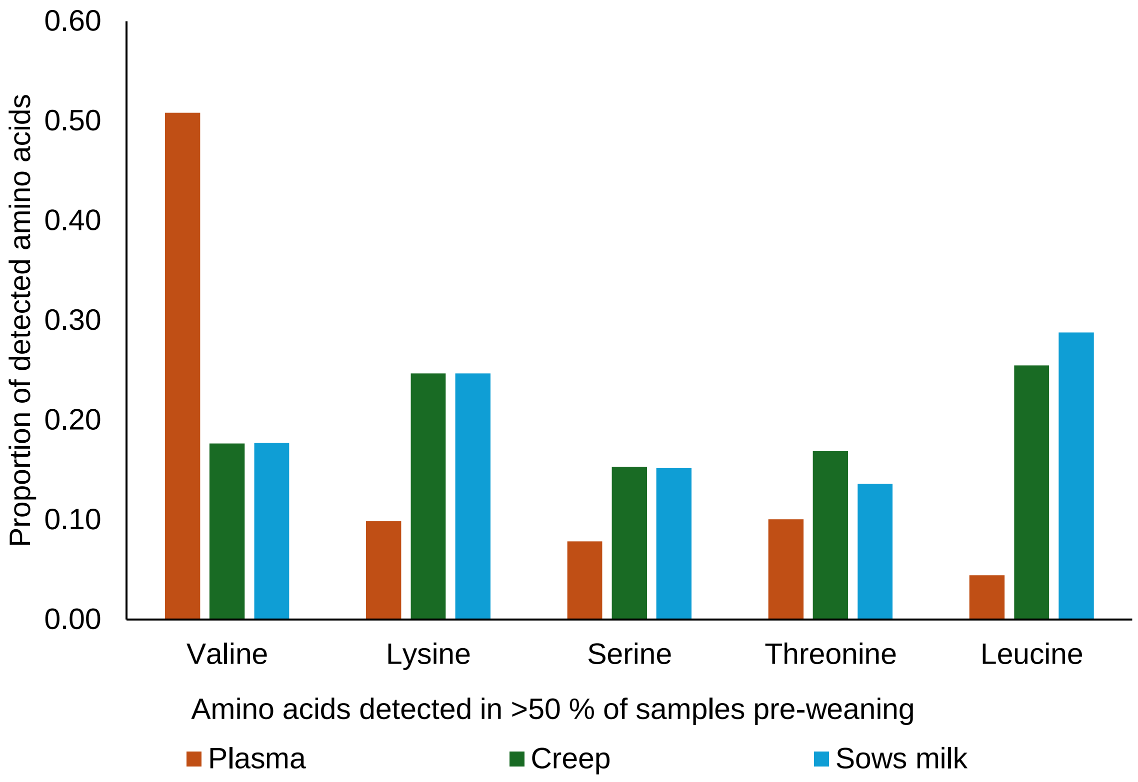

Figure 34: Comparison of proportion of amino acids detected in pig plasma in at least 50 % of samples pre-weaning with those formulated in the pre-weaning creep feed, and predicted in sow's milk at d10 (sows' milk values from Yao *et al.*, 2023).

More amino acids were detected in >50% of samples post-weaning compared to pre-weaning. Lysine was not detected in sufficient samples, but valine, threonine and aspartic acid were all higher in plasma than feed (Figure 35). Leucine, isoleucine, and phenylalanine were lower in plasma than in feed, and serine and proline were similar in plasma and feed.

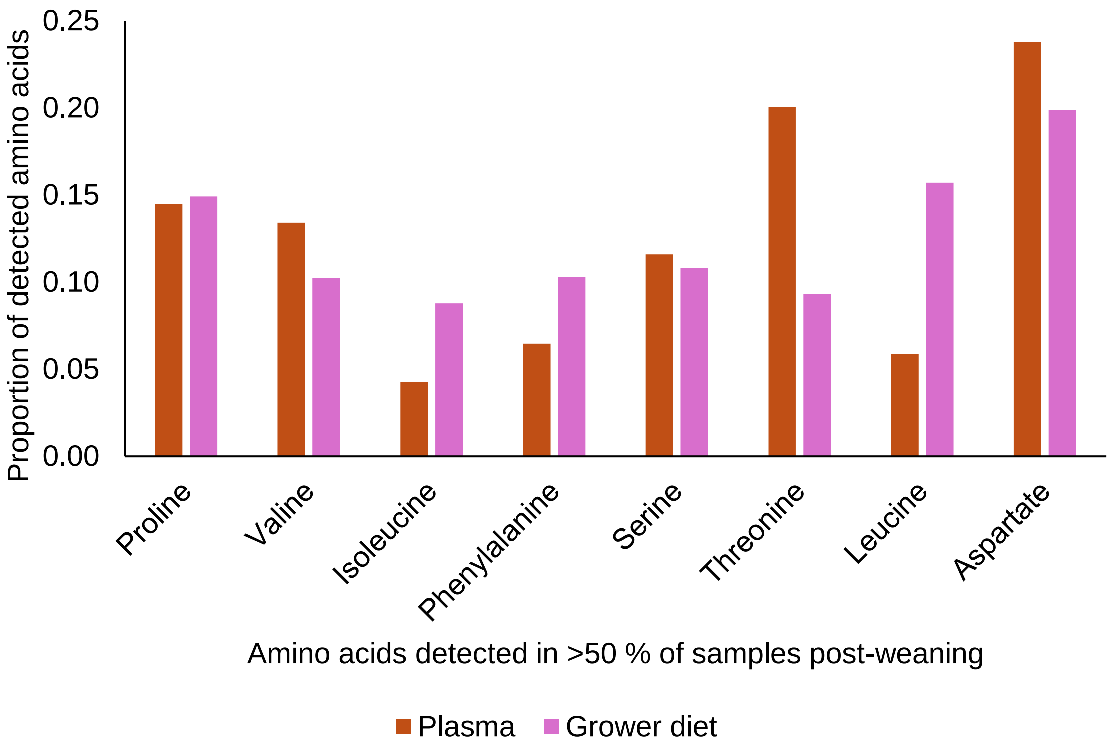

Figure 35: Comparison of proportion of amino acids detected in pig plasma in at least 50 % of samples post-weaning with those formulated in the grower diet.

PCA scores plot revealed distinct separation of pre- and post-weaning metabolome. (Figure 36). The multiple dimensions that resulted from plotting concentrations of metabolites against each other were condensed into a two-dimensional plot. Principle component 1 (the x axis) accounts for 72.4 % of the variance in metabolite concentrations, and PC 2 (the y axis) accounts for 20.6 % of the variance. Each pig is a point on the graph, on day 26 and d55. The coloured disc represents the 95 % confidence limit for each factor, therefore separation of the discs is a graphical representation that the plasma metabolome differed between pre- and post-weaning samples for most pigs.

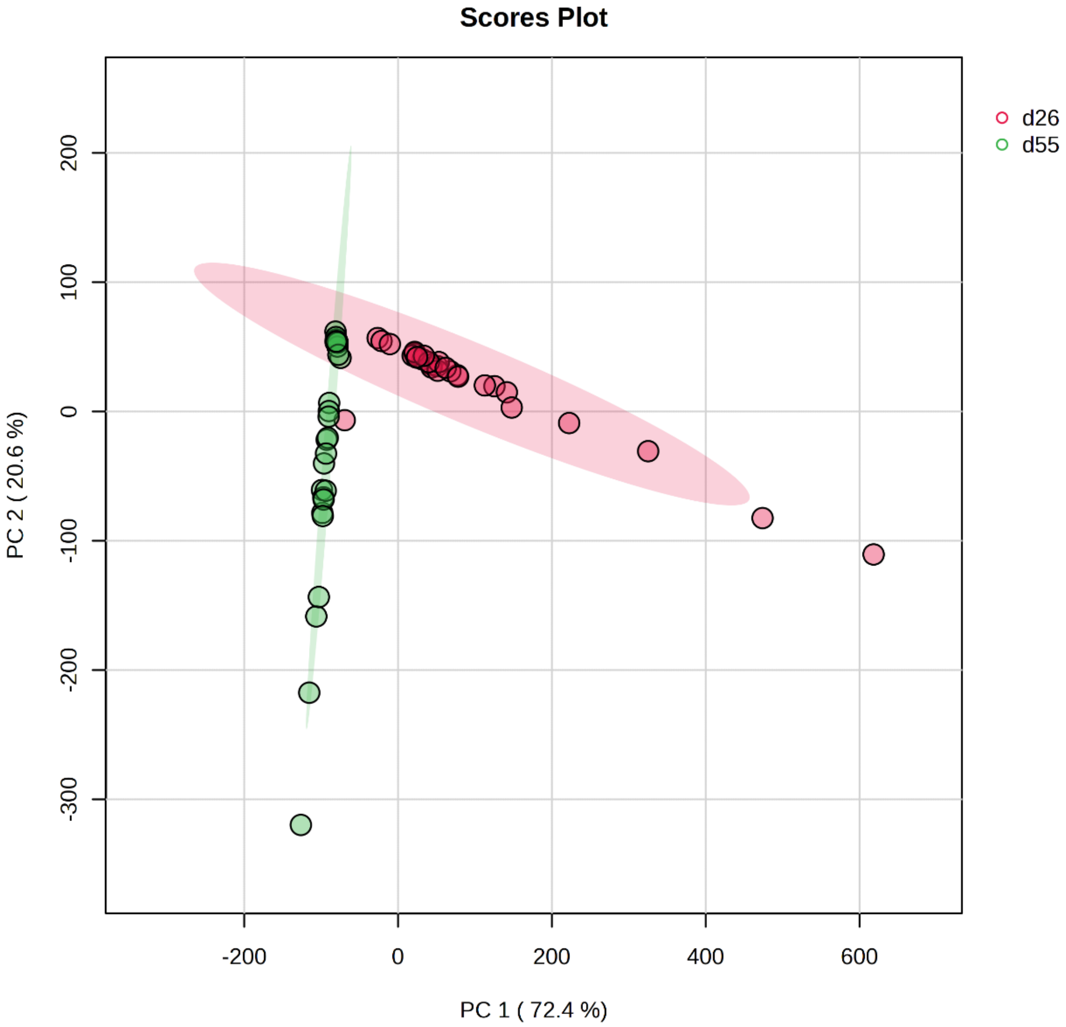

Figure 36: PCA scores plot for metabolites detected in the plasma of pigs pre- (d26) and post-weaning (d55).

Pathway analysis revealed a number of significantly affected pathways: the annotation of Figure 37 and Table 41 show those pathways where *P* <0.1 and at least two metabolites were identified. Other pathways shown in Figure 37 either had only one metabolite identified, or were not significantly affected by weaning (Table 42).

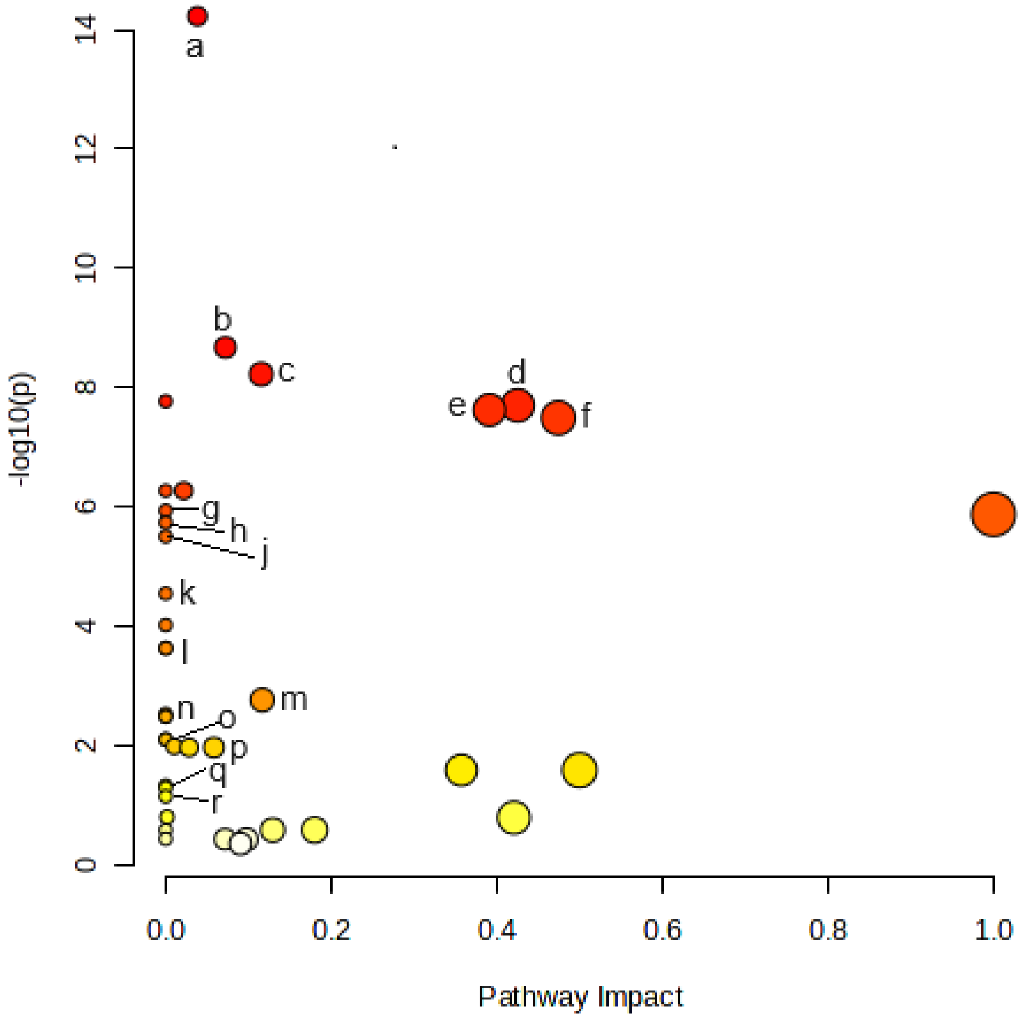

Figure 37: Metabolic pathways in pig plasma impacted by weaning, annotation shows pathways where P <0.10 (-log10(p) >1.0), FDR <0.10 and at least two metabolites identified, see Table 41 for key. Metabolic pathways that are unannotated are in Table 42.

Pathway impact is calculated as the sum of importance of matched metabolites/sum of importance of all metabolites. Importance refers to the positioning of the metabolite within the pathway, and is defined as the number of direct connections to other metabolites within the pathway. Thus, the maximum impact is 1.0, achieved if the detected metabolites are directly connected to all other metabolites within the pathway. The size of the dot indicates the impact of the pathway, with the colour representing significance of enrichment between treatments (stronger red for lower *P*-value). Figure 37 therefore shows both the statistical significance of pathway enrichment due to treatment, and an indication of the biological significance of the metabolites detected within the enriched pathways.

Table 41: Metabolic pathways in pig plasma that were enriched post-weaning compared to pre-weaning with FDR <0.10 and at least two metabolites identified.

Metabolic pathway	Key for Figure 37	*P*-value	FDR
Arginine and proline metabolism	a	5.94×10^{-15}	2.38×10^{-13}
Pentose and glucuronate interconversions	b	2.14×10^{-9}	4.28×10^{-8}
Glutathione metabolism	c	6.02×10^{-9}	8.03×10^{-8}
Starch and sucrose metabolism	d	2.01×10^{-8}	1.60×10^{-7}
Galactose metabolism	e	2.40×10^{-8}	1.60×10^{-7}
Glycine, serine and threonine metabolism	f	3.26×10^{-8}	1.86×10^{-7}
Valine, leucine and isoleucine biosynthesis	g	1.18×10^{-6}	4.17×10^{-6}
Pantothenate and CoA biosynthesis	h	1.86×10^{-6}	6.20×10^{-6}
Valine, leucine and isoleucine degradation	j	3.19×10^{-6}	9.82×10^{-6}
Biosynthesis of unsaturated fatty acids	k	2.88×10^{-5}	8.24×10^{-5}
Glycolysis / Gluconeogenesis	l	2.39×10^{-4}	5.61×10^{-4}
Arginine biosynthesis	m	1.76×10^{-3}	3.91×10^{-3}
Histidine metabolism	n	3.03×10^{-3}	6.37×10^{-3}
Steroid hormone biosynthesis	o	0.011	0.017
Primary bile acid biosynthesis	p	0.011	0.017
D-Amino acid metabolism	q	0.052	0.070
Porphyrin metabolism	r	0.073	0.094

FDR: False discovery rate- *P*-value adjusted for multiple tests.

Table 42: Metabolic pathways in pig plasma affected by weaning, with FDR <0.10 with only one metabolite identified, or unaffected by weaning (FDR >0.10)

Metabolic pathway	*P*-value	FDR
Cysteine and methionine metabolism	5.43×10^{-7}	2.42×10^{-6}
Sphingolipid metabolism	5.43×10^{-7}	2.42×10^{-6}
Linoleic acid metabolism	1.36×10^{-6}	4.94×10^{-6}
Fatty acid degradation	9.73×10^{-5}	2.60×10^{-4}
Pyruvate metabolism	2.37×10^{-4}	5.61×10^{-4}
beta-Alanine metabolism	3.34×10^{-3}	6.37×10^{-3}
Nicotinate and nicotinamide metabolism	3.34×10^{-3}	6.37×10^{-3}
Butanoate metabolism	8.08×10^{-3}	0.014
Nitrogen metabolism	8.08×10^{-3}	0.014
Steroid biosynthesis	0.011	0.017
Phenylalanine, tyrosine and tryptophan biosynthesis	0.026	0.038
Phenylalanine metabolism	0.026	0.038
Purine metabolism	0.048	0.066
Lipoic acid metabolism	0.162	0.201
Alanine, aspartate and glutamate metabolism	0.166	0.201
Glyoxylate and dicarboxylate metabolism	0.267	0.298
Inositol phosphate metabolism	0.268	0.298
Ascorbate and aldarate metabolism	0.268	0.298
Selenocompound metabolism	0.377	0.395
Amino sugar and nucleotide sugar metabolism	0.383	0.395
Fructose and mannose metabolism	0.385	0.395
Citrate cycle (TCA cycle)	0.456	0.456

FDR: False discovery rate- *P*-value adjusted for multiple tests.

5.3.4.2. Two-way interaction SM*creep

5.3.4.2.1. Pre-weaning

Metabolites identified and their relative concentrations pre-weaning according to pre-weaning SM*creep consumption level are shown in Figure 38.

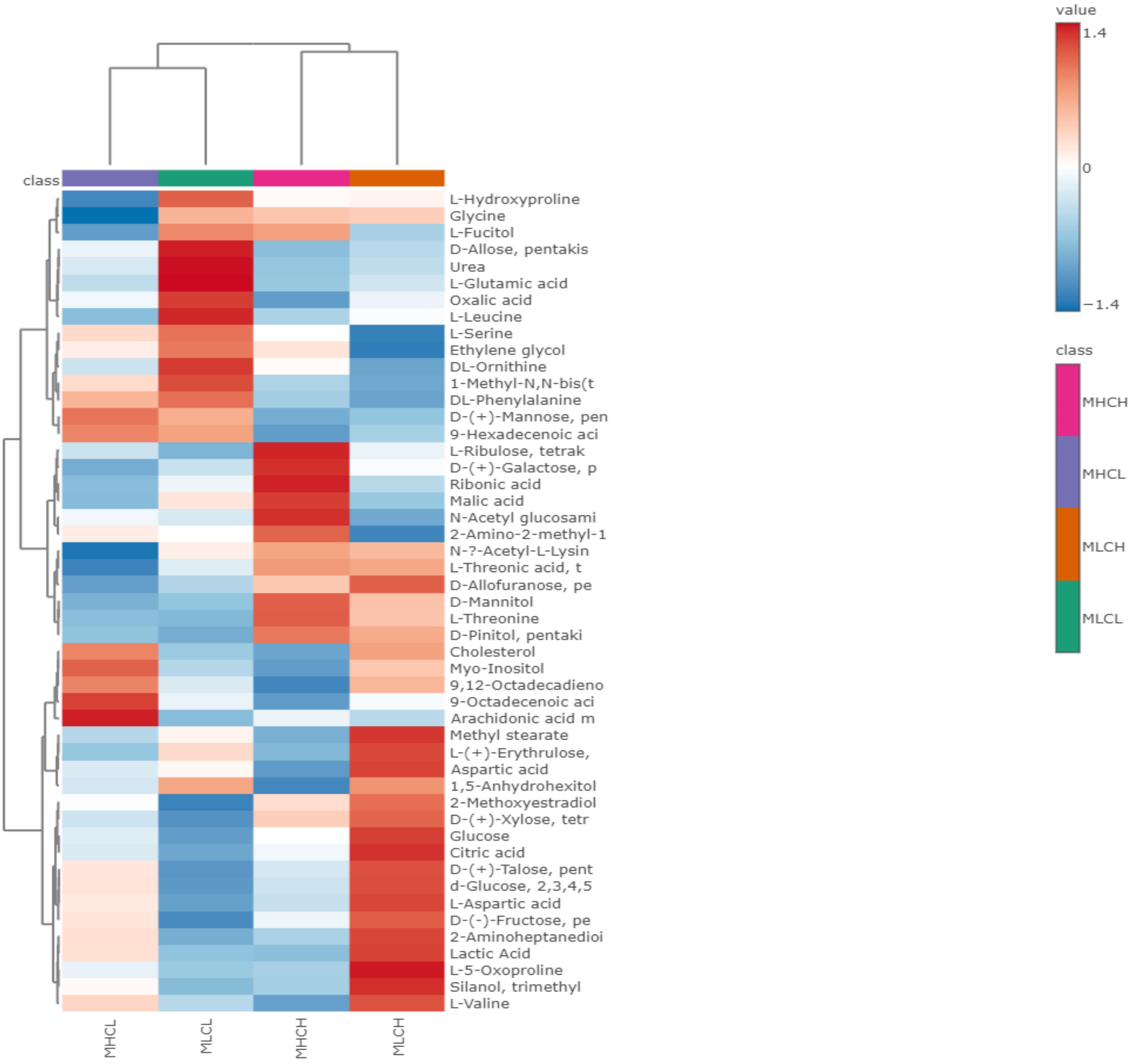

Figure 38: Heatmap of relative concentrations of metabolites detected in plasma of pigs pre-weaning, grouped by low (L) or high (H) pre-weaning supplementary milk (M) and creep (C) consumption level.

Although the heatmap shows similarities in profiles with respect to creep consumption with MLCL and MHCL grouped together, and MLCH and MHCH grouped together, p*ost-hoc* ANOVA revealed there were no differences in concentrations of individual metabolites at *P* <0.05, between pigs grouped according to pre-weaning SM*creep consumption. The PCA analysis also failed to separate the groups (Figure 39).

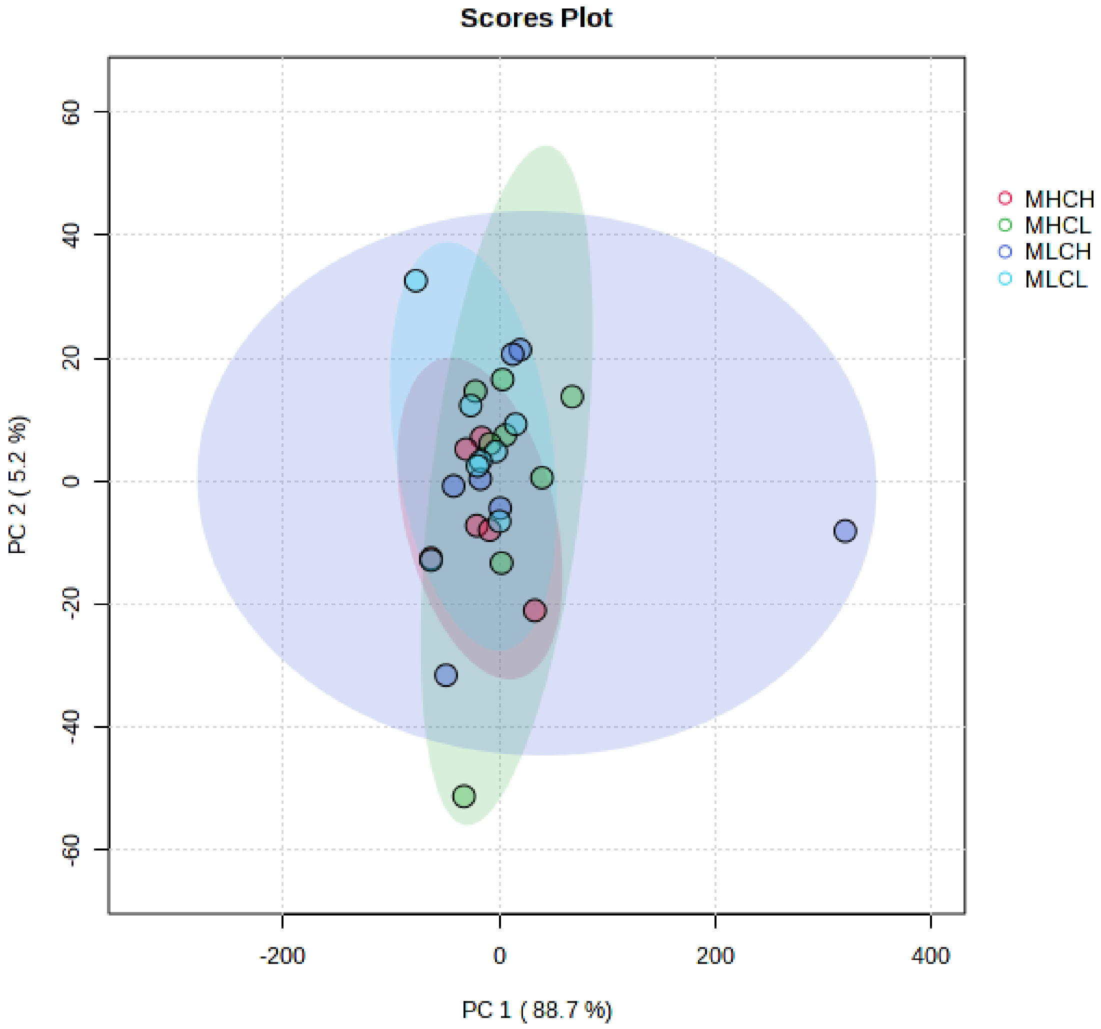

Figure 39: PCA scores plot for concentration of metabolites detected in the plasma of pigs pre-weaning, grouped by low (L) or high (H) pre-weaning supplementary milk (M) and creep (C) consumption level.

Pathway analysis of the SM*creep effect pre-weaning was not performed as having more than two treatments is not well-defined in MetaboAnalyst.

5.3.4.2.2. Post-weaning

Metabolites identified and their relative concentrations post-weaning according to pre-weaning SM*creep consumption level are shown in Figure 40.

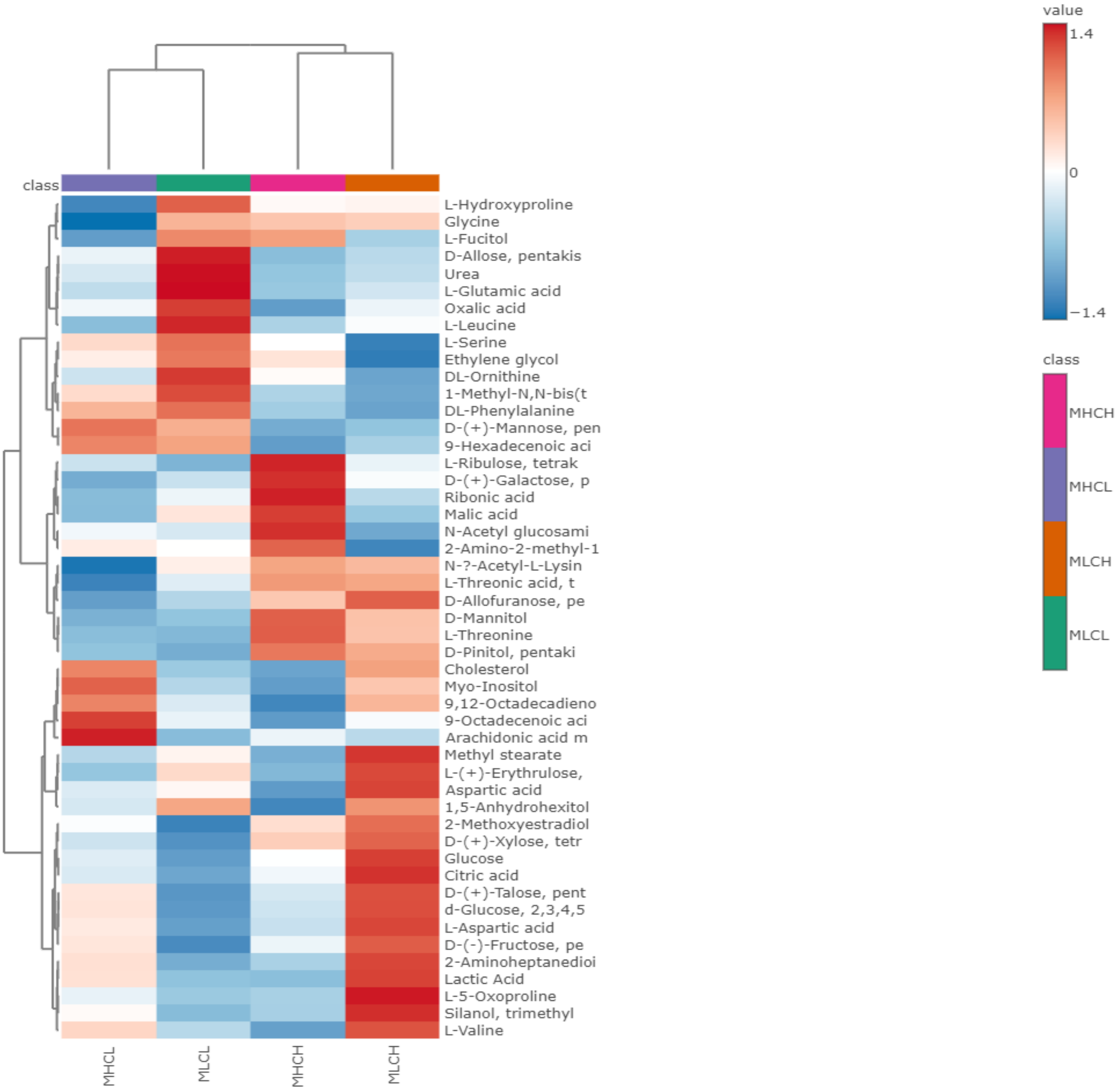

Figure 40: Heatmap of relative concentrations of metabolites detected in plasma of pigs post-weaning, grouped by low (L) or high (H) pre-weaning supplementary milk (M) and creep (C) consumption level.

Similar to pre-weaning, grouping was evident based on creep consumption but not SM consumption. However, *post-hoc* ANOVA revealed there were no differences in concentrations of individual metabolites at *P* <0.05, between pigs grouped according to pre-weaning SM*creep consumption. The PCA analysis also failed to separate the groups (Figure 41).

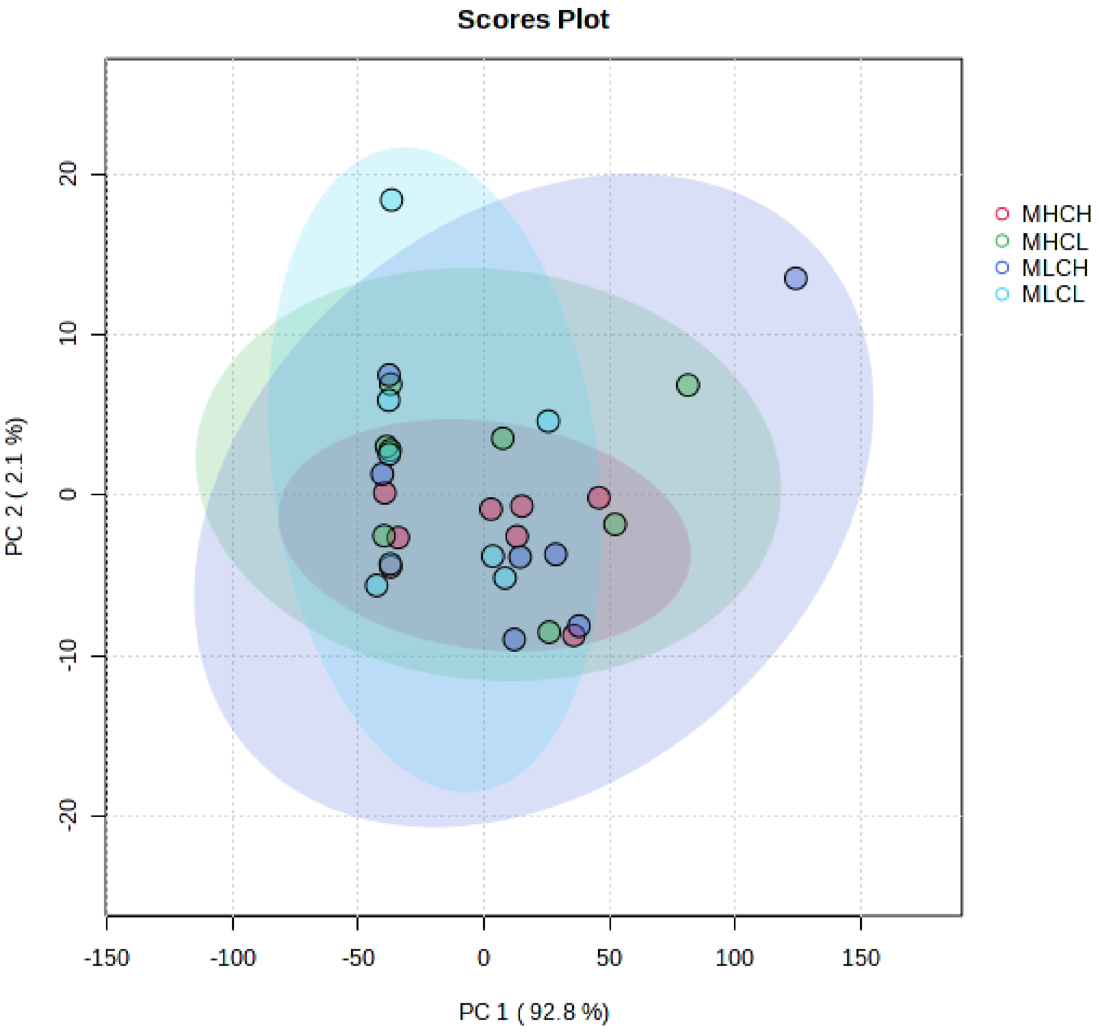

Figure 41: PCA scores plot for concentration of metabolites detected in the plasma of pigs post-weaning, grouped by low (L) or high (H) pre-weaning supplementary milk (M) and creep (C) consumption level.

5.3.4.3. Effect of supplementary milk

5.3.4.3.1. Pre-weaning

Metabolites identified and their relative concentrations pre-weaning according to pre-weaning SM consumption level are shown in Figure 42.

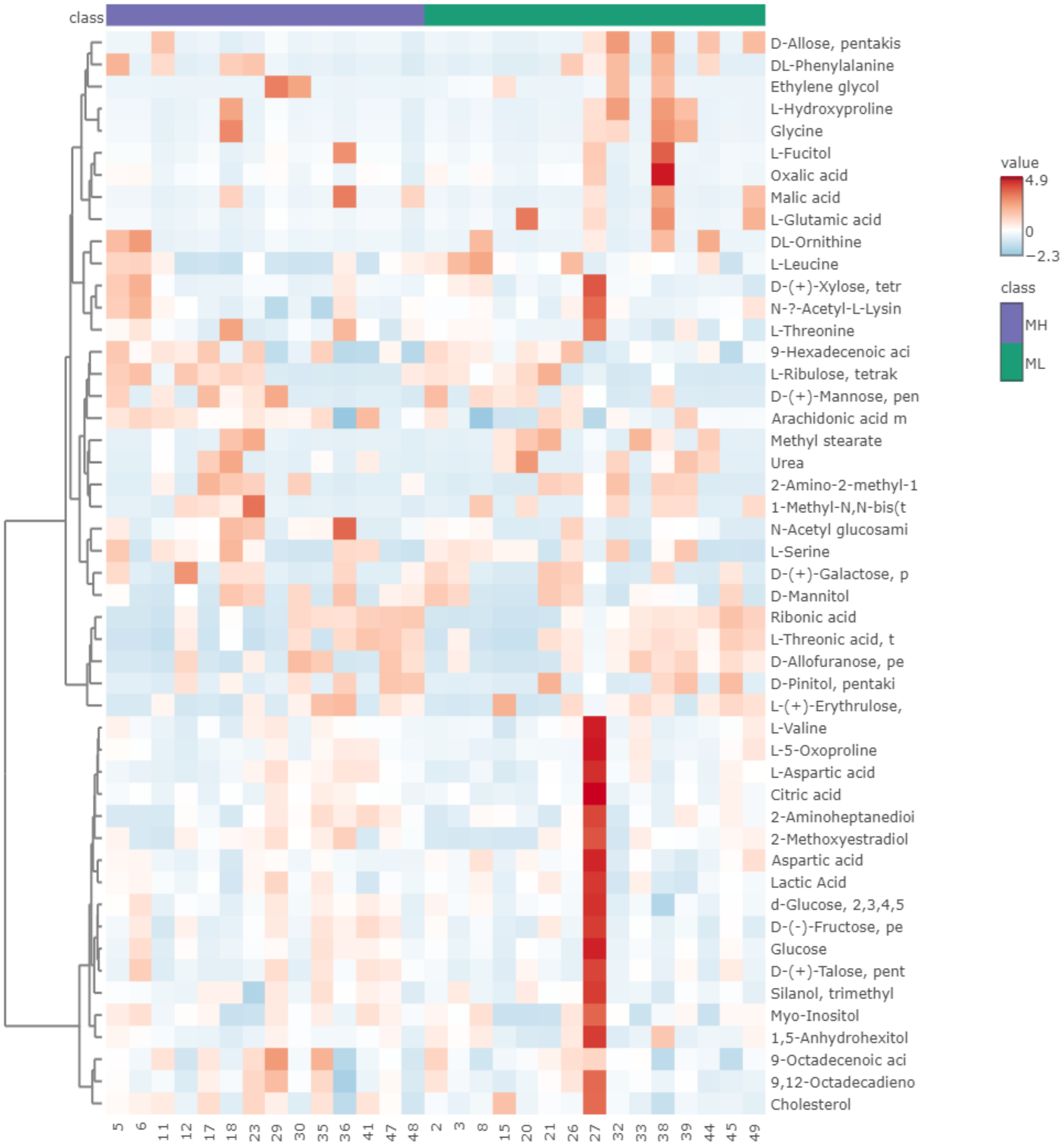

Figure 42: Heatmap of relative concentrations of metabolites detected in plasma of pigs pre-weaning, grouped by low (L) or high (H) pre-weaning supplementary milk (M) consumption level.

Post-hoc t-test revealed there were no differences in concentrations of individual metabolites at *P* <0.05, between pigs grouped according to pre-weaning SM consumption. The PCA analysis also failed to separate the groups (Figure 43).

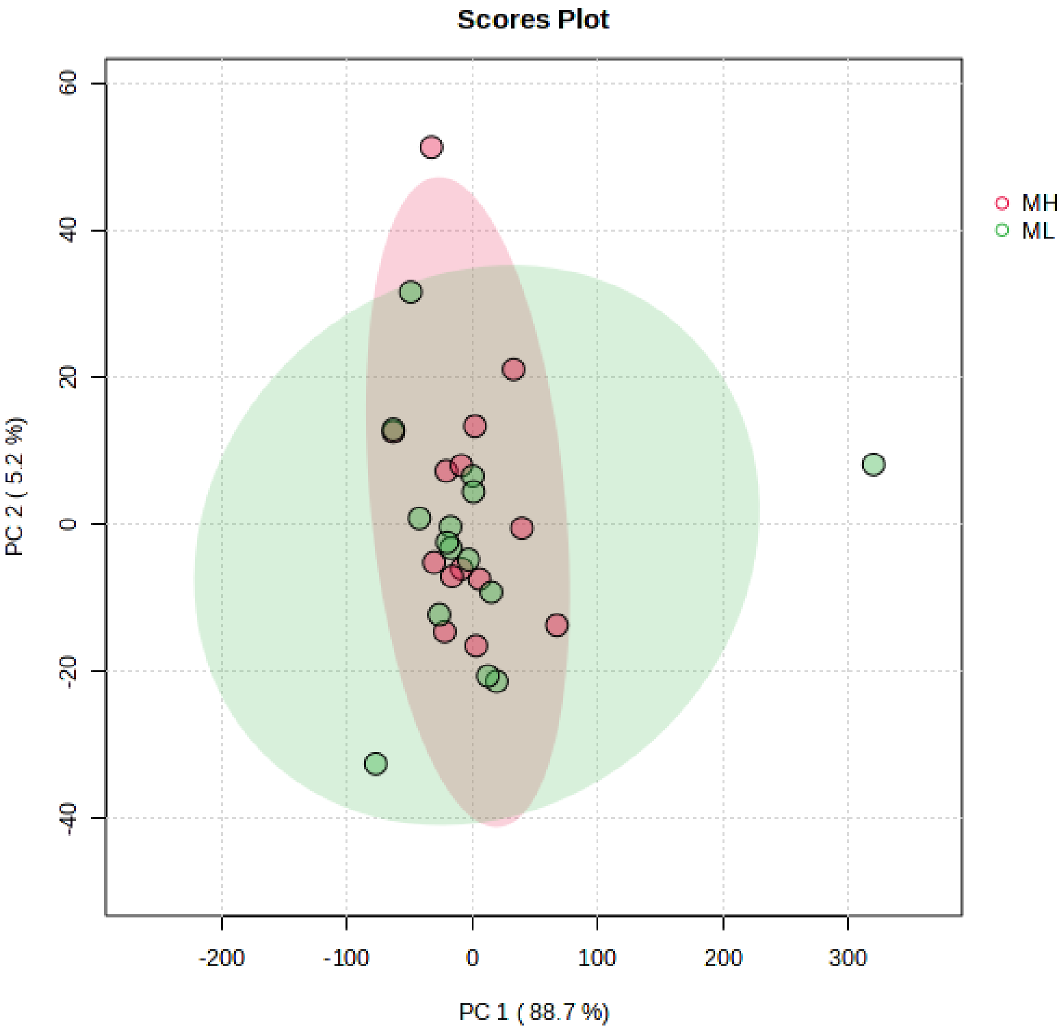

Figure 43: PCA scores plot for concentration of metabolites detected in the plasma of pigs pre-weaning, grouped by low (L) or high (H) pre-weaning supplementary milk (M) consumption level.

There were no metabolic pathways identified pre-weaning that were affected by pre-weaning SM consumption level (*P* >0.05; Table 43).

Table 43: Enrichment of metabolic pathways in pig plasma pre-weaning for high SM consumers compared to low SM consumers.

Metabolic pathway	*P*-value	FDR
Butanoate metabolism	0.102	0.945
Nitrogen metabolism	0.102	0.945
Arginine and proline metabolism	0.201	0.945
Porphyrin metabolism	0.317	0.945
D-Amino acid metabolism	0.336	0.945
Amino sugar and nucleotide sugar metabolism	0.346	0.945
Fructose and mannose metabolism	0.351	0.945
Pentose and glucuronate interconversions	0.446	0.945
Steroid biosynthesis	0.470	0.945
Primary bile acid biosynthesis	0.471	0.945
Steroid hormone biosynthesis	0.471	0.945
Starch and sucrose metabolism	0.492	0.945
Neomycin, kanamycin and gentamicin biosynthesis	0.496	0.945
Galactose metabolism	0.500	0.945
Biosynthesis of unsaturated fatty acids	0.503	0.945
Inositol phosphate metabolism	0.537	0.945
Ascorbate and aldarate metabolism	0.537	0.945
Glycine, serine and threonine metabolism	0.540	0.945
Lipoic acid metabolism	0.560	0.945
Purine metabolism	0.591	0.945
Histidine metabolism	0.627	0.945
beta-Alanine metabolism	0.671	0.945
Nicotinate and nicotinamide metabolism	0.671	0.945
Arginine biosynthesis	0.696	0.945
Glutathione metabolism	0.701	0.945
Linoleic acid metabolism	0.731	0.945
Alanine, aspartate and glutamate metabolism	0.746	0.945
Valine, leucine and isoleucine degradation	0.791	0.945
Valine, leucine and isoleucine biosynthesis	0.807	0.945
Pantothenate and CoA biosynthesis	0.845	0.945
Cysteine and methionine metabolism	0.876	0.945
Sphingolipid metabolism	0.876	0.945
Citrate cycle (TCA cycle)	0.915	0.945
Glyoxylate and dicarboxylate metabolism	0.933	0.945
Glycolysis / Gluconeogenesis	0.945	0.945
Pyruvate metabolism	0.945	0.945

5.3.4.3.2. Post-weaning

Metabolites identified and their relative concentrations post-weaning according to pre-weaning SM consumption level are shown in Figure 44.

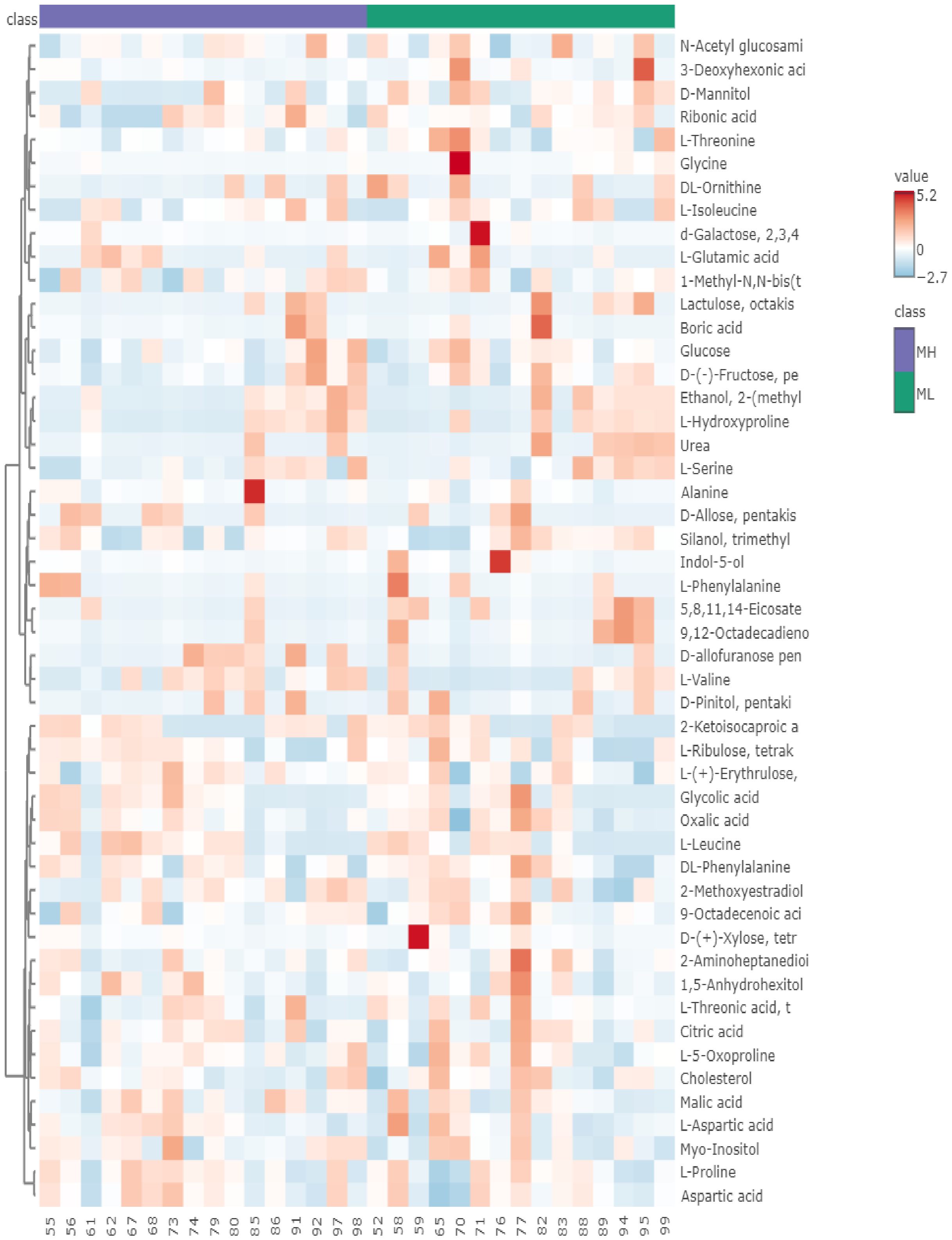

Figure 44: Heatmap of relative concentrations of metabolites detected in plasma of pigs post-weaning, grouped by low (L) or high (H) pre-weaning supplementary milk (M) consumption level.

Post-hoc t-test revealed there were no differences in concentrations of individual metabolites at *P* <0.05, between pigs grouped according to pre-weaning SM consumption. The PCA analysis also failed to separate the groups (Figure 45).

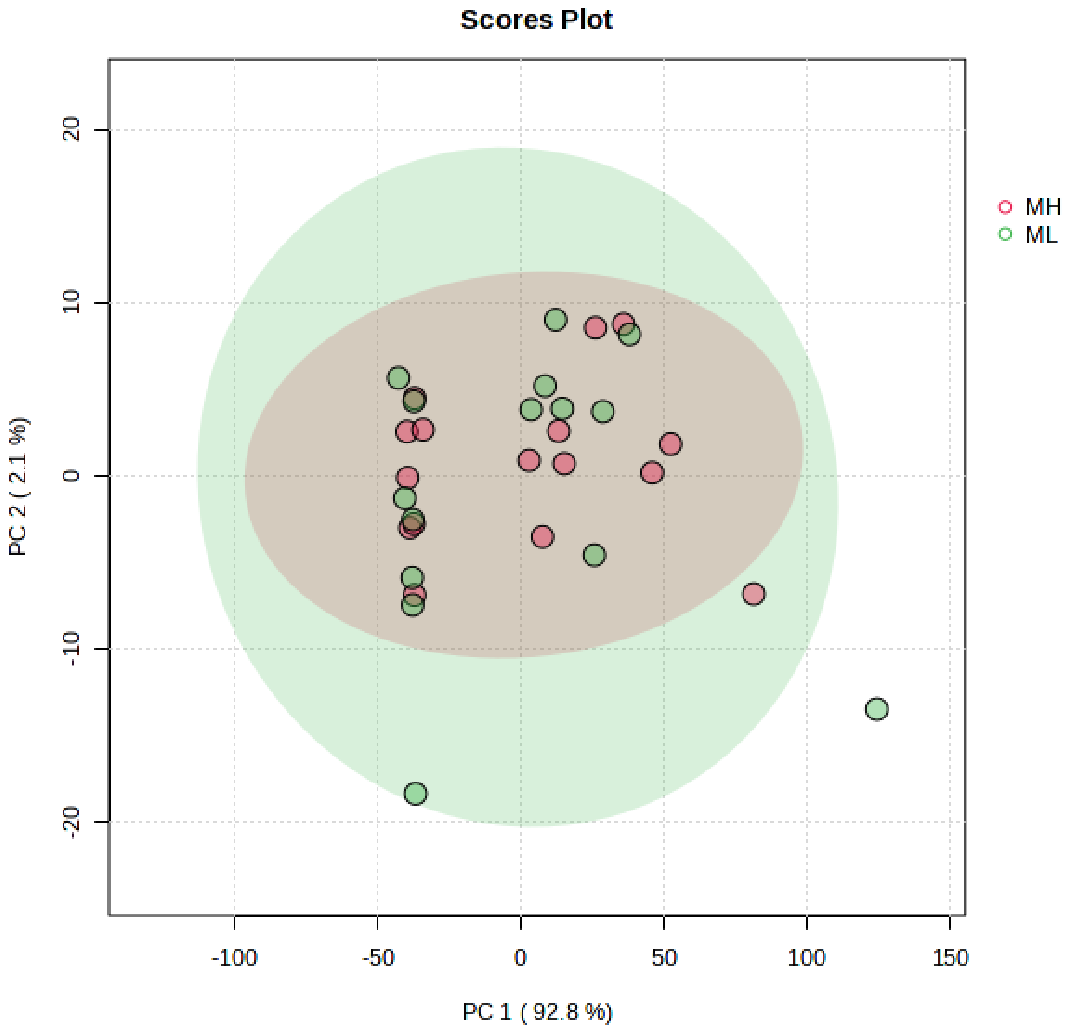

Figure 45: PCA scores plot for concentration of metabolites detected in the plasma of pigs post-weaning, grouped by low (L) or high (H) pre-weaning supplementary milk (M) consumption level.

There were no metabolic pathways identified post-weaning that were affected by pre-weaning SM consumption level (*P* >0.05; Table 44). Although there was a trend for an effect of SM consumption level on linoleic acid metabolism, once this was adjusted for multiple test, it was not significant (FDR >0.05).

Table 44: Enrichment of metabolic pathways in pig plasma post-weaning for high SM consumers compared to low SM consumers.

Metabolic pathway	*P*-value	FDR
Linoleic acid metabolism	0.064	0.967
Purine metabolism	0.139	0.967
Glycine, serine and threonine metabolism	0.177	0.967
Lipoic acid metabolism	0.195	0.967
Porphyrin metabolism	0.205	0.967
Valine, leucine and isoleucine biosynthesis	0.207	0.967
Biosynthesis of unsaturated fatty acids	0.221	0.967
Arginine biosynthesis	0.270	0.967
Cysteine and methionine metabolism	0.294	0.967
Sphingolipid metabolism	0.294	0.967
Glyoxylate and dicarboxylate metabolism	0.348	0.967
Selenocompound metabolism	0.369	0.967
Valine, leucine and isoleucine degradation	0.384	0.967
Primary bile acid biosynthesis	0.448	0.967
D-Amino acid metabolism	0.450	0.967
Fructose and mannose metabolism	0.480	0.967
Starch and sucrose metabolism	0.480	0.967
Amino sugar and nucleotide sugar metabolism	0.480	0.967
Glutathione metabolism	0.503	0.967
Galactose metabolism	0.522	0.967
Steroid biosynthesis	0.548	0.967
Steroid hormone biosynthesis	0.560	0.967
Pantothenate and CoA biosynthesis	0.631	0.970
Citrate cycle (TCA cycle)	0.651	0.970
Inositol phosphate metabolism	0.697	0.970
Ascorbate and aldarate metabolism	0.697	0.970
Glycolysis / Gluconeogenesis	0.740	0.970
Pentose and glucuronate interconversions	0.744	0.970
Phenylalanine, tyrosine and tryptophan biosynthesis	0.795	0.970
Phenylalanine metabolism	0.795	0.970
Fatty acid degradation	0.847	0.970
Alanine, aspartate and glutamate metabolism	0.866	0.970
Arginine and proline metabolism	0.892	0.970
Butanoate metabolism	0.930	0.970
Nitrogen metabolism	0.930	0.970
beta-Alanine metabolism	0.944	0.970
Nicotinate and nicotinamide metabolism	0.944	0.970
Histidine metabolism	0.992	0.992

FDR: False discovery rate- *P*-value adjusted for multiple tests.

5.3.4.4. Effect of creep

5.3.4.4.1. Pre-weaning

Metabolites identified and their relative concentrations pre-weaning according to pre-weaning creep consumption level are shown in Figure 46.

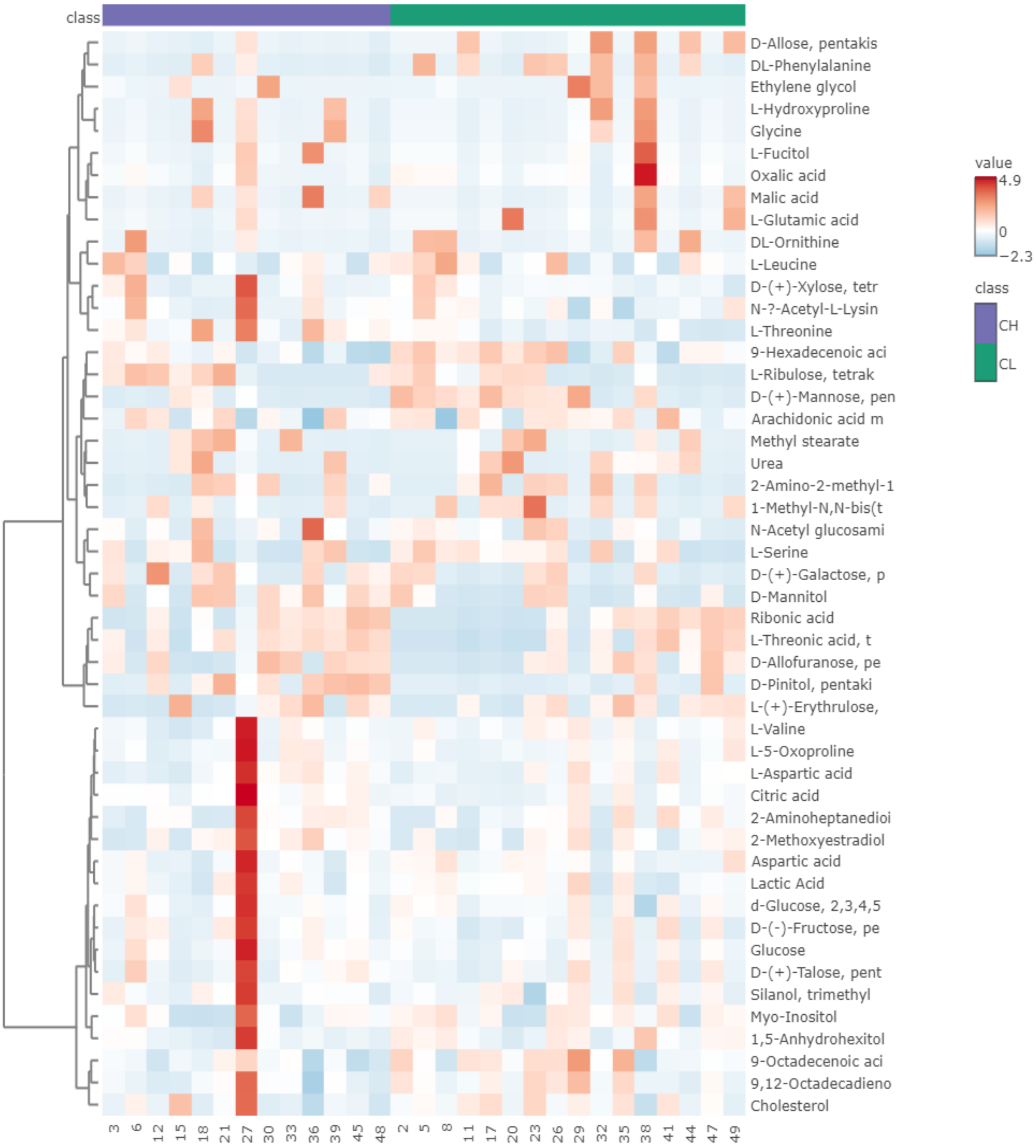

Figure 46: Heatmap of relative concentrations of metabolites detected in plasma of pigs pre-weaning, grouped by low (L) or high (H) pre-weaning creep (C) consumption level.

Post-hoc t-test revealed there were no differences in concentrations of individual metabolites at *P* <0.05, between pigs grouped according to pre-weaning creep consumption. The PCA analysis failed to separate the groups (Figure 47).

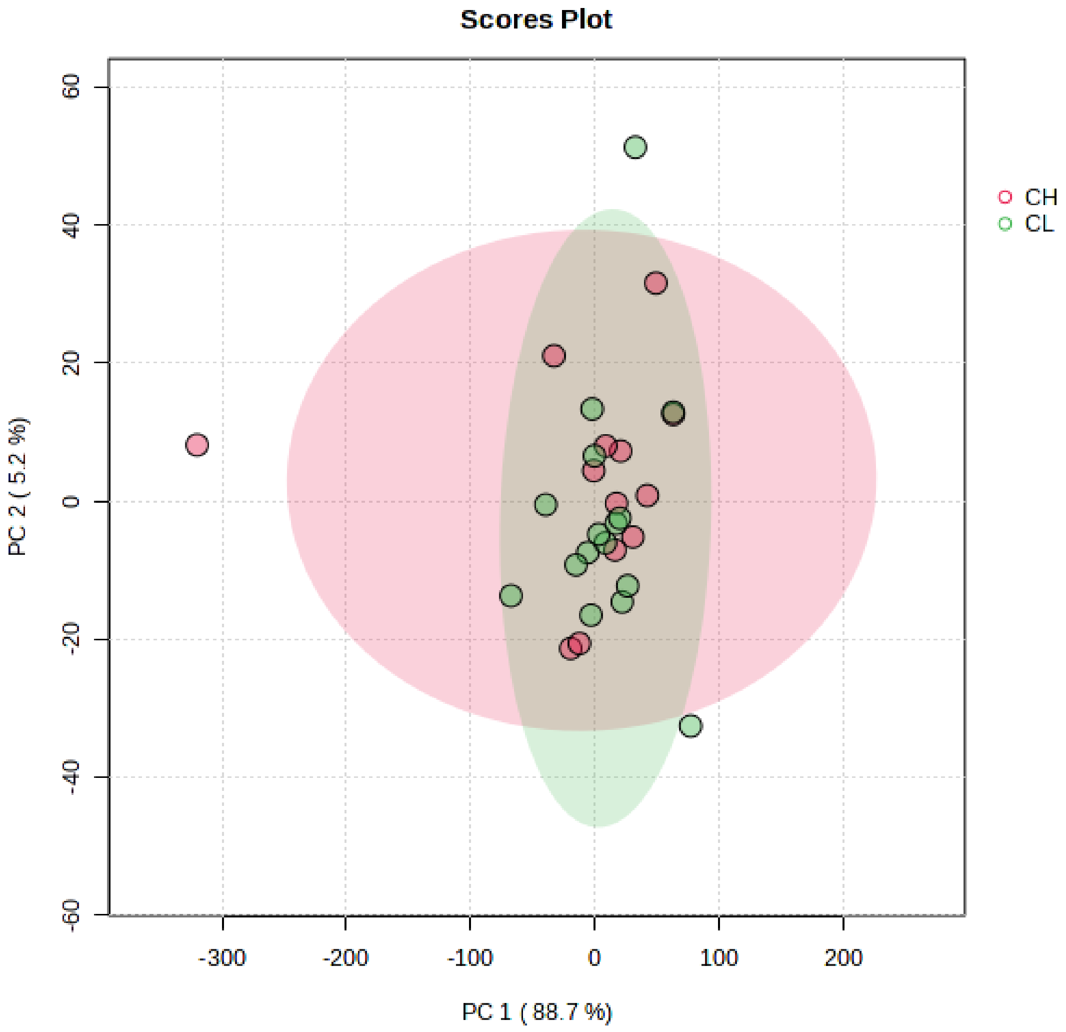

Figure 47: PCA scores plot for concentration of metabolites detected in the plasma of pigs pre-weaning, grouped by low (L) or high (H) pre-weaning creep (C) consumption level.

There were four metabolic pathways affected by level of creep consumption (*P* <0.10; Table 45), with at least two metabolites detected. The FDR was >0.05, and so these should be viewed with caution. As FDR was close to 0.10, it was deemed appropriate to conclude that the glycine, serine and threonine metabolism pathway was the only one enriched by high creep consumption (Figure 48).

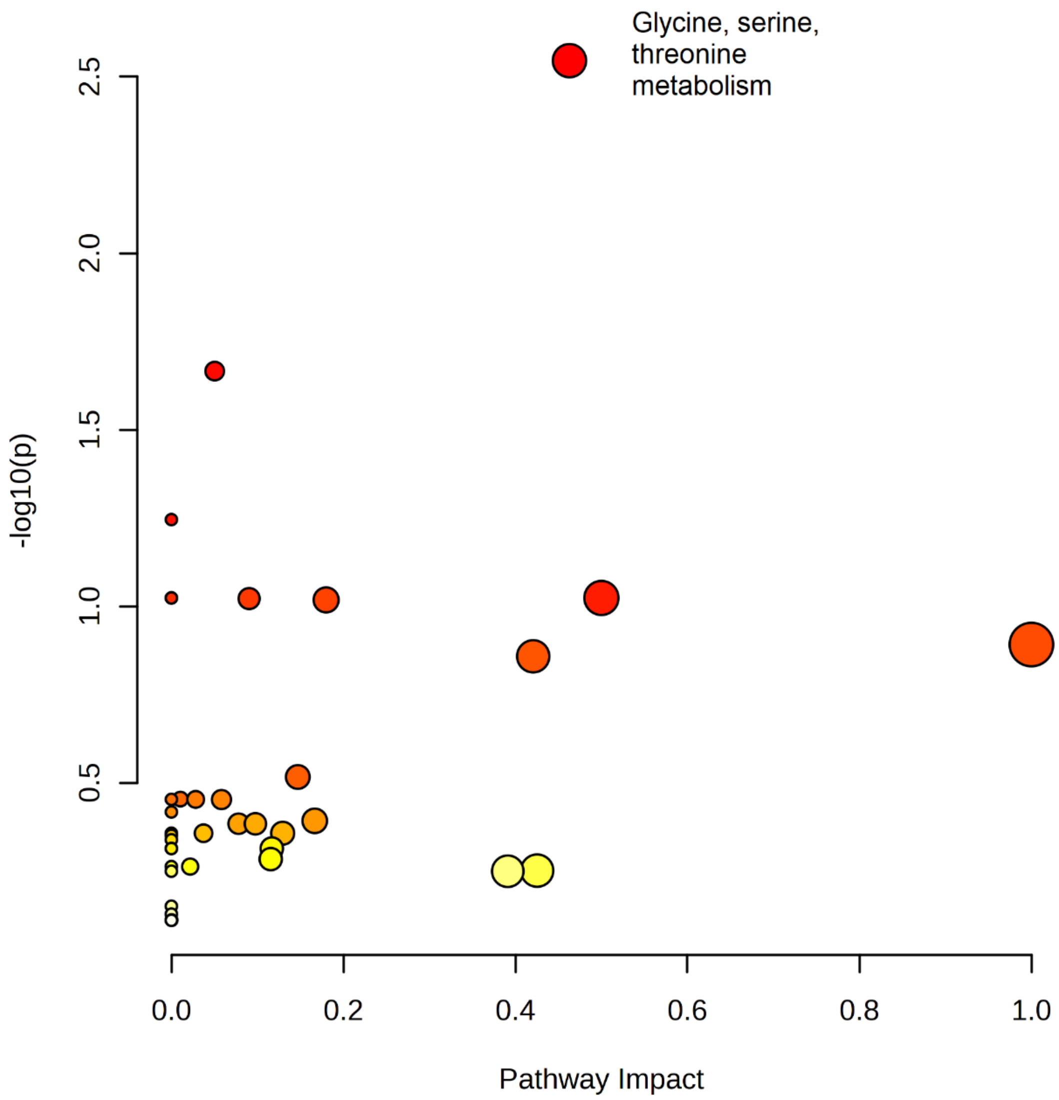

Figure 48: Glycine, serine and threonine metabolism pathway was enriched in pig plasma pre-weaning comparing high to low creep consumers at *P* <0.10 (-log10(p) >1.0) and at least two metabolites identified. Pathway impact: sum of importance of matched metabolites/sum of importance of all metabolites.

Table 45: Enrichment of metabolic pathways in pig plasma pre-weaning for high creep consumers compared to low creep consumers.

Metabolic pathway	Raw *P*	FDR
Glycine, serine and threonine metabolism	0.003	0.105
Amino sugar and nucleotide sugar metabolism	0.022	0.399
Biosynthesis of unsaturated fatty acids	0.057	0.443
D-Glutamine and D-glutamate metabolism	0.095	0.443
Butanoate metabolism	0.095	0.443
Nitrogen metabolism	0.095	0.443
Citrate cycle (TCA cycle)	0.095	0.443
Glyoxylate and dicarboxylate metabolism	0.096	0.443
Linoleic acid metabolism	0.128	0.512
Alanine, aspartate and glutamate metabolism	0.139	0.512
Arginine and proline metabolism	0.304	0.631
Steroid hormone biosynthesis	0.351	0.631
Porphyrin and chlorophyll metabolism	0.352	0.631
Steroid biosynthesis	0.352	0.631
Primary bile acid biosynthesis	0.352	0.631
Valine, leucine and isoleucine biosynthesis	0.382	0.631
Aminoacyl-tRNA biosynthesis	0.405	0.631
Pentose and glucuronate interconversions	0.413	0.631
Fructose and mannose metabolism	0.413	0.631
Inositol phosphate metabolism	0.439	0.631
Phosphatidylinositol signaling system	0.439	0.631
Ascorbate and aldarate metabolism	0.439	0.631
Purine metabolism	0.445	0.631
Histidine metabolism	0.458	0.631
beta-Alanine metabolism	0.485	0.631
Nicotinate and nicotinamide metabolism	0.485	0.631
Arginine biosynthesis	0.485	0.631
Glutathione metabolism	0.520	0.631
Cysteine and methionine metabolism	0.546	0.631
Sphingolipid metabolism	0.546	0.631
Starch and sucrose metabolism	0.560	0.631
Galactose metabolism	0.563	0.631
Pantothenate and CoA biosynthesis	0.707	0.769
Valine, leucine and isoleucine degradation	0.744	0.775
Glycolysis / Gluconeogenesis	0.774	0.775
Pyruvate metabolism	0.775	0.775

Raw *P*: *P*-value before adjustment for multiple tests; FDR: False discovery rate- *P*-value adjusted for multiple tests.

5.3.4.4.2. Post-weaning

Metabolites identified and their relative concentrations post-weaning according to pre-weaning creep consumption level are shown in Figure 49.

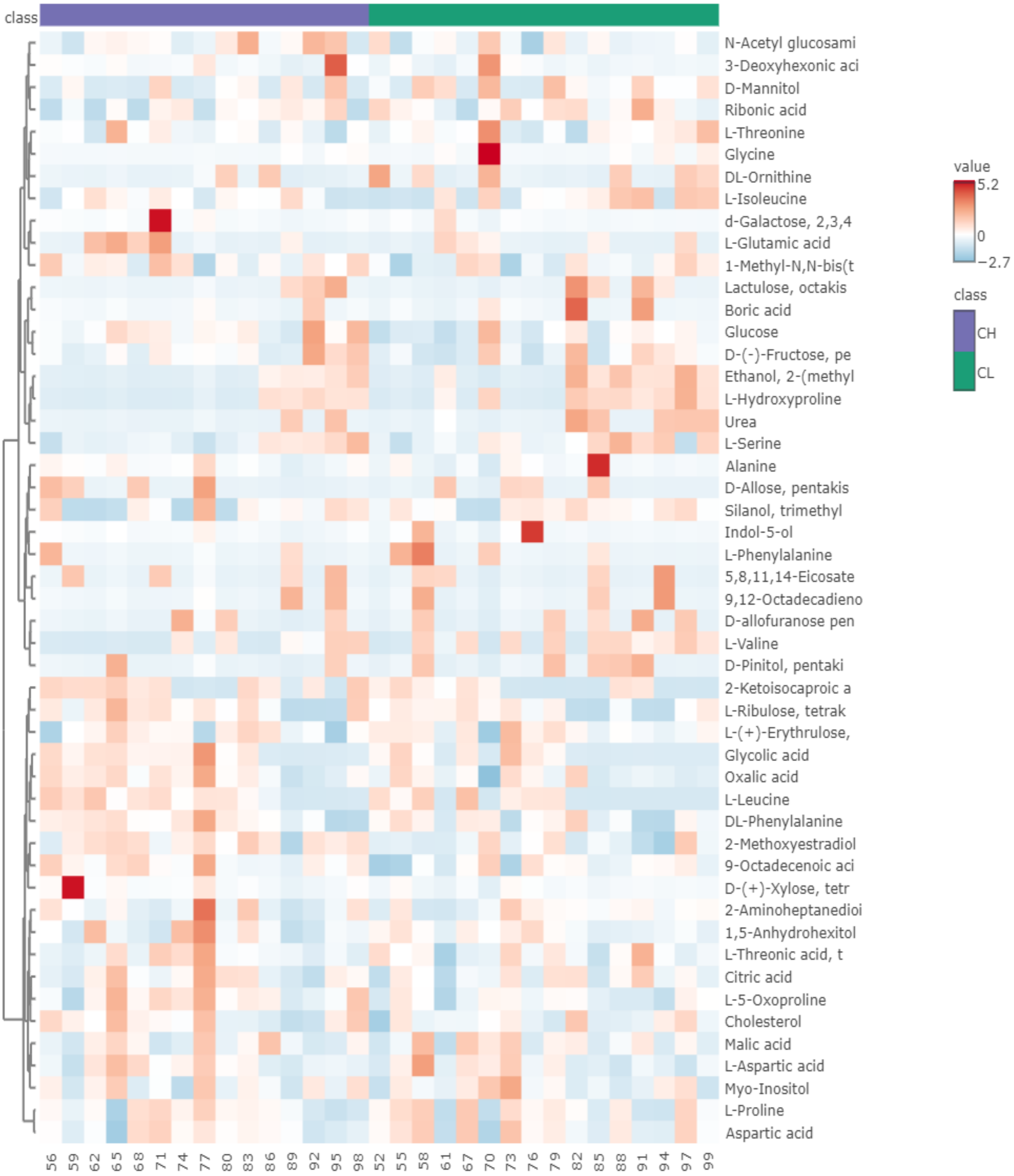

Figure 49: Heatmap of relative concentrations of metabolites detected in plasma of pigs post-weaning, grouped by low (L) or high (H) pre-weaning creep (C) consumption level.

Post-hoc ANOVA revealed there were no differences in concentrations of individual metabolites at *P* <0.05, between pigs grouped according to pre-weaning creep consumption. The PCA analysis also failed to separate the groups (Figure 50)

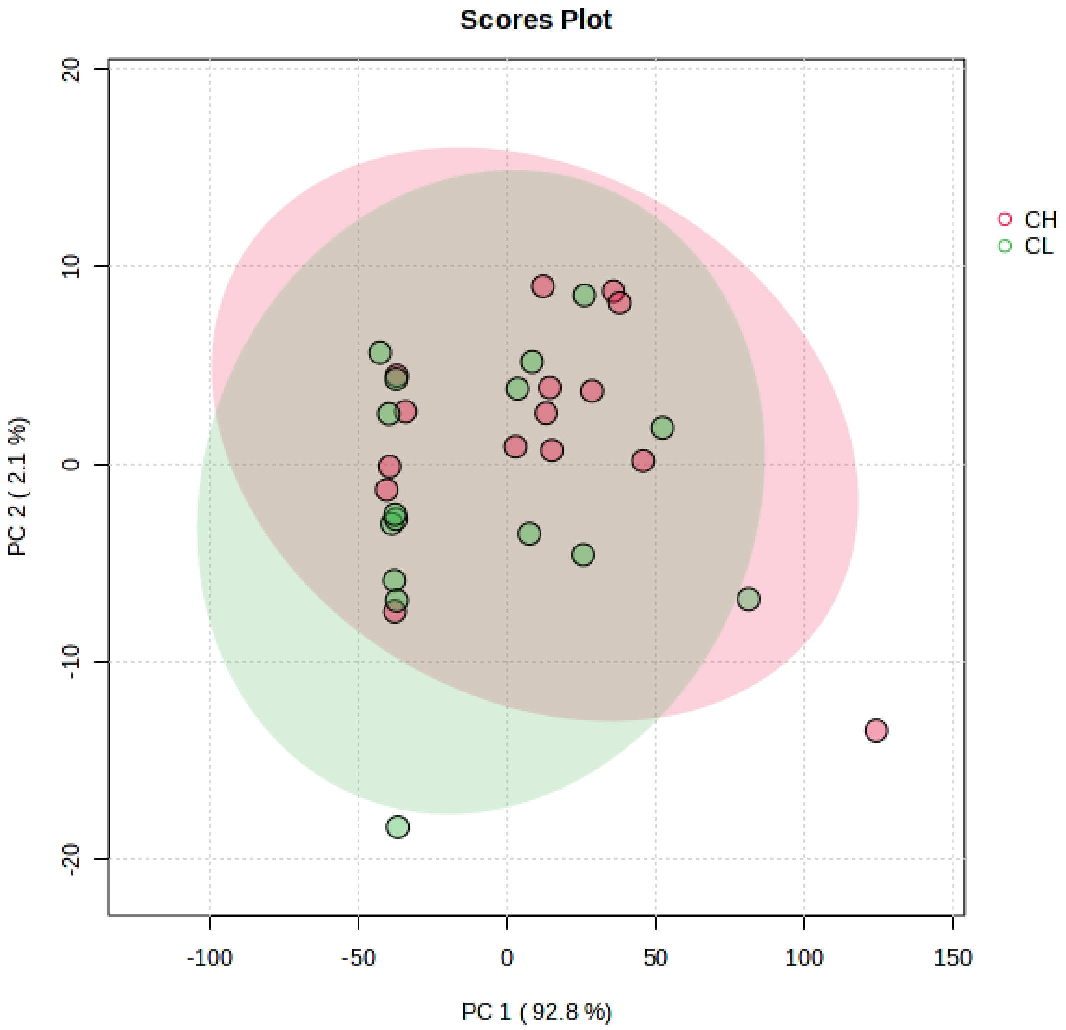

Figure 50: PCA scores plot for concentration of metabolites detected in the plasma of pigs post-weaning, grouped by low (L) or high (H) pre-weaning creep (C) consumption level.

There were no metabolic pathways in post-weaning pig plasma affected by level of pre-weaning creep consumption (FDR >0.10; Table 46).

Table 46: Enrichment of metabolic pathways in pig plasma post-weaning for high creep consumers compared to low creep consumers.

Pathway	Raw *P*	FDR
Glutathione metabolism	0.012	0.462
Biosynthesis of unsaturated fatty acids	0.034	0.478
Glycolysis / Gluconeogenesis	0.038	0.478
Pentose and glucuronate interconversions	0.145	0.633
Steroid hormone biosynthesis	0.153	0.633
Steroid biosynthesis	0.169	0.633
Primary bile acid biosynthesis	0.182	0.633
Valine, leucine and isoleucine degradation	0.183	0.633
Fatty acid degradation	0.184	0.633
D-Glutamine and D-glutamate metabolism	0.241	0.633
Butanoate metabolism	0.241	0.633
Nitrogen metabolism	0.241	0.633
Citrate cycle (TCA cycle)	0.248	0.633
Purine metabolism	0.266	0.633
Porphyrin and chlorophyll metabolism	0.274	0.633
Glyoxylate and dicarboxylate metabolism	0.314	0.633
Fructose and mannose metabolism	0.342	0.633
Starch and sucrose metabolism	0.342	0.633
Pantothenate and CoA biosynthesis	0.350	0.633
Arginine biosynthesis	0.353	0.633
Galactose metabolism	0.366	0.633
Histidine metabolism	0.445	0.633
Alanine, aspartate and glutamate metabolism	0.449	0.633
Phenylalanine, tyrosine and tryptophan biosynthesis	0.450	0.633
Phenylalanine metabolism	0.450	0.633
Glycine, serine and threonine metabolism	0.465	0.633
beta-Alanine metabolism	0.466	0.633
Nicotinate and nicotinamide metabolism	0.466	0.633
Aminoacyl-tRNA biosynthesis	0.501	0.656
Valine, leucine and isoleucine biosynthesis	0.519	0.657
Arginine and proline metabolism	0.538	0.659
Cysteine and methionine metabolism	0.629	0.718
Sphingolipid metabolism	0.629	0.718
Inositol phosphate metabolism	0.680	0.718
Phosphatidylinositol signaling system	0.680	0.718
Ascorbate and aldarate metabolism	0.680	0.718
Selenocompound metabolism	0.922	0.947
Linoleic acid metabolism	0.963	0.963

Raw *P*: *P*-value before adjustment for multiple tests; FDR: False discovery rate- *P*-value adjusted for multiple tests.

5.4. Discussion

5.4.1. Pre-weaning

5.4.1.1. Supplementary milk consumption

The effects of birthweight group and suckling position on performance and SM consumption were similar to Study 1 (Chapter 4), and so will not be discussed again. An analysis of SM consumption relative to ADG revealed that the lightest birthweight piglets (group 1; ≤ 1.04kg) had the highest SM usage relative to their growth rate. This was probably a combination of these piglets having the lowest ADG overall, and the highest proportion of non-sucklers, which had the highest SM usage. It suggests that the light birthweight piglets obtained a disproportionate amount of their nutrition from SM, assuming that efficiency of converting sows' milk to growth did not vary between birthweight groups. Recent work by Hojgaard *et al.* (2020) determined that regardless of bodyweight, piglets required 4.2 g of sow milk for 1 g of growth, and so it seems a fair assumption to make. They concluded that this efficiency was unaffected by piglet weight because although smaller piglets have lower maintenance requirements, they also lose proportionally more energy as heat due to higher surface area:volume ratio.

5.4.1.2. Patterns of creep consumption

Diurnal variation in creep consumption closely mirrored that of SM consumption, discussed in Chapter 4.

Average weighed creep intake per piglet in the current study from d11-18 (11 g DM/piglet) was higher than in that of the dry creep fed piglets (5.43 g DM/piglet) and lower than liquid creep fed piglets (20.07 g DM/piglet) in the study of Byrgesen *et al.* (2020). From d19-weaning, weighed DM intake was higher in the current study (141 g/piglet) than both dry- and liquid-fed piglets (22.9 g/piglet and 31.1 g/piglet respectively) in Byrgesen *et al.* (2020). The litters of Byrgesen *et al.* (2020) contained only 10-14 piglets, whereas in the current study there was an average of 14.3 piglets weaned/litter, with only three litters containing less than 14 piglets. The average piglet weight at weaning was also considerably lighter in Byrgesen *et al.* (2021), with a 24-day-old piglet weighing 5.70-6.01 kg, whereas in the current study piglets were weaned at 27 d old at 8.68 kg. This suggests that demand for supplementary nutrition was higher in the current study due to the higher performance level. Creep DMI in comparison to litter weight gain was low (2.02 kg creep v*s* 94 kg litter weight gain from d4), and so the majority of growth was provided by the sow. Creep intake was higher than in the study of Sulabo *et al.* (2010a), but their piglets were weaned at 20 days old. Comparing similar periods, their litters consumed 514-745 g/litter from d15-20, whereas in the current study, from d12-18 the average litter intake was 123 g/litter. Bruininx *et al.* (2002a) reported creep intakes from d11-28 of 377 g/piglet (as-fed), and Muns and Magowan (2018) of 620 g/piglet, considerably higher than in the current study. It is possible that in the current study creep intake was restricted later in the suckling period, as commonly a multispace feeder would be provided to allow social feeding (as in Bruininx *et al*., 2002a), but this was not possible due to the antenna being capable of only reading one EID tag at once. Social feeding resulting in higher supplementary feed intakes has been proposed by Wattanakull *et al.* (2005) and Kobek-Kjeldager *et al.* (2021b). Creep intake is highly variable both within and between litters (Bruininx *et al.*, 2002a), and may also be affected by fostering protocol, as Huting *et al.* (2017) found that heavy birthweight piglets in uniform weight litters consumed more creep feed than those in mixed litters, or light birthweight piglets in any litters. They concluded that heavy pigs in mixed litters were able to stimulate their teat to produce a greater proportion of the sows' total milk yield through more vigorous suckling and teat stimulation than lighter piglets. This resulted in the sow providing more of their requirements than in uniform litters where they did not have the weight advantage, and consequently required higher creep to satisfy their appetites.

The pattern of increase in creep consumption with a slow increase from d11/12 and a more rapid increase from ~d20 during suckling is similar to that reported by Bruininx *et al.* (2002a), Huting *et al.* (2017) and Byrgesen *et al.* (2021, in dry creep fed piglets), albeit with different magnitudes relating to total intake for the respective studies. De Passillé *et al.* (1989) also found creep duration of feeding and visits increased gradually from d10-20, but the piglets were then weaned on d21. Sulabo *et al.* (2010a) weaned at 20 d old, but varied the timing of creep introduction: for piglets offered creep from d8, the amount consumed/piglet increased from 14.5 g/piglet (d8-14) to 45.8 g/piglet (d15-20), showing an increase in the second week of offering as observed in studies previously mentioned. Piglets offered creep from d14 had a similar total intake (66 g/piglet) to those offered creep from d8, but when creep was offered from d18, intake was lower (31.2 g/piglet). Sulabo *et al.* (2010a) found that the proportion of piglets consuming creep by weaning (eaters) was higher when creep was introduced at d8 (80.2 %) than at d14 (70.0 %) or d18 (70.6 %). This proportion of eaters was higher than reported by Huting *et al.* (2017), who observed that 53.9 % of piglets consumed some creep, with 10.3 % classified as high consumers, but similar to Muns and Magowan (2018), who observed only 6 % non-eaters. In the current study, all piglets would have been classified as eaters at weaning, and the high proportion of piglets consuming creep from introduction contrasts with results of Byyrgesen *et al.* (2021). They experienced a lag in consumption, particularly for the first week of offering creep, with approximately 20 % of their piglets categorised as eaters (in-feed ferric oxide dying faeces) by d18 after creep introduction on d10, and only 50 % by d24. This high proportion of “eaters” from introduction in the current study could be due to higher litter size increasing piglet demand for supplementary nutrition, or could reflect the piglets’ familiarity with supplementary feeding as they already consumed SM, so they were more likely to investigate a new feeder. There could also be a dilution effect in studies using coloured markers, as high milk/liquid intake may dilute the marker to make it indiscernible and return a false negative (Byrgesen *et al.*, 2021; Bruininx *et al*., 2002a).

5.4.1.3. Effect of creep provision on SM consumption

Piglet SM consumption did not reduce as a result of creep introduction, as the pattern of SM consumption over time was similar between study 1 and study 2.

Due to the higher DM content of the creep, although as-fed quantities consumed were lower than those of SM, supplementary DMI as calculated by the calibration equations was 1.98 times higher in study 2 than study 1, from d19-weaning. This may be due to unrecorded differences between the batches, but number of pigs weaned and average weaning weight between the studies were similar, and so it appears that piglets started to consume creep in addition to SM to satisfy their appetite, rather than reducing SM consumption. A similar effect was recorded by Byrgesen *et al.* (2021), whereby piglets fed a dry creep had a higher rate of increase in daily DM consumption after d18 of age, compared to those offered a liquid creep. Maintaining SM consumption may be due to familiarity with the diet, or may reflect the high SM consumption of the non-suckling pigs in both studies having a large influence over total consumption. The reason for the apparent preference for solid supplementary feed above baseline SM consumption is unknown as there is a lack of published material investigating the combination of SM and creep, when both are supplied *ad libitum* during the suckling period. Algers *et al.* (1990) proposed an “optimal foraging theory”, that piglets suckling the least productive teats weaned themselves earlier than those suckling more productive teats as it was beneficial to them to consume more solid feed. Following this theory, in the current Study, it is possible that when creep was provided and piglets were familiar with solid feed, they reduced intake of sow milk accordingly and began to self-wean. This did not occur when solely SM was provided, as sow’s milk is a better source of nutrition for the piglet. Additional work to verify this would include observation of frequency of suckling, and analysis of sow weight and P2 change where creep was provided or not provided.

5.4.1.4. Birthweight effect on creep consumption

Piglets from the lightest birthweight group had the lowest creep consumption as measured by du/d in the final week of suckling. This agrees with the findings of Pajor *et al.* (1991), who reported creep feed intake was positively correlated with birthweight, but appears contradictory to Sulabo *et al.* (2010a), who observed that the lightest birthweight piglets had a (non-significant) higher proportion of creep eaters at weaning. They made no assessment of actual quantity consumed, and no calibration of the faecal colour compared to weighed intake. They also didn't state what constituted a light birthweight piglet. Lower creep intake in lighter birthweight piglets (<1.25 kg birthweight, heavier than the current study) has been reported before by Huting *et al*. (2017), and they proposed several explanations: 1) milk intake from the sow was not limiting due to lower appetite; 2) less mature digestive system meant they did not seek out additional nutrition; 3) competition from heavier littermates reduced opportunity to access the feeder. In the current study there were periods of the day where creep consumption was lower, which would provide opportunities for lighter piglets to feed, and so access to the feeder was not limiting. Consumption of SM relative to ADG was highest in light birthweight piglets, and so it doesn't appear to be the case that their appetites were satisfied by the sow. When creep consumption was analysed relative to ADG, there was no difference between the birthweight groups, so the proportion of growth obtained from creep for the lightest birthweight piglets did not differ from that of the heavier piglets. It appears that the lighter birthweight piglets preferred to obtain a higher proportion of their supplementary nutrition from SM than creep. As these piglets had higher SM consumption relative to ADG from the beginning of the suckling period, they were more accustomed to consuming SM before creep introduction, and so continued with SM as habit rather than switching to creep. Byrgesen *et al.* (2021) observed that until 18 days of age, DM intake of a creep diet was higher when it was mixed with water into a 36 % DM liquid, than when it was fed as a dry meal, suggesting that young piglets prefer a liquid diet, although they didn't investigate the effects of birthweight.

5.4.1.5. Suckling position effect on creep consumption

Huting *et al.* (2017) and Algers *et al*. (1990) found that piglets suckling anterior teats had lower creep intake, but in the current study, amongst piglets with a teat to suckle, there was no effect of suckling position on absolute creep consumption. However, when consumption was analysed relative to ADG, anterior and centre-suckled piglets had the lowest creep:ADG, indicating that a lower proportion of their growth came from creep, and given that ADG was higher in these piglets than posterior-suckled piglets, sow milk yield was less limiting for anterior- and centre-suckled piglets.

Non-suckled piglets consumed the most creep, particularly when related to ADG, and in addition to having the highest SM consumption. They were the smallest piglets, and so would be expected to have the lowest appetites due to having smaller stomachs, therefore the consumption patterns observed validated their non-suckling classification.

5.4.1.6. Effect of SM and creep consumption on performance

Piglets in this study were classified as low or high consumers with no intermediate grouping, and this is probably the reason for the lack of effect of SM consumption on weaning weight, as in study 1 where there was an intermediate classification, the highest consumers were lighter at weaning, due to the influence of the lighter weight non-sucklers. It is a limitation of this Study that only two groupings for each factor were made, as piglets with medium consumption will have been allocated as low or high, but may not differ very much in terms of consumption. For the metabolomic analysis, pigs that fell at the more extreme ends of the distribution for each SM*creep consumption level were selected for sampling in order to attain some separation of the consumption levels of the groups

Piglets with high creep intake tended to be lighter at weaning than those with low creep intake, and this was also observed by Huting *et al.* (2017), and Sulabo *et al* (2010a, eaters compared to non-eaters). Sulabo *et al.* (2010a) concluded that it was due to the lighter birthweight piglets having a higher proportion of eaters, and in the current study it was due to the high consumption of non-sucklers, which were predominantly from birthweight group 1 and 2 (67 % of non-suckers), and were lighter at weaning.

5.4.2. Post-weaning

5.4.2.1. Performance

5.4.2.1.1. Nursery

A low SM score (ML) suggests the piglet obtained most nutrition from suckling the sow, and is therefore most comparable to previous studies with no SM provision. The overall higher ADFI post-weaning observed in MLCH compared to MLCL pigs is broadly in agreement with Bruininx *et al.* (2002a), Sulabo *et al.* (2010a) and Muns and Magowan (2018), although those researchers reported an increase in ADFI in the first week post-weaning, which was not significant in the current study. The fact that MLCL piglets had the lowest ADFI overall may reflect that they were least self-sufficient at weaning as more of their nutrition came from the sow.

In the current study, there was a tendency for higher ADG for MH pigs in the first 7d post-weaning, and a small positive effect of individual SM duration on ADG from weaning to d15, but it did not persist further, and had no overall effect to the end of the weaner period, or to slaughter. In Study 1 there was a positive effect of SM on ADG to the end of the nursery period, and previous studies have reported varying effects of SM on post-weaning performance: Van Oostrum *et al.* (2016) found improvements in ADG from weaning to d28 in piglets supplemented for 5 days pre-weaning. Their study did not continue past d28, so long-term effects are unknown. Miller *et al.* (2012) had no intermediate weights between weaning and 10 weeks old, and reported no significant effects of SM provision on post-weaning performance. Wolter *et al.* (2002) found no effect of SM on ADG or ADFI to 25 kg, but gain:feed of supplemented pigs was lower from weaning to 14 kg than unsupplemented pigs. They reported that ADFI was higher in supplemented pigs than unsupplemented pigs from 25-65 kg, with a tendency for increased ADG.

None of the previous studies had a method for quantifying SM consumption, but were based on SM being available to the litters or not. The methods used in this study therefore represent a refinement to the investigation of the effect of SM on performance. It was suggested by van Oostrum *et al*. (2016) that the longer villus length in piglets fed milk replacer observed in a study by Zijlstra *et al.* (1996) could account for the improved performance recorded by supplemented pigs in their study, but an investigation of gut histomorphology was beyond the scope of the current study.

The use of in-feed markers to colour faeces has allowed a distinction between eaters and non-eaters of creep to be made. Although all piglets in the current study had consumed some creep and so can't be called non-eaters, it is likely that some of the non-eaters in the literature also consumed creep, with faecal colour being diluted by relatively high milk intake (Huting *et al*., 2017; Byrgesen *et al.*, 2021). This is where individual recording utilising EID represents a more accurate assessment of creep intake.

Piglets with high creep consumption were lighter at weaning in agreement with Huting *et al.* (2017), as discussed in previously. However, this is not a universal finding as there is often no difference in weaning weight (Bruininx *et al*., 2002a; Sulabo *et al.*, 2010a; Byrgesen *et* al., 2021).

The higher ADFI from wean-d54 observed from piglets classified as CH compared to CL agrees with the findings of Sulabo *et al.* (2010a) and Muns and Magowan (2018) with eaters and non-eaters, and led to a tendency towards higher ADG for the period. They concluded that creep-feeding improved feed intake due to familiarity with solid feed, and this is supported by Bruininx *et al.* (2002a), who reported that latency to commence feeding post-weaning was lower in eaters than non-eaters, with eaters having a higher proportion of feeder visits that resulted in consumption during the first eight days post-weaning. Bruininx *et al.* (2002a) reported higher ADFI from d0-8 post-weaning for eaters (202 g/d compared to 160 g/d), and a trend for higher gain:feed resulting in higher ADG (125 g/d eaters and 72 g/d non-eaters. They also found that ADG to d34 post-weaning was higher in eaters than non-eaters, although ADFI and gain:feed were both similar. It was therefore probably a combination of numerically improved feed intake and gain:feed that resulted in the higher ADG. The performance of non-eaters was similar to that of piglets not offered creep feed.

Huting *et al.* (2017) observed higher ADG to 61 d old in piglets classified as high creep consumers, but didn't report any feed consumption. Metzler-Zebeli *et al* (2023) reported lower piglet weight seven days post-weaning in creep-fed piglets compared to non-creep-fed piglets. Their creep-fed piglets did not increase in weight between weaning and seven days post-weaning, whereas from 3-7 days post-weaning, the unsupplemented piglets increased in weight. Feed intake was not reported, and so it is unknown whether the lack of growth was caused by poor intake or FCR. The creep feed used by Metzler-Zebeli *et al.* (2023) was SM from d10-26, with starter feed (20.5 % CP) mixed in from d24 and 25, then 100 % starter feed from d26-28 (weaning), and so creep-fed piglets would not have been as familiar with solid feeding as in the current study, and previous ones discussed. No individual intakes were recorded by Metzler-Zebeli *et al.* (2023), although differences in metabolomic profile were observed between creep and non-creep fed piglets, so the piglets must have consumed at least a small amount of creep pre-weaning. They offered no reason for the poor post-weaning performance of the creep-fed piglets.

In the research of Sulabo *et al.* (2010a), ADFI was higher in high-creep piglets in the period immediately post-weaning, which is not the case in the current study. Instead, FCR was lower for CH piglets compared to CL, and suggests a physiological rather than behavioural adaptation in the acute phase of weaning. This is a less common observation than the increase in post-weaning ADFI associated with higher pre-weaning creep consumption, but was also observed by Bruininx *et al.* (2002a). Kuller *et al*. (2004) reported that eaters of creep (defined by green-stained faeces on at least two occasions pre-weaning) had higher net absorption of a solution containing 9 g/L NaCl, 1 g/L glucose and 1 g/L amino acids in perfusion tests on day 4 post-weaning. They did not report any performance results, or offer reasons for the improved absorption (such as enzyme activity or intestinal structure), but it is apparent there was a physiological effect post-weaning of being an “eater” of creep, aside from any difference in appetite. De Passillé *et al.* (1989) found that piglets with longer duration of feeding at the creep feeder had heavier pancreas and stomach, and higher activities of amylase and chymotrypsin, but they concluded that these were related to the piglet's higher growth rate, as the actual amount of creep consumed was very low. Physiological differences between liquid creep-fed (DM 36.1 %) piglets and dry creep fed piglets were observed by Byrgesen *et al.* (2021), with higher sucrase and maltase levels in the dry creep-fed piglets despite them having lower consumption overall. They theorised that form of substrate may also have an effect on gut maturation, but this was beyond the scope of the current study. Enzyme levels between eaters and non-eaters were similar and

Bygesen *et al.* (2021) concluded that this was because of the small amount of creep consumed, or that the definition between eaters and non-eaters was uncertain. The latter explanation seems more likely, due to the fact that a difference in enzyme activity was identified when comparing the creep presentation methods.

5.4.2.1.2. Finisher

The positive effect of weaning weight on ADG to weaner exit and slaughter revealed by the multiple regression equations has been well-documented previously (for example Wolter and Ellis, 2001; Collins *et al.* 2017). In the current study when piglets were assigned birthweight groups, the effect of birthweight group on ADG to slaughter was limited to group 1 being lower than the other three, and was due to performance prior to the finishing phase, as ADFI, ADG and FCR in the finishing phase did not differ.

This is the first time a quantification of SM and creep usage has been included in a post-weaning growth model. Although the treatment classifications were too broad to reveal an SM or creep level of consumption (low *vs* high) effect, multiple regression using individual consumption and weight increased sensitivity. The additional (to bodyweight) positive effect of creep duration persisted longer than that of SM, demonstrating that adaptation to a solid diet had a more beneficial effect in terms of lifetime performance of the pig. This may reflect the higher DM content of the creep compared SM as a supplemental feed (each second of duration consumes more DM) as well as physiological changes. Appetite (ADFI d65-slaughter) in pigs with high creep intake pre-weaning was less affected by weight at d65 pw, indicating that CH pigs reached their peak appetite at a lower weight than CL pigs. This is potentially a continuation of their higher feed intake from weaning to d54 post-weaning. The regression models had relatively low r^2 indicating other influences, such as health, environment, and social interactions/hierarchy also had an effect on post-weaning performance.

5.4.2.2. Metabolomic profile

The number of metabolites able to be identified (67) was lower than in other studies, with Metzler-Zebeli *et al.* (2023) identifying 345 metabolites using HPLC-MS, although they had three different analyses. Luise *et al.* (2020) identified 167 using HPLC-MS, and Southey *et al.* (2021) identified 170 using GCMS. However, these authors did not clarify whether they referred to the number of peaks obtained, or definitive identification. In the current study, over 200 peaks were obtained, but the majority were deemed to be too small to be identified, or were not genuine peaks. It is possible that metabolites were over-screened, as concentration of metabolites in the blood have a wide range from mM (10^{-3}M) to pM (10^{-12}M), with amino acids, urea and cholesterol having highest abundance (Kiseleva *et al.*, 2022).

These highly-abundant metabolites were identified, and so spectral peaks associated with the metabolites found in lower concentrations may have appeared insignificant, and may have been discarded, but this was thought to be less problematic than risking false positives. Metabolic pathways were deemed to be enriched if the FDR was ≤0.10. Although raw *P*-values were also quoted as an indication of significance by Xiong *et al.* (2022), FDR is the usual method to avoid overstating significance, although it may be too conservative and increase the risk of type II errors (Jorge-Smeding *et al.*, 2024).

5.4.2.2.1. Effect of weaning

Weaning had the largest effect on the piglet plasma metabolome, as evidenced by the separation of the PCA scores plot which did not occur when analysing the effects of SM and creep. A separation of PCA scores plot between weaning and 28 days post-weaning was also reported by Wellington *et al.* (2023). This is unsurprising as it is the single largest change that will happen to the pig in its lifetime. It is well-documented that social and nutritional stress at weaning has adverse effects on feed intake (Spreeuwenberg *et* al., 2001; Bruininx *et al.*, 2002a; Muns and Magowan, 2018), gut structure and function (Spreeuwenberg *et* al., 2001; Montagne *et al.*, 2007; Muns and Magowan, 2018), with resultant lower ADG and increased risk of dysbiosis and diarrhoea (Jiang *et al.*, 2020; Meng *et al.*, 2020). This study did not investigate the acute effects of weaning on the metabolome, as may be observed within the first seven days post-weaning, but primarily aimed to determine differences in piglet metabolome at weaning according to relative consumption of SM and/or creep, and whether differences were evident 29 days post-weaning to characterise any lasting effect of pre-weaning nutrition. Therefore, the period of time between sampling points was longer than in experiments which have had the primary aim of assessing the effect of weaning on the piglet's metabolome. Dietary effects on the metabolome are probably more severe in the current study as the grower diet fed was likely to be very different to starter feed supplied for the first week post-weaning in other studies. These would aim to introduce the piglet gradually to a cereal-based diet, and included milk powder, cooked wheat, fishmeal (Byrgesen *et al.*, 2021; not disclosed in Metzler-Zebeli *et al.*, 2023). However, any weaning-stress-related changes should be less apparent, and this study is (to the best of my knowledge) the first time a characterisation of the piglet plasma metabolome at these time points, considering supplementary nutrition pre-weaning, has been performed. Sampling piglets during the acute phase of weaning also risks the confounding effect of low appetite on the metabolic profile, as some amino acids (lysine, threonine, glutamine for example) will be utilised directly by the intestinal tissue without being absorbed when supply is low (Schaart *et al.*, 2005). There may also be catabolism of body tissue for energy during the acute weaning phase. Consequently, plasma levels would be affected by appetite which may be independent of dietary treatment, and not represent any physiological adaptation to weaning.

Metabolic pathways are complex and interlink. In the current study, few metabolites from each significantly enriched pathway were identified, which makes assessing whether pathways were up/down-regulated difficult. It is possible that metabolites were “over-screened” when assessing whether a peak on the spectrograph was a true peak or background “noise”. As an untargeted approach was used, there may be refinements of the methodology that would enable greater identification, targeting for example amino acids and associated metabolites, as the majority of the enriched pathways identified were amino acid-based. These enriched pathways were linked with amino acids in common. A holistic consideration is therefore necessary, rather than treating pathways in isolation. This linkage is exemplified by glutamate, which is a product and substrate for many pathways, and is often oxidised for energy (Liao, 2021), and reinforces the importance of not relying on a single metabolite to establish significance of pathway enrichment. Excess plasma amino acids will be metabolised in the liver into other compounds and may be used for energy generation; plasma circulating levels represent biological necessity (Jorge-Smeding *et al.*, 2024) with the supply combined from diet and biosynthesis. Figure 51 displays the network of the enriched pathways involving amino acids and cholesterol, as this metabolite linked the amino acid and fat-based pathways. The majority of amino acids were found in higher concentrations in the plasma of pigs post-weaning rather than pre-weaning, potentially indicating higher protein turnover and growth; in the seven days pre-weaning piglets gained 327 g/d whereas in the 14 days prior to post-weaning sampling piglets gained 662 g/d. The creep diet fed pre-weaning and the grower diet fed at the point of post-weaning sampling supplied amino acids to the recommended standard, or above (Whittemore *et al.*, 2003).

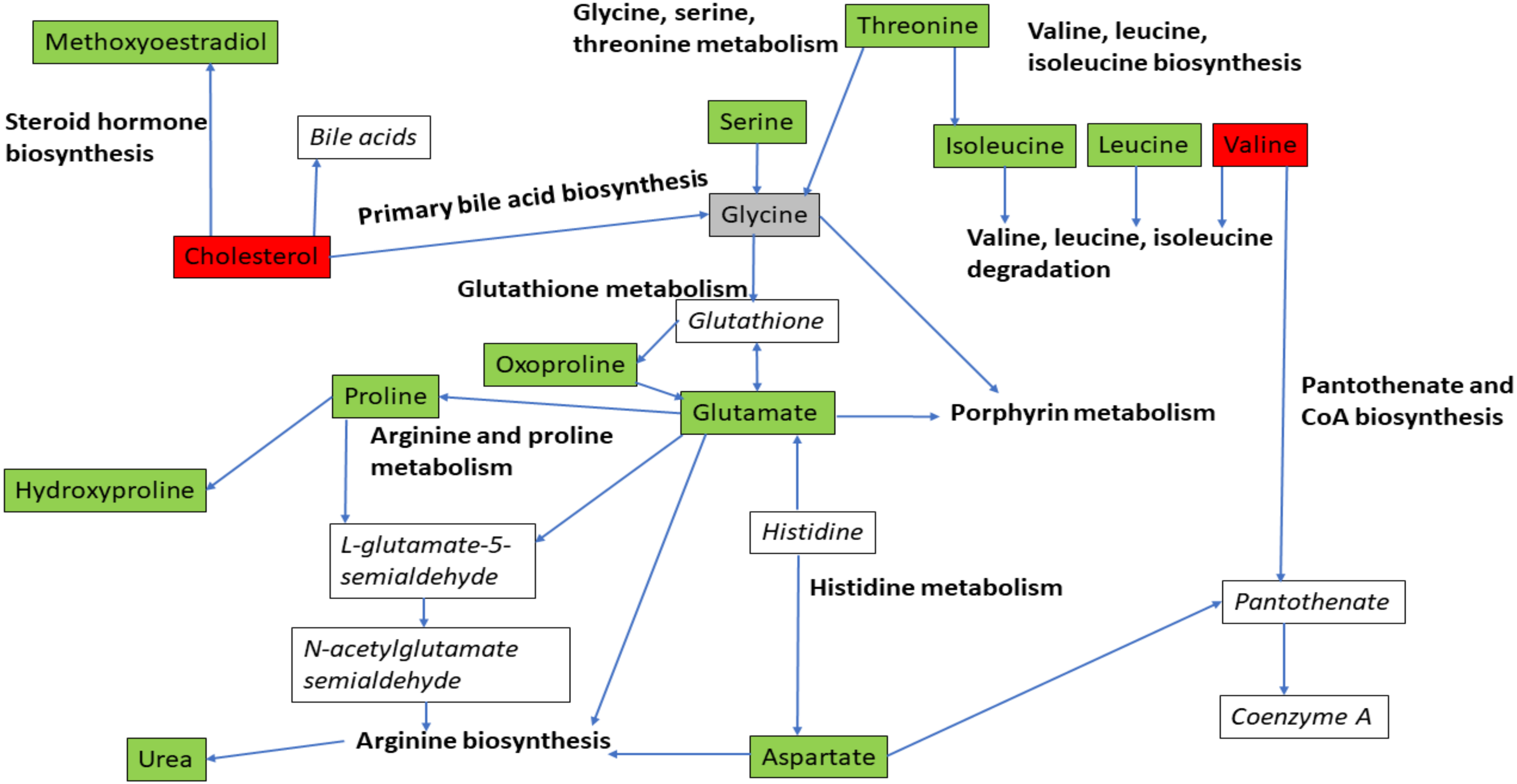

Figure 51: Network of amino acid- and fat-based significantly enriched pathways in piglet plasma post-weaning. Based on KEGG pathways (https://www.genome.jp/kegg/). Green highlight=metabolite increased post-weaning; red highlight=metabolite decreased post-weaning; grey highlight=no difference between pre- and post-weaning. Italics=metabolite not identified in the current study. Bold=significantly enriched pathway post-weaning.

The comparison of amino acids in plasma pre-weaning compared to sow's milk indicated a large oversupply of valine, as it was almost three times higher as a proportion of amino acids detected, than the proportion predicted in sow milk (from Yao *et al.*, 2023), or formulated in creep. The difference between valine in plasma and the grower diet post-weaning was smaller. Valine and aspartate were detected as significant metabolites in the enriched pantothenate and CoA biosynthesis pathway when comparing pre- and post-weaning profile. Pantothenate is a B vitamin (B_5) and is metabolised to CoA, which is involved in energy production through the TCA cycle, fatty acid metabolism and phospholipid biosynthesis (Leonardi and Jackowski 2007). Therefore, the excess valine pre-weaning will probably have been utilised for energy production. Lysine was lower in plasma than in the predicted sow's milk profile pre-weaning, although it was not implicated in any pathway analysis. It was also poorly detected post-weaning. There is no analytical reason for this, as pre- and post-weaning samples were treated identically, and analysis through GC-MS was a continuous run. It is possible that lysine was utilised fully so that plasma levels were undetectable post-weaning, and almost fully utilised pre-weaning. This would reflect that lysine is the first limiting amino acid for pigs, and diets are formulated on the basis of lysine (Whittemore *et al.*, 2003). The majority of "free" lysine is stored in the intracellular space of muscle tissue (Liao *et al.*, 2015), which also may explain the low plasma levels observed. However, in a review by Liao *et al.* (2015), they noted that plasma concentrations of lysine were diet-dependant in several studies, and so the reason for the lack of detection post-weaning remains unknown. Leucine was also lower in plasma than the diet pre- and post-weaning, and together with isoleucine and valine was responsible for the enrichment of the valine, leucine, isoleucine biosynthesis and degradation pathways. These are branched chain amino acids (Wessels *et al.*, 2016) and enrichment of the valine, leucine and isoleucine synthesis and degradation pathways may reflect the higher growth rate of the post-weaning pigs, as these amino acids stimulate protein synthesis in muscle (Escobar *et al.*, 2010; Banerjee *et al.*, 2020; Wellington *et al*., 2023). Leucine, along with arginine activates the mechanistic target of rapamycin (mTOR) pathway (Wessels *et al.*, 2016), thereby increasing protein synthesis by regulating mRNA translation, suppressing catabolism, and promoting glycolysis (Saxton and Sabatini, 2017).

Serine and threonine proportions were also lower in plasma than predicted in milk or formulated in creep, and this may reflect the high degree of "first pass" usage by the intestine. Up to 71 % of threonine utilisation has been reported in the small intestine (Schaart *et al.,* 2005). Threonine is a substrate for mucin (Luise *et al.*, 2020), comprising 30 % of the amino acid content, and affects amylase secretion, with 20 % of this enzyme being threonine (Tang *et al.*, 2021). Koo *et al.* (2020b) reported that addition of threonine to nursery pig diets resulted in increased villus height and number of enterocytes in the jejunum, and increased expression of the tight junction protein occludin. In addition to the barrier function in the small intestine, mucin proceeds intact to the large intestine where it can be metabolised by saccholytic bacteria and so threonine may have benefits to the microbiome (Tang *et al.*, 2021). The positive correlation of plasma threonine level and pre-weaning ADG demonstrates the importance of threonine supply for growth, as it is an essential amino acid (Mahan and Shields, 1998) and has a role in skeletal muscle synthesis by stimulating IGF-1 secretion, and when metabolised into glycine (glycine, serine threonine metabolism pathway) inhibits proteolysis (Tang *et al.*, 2021), and supports glutathione production (Meister, 1988).Threonine also has a role in the immune system, by enabling proliferation of lymphocytes, secretion of immunoglobulins and regulating cytokine responses (Koo *et al.,* 2020a; Tang *et al.*, 2021; Liao, 2021). A higher proportion of threonine in plasma compared to the diet was observed post-weaning and this can be converted into glycine in the glycine, serine, threonine metabolism pathway, which was enriched post-weaning. Glycine levels were not affected by weaning, but glycine was also identified as a substrate in glutathione metabolism and porphyrin metabolism pathways, and so may have been utilised further.

Glutathione is synthesised from glycine, cysteine, and glutamate intracellularly, and as such is a store of cysteine (Meister, 1988). The majority of ingested glutamate is used by the small intestine, making it a key metabolite for gut health (Liao *et al.*, 2021), and therefore plasma glutamate levels are the result of metabolism in the liver (Wu *et al.* 2004). *In vitro* investigations by Liu *et al.* (2018) revealed that addition of glutamate (as L-glutamine) to cell culture reduced oxidative apoptosis of enterocytes by increasing production of intracellular glutathione. Glutathione functions mainly as an antioxidant to regulate redox status intracellularly, by absorbing reactive oxygen species from metabolism in the process of oxidation to glutathione disulphide (Meister, 1988; Wang *et al.* 2009). Glutathione is also involved in protein synthesis and proteolysis, cell proliferation (particularly intestinal epithelium and lymphocytes), DNA synthesis, and cytokine production (Wu *et al.* 2004). Weaning stress increases oxidation of glutathione to glutathione disulphide (Wang *et al.* 2009). Enrichment of the glutathione metabolism pathway therefore could indicate many potential changes in the metabolic status of the pig. It is unlikely to indicate post-weaning stress due to the extended period between weaning and sampling, as cortisol levels of weaned piglets return to normal by six days post-weaning (Kick *et al.*, 2012).

Glutamate was reported to be an important metabolite post-weaning by Metzler-Zebeli *et al.* (2023); in their study glutamate was associated with pathways involved in growth, and in the current study (as well as glutathione metabolism), glutamate was involved in the arginine and proline metabolism, and arginine biosynthesis pathways. Both of these pathways are associated with growth, with arginine also being metabolised into proline, which comprises 10 % of collagen (Liaubet *et al*., 2023). Arginine has been shown to activate pathways stimulating secretion of IGF-1 by activating the mTOR pathway and thus increasing growth in skeletal muscle (Yao *et al.*, 2008, 2011). In the small intestine, arginine supplementation increased expression of vascular endothelial growth factor, leading to heavier relative weight of intestine and higher villus length and shallower crypts in the duodenum, jejunum and ileum (Yao *et al.*, 2011). Arginine was not detected in the current study, but arginine biosynthesis pathway was enriched by urea, aspartate and glutamate which were all found in higher concentrations post-weaning. Arginine is also a stimulator of insulin secretion (Yao *et al.*, 2011) and so enrichment of this pathway links with carbohydrate metabolism and may explain the lower levels of plasma glucose observed in the current study.

Histidine metabolism was enriched due to the presence of higher levels of aspartate and glutamate. They are both products of histidine degradation so the assumption can be made that dietary histidine supply was in excess of that required for growth and production of carnosine, haemoglobin, histamine, trypsin, and chymotrypsin (Cheng *et al.*, 2023), although histidine was not detected in the current study.

The connection between the enriched carbohydrate metabolic pathways is shown in Figure 52, with glucose linking them all, and all contributing to the pentose and glucoronate interconversion pathway. Although only xylose and ribulose were identified as significant metabolites for this pathway, the other enriched pathways were linked by UDP glucose (galactose metabolism, starch and sucrose metabolism) or α D glucose 1P (glycolysis/gluconeogenesis). The lower plasma concentration of galactose resulting in down-regulation of galactose metabolism post-weaning was expected as galactose is predominantly found in milk as part of lactose, and so post-weaning carbohydrate metabolism changes to solid feed (Southey *et al.*, 2021), as indicated by the enrichment of the starch and sucrose metabolism pathway. Metzler-Zebeli *et al.* (2023) reported that a higher rate of glycolysis due to higher starch produced more acetyl-CoA to be transported by acylcarnitines, but neither metabolite was detected here. The lower levels of lactate post-weaning (enriching the glycolysis/gluconeogenesis pathway) may be an indication of the energy metabolism shifting from fat-based to carbohydrate-based, as Liaubet *et al*. (2023) reported that higher lactate (and pyruvate, not detected) indicated a higher rate of gluconeogenesis from non-carbohydrate sources.

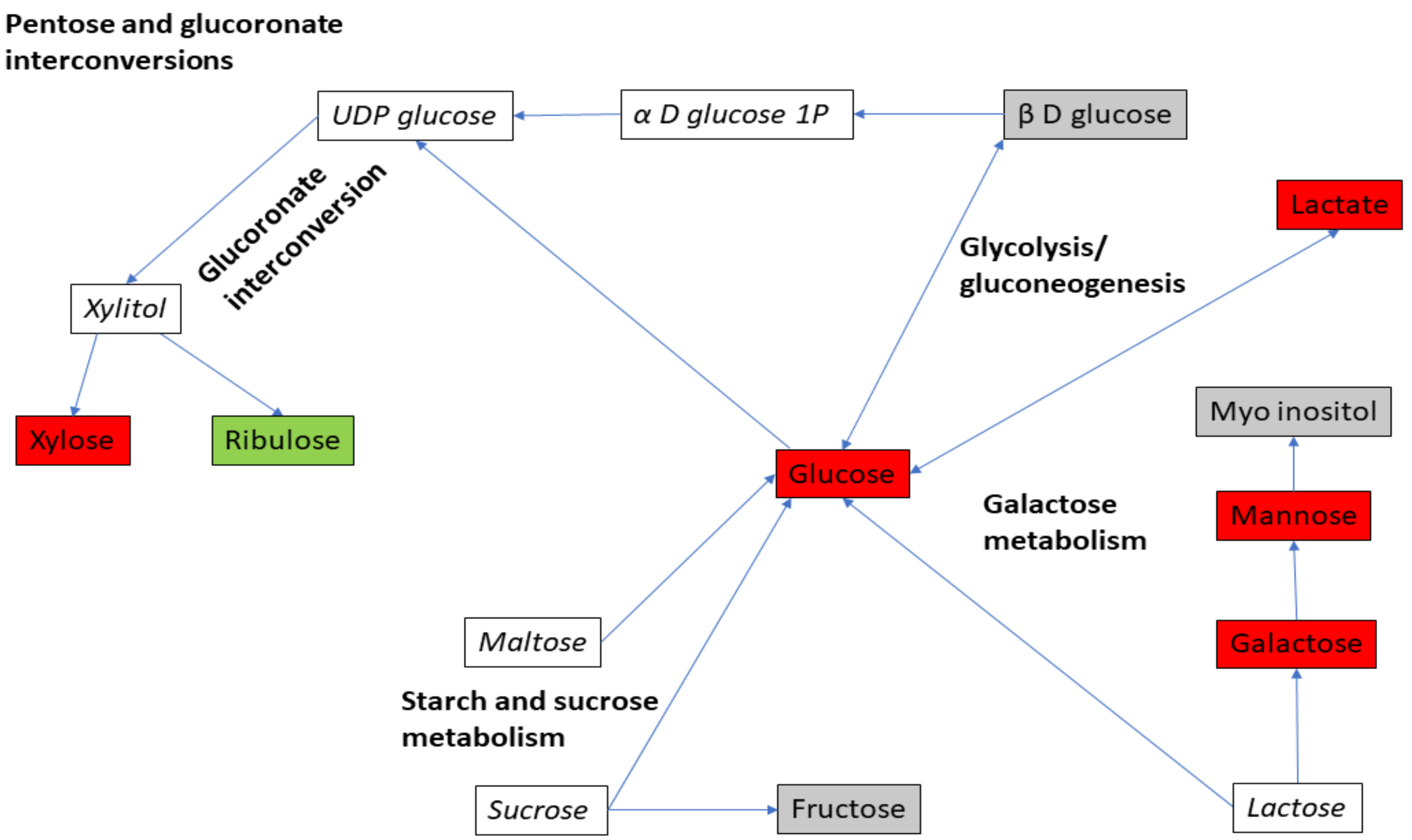

Figure 52: Network of carbohydrate-based significantly enriched pathways in piglet plasma post-weaning. Based on KEGG pathways (https://www.genome.jp/kegg/). Green highlight=metabolite increased post-weaning; red highlight=metabolite decreased post-weaning; grey highlight=no difference between pre- and post-weaning. Italics=metabolite not identified in the current study. Bold=significantly enriched pathway post-weaning.

The changing energy source from pre- to post-weaning resulted in the down-regulation of four fatty acids post-weaning from the five identified in the plasma, reflecting the higher fat content of sow's milk compared to the post-weaning diet (Metzler-Zebeli *et al.*, 2023). This resulted in the downregulation of the biosynthesis of unsaturated fatty acids pathway. Metzler-Zebeli *et al.* (2023) also reported reductions in plasma triglycerides (TG) post-weaning, but no TG were identified in the current study. To detect TG using GCMS, a different preparation method would have been required, and the temperature of the GCMS would have needed to be increased further to 330 °C (Garcés *et al.* 2023).

Primary bile acid biosynthesis was also enriched post-weaning in the current study. Metzler-Zebeli *et al.* (2023) found that two bile acids (taurohyodeoxycholic acid and taurochenodeoxycholic acid) were lower in creep-fed piglets compared to solely suckled piglets, and they concluded that this was because bile acids are required for digesting fat so are required in lower concentrations in a lower-fat diet. In the current study, bile acids were not identified, and the pathway enrichment was due to higher levels of cholesterol in pre-weaning pigs, due to the higher fat content of the diet. The only fatty acid to be upregulated post-weaning was ketoisocaproic acid (KIC), a metabolite derived from leucine (Moghei *et al.*, 2016), but KIC was not highlighted as having a significant role in either biosynthesis or degradation of valine, leucine and isoleucine pathways.

5.4.2.2.2. Effect of SM*creep

The lack of interaction in relative metabolite concentrations both pre- and post-weaning, and the heatmap grouping by creep intake demonstrated that the effect of creep-feeding on the metabolome was stronger than the effect of SM, regardless of consumption level. This is probably due to SM being relatively more similar in composition to sows' milk than creep is, and so induced less of a change in the metabolome.

5.4.2.2.3. Effect of SM

The absence of a relationship between SM consumption classification and metabolomic profile at weaning, with no consistency of the heatmap, and no significantly affected metabolites or pathways, at first seems contradictory to the work of Rosa *et al.* (2020) who reported differences in metabolome between human-milk and bovine-milk reared piglets, and Amdi *et al.* (2022) who observed differences in metabolic profile between AR and sow-reared piglets. The metabolism of the human-milk fed piglets in the study of Rosa *et al.* (2020) shifted from fat-based to carbohydrate-based as an energy source once the piglets had been weaned onto solid feed, whereas the bovine-milk fed piglets already had a carbohydrate-based energy metabolism at weaning. A similar effect could have been predicted in the current study, as sow milk is higher in fat than bovine milk, which is the basis for commercial milk replacer powders. The AR piglets in the study of Amdi *et al.* (2022) had a lower growth rate than the sow-reared piglets, which they concluded was indicated by the difference in metabolic profile, with higher creatine and alanine amino transferase as direct markers of metabolic activity. The lack of effect of SM consumption level on metabolome in the current study may be due to both ML and MH piglets having access to the sow (rather than being artificially reared on sow milk/bovine milk), and suckling sow milk by preference (Kobek-Kjeldager *et al.*, 2020a). In the final week prior to weaning when SM consumption was highest, average SM DMI was estimated at 37 g/piglet per day, and piglets were growing at 302 g/d, so the contribution of SM to growth for the majority of piglets was low. Even MH piglets are likely to have obtained most of their nutrition from the sow, with only three of these 16 piglets not observed suckling. This similarity in post-weaning metabolome between low and high consumers of SM supports the performance findings, that SM had little effect on growth.

5.4.2.2.4. Effect of creep

Although the pre-weaning creep consumption heatmap was inconclusive, the SM*creep heatmap demonstrated grouping of the metabolomic profile on the basis of creep consumption, rather than SM consumption. The apparent difference in metabolomic profile at weaning between creep consumption classifications would appear to support a physiological adaptation to weaning due to higher creep intake. Although when considered singularly there was no difference in plasma metabolite concentrations, the glycine, serine, threonine metabolism pathway was enriched by higher creep intake, with threonine level being most enhanced in CH pigs compared to CL pigs. As already discussed, threonine is an important component of mucin and may increase villus height, improving absorptive capacity of the small intestine. As dietary threonine use is prioritised by the small intestine (Tang *et al.*, 2021), the higher plasma threonine levels found in CH piglets may indicate that intestinal structure was optimised, with additional dietary threonine then available for protein synthesis and so it is possible that this is the mechanism for the improved FCR and ADG in CH pigs observed immediately post-weaning. Threonine also regulates the mTOR signalling pathway, reducing proteolysis, as a precursor to glycine (Tang *et al.*, 2021),

The lack of a residual effect of pre-weaning supplementation on metabolome at d28 post-weaning (with no enriched pathways) is similar to that reported by Rosa *et al.* (2020) They found that the difference in metabolism of human-milk fed piglets compared to bovine-milk fed piglets was less evident at d30 post-weaning than at weaning.

The lack of effect of creep consumption on carbohydrate and lipid metabolism is surprising, but it is possible that the amounts consumed were too low to have made a detectable difference. These findings appear to contradict the work of Metzler-Zebeli *et al.* (2023), who found that fat and carbohydrate metabolism varied between sow-fed and creep-fed piglets. This was indicated by creep-fed piglets having higher levels of acylcarnitines in plasma, required for transport of acetyl CoA from a higher rate of glycolysis, and enrichment of the pyruvate and glycolysis/gluconeogenesis pathways. Sugiharto *et al.* (2014) reported a reduction in acylcarnitine post-weaning, theorising that this was due to low energy intake. Considering that all piglets consumed some creep in the current study, with 97 % in the final week before weaning, it is likely that carbohydrate metabolism had begun to develop in all piglets pre-weaning, and this is the source of the contradiction. However, Byrgesen *et al.* (2021) reported differing cholesterol levels between wet- and dry-creep fed piglets, with cholesterol tending to be higher in piglets fed dry creep, but offered no explanation. It is possible that the higher cholesterol levels were due to higher sow milk consumption, as creep intake was lower and weaning weight was similar in the dry-fed compared to wet-fed piglets, and cholesterol is associated with lipid-based metabolism. Cholesterol was detected in the current study, but with no difference between CH and CL piglets. Although overall creep consumption was higher than that of Byrgesen *et al.* (2021), it is possible that there was insufficient separation between creep consumption of CL and CH piglets to observe an effect of creep consumption level on plasma carbohydrate- and lipid-based metabolome.

5.5. Conclusion

Although SM is a useful and important tool to reduce pre-weaning mortality in hyperprolific litters and eliminates the social stress associated with artificial rearing, increasing creep consumption pre-weaning had a greater effect on post-weaning performance and appears to have improved physiological adaptation to weaning, demonstrated by a lower FCR for CH pigs compared to CL pigs in the first seven days post-weaning. Limited effects of creep consumption on the plasma metabolome were observed, but the glycine, serine, threonine metabolism pathway was enriched in CH pigs compared to CL pigs at weaning which may have optimised intestinal structure and activated protein synthesis pathways post-weaning.

5.6. Plan for study 3

The focus of study 3 was to examine the effect of creep consumption level and post-weaning diet on performance and duodenal morphology. In order to refine the use of SM and creep a pilot trial was undertaken to determine the effect of early withdrawal of SM provision on creep intake.

5.7. Pilot study: Early withdrawal of SM

5.7.1. Introduction

Studies 1 and 2 demonstrated that SM was effective in rearing supernumerary piglets compared to sows' functional teats. Study 2 showed that creep intake increased more rapidly from d19-weaning than from d12-19. Study 2 also determined that piglets with high creep intake had improved post-weaning performance, and that the beneficial effect of higher creep intake lasted longer than that of SM. Restricting piglet nutrition from the sow has been shown to increase creep intake but has negative effects on piglet performance (Kuller *et al.*, 2004; Middelkoop *et al.*, 2019). If DM provided by SM can be substituted for creep, based on findings in Study 2, creep intake will almost double. The aim of this pilot trial was to determine whether withdrawing SM seven days prior to weaning would further stimulate the increase in creep intake, and to assess effects on performance and mortality.

5.7.2. Materials and methods

This study employed no regulated procedures under the ASPA 1986 legislation, but was approved by the Harper Adams University ethics committee, project number 0479-202208-PGMPHD.

Twenty-eight litters from a single farrowing batch of 32 sows were selected eight days prior to weaning. These comprised all the litters of the F1 dam x terminal sire (TN70 x Tempo) in the batch, with the remaining four litters being from Grandparent sows and so ineligible for selection. For details of standard management procedures see Chapter 3. The litters had not previously been participating in a study, and had received SM from birth and creep for six days at the point of selection. All piglets were individually weighed, and litters were allocated to Control (SM withdrawn two days prior to weaning) or Early (SM withdrawn seven days prior to weaning) treatments, balanced for number of piglets and average piglet weight. The following day, SM provision was removed from litters receiving Early treatment, creep hoppers were emptied from all litters and refilled with a weighed amount of creep. Creep was provided to all litters *ad libitum* until weaning, and SM was provided to Control litters until two days prior to weaning. Piglets were weighed at weaning, and creep remaining in the hopper was weighed back out. Supplementary milk consumption was recorded on a batch basis.

5.7.3. Results

Litter weight, number of piglets/litter and average piglet weight were similar at the start by design (*P* >0.05; Table 47). At weaning, there was no difference between Early and Control litters in with regards to litter weaning weight, number of piglets weaned/litter, average weaning weight or litter ADG (*P* >0.05). Early litters had higher creep intake/litter and per pig weaned ($P < 0.05$). Total SM+creep DMI was higher for Control than Early, but this was unable to be analysed statistically as SM consumption was recorded on a batch basis. There was no difference in sow feed intake over lactation (301.4 kg Early compared to 302.0 kg Control).

Table 47: Effect of withdrawing supplementary milk seven days prior to weaning (Early) or maintaining provision until two days prior to weaning (Control) on litter performance and creep intake.

	Early	Control	s.e.m	*P*-value
n	*14*	*14*		
Litter weight start (wn-7; kg)	92.37	93.52	2.199	0.715
No. pigs start	14.50	14.64	0.402	0.803
Average weight start (kg)	6.40	6.42	0.157	0.913
Litter weight wean (kg)	117.09	119.87	2.665	0.467
No. pigs weaned	14.43	14.57	0.416	0.810
Average weight weaned (kg)	8.17	8.28	0.213	0.700
Final week litter weight gain (kg)	25.17	26.44	0.858	0.306
Final week litter ADG (kg)	3.60	3.78	0.122	0.306
Final week creep/litter (kg)	5.70	3.13	0.606	0.006
Final week creep/pig weaned (g)	390	219	38.6	0.004
Final week SM+creep DMI (kg/d)	0.717	0.950		

On average, SM consumption was 33.3 L/litter for the final five days. Using costs of £2500/T for milk powder and £1500/T for creep a saving of 47 p/piglet weaned was made by withdrawing SM provision early (Table 48).

Table 48: Economic effect of withdrawing SM seven days prior to weaning (Early) or maintaining provision until two days prior to weaning (Control)

	Early	Control
SM consumed/litter (kg)		33.3
Milk powder/litter (0.15 kg/L)		4.33
Cost of milk powder at £2500/T		10.82
Creep consumed/litter (kg)	5.70	3.13
Cost of creep at £1500/T	8.55	4.70
Total cost supplementary feeding (£/kg)	8.55	15.52
Cost/pig weaned (£)	0.59	1.07
Saving/pig weaned (£)	0.47	

5.7.4. Conclusion

In agreement with Kuller *et al.* (2004) and Middelkoop *et al*. (2019), restricting nutrition resulted in higher creep consumption. Contradictory to those studies, there was no loss in growth performance by withdrawing SM to encourage creep intake, even though supplementary DMI was lower, probably because the majority of piglets still obtained most of their nutrition from the sow. Early SM withdrawal was an effective method of reducing the cost of supplementary feeding, and did not increase mortality. In Study 2, higher creep intake pre-weaning improved performance post-weaning; therefore, this regime will be utilised in Study 3.

6. Study 3-Effect of creep consumption and post-weaning feeding regime on performance and duodenal histomorphology

6.1. Introduction

In study 2, utilisation of EID and an antenna system enabled a quantification of the pre-weaning creep intake of individual piglets, with continuous recording during the suckling period. The enhanced precision of measurement, and use of metabolomic analysis of blood plasma, identified that high creep consumers may have improved adaptation to weaning, with lower FCR, higher ADG, and indicated potential enrichment of the glycine, serine, threonine metabolism pathway. Previously, improvements in piglet performance post-weaning due to higher creep intake have been attributed to higher feed intake, due to familiarity with the diet (Sulabo *et al.,* 2010a; Muns and Magowan, 2018), rather than improved physiological adaptation to consuming a cereal-based diet. Investigating the effect of level of pre-weaning creep consumption on duodenal histomorphology may reveal whether maintenance of intestinal structure is a causative factor in the improved post-weaning performance of piglets with high pre-weaning creep consumption.

Homeostasis of intestinal structure is maintained by intestinal stem cells (ISCs) within the crypts, which produce transit amplifying (TA) cells that undergo mitosis up to six times. These TA cells either migrate to the crypt-villus junction and differentiate into enterocytes, goblet cells, and enteroendochrine cells, which comprise the villus epithelium, or differentiate into secretory Paneth cells located in the base of the crypt (Rao and Wang, 2010). Proliferation is stimulated by IGF-1, epidermal growth factor (EGF), and keratinocyte growth factor (Baldassano and Amato, 2014). It is well-documented that at weaning, the change in diet and reduced feed intake leads to villus atrophy (and hence reduced absorptive surface area) and reduced villus height:crypt depth (vh:cd) ratio (Pluske *et al.*, 1995; van Beers-Schreurs *et al.*, 1998; Muns and Magowan 2018). The mechanism for increased villus atrophy post-weaning is thought to be a lack of nutrients directly available to the enterocytes thereby increasing cell death (Pluske *et al.* 1997), and a negative effect of low feed intake on secretion of glucagon-like peptide-2 (GLP-2). Secretion of GLP-2 from L cells in the ileum is initiated by neural, hormonal, and nutritional stimuli, with nutrient intake the most important factor (Burrin *et al.*, 2003). Glucagon-like peptide-2 binds with receptors on enteric neurones and myofibroblasts, causing the secretion of IGF-1, EGF, and keratinocyte growth factor (Baldassano and Amato, 2014) and also increases cell hypertrophy (Litvak *et al*., 1998). In 2013, Ipharraguerre reported that primary bile acids may be responsible for stimulating the secretion of GLP-2, as piglets fed additional chenodeoxycholic acid (CDC) had higher GLP-2 plasma levels than those fed deionised water. Although there was no subsequent effect on jejunal or ileal morphology, the authors concluded this was due to the method of delivery (once per day), with evidence of this increasing inflammation, as lymphocytes and IL-6 were higher in the ileum in CDC-fed piglets. There is recent evidence that the primary bile acid biosynthesis pathway is down-regulated post-weaning due to reduced lipid content of the diet (Metzler-Zebeli *et al.*, 2023), and so this may be a contributory factor for GLP-2 mediated intestinal growth. Deng *et al.* (2016) demonstrated that daily injections of GLP-2 analogue protected against damage to villus integrity caused by *E. coli* lipopolysaccharide (LPS) challenge.

The effect of reduced feed intake at weaning on intestinal structure appears to be transient: Christensen and Huber (2022) found that jejunum villus height (vh) was lowest seven days post-weaning, but had recovered by 28 days post-weaning; Engelsmann *et al.* (2023b) observed no difference 28 days post weaning in vh, crypt depth (cd) or vh:cd between pigs with low post-weaning feed intake (35.7 g/d for first four days) and pigs with high post-weaning feed intake (181 g/d for first four days). The pigs with low initial feed intake remained at a lower feed intake level until the end of the study at 28 days post-weaning, with resultant lower ADG, and higher FCR which suggests a physiological difference. Engelsmann *et al*. (2023a) reported that the area of acid mucins in the crypts, villi and overall was higher in the pigs with high post-weaning feed intake, and that as these confer more protection against bacteria than neutral mucins there was a lasting benefit associated with higher feed intake.

Combined with reduced absorptive area, activity of brush border enzymes is reduced with increasing villus atrophy, compounding the negative effect of weaning on digestive function (Pluske *et al.*, 1997). Creep feeding has been proposed to ameliorate this effect, as piglets consuming creep should have commenced the transition from a milk-based to a cereal-based diet, and suffer less with post-weaning anorexia (Bruininx *et al.*, 2002a). So far, there is conflicting evidence, with Muns and Magowan (2018) reporting that although eaters of creep had higher ADFI than piglets that were not offered creep, at one week post-weaning the effects of creep intake on intestinal structure were inconsistent: there was no difference in duodenal gut structure, and in the jejunum, eaters had a lower vh:cd ratio, whereas in the ileum no creep piglets had a higher vh:cd. Bruninx *et al.* (2004) also found that creep intake of eaters compared to non-eaters failed to mitigate the structural damage observed at weaning. However, Kuller *et al.* (2007) reported an increase in absorption from the small intestine for eaters compared to non-eaters, increasing substrates available for growth, although they did not measure morphology. As previously mentioned in study 2, the use of dietary markers to colour the faeces is a somewhat imprecise method to classify eaters of creep, and it is hoped that the increased accuracy of classification afforded by the PigTrack ® system will enable effects of creep intake on duodenal morphology to be identified.

To further ease the transition between sows' milk and cereal-based solid diets, high quality post-weaning diets are commonly fed; they contain highly digestible ingredients such as fishmeal, whey (Levesque *et al.*, 2012; Skinner *et al.*, 2014), acidifiers, flavouring and cereals that have been processed to be more easily digestible (Skinner *et al.*, 2014). Engelsmann *et al.* (2022), found that standard ileal digestible (SID) CP digestibility coefficients for weaned pigs were highest for diets with casein (0.78) or wheat (0.74) as the protein source, and lowest for enzyme-treated soyabean meal (0.46). Processing includes heating under steam and pressure to increase starch gelatinisation (Berrocoso *et al.*, 2012), or grinding to a very fine powder to increase surface area for enzymes to work (micronisation, Kim *et al.*, 2002). Heating breaks down cell walls and protein structures, and gelatinisation cleaves some of the glycosidic bonds between glucose molecules, improving access for α-amylase. This increases the proportion of rapidly digestible starch absorbed by the proximal small intestine (Zurak *et al.*, 2023), but the degree of gelatinisation, and thus improvement in digestibility, depends on the processing technique (Doucet *et al.*, 2009). Heating the diet was shown by Lundblad *et al.* (2011) to increase the total tract digestibility of DM and gain:feed from weaning-d36 of pigs compared to feeding a raw mash diet, although starch digestibility was only higher in diets that had been extruded compared to expanded, with no difference between cooked *vs* raw.

Some high-quality diets may also contain spray dried plasma and other blood products (Mavromichalis *et al.*, 2001b; Levesque *et al.*, 2012; Skinner *et al.*, 2014) but this is not currently permitted under quality assurance schemes in the UK. Low quality diets are predominantly cereal-based: maize (for example Sulabo *et al.*, 2010b; Levesque *et al.*, 2012; Skinner *et al.*, 2014) or wheat (for example Collins *et al.*, 2017; Koo *et al.*, 2017) with soyabean meal as protein and lysine source, and a small amount of fishmeal (Mavromichalis *et al.*, 2001b; Levesque *et al.*, 2012; Skinner *et al.*, 2014). Diets containing high quantities of soya contain antinutritive factors such as non-starch polysaccharides (NSP), and antigenic factors glycinin and β-conglycinin (Koo *et al.* 2020a), which may irritate the immature intestine in the acute weaning phase, with Mahan *et al.* (2004), Skinner *et al.* (2014) and Koo *et al.* (2017) proposing this as a reason for the poor performance of pigs fed low quality diets. The structure of the protein also differs between animal- and plant-derived protein, with plant-derived protein having a higher proportion of β-formation, which is typically less digestible due to being more hydrophobic than α-formation, which predominates in animal-derived protein (Pieper *et al.*, 2016b). Alpha formation results from hydrogen bonds between amino acids within a protein molecule to twist the single molecule into a helix, whereas β-formation results from hydrogen bonds between protein molecules to form a sheet of multiple molecules. Therefore, proteins in α-formation have greater surface area for digestion.

High-quality diets (complex diets) are designed to ease the weaning transition to solid feed, compared to low quality diets (or simple diets), but are more expensive (Muns and Magowan, 2018, Wang *et al.*, 2018). Higher ADFI, gain:feed and ADG post-weaning using high quality diets compared to low quality diets has been reported widely (for example Mavromichalis *et al.*, 2001b; Skinner *et al.*, 2014; Koo *et al*., 2017). The most beneficial effect of high-quality diets on pig performance occurs in the initial period post-weaning, with Skinner *et al.* (2014) reporting ADG during the first week was 30 % higher in pigs fed high quality compared to low quality diets. The differences in ADFI, ADG and gain:feed observed after this point were of a lower magnitude, and ceased after six weeks post-weaning. Mavromichalis *et al.* (2001b) also observed higher ADFI, ADG, gain:feed in pigs fed high quality diets for the first 10 days post-weaning, but not for the remaining 20 days of their study. It is possible that the diets in the second period of the study by Mavromichalis *et al.* (2001b) were insufficiently different to produce a performance response, as there was a smaller difference in protein content, soyabean meal, whey content and no plasma was included in the high-quality diet. Mavromichalis *et al.* (2001b) calculated that DM and N digestibility at day 10 was higher in pigs fed high quality diets. The 3 % higher DM and 5 % higher N digestibility translated into a 6 % higher gain:feed, and 26 % higher ADG. Improvements in digestibility of DM, N and GE were reported by Kim *et al.* (2002) when particle size of maize was reduced from 1000-500 µm, with the higher surface area providing more contact with enzymes, and resulting in higher ADG and gain:feed, particularly in the first 10 days post-weaning. Digestibility of high-quality diets was similar to that of low-quality diets at d14 in the work of Koo *et al.* (2017), but higher at d28 for high-quality diets. Average daily feed intake was higher to d14 in high-quality diet fed piglets, and Koo *et al.* (2017) speculated that the lower feed intake for pigs fed low-quality diets may have been due to antinutritive factors (NSP) in soyabean meal, as lymphocytes were higher in these pigs on d14, indicating an inflammatory response. This was a short-term effect, as lymphocyte levels were not different at d28 post-weaning. Higher feed intake translated into vh in the ileum tending to be higher, and higher vh:cd on d28 post-weaning in pigs fed high-quality diets. Christensen and Huber (2022) also reported that highly digestible ingredients and higher ADFI reduced the negative effects of weaning on intestinal histomorphology, with piglets that received high-quality diets having a lower reduction in jejunal vh on d28 post-weaning than those that received low-quality diets. Both these studies examined histomorphology at 28 days post-weaning, which is outside of the period where performance was beneficially affected by feeding high quality diets, and so it is possible that had they sampled earlier there may have been a more noticeable effect. It appears that high quality diets may only be required for a short period post-weaning; as already discussed, continuing with high-quality regimes has diminishing returns over time.

High-quality diets may have negative effects on piglet gut health if protein level is too high. Berrocoso *et al.* (2012) reported that high-quality diets using cooked maize had no effect on performance, but increased diarrhoea incidence. They concluded that this was because the diet contained 12 g/kg more CP than planned, which caused dysbiosis due to hindgut fermentation of the protein. Protein content (specification) of the diet is increasingly of interest due to the EU ban on antibiotic growth promoters and pharmaceutical levels of ZnO in pig diets, and expectations of similar in the rest of the world (Wellock *et al.*, 2008; Batson *et al.*, 2021; Faba *et al.*, 2024). This has necessitated a greater focus on maintaining gut health by nutritional means. Protein is necessary for growth, but particularly at weaning when the intestine is immature and enzyme function is reduced, if fed in excess, amino acids will be fermented in the distal intestine, producing NH_3 and leading to conditions (higher pH) that favour pathogenic bacteria (Nyachoti *et al.*, 2006; Pieper *et al.*, 2016b) such as *E. coli, Kebsiella sp, Campylobachter sp, Streptococcus sp, C. perfringens, C. difficile, Bacteroides fragilis* (Pieper *et al.*, 2016b). Excess amino acids will also be decarboxylated at low pH (4-6) in the proximal small intestine, which results in biogenic amines (Pieper *et al.*, 2016b). Although these compounds are involved in maintaining intestinal barrier function and may be involved in stimulating intestinal maturity, an excess can be harmful (Bekebrede *et al.*, 2020). Of the biogenic amines (also referred to as polyamines) produced by microbial fermentation of protein, histamine (from histidine) is the most widely studied; in the intestine histamine stimulates chloride secretion in an inflammatory reaction, and damages the mucosa, causing a reduction in water absorption and resulting in diarrhoea (Pieper *et al.*, 2016b; Xia *et al.*, 2021). Cadaverine (from lysine) and putrescine (from ornithine) are thought to reduce histamine metabolism (Pieper *et al.*, 2016b). Putrescine can be converted to succinate and used for energy production in enterocytes, but this can reduce acetyl CoA levels. Putrescine can also contribute to apoptosis, through the production of toxic aldehydes 3-acetamidopropanal and 3-aminopropanal and H_2O_2 when catabolised (Bekebrede *et al.*, 2020). In healthy pigs, enzymes will catabolise biogenic amines such as histamine, cadaverine, putrescine, but enzyme activity is reduced in pigs immediately post-weaning due to villus atrophy (Faba *et al.*, 2024). To model the effects of CP level and dysbiosis on performance, the effect of dietary CP level on pig performance during an induced bacterial challenge has been investigated, with Wellock *et al.* (2008) reporting a greater reduction in ADG for pigs fed high CP diets (42 % reduction) compared to low CP diets (25 % reduction), when experimentally challenged with *E Coli*. In contrast, Heo *et al.* (2009) found no effect of CP level on performance after challenge with *E Coli*, although incidence of diarrhoea was lower in pigs fed low CP diets. Differences between studies may be due to differences in definition of low and high CP; for example, in Wellock *et al.* (2008) CP content was 130 g/kg and 230 g/kg for low and high respectively. For Heo *et al* (2009), CP content was 175 g/kg and 256 g/kg for low and high respectively.

Reducing CP level of the diet without compromising performance is possible with the use of additional synthetic amino acids. Nyachoti *et al.* (2006) investigated the effect of feeding diets containing 170, 190, 210, 230 g/kg CP to pigs weaned at 18 days old, for 21 days post-weaning. They reported a decrease in ADFI, ADG and gain:feed with reduction in CP level, but concluded that this may be due to insufficient valine. Wellock *et al.* (2008) balanced amino acids within protein, and fed 130 g/kg or 230 g/kg CP. They observed that pigs fed low CP had higher ADFI for the first six days post-weaning (365 *vs* 322 g/d), but ADG tended to be higher for pigs fed the high CP diet, and was significantly higher from d6-14, as was gain:feed. This study consisted of a very large reduction in CP, but pigs were a similar weight at 10 weeks old (35.08 kg *vs* 35.27 kg). Heo *et al.* (2009) reported no difference in weight 28 days post-weaning between pigs fed 256 g/kg or 175 g/kg CP, when valine and isoleucine were added to the lower protein diets. Zhang *et al.* (2013) added valine, leucine and isoleucine (branched chain amino acids; BCAA) to 171 g/kg CP diets to maintain a similar amino acid profile compared to a diet containing 209 g/kg CP, and reported that over the 14-day feeding period, ADFI, ADG and FCR were similar between pigs fed these diets, and were higher than for pigs fed a 171 g/kg CP diet without additional BCAA. Serum levels of valine, leucine and isoleucine were similarly affected, although arginine and histidine were higher in the pigs fed high CP compared to both low CP diets, which did not differ. Zhang *et al.* (2013) hypothesised that BCAA may regulate the expression of the peptide transporter PepT1, which is responsible for transporting dipeptides and tripeptides from the intestinal lumen into the enterocytes, as it was found in higher levels in the pigs fed low CP diets with additional BCAA. They concluded that as PepT1 levels had previously been shown to increase with decreasing feed intake, this was a mechanism for improving bioavailability of amino acids when protein supply is low.

Efficiency of protein usage can be assessed by plasma urea nitrogen (PUN) and faecal NH_3-N levels. Nyachoti *et al.* (2006) found that PUN levels were lower in pigs fed diets containing 170 and 190 g/kg CP compared to 230 g/kg on d7 post-weaning, and reduced as dietary CP reduced on d14 and d21, but with no difference between pigs fed diets containing 170 or 190 g/kg CP. They concluded that plasma PUN had reached its minimum level at 190 g/kg CP and there was no further increase in efficiency after this point. Plasma PUN was also lower in pigs fed low protein diets in the study of Heo *et al.* (2009), as was faecal NH_3-N level. As feed intake didn't differ between pigs fed low or high CP levels, Heo *et al.* (2009) concluded that lower faecal NH_3-N reflected lower microbial fermentation of protein in the distal intestine, and this was also observed by Yu *et al.* (2019) in the colon. Higher PUN in pigs fed high protein diets indicated higher microbial fermentation, or catabolism of excess amino acids, but Heo *et al.* (2009) considered this was probably due to microbial fermentation as gain:feed was unaffected by CP level; growth would have been less efficient due to the energy expended by the pig during catabolism, whereas microbial fermentation has no energy investment from the pig. The improved efficiency of nitrogen utilisation is also of interest due to the environmental benefits of reduced N excretion (Opapeju et al., 2008; Yue and Qiao, 2008; Larsen et al. 2021).

Dietary protein level was shown to have variable effects on post-weaning intestinal structure, with Nyachoti *et al.* (2006) finding no effect of CP level on duodenal or ileal morphology, but in the jejunum, there was a negative association between CP level and vh. Bikker *et al.* (2007), comparing the jejunum of pigs fed 150 g/kg or 220 g/kg CP, reported no effects of dietary CP level on histomorphology seven days post-weaning. Zhang *et al.* (2013) found that 14 days post-weaning, pigs fed diets containing 209 g/kg CP had longer villi and deeper crypts in the duodenum than pigs fed 171 g/kg CP, and longer villi in the jejunum and ileum. The vh:cd ratio was unaffected by dietary CP level. They concluded that the higher ADG and gain:feed observed in pigs fed the high protein diet was due to the greater absorptive surface area. Yu *et al.* (2019) examined intestinal morphology at the end of a 45-d study, finding that pigs fed 200 g/kg CP had longer villi and higher vh:cd ratio in the duodenum and jejunum than those fed 170 g/kg or 140 g/kg CP. Crypt depth in the duodenum was shallower in the pigs fed 200 g/kg CP compared to 140 g/kg CP. They attributed the lower villus height of pigs fed a low protein diet to a reduction in intestinal stem cells.

This study investigated the effects of pre-weaning creep consumption, post-weaning diet specification (protein and lysine level) and post-weaning diet quality on post-weaning performance and duodenal histomorphology. Any effects of weaning on intestinal histomorphology are more likely to be observed at the proximal region because epithelium rely on luminal rather than circulatory nutrition (Montagne *et al.*, 2007; Modina *et al.*, 2021).

The hypotheses for this Study were:

- Beneficial effects of higher creep consumption on post-weaning pig performance are due to a reduction in weaning-induced duodenal villous atrophy.
- Piglets with high pre-weaning creep consumption can maintain performance when provided with post-weaning diets of lower quality and lower lysine level.

6.2. Study materials and methods

This study employed no regulated procedures under the ASPA 1986 legislation, but was approved by the Harper Adams University ethics committee, project number 0480-202208-PGMPHD.

6.2.1. Pre-weaning

Two batches farrowing six weeks apart, with 28 sows/batch were utilised. Sows farrowed over a period of four days (batch 1) and seven days (batch 2). Farrowing performance of the two batches utilised is summarised in Table 49.

Table 49: Farrowing performance of batches utilised in study 3.

Parameter	Mean	s.d
Number of litters	*63*	
Litters/batch	31.5	0.50
Sow parity	3.08	0.14
Total born	16.8	0.16
Born alive	16.0	0.33
Piglet birth weight (kg)	1.46	0.01
Number weaned	14.6	0.26
Piglet weaning weight (kg)	8.73	0.15
Litter weaning age (d)	26.7	0.98

Details of animals used, management, accommodation, piglet and sow nutrition, weighing, feed recording, and the PigTrack® system used to record creep consumption can be found in Chapter 3.

Piglets had access to supplementary milk (SM; Faramate, Volac UK) *ad libitum* from birth to seven days prior to weaning (wn-7), provided through a bowl in the crate, constantly supplied from a single tank per batch. Consumption of SM was not measured in this study, due to it having no persistent effect on post-weaning performance, or the metabolome (Study 2). Creep was introduced at 14 days prior to weaning (wn-14), fed through a single space feeder. Weight of creep consumed per litter was recorded. Individual piglet creep consumption was measured using the PigTrack ® system (Asserva, France). Piglets were individually weighed on day 21 prior to weaning (wn-21), wn-14, wn-7, wn-1 and weaning (average 26 days old). Suckling was observed on wn-14 to determine which piglets were without a teat (designated non-sucklers).

6.2.2. Experimental design

This study was performed as a 2x2x2 factorial, with pre-weaning creep utilisation (low CL/high CH), post-weaning diet specification (low SL/high SH) and post-weaning diet quality (low QL/high QH) as factors (diet specifications in Table 50 and Table 51 and ingredients in Table 52 and 53). Experimental diets were fed in a two-stage regime from weaning to day nine (d9) post-weaning and d9-d22 post-weaning. Specification was formulated based on standard ileal digestible (SID) lysine content, with a corresponding low or high CP level. The ratio of SID lysine:DE was maintained. Standard ileal digestible amino acid balance with regards to lysine was consistent between diets, and was equal to or in excess of minimum requirements published by BSAS (2003). Vitamin and mineral content of the diets did not vary. The diets were not formulated to have large differences in specification, as the desired outcome of this study was to inform commercial practice, rather than to test physiological limits. For the first stage diets, although lysine level was constant between QL and QH, CP varied as a result of the quality-based formulation, due to the ingredients available. As there was a smaller difference in quality between QL and QH for the second stage diets, CP level was able to be standardised. The SLQH diet was considered the baseline, as it conformed to minimum lysine and DE requirements for pigs 10-30 kg (BSAS, 2003), and utilised ingredients commonly found in initial post-weaning diets (cooked cereals, fishmeal, skimmed milk powder). The low-quality diets were designed to test whether the pigs with high pre-weaning creep consumption had better adaptation to cereal-based diets, and so could maintain performance on a lower cost regime; they contained lower cooked cereal and fishmeal content, no skinned milk powder and a consequential increase in soya content. The high-specification diets had higher lysine content to determine whether high creep consumers could utilise extra lysine efficiently, which would result in improved growth.

Table 50: Specification of post-weaning pig diets with low (L) or high (H) specification (S) and quality (Q) fed from weaning-d9 post-weaning (first stage).

	SL		SH	
Constituent	QL	QH	QL	QH
Dry matter (g/kg)	906	906	914	914
Moisture (g/kg)	94	94	86	86
Oil A (g/kg)	39.1	34.6	85.5	82.3
Oil B (g/kg)	46.8	42.7	93.0	90.2
Protein (g/kg)	196	205	204	216
Fibre (g/kg)	19.7	18.6	18.7	17.8
Ash (g/kg)	57.0	55.6	57.8	56.8
Lysine (g/kg)	13.5	13.5	15.0	15.0
Salt (g/kg)	11.1	10.2	11.5	10.8
Ca (g/kg)	8.4	8.3	8.4	8.3
P (g/kg)	7.2	6.9	7.2	6.9
Digestible P (g/kg)	4.5	4.5	4.5	4.5
Na (g/kg)	3.0	3.0	3.0	3.0
K (g/kg)	10.2	9.5	10.4	9.9
Zn (mg/kg)	133	136	132	136
Cu (mg/kg)	145	144	145	144
Mn (mg/kg)	60.4	59.4	59.7	58.9
Se (mg/kg)	0.4	0.4	0.4	0.4
NDF (%)	6.4	6.3	5.9	5.8
DE (MJ/kg)	15.0	14.9	16.1	16.2
Starch (g/kg)	32.6	34.2	28.0	29.0
Sugars (g/kg)	17.1	16.5	17.2	16.6
Vitamin A (iu)	12500	12500	12500	12500
Vitamin D3 (iu)	2000	2000	2000	2000
Vitamin E (iu)	250	250	250	250

Table 51: Specification of post-weaning pig diets with low (L) or high (H) specification (S) and quality (Q) fed from d9-d22 post-weaning (second stage).

	SL		SH	
Constituent	QL	QH	QL	QH
Dry matter (g/kg)	895	895	895	895
Moisture (g/kg)	105	105	105	105
Oil A (g/kg)	29.6	29.6	29.6	29.6
Oil B (g/kg)	37.9	37.9	37.9	37.9
Protein (g/kg)	201	201	201	201
Fibre (g/kg)	24.9	24.9	24.9	24.9
Ash (g/kg)	53.8	53.8	53.8	53.8
Lysine (g/kg)	12.5	12.5	14.0	14.0
Salt (g/kg)	7.4	7.4	7.4	7.4
Ca (g/kg)	8.4	8.4	8.4	8.4
P (g/kg)	7.5	7.5	7.5	7.5
Digestible P (g/kg)	4.5	4.5	4.5	4.5
Na (g/kg)	2.0	2.0	2.0	2.0
K (g/kg)	9.4	9.4	9.4	9.4
Zn (mg/kg)	134	134	134	134
Cu (mg/kg)	145	145	145	145
Mn (mg/kg)	62.9	62.9	62.9	62.9
Se (mg/kg)	0.3	0.3	0.3	0.3
NDF (%)	8.1	8.1	8.1	8.1
DE (MJ/kg)	14.6	14.6	14.6	14.6
Starch (g/kg)	37.1	37.1	37.1	37.1
Sugars (g/kg)	10.7	10.7	10.7	10.7
Vitamin A (iu)	12500	12500	12500	12500
Vitamin D3 (iu)	2000	2000	2000	2000
Vitamin E (iu)	250	250	250	250

Table 52: Ingredients of post-weaning pig diets with low (L) or high (H) specification (S) and quality (Q) fed from weaning-d9 post-weaning (first stage).

	SL		SH	
Ingredient	QL	QH	QL	QH
Lactose (g/kg)	150.00	150.00	150.00	150.00
Milk protein (g/kg)	23.05	35.91	23.05	35.91
Cooked cereals (g/kg)	150.00	300.00	150.00	300.00
Micronised barley (g/kg)	50.00	100.00	50.00	100.00
Micronised wheat (g/kg)	50.00	100.00	50.00	100.00
Micronised oats (g/kg)	50.00	100.00	50.00	100.00
SID lysine (g/kg)	13.50	13.50	15.00	15.00
Barley 62kg/hl	0.00	0.00	0.00	0.00
Raw wheat wholemeal (g/kg)	366.92	256.09	289.31	167.02
Fishmeal (g/kg)	40.00	80.00	40.00	80.00
Soya Hypro (g/kg)	177.73	108.73	202.04	144.83
Dried skim milk powder (g/kg)	0.00	50.00	0.00	50.00
Whey powder (g/kg)	202.16	166.31	202.16	166.31
Amino acid balance (g/kg)	15.17	9.48	20.76	13.63
Vitamin E (g/kg)	0.20	0.20	0.20	0.20
Sucram (g/kg)	0.10	0.10	0.10	0.10
Benzoic acid (g/kg)	5.00	5.00	5.00	5.00
Dicalcium phosphate (g/kg)	13.18	6.91	13.32	6.92
Salt (g/kg)	2.24	0.40	2.24	0.83
Sodium Carbonate (g/kg)	0.00	0.41	0.00	0.01
Soya oil (g/kg)	22.15	11.23	69.72	60.00

Table 53: Ingredients of post-weaning pig diets with low (L) or high (H) specification (S) and quality (Q) fed from d9-d22 post-weaning (second stage).

	SL		SH	
Ingredient	QL	QH	QL	QH
Lactose (g/kg)	75.00	75.00	75.00	75.00
Milk protein (g/kg)	11.52	17.95	11.52	17.95
Cooked cereals (g/kg)	0.00	200.00	0.00	200.00
Micronised barley (g/kg)	0.00	100.00	0.00	100.00
Micronised wheat (g/kg)	0.00	50.00	0.00	50.00

Micronised oats (g/kg)	0.00	50.00	0.00	50.00
SID lysine (g/kg)	12.50	12.50	14.00	14.00
Barley 62kg/hl	100.00	0.00	100.00	0.00
Raw wheat wholemeal (g/kg)	488.37	418.31	405.75	310.31
Fishmeal (g/kg)	25.00	50.00	25.00	50.00
Soya Hypro (g/kg)	229.59	185.29	250.00	231.58
Dried skim milk powder (g/kg)	0.00	25.00	0.00	25.00
Whey powder (g/kg)	101.08	83.15	101.08	83.15
Amino acid balance (g/kg)	10.12	7.89	16.11	10.40
Vitamin E (g/kg)	0.20	0.20	0.20	0.20
Sucram (g/kg)	0.10	0.10	0.10	0.10
Benzoic acid (g/kg)	5.00	5.00	5.00	5.00
Dicalcium phosphate (g/kg)	17.53	13.83	17.77	13.80
Salt (g/kg)	1.64	0.38	1.46	1.13
Sodium Carbonate (g/kg)	0.35	0.70	0.52	0.01
Soya oil (g/kg)	15.87	5.00	71.86	64.17

From d22 to d61 post-weaning, a common grower diet was fed to all treatments; from d61 to 75 post-weaning this was blended with a common finisher diet in a gradually decreasing grower:finisher ratio. The finisher diet was fed from d75 post-weaning to slaughter (specifications in Table 54).

Table 54: Specification of common grower and finisher diets fed to all pigs.

Constituent	Grower	Finisher
Crude protein (g/kg)	192	173
Crude fibre (g/kg)	43	58
Crude oil and fats (g/kg)	38	40
Crude ash (g/kg)	53	53
Lysine (g/kg)	13	11
Methionine (g/kg)	3.0	2.8
Ca (g/kg)	7.0	7.0
Na (g/kg)	2.3	2.6
P (g/kg)	4.8	5.0
Vitamin A (iu/kg)	9500	6500
Vitamin D3 (iu/kg)	1850	1650
Vitamin E (iu/kg)	75	35

The grower and finisher diets were standard commercial diets, and so quantified composition is unavailable. Ingredients were (in order of inclusion rate):

- Grower diet: wheat, barley, soyabean meal, wheatfeed, rapeseed expeller, maize germ, calcium carbonate, sodium chloride, vegetable oil and fat (soya), dicalcium phosphate,sodium bicarbonate
- Finisher diet: barley, wheat, wheatfeed, rape seed expeller, sunflower seed meal, soyabean meal, cane molasses, calcium carbonate, beet molasses, sugar beet molasses (partially desugared/debetainised), vegetable oil and fat (soya), sodium chloride

6.2.2.1. Selection

All piglets, except those identified as non-sucklers, were eligible for selection. Pigs were weighed on wn-1, and as in Study 2, from the PigTrack® data and weighing data from wn-14 to wn-1, a creep score was calculated, intended to be a measure of how important creep consumption was to the individual piglet's growth. This was calculated as:

$$creep\ score\ =\ total\ duration\ of\ visits\ (s)/average\ daily\ growth\ rate\ (ADG;\ kg/d)$$

Each batch comprised 96 male and 96 female piglets, in 32 pens (3 male and 3 female/pen). Piglets were selected from the first and third quartiles for creep score, designated CL and CH respectively. In batch 2, a further eight piglets each from Q1 and Q3 (four male and four female) were selected to be sacrificed on wn-1 to collect baseline histological data.

Within creep consumption treatments, dietary treatments (specification and quality) were allocated, with average pig weight on wn-1 equalised between dietary treatments and pens (Figure 53). The difference in average pig weight on wn-1 according to creep score was maintained for post-weaning selection.

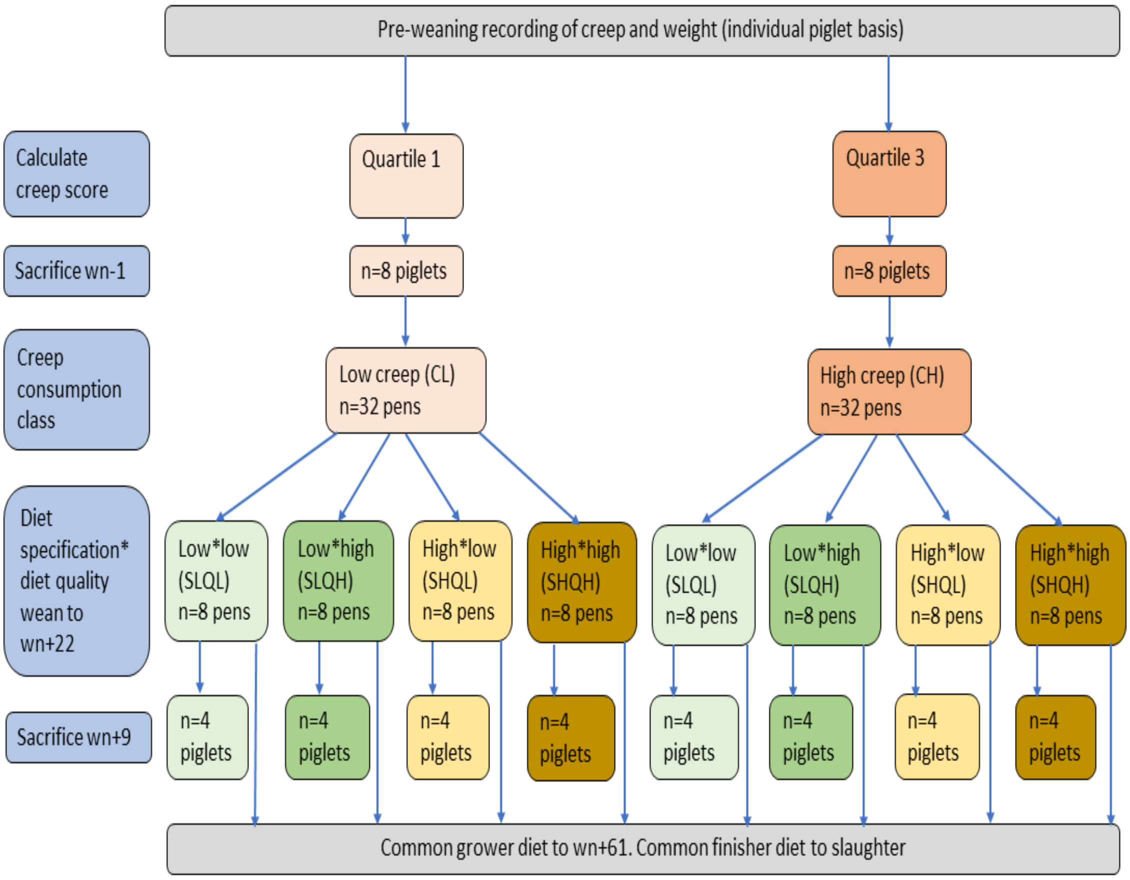

Figure 53: Schematic of study 3 experimental design.

Over the two batches, for main effects of creep consumption, diet specification and diet quality n=32 pens (performance) and n=16 pigs (histology). For three-way creep*specification*quality interaction n=8 pens (performance) and n=4 pigs (histology).

6.2.3. Post-weaning

Details of nursery accommodation and commercial finishing accommodation can be found in Chapter 3

6.2.3.1. Weighing

Piglets were individually weighed, and pen feed intake recorded on d3, 6, 9, 12, 15, 22, 36 and 54 post-weaning (nursery exit). Pigs were also weighed at 19 weeks old, before the first slaughter draw. Procedures for weighing are detailed in Chapter 3.

6.2.3.2. Sample collection

In batch 2, eight pigs each (four males and four females) from Q1 and Q3 for creep consumption and within 1 s.d of the mean weight were sacrificed on wn-1. One pig per pen was sacrificed on d9 post-weaning. This was the pig with the median weight and a positive growth rate at d6 post-weaning, ensuring there were two males and two females each consumption*diet specification*diet quality treatment. The same euthanasia and sampling procedures were followed for the pigs sacrificed on wn-1 and wn+9. Pigs were restrained by holding the shoulders securely and euthanised using a captive bolt gun and subsequent pithing, by a WATOK-certified operator. Death was confirmed by testing for absence of the corneal reflex. Sample collection commenced immediately after confirmation of death, and concluded within 15 minutes. An incision was made ventrally from sternum to groin to access the intestines. A 2cm cross-section was taken from the duodenum 30 cm from the pyloric sphincter. This section was selected as the proximal region of the intestine is most-susceptible to villus atrophy due to inadequate feed intake. The intestine was held using forceps applied gently to the exterior wall and a transverse incision made using a scalpel, severing the intestine. The incision was repeated 2 cm along the intestine, and the resulting section was immersed in 10 % neutral buffered formalin (Sigma Aldritch UK), and stored at room temperature until slides were prepared for histology. The sections were handled carefully and held using forceps from the outside or edge only to prevent damage to intestinal microstructure.

6.2.3.3. Slide preparation

Working in a fume hood, the end of the formalin-preserved sample where the tissue curled back on itself was removed and discarded. A 2 mm transverse cross-section was cut from the remaining sample and placed in a lidded histology cassette (Thermoscientific, UK), with the sample identification written on the cassette in pencil, as this would not be dissolved by formalin or xylene. The lidded cassettes were immersed in formalin until all samples had been cut and placed in cassettes. The cassettes were placed into a Leica HistoCore PEARL tissue processor overnight to dehydrate the samples, and to impregnate the samples with paraffin wax. This was achieved by:

1. Fixation in formalin for 60 minutes at 37 °C
2. Rinse in deionised water for two minutes
3. Dehydration in increasing ethanol concentrations at 45 °C:
    3.1. 70 % ethanol for 40 minutes
    3.2. 80 % ethanol for 40 minutes
    3.3. 95 % ethanol for 40 minutes
    3.4. 100 % ethanol for 60 minutes, this stage repeated twice
4. Removal of ethanol from tissue by xylene for 60 minutes at 45 °C, this stage repeated twice
5. Impregnation of tissue with paraffin wax at 65 °C for 60 minutes, repeated once for 60 minutes and once for 90 minutes.

Repetition of stages used fresh chemicals each time. After tissue impregnation, stages of the method for preparing the slides are shown in Figure 54.

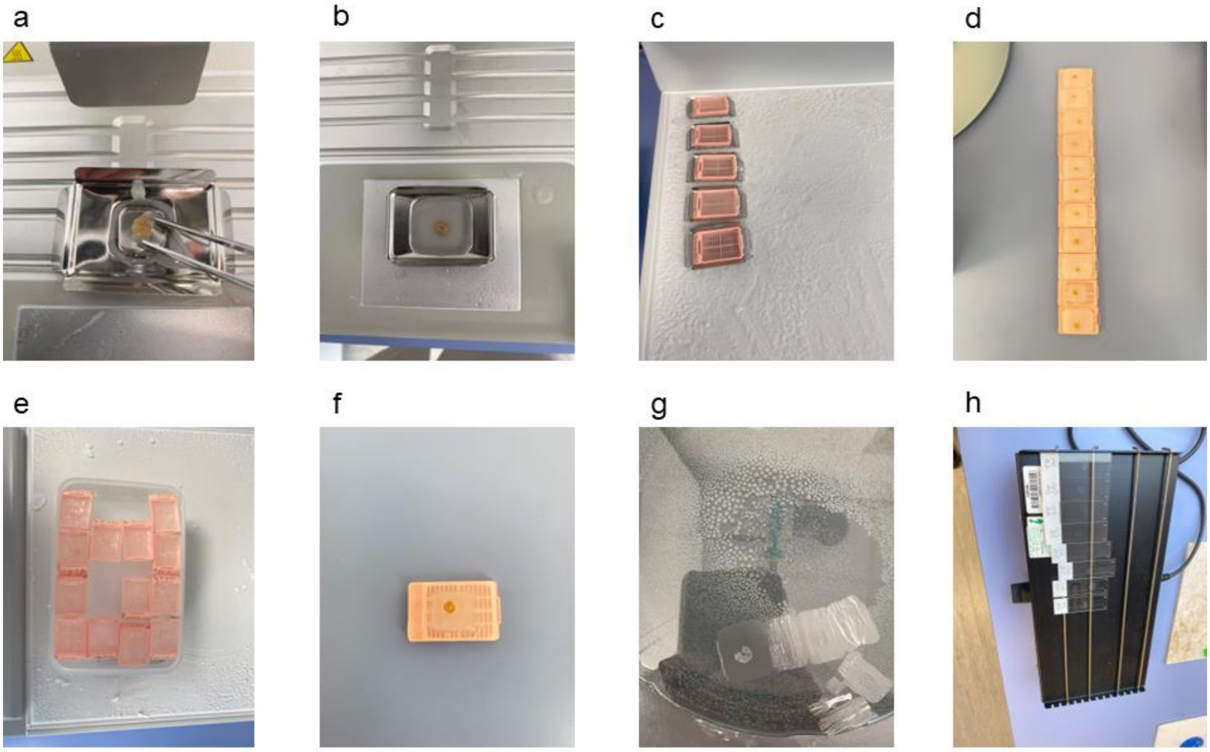

Figure 54: Stages of preparation of histology slides from sample embedding to drying in preparation for staining. a: embedding sample in liquid paraffin wax; b: sample setting in wax before filling of mould; c: chilling of wax-filled moulds; d: trimmed blocks; e: wax blocks on ice before full-facing; f: full-faced block with sample exposed; g: slices in the flotation bath; h: slides drying on the rack.

Samples were set in a block of paraffin wax using the Leica HistoCore Arcadia H, and cooled on the Leica HistoCore Arcadia C: a mould of appropriate size was selected, ensuring it was sufficiently large so that the sample did not touch the sides of the mould. A small amount of liquid paraffin wax was dispensed into the mould, and the sample was removed from the cassette using forceps and carefully pushed into the wax (a), ensuring that the cut edge was orientated parallel to the surface of the mould. Whilst maintaining gentle downwards pressure on the sample with the forceps to ensure the sample stayed flat and in position, the mould was slowly pulled back towards the cooling area to set the sample (b). The cassette (without the lid) was replaced on top of the mould and the combination was filled to the top of the cassette with liquid paraffin wax to form a wax block. The mould containing the block was placed onto the cold plate to fully solidify (45 minutes), ensuring that it felt icy to the touch before proceeding (c). The mould was gently removed to leave the wax block sealed to the cassette. Excess wax was trimmed from the narrow sides of the cassette using a block trimmer set at 65 °C (d; CellTec Block Trimmer+, CellPath). The sample was full-faced (wax trimmed to reveal the sample) using the Leica rotary microtome RM2235 set at 15 microns initially, reducing to 5 microns as the sample was revealed (e). Following this, the sample was chilled face-down on an ice block, moving it occasionally to ensure it didn't adhere to the ice surface (f). The microtome was set at 3 microns to cut slices for the slides, ensuring the wax block was orientated the same way as when full-facing. Slices were cut slowly to prevent wrinkling (a ribbon of at least three slices per sample), and placed in a flotation bath (CellPath) at 45 °C to flatten (g). Slices were separated gently using tweezers, and the flattest slice that contained the entire sample was selected. This slice was picked out of the bath using a glass slide (labelled in pencil with the sample number and 1). Ten more slices were cut from the block and discarded to ensure the location was different from the first slice, and another ribbon of 3-micron slices was cut. These were placed in the flotation bath and the best slice was picked out on a different glass slide (labelled in pencil with the sample number and 2). This process was repeated a third time for sample number 3. The glass slides were placed on a heated rack (Cole Parmer slide drying bench) to dry and melt the remaining wax (h) before being placed in a slide holder (capacity 25 slides). At this point they were stable for storage at room temperature, before staining.

6.2.3.4. Staining

In a fume hood, the slide holder was immersed in two coplin jars of xylene for three minutes each jar to de-wax the slides. Slides 1 and 2 for each sample were stained using the haematoxlin and eosin (h+e) method, to identify and allow measurement of villi and crypts. Slide 3 for each sample was stained using the alcian blue-periodic acid Schiff's reagent (AB-PAS) method, to identify mucin-secreting goblet cells.

6.2.3.4.1. Haematoxylin Z and eosin Y (h+e) staining

The slide holder was immersed in 100 % ethanol for three minutes, 80 % ethanol for three minutes, and methylated spirits for ninety seconds. It was then rinsed under tap water for two minutes before staining. The stain was applied with immersion of the slides in the following sequence:

1. Alcian blue solution for five minutes
2. Distilled water for three minutes
3. Haematoxylin Z for five minutes
4. Rinsed in tap water until it ran clear
5. Dipped in acid alcohol four times rapidly
6. Rinsed in tap water for one minute
7. Scotts tapwater for one minute
8. Rinsed in tap water for one minute
9. Eosin Y for one minute
10. Rinsed in tap water for one minute
11. Methylated spirits for one minute
12. 75 % ethanol for two minutes
13. 95 % ethanol for two minutes
14. 100 % ethanol for two minutes
15. Two containers of xylene for two minutes per container

6.2.3.4.2. Alcian blue-Periodic acid-Schiff (AB-PAS) staining

The slide holder was immersed in xylene for five minutes minutes, 100 % ethanol for two minutes, and methylated spirits for ninety seconds. It was then rinsed under tap water for two minutes before staining. The stain was applied with immersion of the slides in the following sequence:

1. Alcian blue solution for five minutes
2. Rinsed in tap water for five minutes
3. Dipped in distilled water
4. Immersed in 1 % periodic acid for five minutes
5. Rinsed in tap water for five minutes
6. Dipped in distilled water
7. Immersed in Schiffs reagent for 15 minutes
8. Rinsed in tap water for 10 minutes
9. Haematoxymin Z for two minutes

10. Two quick dips in 1 % acid alcohol
11. Rinsed in tap water for three minutes
12. 100 % ethanol for two minutes
13. Two containers of xylene for five minutes each container

All stains were from CellPath UK. Other reagents were from Thermofisher UK.

For both stains, slides remained immersed in xylene until coverslipped using mounting medium and a 22 mm x 22 mm glass coverslip (maximum xylene immersion of 1 hour to avoid damage to sample). Slides were left to dry overnight before image capture.

6.2.3.5. Image capture

A Zeiss AX10 imager M2 microscope (Carl Zeiss Microscopy Ltd, Cambourne, UK) connected to Zeiss Axiocam 208 colour camera was used to visualise and photograph the slides at 20 x magnification, using the Zeiss Zen 3.5 (blue edition) software. The microscope was instructed to perform its calibration routine prior to scanning slides. Auto-exposure was used, with transmitted light (TL) set at 25 %. White balance was achieved by assessment of the RGB histogram, and auto re-balancing on an area of slide with no sample present where necessary. Shading was removed using the same blank area of slide. The tissue was located, and the microscope focussed on the edge. The acquisition area was obtained by tracing the edge of the sample using the mouse and clicking to define the minimum and maximum x and y points. This produced a grid of 200-924 tiles (depending on sample dimensions), with one tile being the field of view of the microscope at 20 x magnification. Twenty-five evenly distributed tiles within this grid were utilised to set the z co-ordinate and thus focus the microscope over the entire topography of the tissue. The camera captured an image of each tile, and these images were combined by the software to form a high-resolution image of the entire sample.

6.2.3.6. Haematoxylin+eosin-stained slides

Similar to the method of King *et al* (2008), measurements were taken from the 10 tallest and most well-oriented villi and associated crypts per sample. Five measurements per slide were taken; to ensure that different villi were measured between the slides, the proposed villi were marked on each image, and a side-by-side visual comparison performed before measurement commenced. The measuring function in the Zen 3.5 (blue edition) was utilised for measurements and the data table was exported as a csv file for each slide. As shown in Figure 55, villus height (vh) was measured from the base of the villus to the tip, and crypt depth (cd) from the villus-crypt junction to the base of the crypt. Villus width (vw) was measured at the base of the villus.

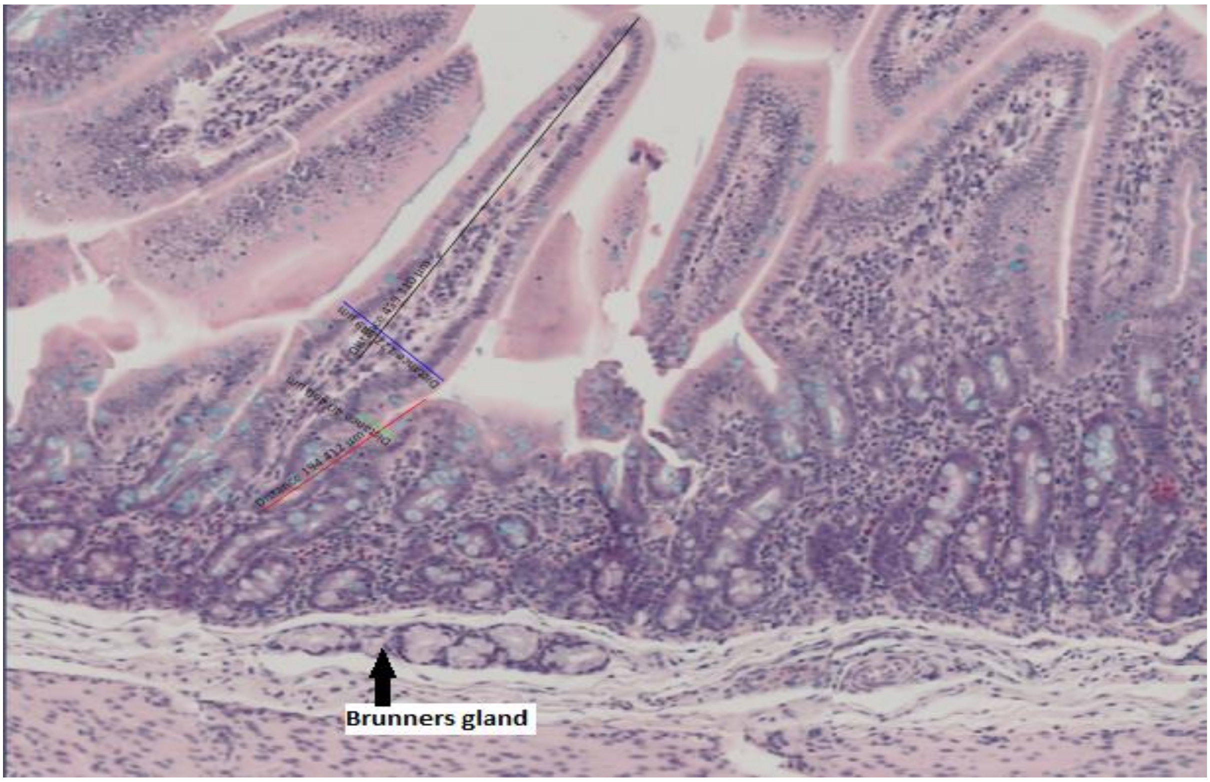

Figure 55: Histomorphological measurements taken from pig duodenum. Black=villus length, blue=villus width, red=crypt depth, green=crypt width.

Mucosal thickness (MT) was measured on at least three folds per sample. Thickness was measured perpendicular to the tip of the fold, and at the base before the mucosal fold turned (Figure 56, points a and b respectively). The mean of the two measurements was calculated and designated as MT.

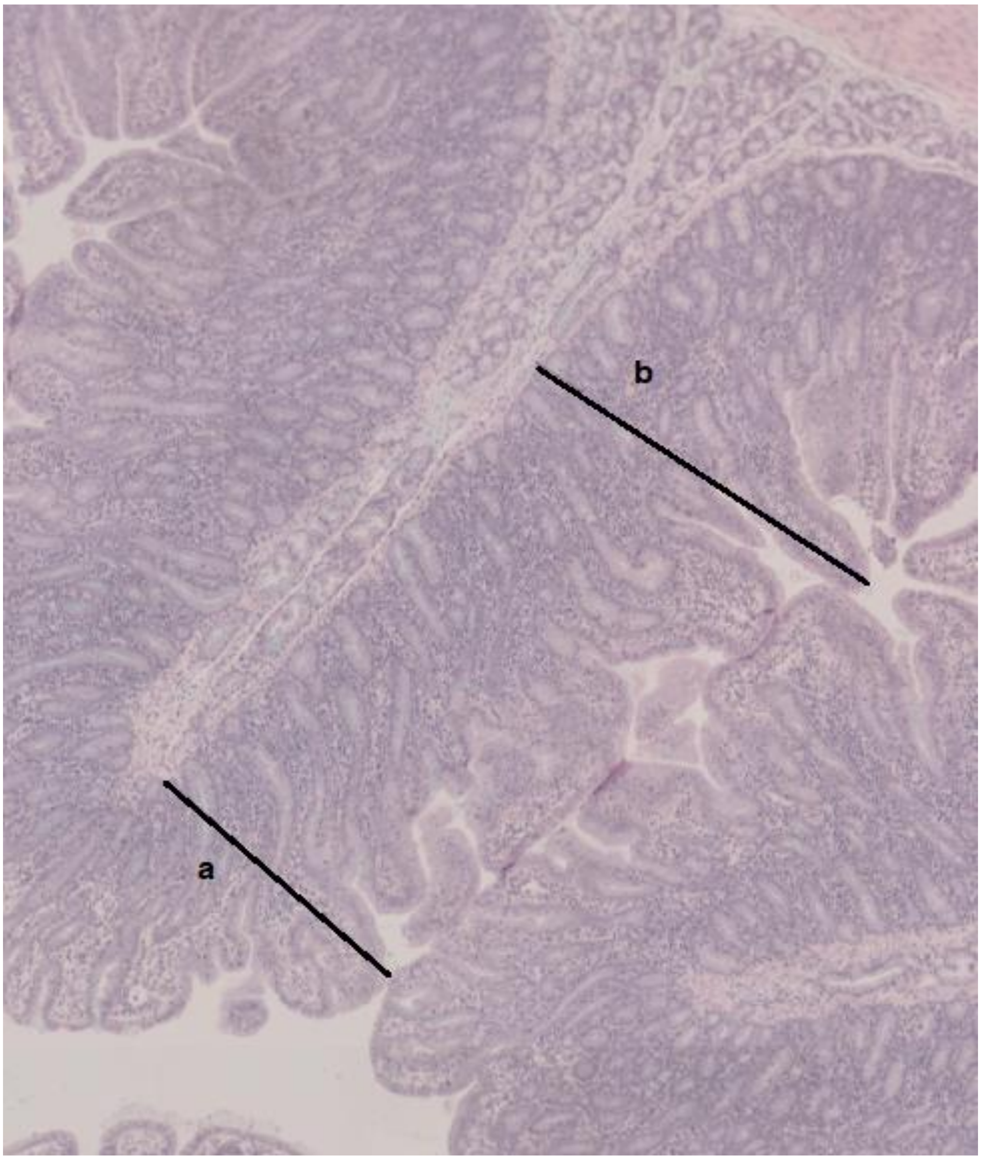

Figure 56: Measuring mucosal thickness of the piglet duodenum.

The resultant csv files for each slide were combined into a single Excel workbook using the Power Query function. For each region measured, villus height:crypt depth (vh:cd) and villus height:villus width was calculated.

6.2.3.7. Alcian blue and Periodic acid-Schiff stained slides

Acid- (blue) and neutral- (magenta) mucin-producing goblet cells were counted on five well-orientated villi and their associated crypts per sample, and reported per mm^2 of epithelium, using the measuring function in the Zen 3.5 (blue edition).

6.2.4. Statistical Analysis

All analyses were performed in Genstat 23rd edition (UK). Pen performance from weaning to d54 post-weaning was analysed by ANOVA in a 2*2*2 factorial design. Where a repeated measures model was utilised, models were assessed in the order: antedependence order 1, antedependence order 1 with uniform correlations within subject, antedependence order 2, antedependence order 2 with uniform correlations within subject. After each model change the deviance and Akaike Information Criterion (AIC) were checked, and if these had not decreased by at least the critical chi-square value for the reduction in degrees of freedom, the previous model was accepted as the best-fitting. Significant differences between treatments were determined by *post-hoc* Bonferroni test.

Individual piglet growth rate to 19 weeks old was analysed by multiple regression. The maximal model was fitted initially, including weight (birth, weaning, d54 post-weaning in separate models), ADG (birth to weaning, weaning to d54 post-weaning, d54 post-weaning to 19 weeks old in separate models), duration of creep visits and creep score. Grouping factors assessed for significance were sex, creep consumption classification, diet specification and quality (in separate models). For each grouping factor, the maximal model was fitted, then each model was subjected to step-wise elimination of non-significant terms to obtain the minimal model. Between the grouping factors, the minimal model with the highest variance accounted for was selected as the final model.

The average of the measurement locations per sample of the histological parameters of villus height, villus width, crypt depth, villus height:crypt depth, villus height:villus width, mucosal thickness, mucin-secreting goblet cells/mm^2 of epithelium and absorptive capacity were analysed by ANOVA (individual pig as a representative of the pen was the experimental unit). Significant differences between treatments were determined by *post-hoc* Bonferroni test. Correlations between histomorphological measurements, ADG and creep score were analysed by regression, with grouping factors of pre-weaning creep consumption, post-weaning diet specification and post-weaning diet quality.

6.3. Results

6.3.1. Pre-weaning comparison with study 1 and 2

There were no pre-weaning treatments were applied in study 3, but performance and creep consumption data were collected to inform selection for the post-weaning study.

6.3.1.1. Performance

A comparison of overall pre-weaning performance between studies is presented in Table 55. Study 2 had slightly higher number of piglets born alive than studies 1 and 3, and consequently lower average birthweight. Study 3 had higher average number of piglets weaned than studies 1 and 2, due to lower pre-weaning mortality. Piglets in Study 3 were not weighed at day 4, but were marginally heavier at other weigh points. From d11-18, ADG of piglets in Study 3 was higher than in the previous studies, but was lower from d18-weaning.

Table 55: Comparison of piglet pre-weaning performance between Studies 1, 2 and 3.

	Study 1	Study 2	Study 3
n (individual piglets at d4)	*638*	*517*	*788*
Age at weaning	27.2	27.4	27.2
Average birthweight	1.45	1.38	1.47
Average weight d4 (kg)	2.10	2.07	
Average weight d11 (kg)	3.92	3.93	4.30
Average weight d18 (kg)	6.00	6.26	6.83
Average weight weaning (kg)	8.54	8.68	8.82
ADG birth-d4 (g/d)	125	127	
ADG d4-d11 (g/d)	252	265	
ADG d11-18 (g/d)	296	288	317
ADG d18-weaning (g/d)	295	302	282
ADG birth-weaning (g/d)	253	261	271
ADG d4-weaning (g/d)	284	287	
n (litter)	*45*	*36*	*53*
Born alive	16.7	17.4	17.0
Stills	1.1	1.1	0.7
Number of pigs weaned	14.2	14.3	14.7
Pre-weaning mortality (%)*	14.8	17.8	8.7

*Mortality may not equal 100x(born alive-weaned)/born alive as piglets may have been fostered to non-trial sows.

Causes of pre-weaning mortality for each study are presented in Figure 57. Study 3 had a lower percentage of piglets that starved or were considered not viable than Study 1 and 2. There was a higher percentage of piglets laid on in Study 1 and 3 compared to Study 2.

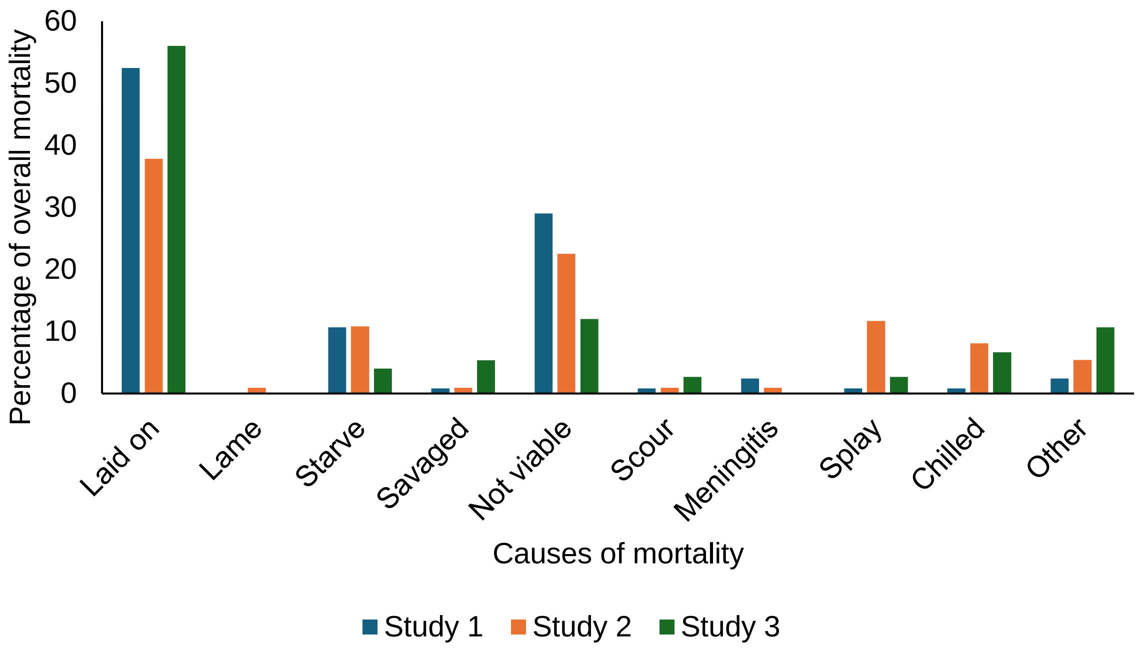

Figure 57: Comparison of causes of pre-weaning mortality between studies 1, 2 and 3.

Mortality was lower in Study 3, mainly due to improved survival of piglets with the lightest 50 % of birthweights (Figure 58).

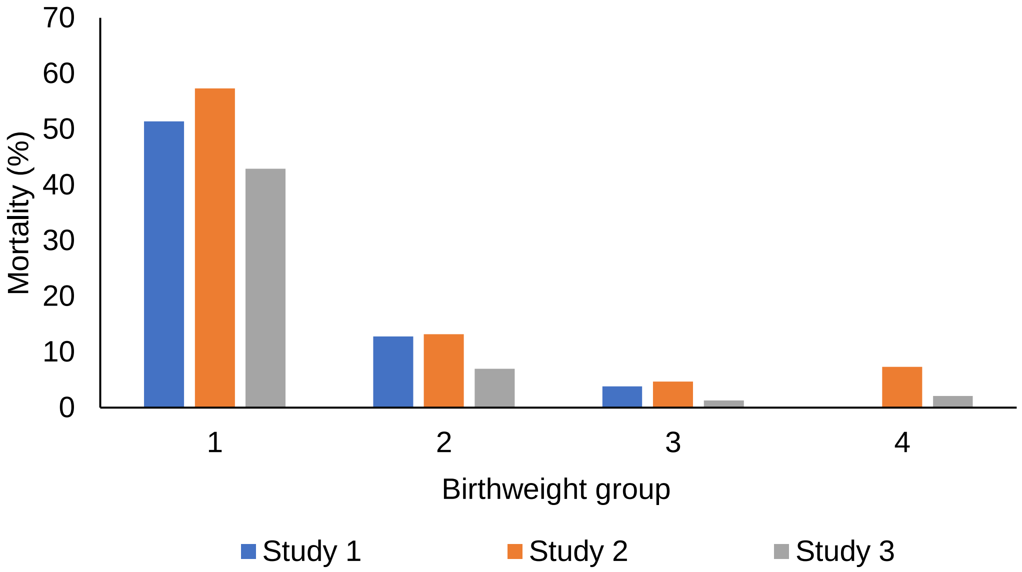

Figure 58: Comparison of mortality according to birthweight group between studies 1, 2 and 3. Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg.

6.3.1.2. Creep consumption during the suckling period

Creep consumed/litter and per pig was lower from d11-18, but higher from d19-weaning in Study 3 compared to Study 2 (Table 56).

Table 56: Comparison of piglet pre-weaning creep consumption between Studies 2 and 3

	Study 2	Study 3
n (litter)	*36*	*53*
Creep consumed/litter d11-18 (kg)	0.189	0.130
Creep consumed/pig d11-18 (kg)	0.013	0.009
Creep consumed/litter d19-weaning (kg)	2.237	3.610
Creep consumed/pig d19-weaning (kg)	0.155	0.244

In both Studies 2 and 3, duration/visit didn't vary, and so patterns of usage were identical when considering duration and number of visits. Due to duration of visits being utilised in the usage model, the following comparisons are based on this parameter.

Daily average duration of visits to the creep feeder/pig was similar between Studies 2 and 3 until d18 (Figure 59). After this point, in Study 2 duration increased gradually, whereas in Study 3 there was a more rapid increase in duration of visits (P <0.001, s.e =275, r^2 =0.199).

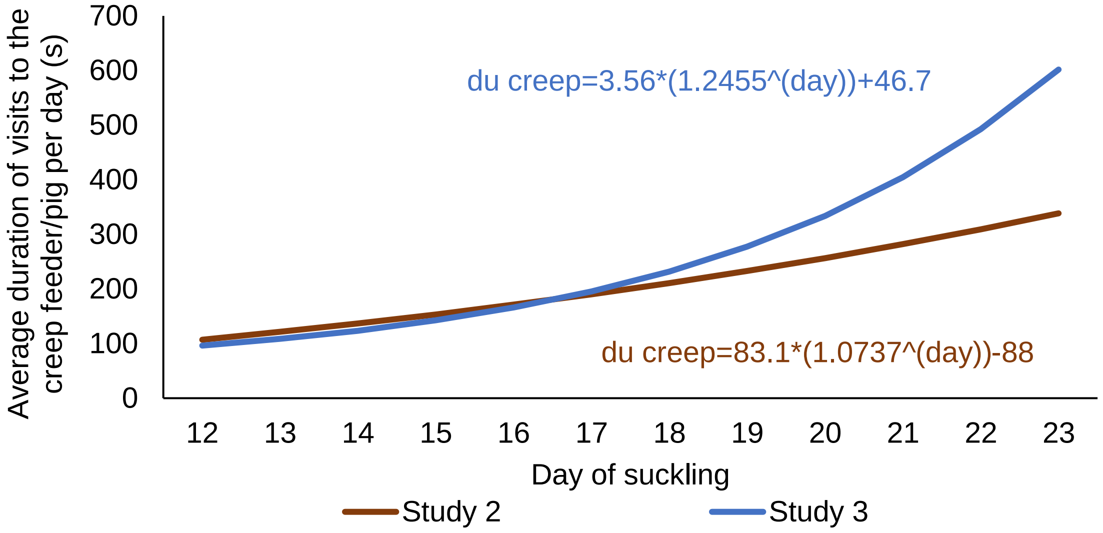

Figure 59: Comparison of average duration of visits to the creep feeder/pig per day between Studies 2 and 3.

The following equation represents the minimal model of the relationship between litter creep intake for the final week pre-weaning, duration of visits and piglet weight, combining data from Studies 2 and 3, with an r^2 of 0.684:

$$Litter\ creep = (0.00011807 * du) - (1.892 * avewt18) + (2.055avewtwn) - 6.53$$

Standard error: 1.44, r^2: 0.684. du: duration of visits per litter d19-weaning; avewt19: average piglet weight at d18; avewtwn: average piglet weight at weaning.

6.3.1.3. Diurnal variation in creep consumption

In both studies there were two distinct peaks in creep usage during the day: from 07:00-09:00, and subsequently from 15:00-17:00 (Figure 60).

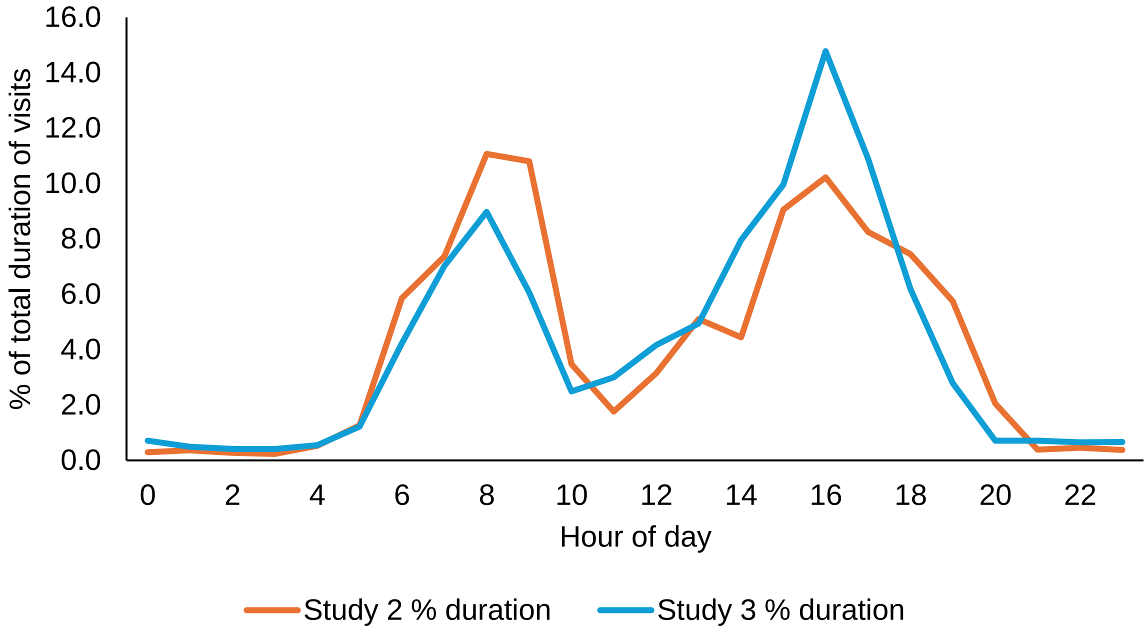

Figure 60: Diurnal variation in percentage of total duration of visits to the creep feeder during Study 2 and Study 3.

6.3.1.4. Comparison of creep consumption groupings

Piglets selected post-weaning in Study 3 were heavier than those selected post-weaning in Study 2 (Table 57). This was likely due to piglets identified as not suckling being ineligible for Study 3. In both studies, piglets classified as CH were lighter at weaning than those classified as CL.

Table 57: Comparison of creep score (duration of visits/ADG for the final week pre-weaning) and weaning weight between piglets classified as low (L) and high (H) creep (C) consumers, between Studies 2 and 3.

	Study 2		Study 3	
	CL	CH	CL	CH
Average creep score (s/kg/d)	8542	13559	6663	14893
Average weight at weaning (kg)	9.06	8.45	9.27	8.97

In Study 2, piglets were split into two halves according to creep score, whereas in study 3, pigs in the CL classification were in the bottom quartile for creep score and pigs classified CH were in the upper quartile for creep score. This resulted in the desired outcome of a greater difference in creep score between the low and high classifications in Study 3 compared to Study 2. The equation linking litter creep intake with duration and weight was deemed sufficiently accurate to approximate creep intake for the final week prior to weaning for individual piglets. Individual piglet contribution to the litter total was calculated as a proportion, and multiplied by the litter creep intake (which was weighed). Average creep intake for the final week prior to weaning for CL was calculated as 0.120 kg/piglet (±0.07 standard deviation), and for CH was 0.331 kg/piglet (±0.15 standard deviation).

6.3.2. Pre-weaning performance

There was no difference in birthweight between CL and CH piglets (P >0.05; Table 58). By d11, when creep was introduced, CH piglets had lower ADG from birth (P =0.039) and tended to be 0.17 kg lighter than CL piglets (P =0.060). By weaning the difference in weight was 0.30 kg, with ADG from birth-weaning 10 g/d lower in CH piglets (P =0.045).

Table 58: Pre-weaning performance of piglets in the first quartile (CL) and third quartile (CH) for pre-weaning creep consumption.

	CL	CH	s.e.m	*P*-value
n	*96*	*96*		
Birthweight (kg)	1.48	1.48	0.022	0.852
Weight d11 (kg)	4.50	4.33	0.063	0.060
Weight d18 (kg)	7.22	6.92	0.088	0.018
Weight wean (kg)	9.27	8.97	0.106	0.047
ADG birth-d11 (g/d)	246	235	3.8	0.039
ADG d11-18 (g/d)	340	324	4.2	0.007
ADG d18-wean (g/d)	293	292	4.7	0.955
ADG birth-wean (g/d)	286	276	3.4	0.045

6.3.3. Pre-weaning creep consumption

Piglets classified as CH had higher creep consumption when measured as average du/d or total du for the period, from d11-18 and d19-weaning (*P* <0.001; Table 59).

Table 59: Weekly creep consumption measured by duration of visits (du) of piglets in the first quartile (CL) and third quartile (CH) for pre-weaning creep consumption.

	CL	CH	s.e.m	*P*-value
Average du/d d11-18 (s)	112	193	5.54	<0.001
Average du/d d19-wean (s)	263	587	10.62	<0.001
Total du d11-18 (s)	680	1294	38.4	<0.001
Total du/d d19-wean (s)	1559	3517	63.9	<0.001

As shown in Figure 61**,** when creep consumption was analysed by birthweight group, piglets from groups 1 and 4 had the highest creep consumption from d20 onwards (*P* =0.002; antedependence order 2 with additional uniform correlations).

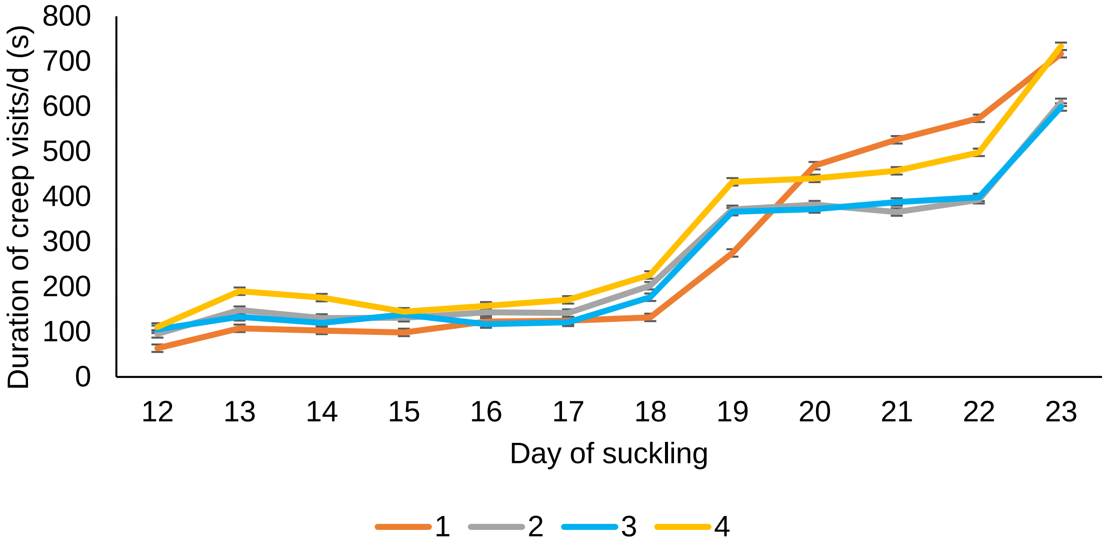

Figure 61: Effect of birthweight group on piglet pre-weaning duration of visits to the creep feeder**.** Group 1 ≤1.04 kg; Group 2 1.06-1.52 kg; Group 3 1.54-1.90 kg; Group 4 ≥1.92 kg. Error bars denote pooled s.e.m

Piglets without a teat to suckle had a greater increase in creep consumption from d20 than suckled piglets (*P* <0.001; Figure 62).

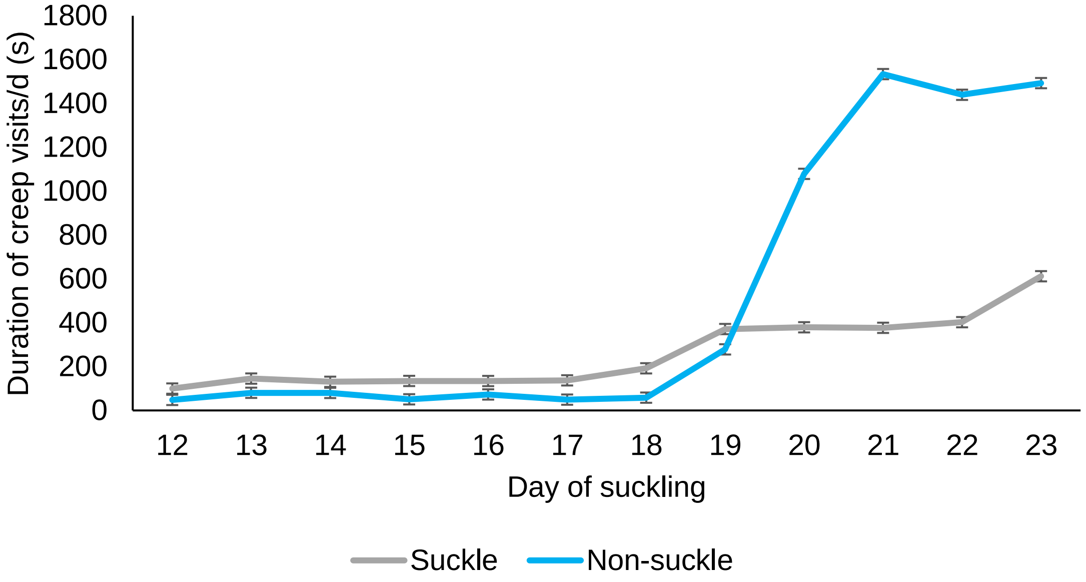

Figure 62: Effect of suckling or not suckling on piglet pre-weaning duration of visits to the creep feeder. Error bars denote pooled s.e.m

The ADG of non-suckling piglets was higher from d19-wean (142 g/d) than from d11-19 (133 g/d), whereas the ADG of suckled piglets was lower from d19-wean (285 g/d) than from d11-19 (320 g/d).

6.3.4. Post-weaning performance

6.3.4.1. Comparison with Study 1 and 2

Pigs were fed differing dietary regimes between the three studies, but overall performance between weaning and d54 post-weaning is presented in Table 60. Although pigs were heavier at weaning in study 3, their post-weaning ADG was lower than pigs in Studies 1 and 2. Average daily feed intake and FCR were highest in Study 1.

Table 60: Comparison of pig performance from weaning to d54 post-weaning between Studies 1, 2 and 3.

	Study 1	Study 2	Study 3
Weight at weaning (kg)	8.70	8.76	9.12
Weight at d54 (kg)	42.6	44.9	43.3
ADFI weaning-d54 (kg/d)	1.17	1.06	0.98
ADG weaning-d54 (g/d)	671	664	603
FCR weaning-d54	1.75	1.59	1.64

6.3.4.2. Overall effect of weaning

Although CL were heavier than CH at weaning, (Table 57), there was no difference in ADG from d19-weaning (*P* >0.05). The negative effect of weaning on ADG was more severe for CL than CH, with a 60 % reduction compared to 36 % reduction in ADG for the first 3 days post-weaning (*P* <0.001; Figure 63).

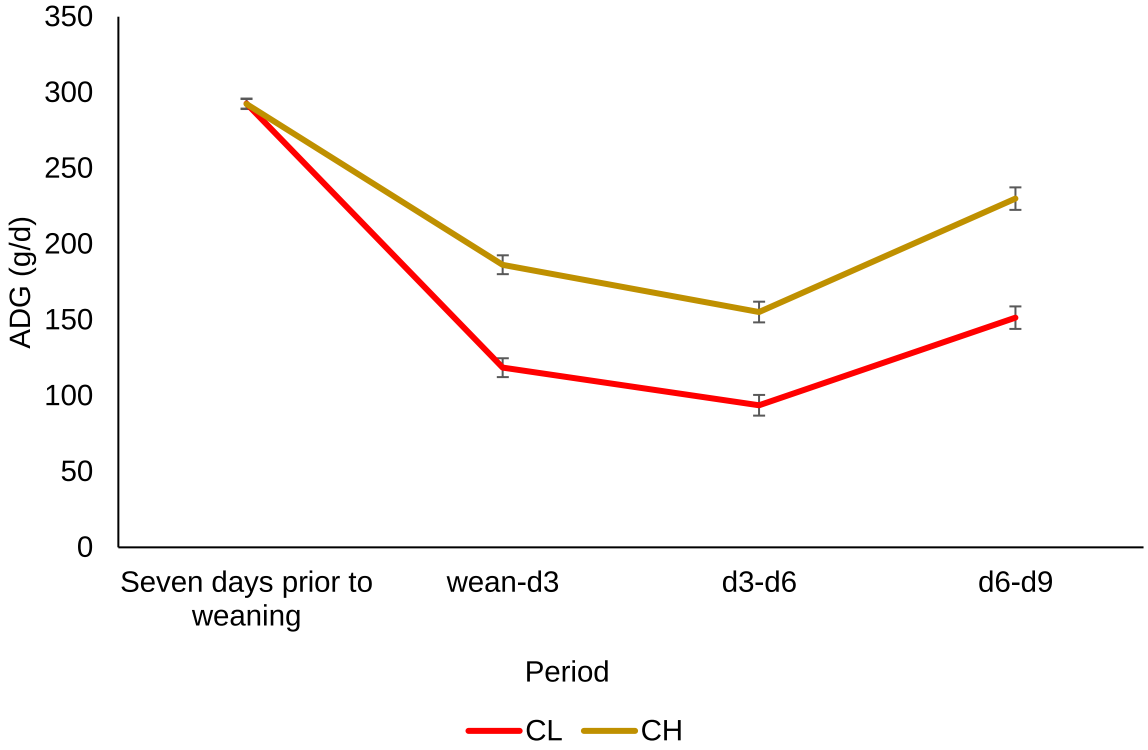

Figure 63: Difference in reduction of ADG post-weaning between piglets in the first quartile (CL) and third quartile (CH) for pre-weaning creep consumption**.** Error bars represent s.e.m. *Denotes significance within day at *P* <0.001.

6.3.4.3. Three-way interaction

From weaning to d54 post-weaning there was no three-way interaction effect of creep*diet specification*diet quality (*P* >0.05) on average weight (Table 61), ADFI (Table 62), ADG (Table 63) or FCR (Table 64). There was a trend for weight of SHQH pigs to be higher in CL pigs than CH pigs at day 3 (*P* =0.085).

Table 61: Effect of low (L) or high (H) pre-weaning creep consumption level (C), post-weaning diet specification (S) and post-weaning diet quality (Q) on pig average weight from weaning to d54 post-weaning.

	CL				CH					
	SL		SH		SL		SH			*P*-value
	QL	QH	QL	QH	QL	QH	QL	QH	s.e.m	C*S*Q
n (pens)	*8*	*8*	*8*	*8*	*8*	*8*	*8*	*8*		
Average creep score (s/kg/d)	*6633*	*6863*	*6587*	*6569*	*14532*	*14618*	*15528*	*14895*		
Weight at weaning (d0; kg)	9.26	9.25	9.28	9.28	8.94	8.96	8.98	8.99	0.022	0.777
Weight d3 (kg)	9.57	9.54	9.78	9.59	9.51	9.41	9.59	9.59	0.053	0.085
Weight d6 (kg)	9.89	9.78	10.15	9.80	9.94	9.84	10.19	10.01	0.073	0.449
Weight d9 (kg)	10.43	10.12	10.74	10.21	10.64	10.41	10.91	10.76	0.106	0.323
Weight d12 (kg)	11.15	10.8	11.59	11.01	11.46	11.27	12.13	11.75	0.166	0.953
Weight d15 (kg)	12.51	11.92	13.01	12.24	12.82	12.49	13.53	13.09	0.219	0.918
Weight d22 (kg)	16.5	15.4	17.0	16.4	16.9	16.0	17.1	17.4	0.34	0.469
Weight d36 (kg)†	26.1	24.7	27.2	25.6	26.4	25.4	27.1	27.5	0.53	0.302
Weight exit (d54; kg) †	43.5	41.1	45.1	43.4	43.5	42.0	44.3	43.5	0.78	0.998

Average creep score: Creep score calculated as duration of visits (s)/ADG (g/d) for the final week pre-weaning. †Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54.

Table 62: Effect of low (L) or high (H) pre-weaning creep consumption level (C), post-weaning diet specification (S) and post-weaning diet quality (Q) on pig ADFI from weaning to d54 post-weaning.

	CL				CH					*P*-value
	SL		SH		SL		SH			
	QL	QH	QL	QH	QL	QH	QL	QH	s.e.m	C*S*Q
n (pens)	*8*	*8*	*8*	*8*	*8*	*8*	*8*	*8*		
Average creep score (s/kg/d)	*6633*	*6863*	*6587*	*6569*	*14532*	*14618*	*15528*	*14895*		
ADFI wean-d3 (kg/d)	0.14	0.14	0.16	0.14	0.19	0.17	0.21	0.2	0.009	0.162
ADFI d3-d6 (kg/d)	0.21	0.20	0.23	0.21	0.26	0.26	0.26	0.24	0.015	0.994
ADFI d6-d9 (kg/d)	0.24	0.23	0.26	0.22	0.29	0.27	0.31	0.29	0.012	0.668
ADFI wean-d9 (kg/d)	**0.20**	**0.19**	**0.22**	**0.19**	**0.25**	**0.23**	**0.26**	**0.24**	**0.009**	**0.515**
ADFI d9-12 (kg/d)	0.36	0.33	0.38	0.33	0.40	0.40	0.45	0.40	0.019	0.374
ADFI d12-15 (kg/d)	0.56	0.54	0.56	0.52	0.58	0.57	0.58	0.59	0.018	0.376
ADFI d15-22 (kg/d)	0.72	0.67	0.67	0.69	0.71	0.64	0.65	0.70	0.022	0.482
ADFI d9-22 (kg/d)	**0.60**	**0.56**	**0.58**	**0.57**	**0.61**	**0.57**	**0.59**	**0.60**	**0.018**	**0.650**
ADFI d22-36 (kg/d) †	1.10	1.04	1.11	1.06	1.08	1.08	1.12	1.15	0.030	0.907
ADFI d36-exit (d54; kg/d) †	1.59	1.52	1.57	1.56	1.60	1.63	1.61	1.57	0.058	0.369
ADFI wean-exit (kg/d) †	0.98	0.93	0.97	0.95	0.99	1.00	1.01	1.00	0.026	0.641

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning.

†Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold.

Table 63: Effect of low (L) or high (H) pre-weaning creep consumption level (C), post-weaning diet specification (S) and post-weaning diet quality (Q) on pig ADG from weaning to d54 post-weaning.

	CL				CH					
	SL		SH		SL		SH			*P*-value
	QL	QH	QL	QH	QL	QH	QL	QH	s.e.m	C*S*Q
n (pens)	*8*	*8*	*8*	*8*	*8*	*8*	*8*	*8*		
Average creep score (s/kg/d)	*6633*	*6863*	*6587*	*6569*	*14532*	*14618*	*15528*	*14895*		
ADG wean-d3 (g/d)	105	97	170	103	189	152	204	201	17.6	0.067
ADG d3-d6 (g/d)	105	78	120	70	144	141	198	138	19.5	0.535
ADG d6-d9 (g/d)	181	114	198	108	235	192	242	251	19.2	0.168
ADG wean-d9 (g/d)	**130**	**97**	**163**	**103**	**189**	**162**	**215**	**197**	**12.1**	**0.308**
ADG d9-12 (g/d)	246	243	293	229	271	293	390	317	34.0	0.728
ADG d12-15 (g/d)	451	374	472	413	450	404	466	448	32.2	0.896
ADG d15-22 (g/d)	576	499	563	586	584	498	506	611	28.8	0.264
ADG d9-22 (g/d)	**471**	**411**	**480**	**464**	**481**	**429**	**470**	**506**	**24.4**	**0.521**
ADG d22-36 (g/d) †	686	660	712	660	681	672	717	725	23.5	0.525
ADG d36-exit (d54; g/d) †	938	886	989	948	923	894	929	862	24.0	0.462
ADG wean-exit (g/d) †	601	570	635	597	609	579	622	606	15.0	0.630

Average creep score: Creep score calculated as duration of visits to the creep feeder(s)/ADG (g/d) for the final week pre-weaning.

†Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold.

Table 64: Effect of low (L) or high (H) pre-weaning creep consumption level (C), post-weaning diet specification (S) and post-weaning diet quality (Q) on pig FCR from weaning to d54 post-weaning

	CL				CH					
	SL		SH		SL		SH			*P*-value
	QL	QH	QL	QH	QL	QH	QL	QH	s.e.m	C*S*Q
n (pens)	*8*	*8*	*8*	*8*	*8*	*8*	*8*	*8*		
Average creep score (s/kg/d)	*6633*	*6863*	*6587*	*6569*	*14532*	*14618*	*15528*	*14895*		
FCR wean-d3*	1.47	1.46	0.99	1.66	1.09	1.20	1.05	1.11		0.106
FCR d3-d6*	2.56	2.41	1.98	3.10	2.14	1.93	1.31	1.92		0.951
FCR d6-d9*	1.48	2.45	1.39	2.50	1.27	1.44	1.32	1.17		0.211
FCR wean-d9	**1.68**	**2.16**	**1.38**	**1.97**	**1.32**	**1.47**	**1.22**	**1.26**	**0.123**	**0.514**
FCR d9-12*	1.62	1.58	1.38	1.58	1.39	1.44	1.30	1.33		0.521
FCR d12-15	1.27	1.35	1.23	1.29	1.28	1.50	1.24	1.32	0.071	0.531
FCR d15-22	1.25	1.37	1.21	1.22	1.22	1.30	1.31	1.15	0.056	0.372
FCR d9-22	**1.28**	**1.40**	**1.22**	**1.26**	**1.27**	**1.34**	**1.27**	**1.19**	**0.052**	**0.652**
FCR d22-36 †	1.61	1.58	1.58	1.61	1.59	1.61	1.58	1.59	0.033	0.420
FCR d36-exit (d54) †	1.69	1.71	1.71	1.65	1.74	1.83	1.74	1.84	0.050	0.538
FCR wean-exit †	1.64	1.65	1.60	1.60	1.63	1.72	1.62	1.65	0.030	0.634

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. †Experimerntal diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. *Required log 10 transformation, actual means presented without s.e.m due to non-normal distribution.

6.3.4.4. Two-way interaction

6.3.4.4.1. Pre-weaning creep consumption*diet specification

From weaning-d54 post-weaning there was no creep*diet specification interaction effect (*P* >0.05) on average weight or ADFI (Table 65).

Table 65: Effect of low (L) or high (H) pre-weaning creep consumption level (C) and post-weaning diet specification (S) on pig average weight and ADFI from weaning to d54 post-weaning.

	CL		CH			*P*-value
	SL	SH	SL	SH	s.e.m	C*S
n (pens)	*16*	*16*	*16*	*16*		
Average creep score (s/kg/d)	*6748*	*6578*	*14575*	*15211*		
Weight at weaning (d0; kg)	9.26	9.28	8.95	8.99	0.015	0.627
Weight d3 (kg)	9.56	9.69	9.46	9.59	0.038	0.962
Weight d6 (kg)	9.83	9.97	9.89	10.1	0.052	0.502
Weight d9 (kg)	10.28	10.47	10.53	10.84	0.075	0.466
Weight d12 (kg)	10.98	11.3	11.37	11.94	0.116	0.289
Weight d15 (kg)	12.21	12.62	12.65	13.31	0.154	0.426
Weight d22 (kg)	15.98	16.65	16.44	17.22	0.242	0.820
Weight d36 (kg) †	25.4	26.38	25.91	27.31	0.377	0.583
Weight exit (d54; kg) †	42.3	44.2	42.7	43.9	0.55	0.466
ADFI wean-d3 (kg/d)	0.14	0.15	0.18	0.2	0.006	0.538
ADFI d3-d6 (kg/d)	0.21	0.22	0.26	0.25	0.011	0.302
ADFI d6-d9 (kg/d)	0.24	0.24	0.28	0.3	0.009	0.266
ADFI wean-d9 (kg/d)	**0.19**	**0.20**	**0.24**	**0.25**	**0.006**	**0.900**
ADFI d9-12 (kg/d)	0.35	0.36	0.40	0.42	0.014	0.631
ADFI d12-15 (kg/d)	0.55	0.54	0.57	0.58	0.012	0.572
ADFI d15-22 (kg/d)	0.69	0.68	0.67	0.67	0.015	0.728
ADFI d9-d22 (kg/d)	**0.58**	**0.57**	**0.59**	**0.60**	**0.012**	**0.624**
ADFI d22-36 (kg/d) †	1.07	1.08	1.08	1.14	0.021	0.376
ADFI d36-exit (kg/d) †	1.55	1.57	1.62	1.59	0.041	0.617
ADFI wean-exit (kg/d) †	0.96	0.96	0.99	1.00	0.019	0.866

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. **†**Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold.

From d36-exit, ADG was higher in CL pigs when they were previously fed SH diets than CH pigs that were previously fed SH diets (*P* =0.043; Table 66). There were no other creep*diet specification interactions (*P* >0.05).

Table 66: Effect of low (L) or high (H) pre-weaning creep consumption level (C) and post-weaning diet specification (S) on pig ADG and FCR from weaning to d54 post-weaning.

	CL		CH			*P*-value
	SL	SH	SL	SH	s.e.m	C*S
n (pens)	*16*	*16*	*16*	*16*		
Average creep score (s/kg/d)	*6748*	*6578*	*14575*	*15211*		
ADG wean-d3 (g/d)	101	136	170	202	12.7	0.881
ADG d3-d6 (g/d)	92	95	142	168	13.7	0.424
ADG d6-d9 (g/d)	147	153	214	247	13.7	0.314
ADG wean-d9 (g/d)	**113**	**128**	**175**	**206**	**8.9**	**0.391**
ADG d9-12 (g/d)	245	261	282	353	23.9	0.256
ADG d12-15 (g/d)	412	443	427	457	22.6	0.987
ADG d15-22 (g/d)	538	575	541	559	20.4	0.635
ADG d9-22 (g/d)	**441**	**472**	**455**	**488**	**17.2**	**0.954**
ADG d22-36 (g/d) †	673	686	676	721	16.6	0.349
ADG d36-exit (d54; g/d) †	912^{ab}	968^{a}	909^{ab}	895^{b}	16.9	0.043
ADG wean-exit (g/d) †	586	616	594	614	10.5	0.637
FCR wean-d3*	1.47	1.32	1.14	1.08		0.769
FCR d3-d6*	2.49	2.54	2.04	1.61		0.103
FCR d6-d9*	1.96	1.94	1.36	1.25		0.579
FCR wean-d9	**1.92**	**1.68**	**1.42**	**1.24**	**0.087**	**0.632**
FCR d9-12*	1.60	1.48	1.42	1.32		0.827
FCR d12-15	1.31	1.26	1.39	1.28	0.050	0.562
FCR d15-22	1.31	1.21	1.26	1.23	0.039	0.391
FCR d9-22	**1.34**	**1.24**	**1.30**	**1.23**	**0.037**	**0.677**
FCR d22-36 †	1.60	1.59	1.60	1.58	0.023	0.773
FCR d36-exit †	1.70	1.68	1.78	1.79	0.035	0.687
FCR wean-exit †	1.64	1.6	1.68	1.64	0.021	0.940

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. **†**Performance for each experimental dietary stage is in bold. *Required log10 transformation, actual means presented without s.e.m due to non-normal distribution. Superscripts denote significance at *P* <0.05.

6.3.4.4.2. Pre-weaning creep consumption*diet quality

From weaning-d54 post-weaning there was no creep*diet quality interaction effect (*P* >0.05) on average weight or ADFI (P >0.05; Table 67).

Table 67: Effect of pre-weaning low (L) or high (H) creep consumption level (C) and post-weaning diet quality (Q) on pig average weight and ADFI from weaning to d54 post-weaning.

	CL		CH			*P*-value
	QL	QH	QL	QH	s.e.m	C*Q
n (pens)	*16*	*16*	*16*	*16*		
Average creep score (s/kg/d)	*6610*	*6716*	*15030*	*14756*		
Weight at weaning (d0; kg)	9.27	9.27	8.96	8.97	0.015	0.675
Weight d3 (kg)	9.68	9.57	9.55	9.5	0.038	0.394
Weight d6 (kg)	10.02	9.79	10.06	9.92	0.052	0.412
Weight d9 (kg)	10.58	10.17	10.78	10.59	0.075	0.135
Weight d12 (kg)	11.37	10.9	11.8	11.51	0.116	0.435
Weight d15 (kg)	12.76	12.08	13.17	12.79	0.154	0.349
Weight d22 (kg)	16.74	15.88	16.99	16.67	0.242	0.264
Weight d36 (kg) †	26.7	25.1	26.8	26.5	0.38	0.114
Weight exit (d54; kg) †	44.3	42.2	43.9	42.7	0.55	0.435
ADFI wean-d3 (kg/d)	0.15	0.14	0.20	0.19	0.006	0.817
ADFI d3-d6 (kg/d)	0.22	0.21	0.26	0.25	0.011	0.820
ADFI d6-d9 (kg/d)	0.25	0.22	0.30	0.28	0.009	0.588
ADFI wean-d9 (g/d)	**0.21**	**0.19**	**0.25**	**0.24**	**0.006**	**0.650**
ADFI d9-12 (kg/d)	0.37	0.33	0.42	0.4	0.014	0.518
ADFI d12-15 (kg/d)	0.56	0.53	0.58	0.58	0.012	0.196
ADFI d15-22 (kg/d)	0.69	0.68	0.68	0.67	0.015	0.879
ADFI d9-22 (kg/d)	**0.59**	**0.56**	**0.60**	**0.59**	**0.012**	**0.567**
ADFI d22-36 (kg/d) †	1.10	1.05	1.10	1.12	0.021	0.106
ADFI d36-exit (kg/d) †	1.58	1.54	1.61	1.6	0.041	0.701
ADFI wean-exit (kg/d) †	0.98	0.94	1.00	1.00	0.019	0.372

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. **†**Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold.

From d6-9, CL pigs fed QH had lower ADG and higher FCR than the other treatments (*P* <0.05; Table 68). This was due to 19 of the 96 pigs losing weight during this period, compared to 12 CLQL, three CHQL and two CHQH pigs that lost weight. This also resulted in FCR for the period wean-d9 (stage 1 diet) being higher in pigs fed SLQH than other regimes (*P* =0.014).

Table 68: Effect of low (L) or high (H) pre-weaning creep consumption level (C) and post-weaning diet quality (Q) on pig ADG and FCR from weaning to d54 post-weaning.

	CL		CH			*P*-value
	QL	QH	QL	QH	s.e.m	C*Q
n (pens)	*16*	*16*	*16*	*16*		
Average creep score (s/kg/d)	*6610*	*6716*	*15030*	*14756*		
ADG wean-d3 (g/d)	137	100	196	176	12.7	0.498
ADG d3-d6 (g/d)	113	74	171	139	13.7	0.806
ADG d6-d9 (g/d)	189^{a}	111^{b}	238^{a}	222^{a}	13.7	0.038
ADG wean-d9 (g/d)	**146**	**95**	**202**	**179**	**8.9**	**0.112**
ADG d9-12 (g/d)	270	236	330	305	23.9	0.855
ADG d12-15 (g/d)	462	394	458	426	22.6	0.431
ADG d15-22 (g/d)	569	546	545	555	20.4	0.376
ADG d9-22 (g/d)	**475**	**438**	**475**	**467**	**17.2**	**0.387**
ADG d22-36 (g/d) †	699	660	699	698	16.6	0.256
ADG d36-exit (d54; g/d) †	963	917	926	878	16.9	0.972
ADG wean-exit (g/d) †	618	583	616	593	10.5	0.568
FCR wean-d3*	1.23	1.57	1.07	1.15		0.140
FCR d3-d6*	2.27	2.76	1.73	1.92		0.641
FCR d6-d9*	1.43^{a}	2.47^{b}	1.30^{a}	1.31^{a}		0.002
FCR wean-d9	**1.53^{a}**	**2.06^{b}**	**1.27^{a}**	**1.36^{a}**	**0.087**	**0.014**
FCR d9-12*	1.50	1.58	1.35	1.38		0.905
FCR d12-15	1.25	1.32	1.26	1.41	0.050	0.445
FCR d15-22	1.23	1.29	1.26	1.22	0.039	0.186
FCR d9-22	**1.25**	**1.33**	**1.27**	**1.27**	**0.037**	**0.238**
FCR d22-36 †	1.59	1.60	1.58	1.60	0.023	0.747
FCR d36-exit †	1.70	1.68	1.74	1.83	0.035	0.101
FCR wean-exit †	1.62	1.62	1.63	1.68	0.021	0.213

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. †Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. *Required log10 transformation, actual means presented without s.e.m due to non-normal distribution. Superscripts denote significance at $P < 0.05$.

6.3.4.4.3. Diet specification*diet quality

At d22 post-weaning there was a trend (*P* =0.074) for SLQH pigs to be lighter than SLQL pigs, but SHQL and SHQH pigs were more similar in weight (Table 69). This was a result of the ADFI of SLQH pigs being lower than the other treatments for this period (*P* =0.005).

Table 69: Interactive effect of low (L) or high (H) post-weaning diet specification (S) and diet quality (Q) on pig average weight and ADFI from weaning to d54 post-weaning.

	SL		SH			*P*-value
	QL	QH	QL	QH	s.e.m	S*Q
n (pens)	*16*	*16*	*16*	*16*		
Average creep score (s/kg/d)	*10583*	*10741*	*11057*	*10732*		
Weight at weaning (d0; kg)	9.10	9.10	9.13	9.14	0.015	0.880
Weight d3 (kg)	9.54	9.48	9.69	9.59	0.038	0.657
Weight d6 (kg)	9.91	9.81	10.17	9.90	0.052	0.139
Weight d9 (kg)	10.54	10.27	10.83	10.49	0.075	0.645
Weight d12 (kg)	11.31	11.03	11.86	11.38	0.116	0.371
Weight d15 (kg)	12.66	12.20	13.27	12.67	0.154	0.648
Weight d22 (kg)	16.72	15.69	17.01	16.86	0.242	0.074
Weight d36 (kg)[†]	26.3	25.0	27.1	26.6	0.38	0.366
Weight exit (d54; kg)[†]	43.5	41.5	44.7	43.4	0.55	0.503
ADFI wean-d3 (kg/d)	0.17	0.15	0.19	0.17	0.006	0.817
ADFI d3-d6 (kg/d)	0.24	0.23	0.25	0.23	0.011	0.597
ADFI d6-d9 (kg/d)	0.27	0.25	0.29	0.25	0.009	0.323
ADFI wean-d9 (kg/d)	**0.22**	**0.21**	**0.24**	**0.22**	**0.006**	**0.412**
ADFI d9-12 (kg/d)	0.38	0.37	0.41	0.37	0.014	0.276
ADFI d12-15 (kg/d)	0.57	0.55	0.57	0.55	0.012	0.899
ADFI d15-22 (kg/d)	0.71^{a}	0.65^{b}	0.66^{ab}	0.69^{ab}	0.015	0.005
ADFI d9-22 (kg/d)	**0.60**	**0.56**	**0.58**	**0.59**	**0.012**	**0.105**
ADFI d22-36 (kg/d)[†]	1.09	1.06	1.12	1.10	0.021	0.805
ADFI d36-exit (kg/d)[†]	1.60	1.58	1.59	1.57	0.041	0.957
ADFI wean-exit (kg/d)[†]	0.99	0.96	0.99	0.97	0.019	0.845

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. [†]Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. Superscripts denote significance at *P* <0.05.

Due to ADFI being low, intake of the majority of essential amino acids was lowest in SLQH from weaning-d22 (Figure 64).

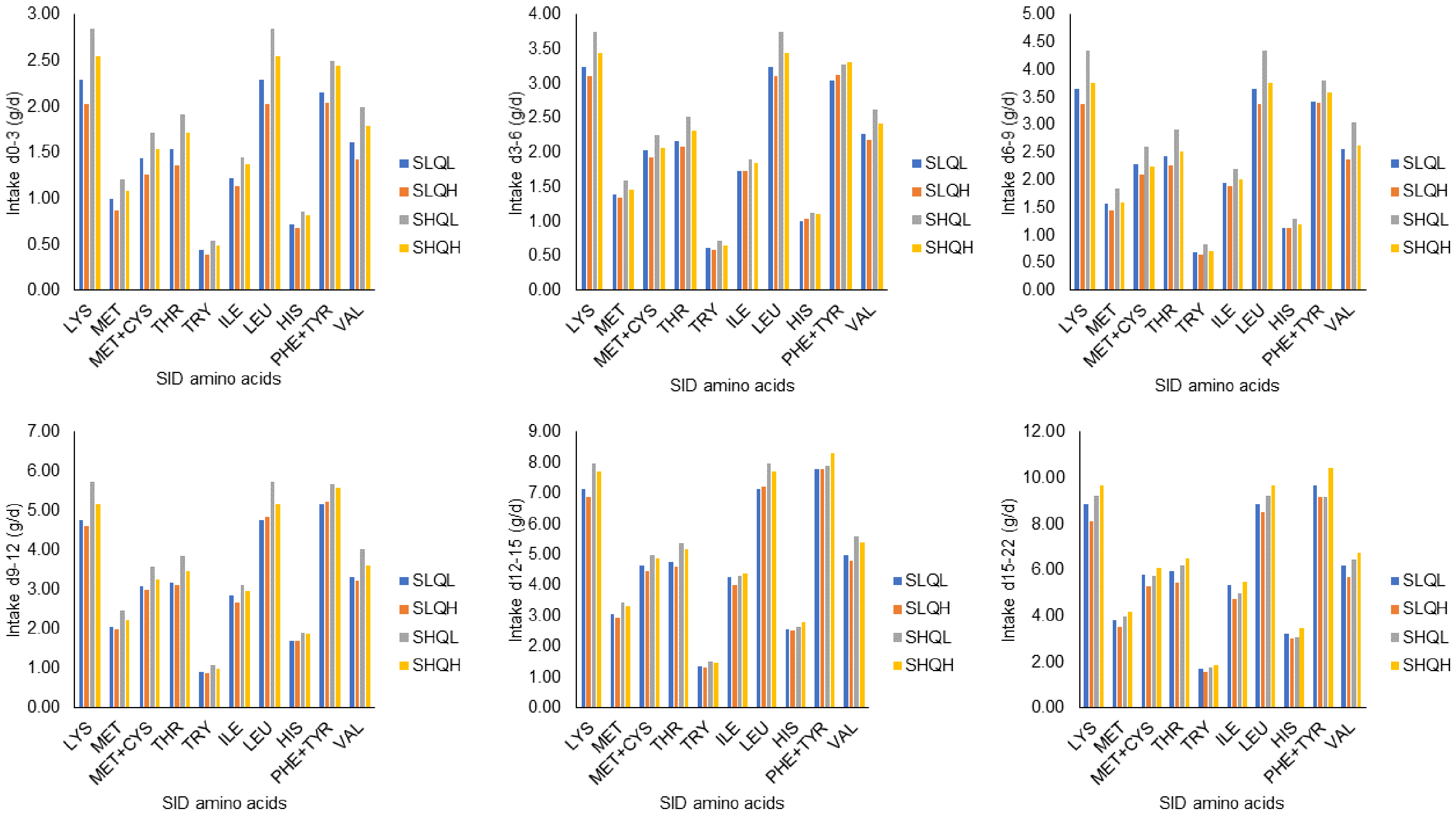

Figure 64: Average daily intake of essential amino acids for pigs fed post-weaning diets with low (L) or high (H) specification (S) or quality (Q) to d22 post-weaning.

Although there were no other diet specification*diet quality effects on average weight, pigs fed SH had similar weight regardless of diet quality, but for those fed SL, the difference in weight between those fed QL and QH increased over time (*P* =0.014, antedependence order 1; Figure 65). Pigs fed SLQL and SHQH were nearly identical in weight throughout.

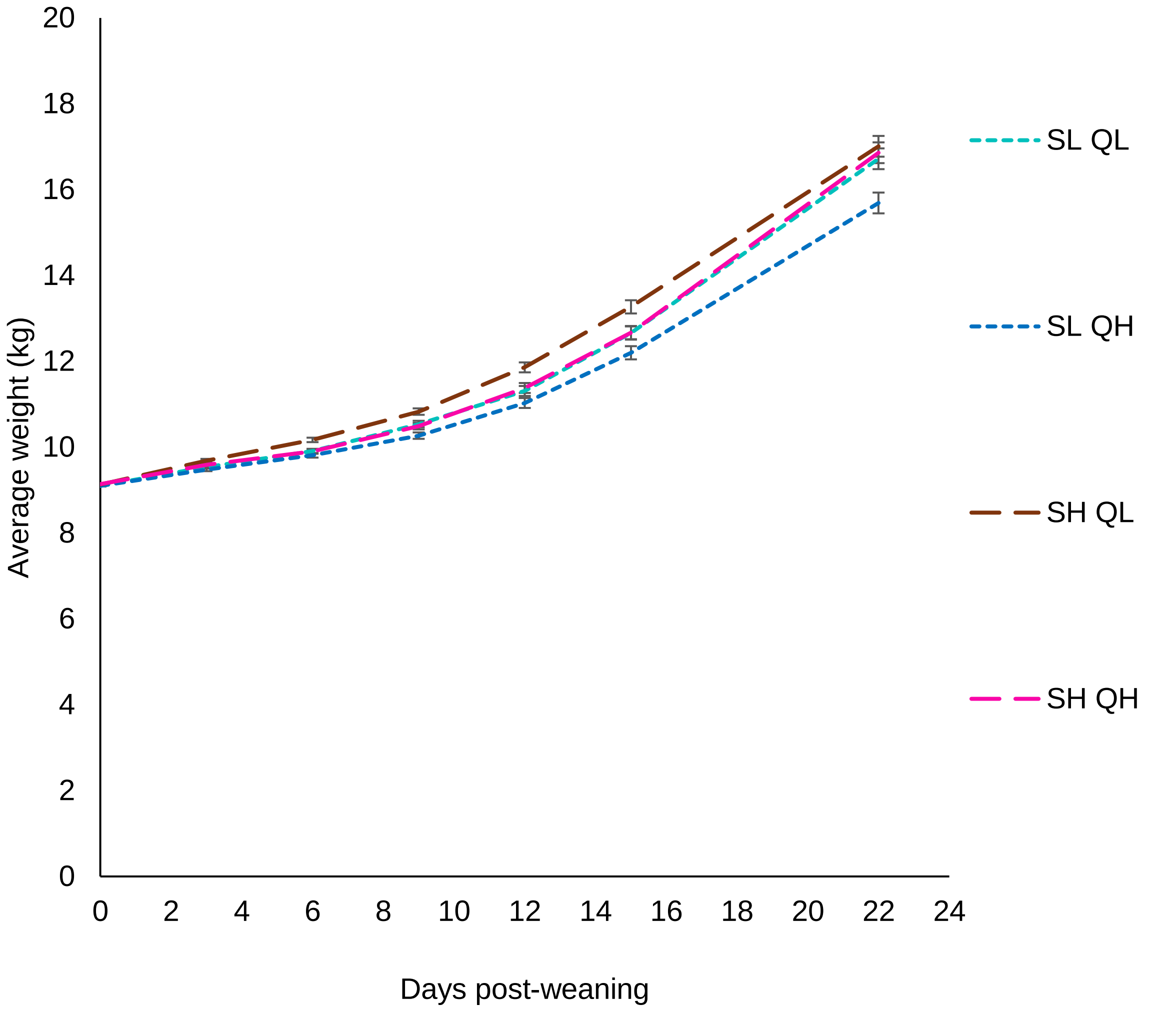

Figure 65: Change in effect of low (L) or high (H) post-weaning dietary specification (S)*diet quality (Q) on pig average weight from weaning to d22 post-weaning. Error bars represent pooled s.e.m.

The lower ADFI from d15-22 of SLQH pigs reported in Table 69, and higher FCR (*P* =0.024; Table 70) resulted in lower ADG for these pigs for this period, and a trend for lower ADG for d9-22 (stage 2 diet; *P* =0.061). From d3-6, pigs fed SHQL had lower FCR than those fed SHQH, with SLQL and SLQH intermediate (*P* <0.001).

Table 70: Interactive effect of low (L) or high (H) post-weaning diet specification (S) and diet quality (Q) on pig ADG and FCR from weaning to d54 post-weaning.

	SL		SH			*P*-value
	QL	QH	QL	QH	s.e.m	S*Q
n (pens)	*16*	*16*	*16*	*16*		
Average creep score (s/kg/d)	*10583*	*10741*	*11057*	*10732*		
ADG wean-d3 (g/d)	147	124	187	152	12.7	0.615
ADG d3-d6 (g/d)	124	110	159	104	13.7	0.145
ADG d6-d9 (g/d)	208	153	220	180	13.7	0.603
ADG wean-d9 (g/d)	**160**	**129**	**189**	**145**	**8.9**	**0.466**
ADG d9-12 (g/d)	258	268	342	273	23.9	0.104
ADG d12-15 (g/d)	451	389	469	431	22.6	0.614
ADG d15-22 (g/d)	580^{a}	499^{b}	534^{ab}	599^{a}	20.4	<0.001
ADG d9-22 (g/d)	**476**	**420**	**475**	**485**	**17.2**	**0.061**
ADG d22-36 (g/d) †	683	666	714	692	16.6	0.896
ADG d36-exit (d54; g/d) †	931	890	959	905	16.9	0.676
ADG wean-exit (g/d) †	605	575	629	602	10.5	0.857
FCR wean-d3*	1.27	1.34	1.02	1.38		0.300
FCR d3-d6*	2.35^{ab}	2.17^{ab}	1.65^{a}	2.51^{b}		0.024
FCR d6-d9*	1.38	1.95	1.35	1.84		0.575
FCR wean-d9	**1.50**	**1.81**	**1.30**	**1.61**	**0.087**	**0.984**
FCR d9-12*	1.51	1.51	1.34	1.46		0.408
FCR d12-15	1.27	1.43	1.24	1.30	0.050	0.381
FCR d15-22	1.24^{ab}	1.33^{a}	1.26^{ab}	1.18^{b}	0.039	0.032
FCR d9-22	**1.27**	**1.37**	**1.24**	**1.23**	**0.037**	**0.124**
FCR d22-36 †	1.60	1.60	1.58	1.60	0.023	0.612
FCR d36-exit †	1.72	1.77	1.73	1.74	0.035	0.599
FCR wean-exit †	1.64	1.68	1.61	1.62	0.021	0.415

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. **†**Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. *Required log10 transformation, actual means presented without s.e.m due to non-normal distribution. Superscripts denote significance at *P* <0.05.

The effect of diet specification*diet quality on ADG varied over time (*P* =0.024, antedependence order 1 with additional uniform correlations within subject; Figure 66).

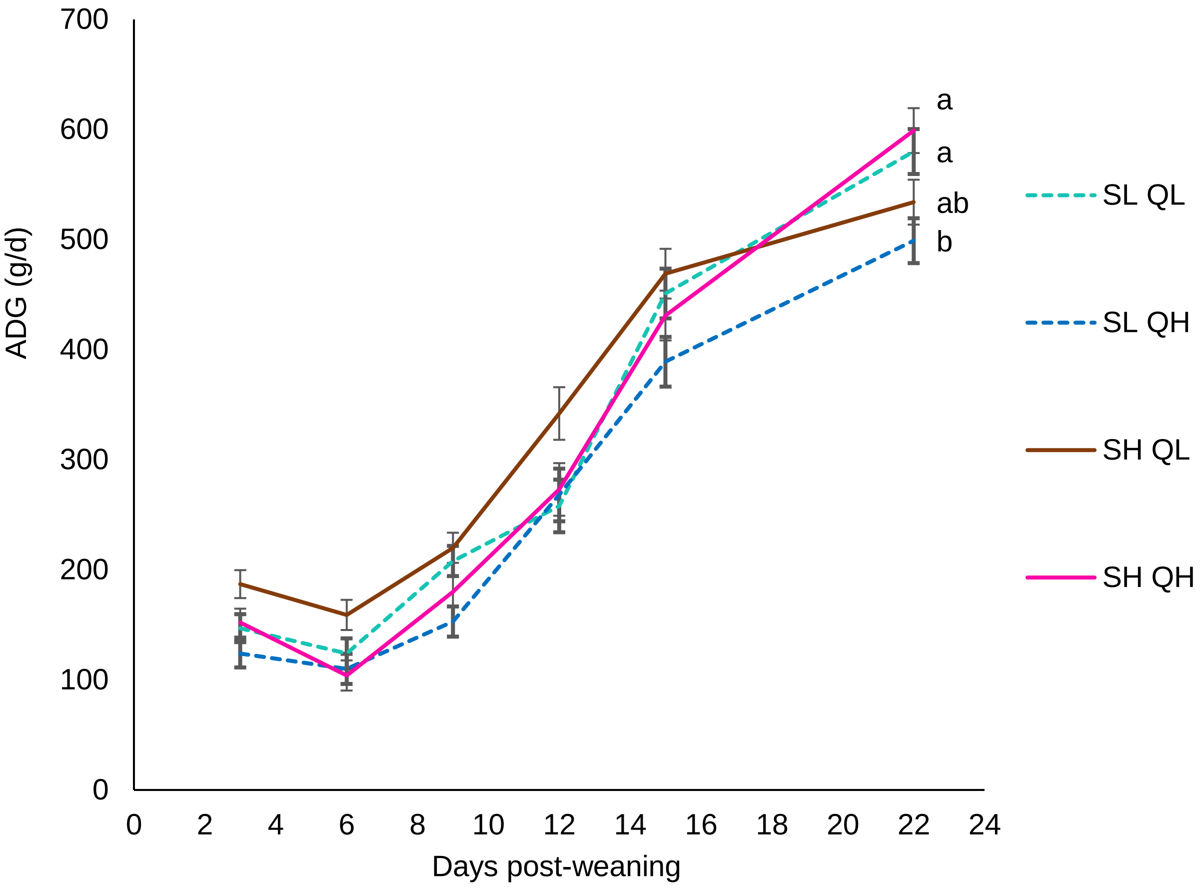

Figure 66: Change in effect of low (L) or high (H) post-weaning dietary specification (S)*diet quality (Q) on pig ADG from weaning to d22 post-weaning**.** Error bars represent s.e.m. Superscripts denote significance at $P < 0.05$.

A meta-analysis by Rocha *et al.* (2023) identified that the ratio of SID lysine:CP (the proportion of SID lysine in the CP content of the diet) may be a better indicator of performance than either lysine or CP level considered separately . As CP level was different in each diet for stage 1, the diets were analysed separately (not as a factorial). Table 71 displays the weight and ADFI of each diet analysed in this way, with the diets displayed in ascending order of SID lysine:CP. From d6, pigs fed SHQL were heaviest, and pigs fed SLQH were lightest ($P < 0.05$).

Table 71: Effect of low (L) or high (H) post-weaning diet specification (S) and diet quality (Q) ordered by SID lysine:CP ratio of the diet on pig average weight and ADFI from weaning to d54 post-weaning.

	SLQH	SLQL	SHQH	SHQL	s.e.m	*P*-value
n (pens)	*16*	*16*	*16*	*16*		
Average creep score (s/kg/d)	*10741*	*10583*	*10732*	*11057*		
Stage 1 SIDlys:CP (%)	*6.57*	*6.87*	*6.93*	*7.34*		
Stage 2 SIDlys:CP (%)	*6.07*	*6.23*	*6.40*	*6.79*		
Weight at weaning (d0; kg)	9.10	9.10	9.14	9.13	0.048	0.938
Weight d3 (kg)	9.48	9.54	9.59	9.69	0.062	0.116
Weight d6 (kg)	9.81^{a}	9.91^{a}	9.90^{a}	10.17^{b}	0.059	<0.001
Weight d9 (kg)	10.27^{a}	10.54^{ab}	10.49^{a}	10.83^{b}	0.087	<0.001
Weight d12 (kg)	11.03^{a}	11.31^{ab}	11.38^{ab}	11.86^{b}	0.145	0.002
Weight d15 (kg)	12.2^{a}	12.66^{ab}	12.67^{ab}	13.27^{b}	0.177	0.001
Weight d22 (kg)	15.69^{a}	16.72^{b}	16.86^{b}	17.01^{b}	0.263	0.003
Weight d36 (kg) †	25.02^{a}	26.29^{ab}	26.55^{b}	27.14^{b}	0.391	0.003
Weight exit (d54; kg) †	41.5^{a}	43.5^{ab}	43.4^{ab}	44.4^{b}	0.67	0.025
ADFI wean-d3 (kg/d)	0.15	0.17	0.17	0.19	0.01	0.175
ADFI d3-d6 (kg/d)	0.23	0.24	0.23	0.25	0.012	0.632
ADFI d6-d9 (kg/d)	0.25	0.27	0.25	0.29	0.011	0.103
ADFI wean-d9 (kg/d)	0.21	0.22	0.22	0.24	0.009	0.149
ADFI d9-12 (kg/d)	0.37	0.38	0.37	0.41	0.016	0.134
ADFI d12-15 (kg/d)	0.55	0.57	0.55	0.57	0.018	0.899
ADFI d15-22 (kg/d)	0.65^{a}	0.71^{b}	0.69^{ab}	0.66^{a}	0.015	0.029
ADFI d9-22 (kg/d)	0.56	0.60	0.58	0.59	0.012	0.205
ADFI d22-36 (kg/d) †	1.06	1.09	1.10	1.12	0.024	0.417
ADFI d36-exit (kg/d) †	1.58	1.60	1.57	1.59	0.045	0.961
ADFI wean-exit (kg/d) †	0.96	0.99	0.97	0.99	0.020	0.752

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. †Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. Superscripts denote significance at $P < 0.05$.

Similar to the interaction analysis, the only period in which ADFI differed between diets was from d15-22, when pigs fed SLQL had higher ADFI than pigs fed SLQH and SHQL ($P = 0.029$). As shown in Table 72, during the periods d6-d9 and d15-d22, pigs fed SHQL had the highest ADG with pigs fed SLQH the lowest ($P < 0.05$), with the same result for each feeding period (weaning-d9 and d9-22). For these periods overall, FCR was lowest in pigs fed SHQL and highest in pigs fed SLQH (*P* <0.05).

Table 72: Effect of low (L) or high (H) post-weaning diet specification (S) and diet quality (Q) ordered by SID lysine:CP ratio of the diet on pig ADG and FCR from weaning to d54 post-weaning.

	SLQH	SLQL	SHQH	SHQL	s.e.m	*P*-value
n (pens)	*16*	*16*	*16*	*16*		
Average creep score (s/kg/d)	*10741*	*10583*	*10732*	*11057*		
Stage 1 SIDlys:CP (%)	*6.57*	*6.87*	*6.93*	*7.34*		
Stage 2 SIDlys:CP (%)	*6.07*	*6.23*	*6.40*	*6.79*		
ADG wean-d3 (g/d)	124	147	152	187	17.2	0.091
ADG d3-d6 (g/d)	110	124	104	159	16.8	0.100
ADG d6-d9 (g/d)	153^{a}	208^{b}	180^{ab}	220^{b}	17.4	0.041
ADG wean-d9 (g/d)	129^{a}	160^{ab}	153^{ab}	189^{b}	12.1	0.011
ADG d9-12 (g/d)	268	258	273	342	27.1	0.127
ADG d12-15 (g/d)	389	451	431	469	22.0	0.075
ADG d15-22 (g/d)	499^{a}	580^{b}	599^{b}	534^{ab}	20.5	0.004
ADG d9-22 (g/d)	420^{a}	476^{b}	475^{b}	485^{b}	16.9	0.037
ADG d22-36 (g/d) †	666	683	692	714	20.5	0.419
ADG d36-exit (d54; g/d) †	890	931	905	954	21.8	0.183
ADG wean-exit (g/d) †	575^{a}	605^{ab}	602^{ab}	628^{b}	10.2	0.006
FCR wean-d3	1.31	1.12	1.28	1.02	0.086	0.065
FCR d3-d6	2.08	2.31	2.51	1.60	0.260	0.089
FCR d6-d9	1.95	1.38	1.84	1.35	0.189	0.058
FCR wean-d9	1.81^{a}	1.50^{ab}	1.60^{ab}	1.30^{b}	0.111	0.017
FCR d9-12	1.51	1.50	1.46	1.34	0.031	0.572
FCR d12-15	1.42	1.27	1.30	1.24	0.057	0.122
FCR d15-22	1.33	1.24	1.18	1.26	0.041	0.080
FCR d9-22	1.37^{a}	1.27^{ab}	1.24^{ab}	1.23^{b}	0.036	0.036
FCR d22-36 †	1.60	1.60	1.60	1.58	0.026	0.894
FCR d36-exit †	1.77	1.72	1.74	1.73	0.037	0.744
FCR wean-exit †	1.68	1.64	1.62	1.61	0.026	0.256

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. †Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. Superscripts denote significance at *P* <0.05.

6.3.4.5. Main effects

6.3.4.5.1. Pre-weaning creep consumption

At weaning and d3 post-weaning, pigs with high pre-weaning creep consumption were lighter than those with low pre-weaning creep consumption (*P* <0.001 and *P* =0.013 respectively; Table 73).

Table 73: Effect of low (L) or high (H) pre-weaning creep consumption level (C) on pig average weight and ADFI from weaning to d54 post-weaning.

	CL	CH	s.e.m	*P*-value C
n (pens)	*32*	*32*		
Average creep score (s/kg/d)	*6663*	*14893*		
Weight at weaning (d0; kg)	9.27	8.97	0.011	<0.001
Weight d3 (kg)	9.62	9.53	0.026	0.013
Weight d6 (kg)	9.90	9.99	0.037	0.091
Weight d9 (kg)	10.37	10.68	0.053	<0.001
Weight d12 (kg)	11.14	11.65	0.082	<0.001
Weight d15 (kg)	12.42	12.98	0.107	<0.001
Weight d22 (kg)	16.31	16.83	0.173	0.039
Weight d36 (kg) †	25.9	26.6	0.27	0.063
Weight exit (d54; kg) †	43.3	43.3	0.38	0.899
ADFI wean-d3 (kg/d)	0.15	0.19	0.004	<0.001
ADFI d3-d6 (kg/d)	0.22	0.26	0.008	<0.001
ADFI d6-d9 (kg/d)	0.24	0.29	0.006	<0.001
ADFI wean-d9 (kg/d)	**0.20**	**0.25**	**0.004**	**<0.001**
ADFI d9-12 (kg/d)	0.35	0.41	0.010	<0.001
ADFI d12-15 (kg/d)	0.55	0.58	0.009	0.013
ADFI d15-22 (kg/d)	0.69	0.67	0.011	0.456
ADFI d9-d22 (kg/d)	**0.58**	**0.59**	**0.009**	**0.229**
ADFI d22-36 (kg/d) †	1.08	1.11	0.015	0.143
ADFI d36-exit (kg/d) †	1.56	1.60	0.028	0.258
ADFI wean-exit (kg/d) †	0.96	1.00	0.013	0.039

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. **†**Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold.

From weaning to d22 post-weaning, CH pigs were heavier than CL pigs (*P* <0.05, except for d6, *P* =0.091; Figure 67). The effect of pre-weaning creep consumption on weight decreased over time (antedependence order 1; *P* <0.001): CH pigs tended to be heavier at d36 (P =0.063), but by d54, there was no effect of pre-weaning creep consumption level on average weight (*P* >0.05).

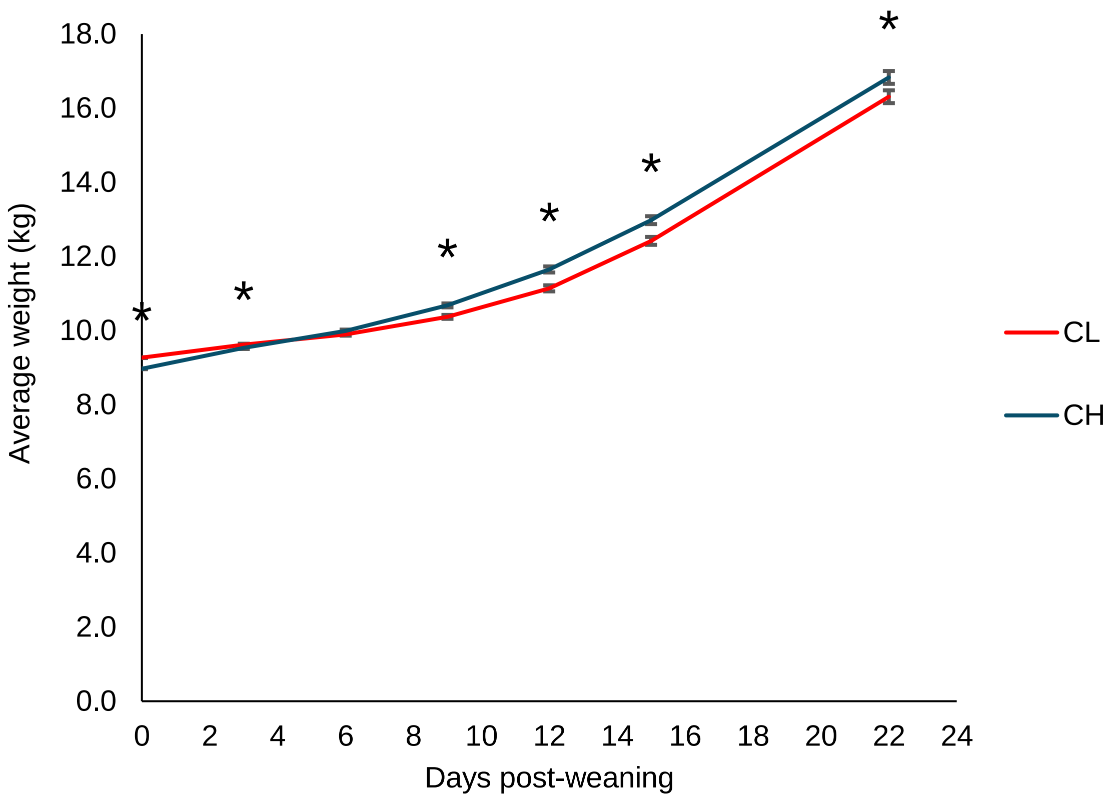

Figure 67: Change in effect of low (L) or high (H) pre-weaning creep consumption (C) level on pig average weight from weaning to d22 post-weaning. Error bars represent s.e.m. * Denotes significance within day at P <0.050.

Average daily food intake was higher in CH than CL from weaning to d15 (P< 0.05; Figure 68), and overall from weaning to exit (*P* <0.05). The effect of pre-weaning creep consumption on ADFI reduced over time (P =0.012, antedependence order 1).

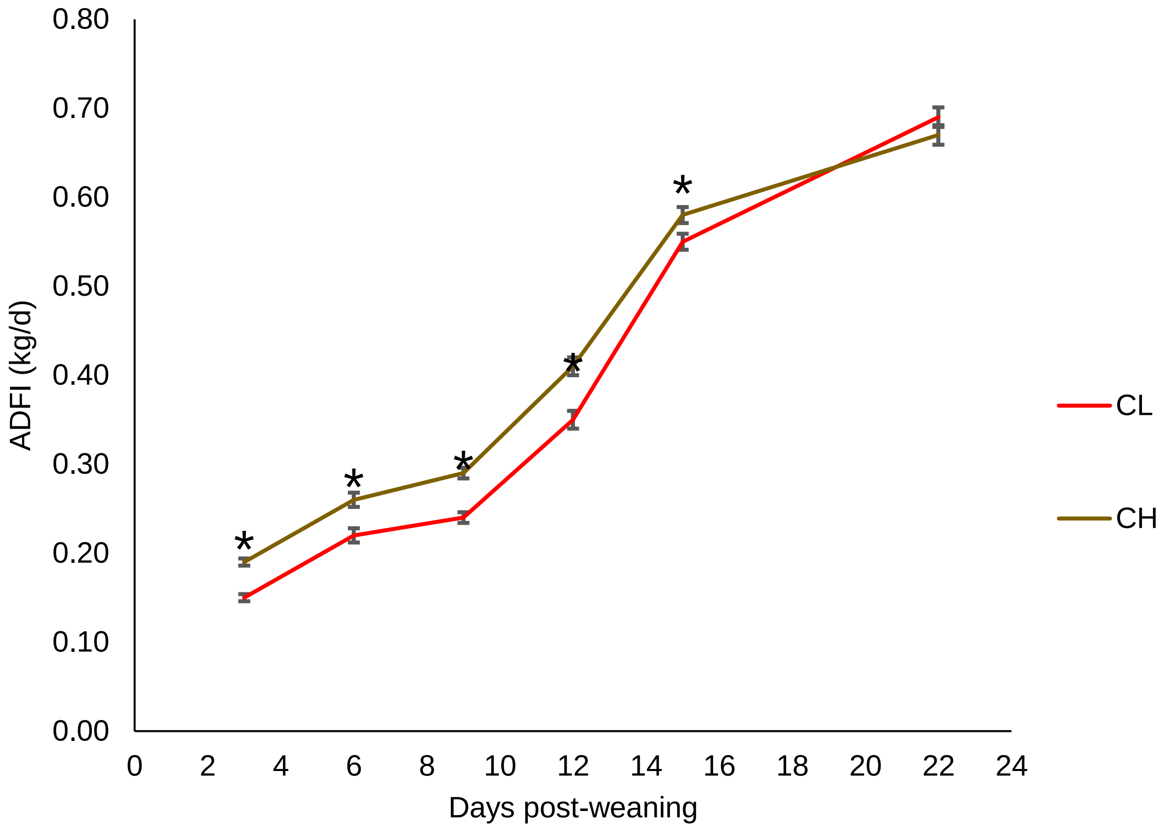

Figure 68: Change in effect of low (L) or high (H) pre-weaning creep consumption (C) level on pig ADFI from weaning to d22 post-weaning. Error bars represent s.e.m. * Denotes significance within day at $P < 0.050$.

From weaning to d12 post-weaning, ADG was highest in CH pigs ($P < 0.05$; Table 74), but there was no effect of pre-weaning creep consumption level on ADG overall from weaning to exit ($P > 0.05$).

Table 74: Effect of low (L) or high (H) pre-weaning creep consumption level (C) on pig ADG and FCR from weaning to d54 post-weaning.

	CL	CH	s.e.m	*P*-value C
n (pens)	*32*	*32*		
Average creep score (s/kg/d)	*6663*	*14893*		
ADG wean-d3 (g/d)	118	186	8.8	<0.001
ADG d3-d6 (g/d)	94	155	9.7	<0.001
ADG d6-d9 (g/d)	150	230	9.9	<0.001
ADG wean-d9 (g/d)	**121**	**191**	**6.3**	**<0.001**
ADG d9-12 (g/d)	253	318	17.0	0.009
ADG d12-15 (g/d)	428	442	15.7	0.519
ADG d15-22 (g/d)	556	550	15.7	0.781
ADG d9-d22 (g/d)	**456**	**471**	**12.3**	**0.392**
ADG d22-36 (g/d) †	679	698	11.6	0.251
ADG d36-exit (d54; g/d) †	940	902	12.1	0.032
ADG wean-exit (g/d) †	601	604	7.3	0.727
FCR wean-d3*	1.40	1.11		0.017
FCR d3-d6*	2.52	1.82		0.002
FCR d6-d9*	1.95	1.30		<0.001
FCR wean-d9	**1.79**	**1.32**	**0.063**	**<0.001**
FCR d9-12*	1.54	1.37		0.110
FCR d12-15	1.29	1.33	0.035	0.322
FCR d15-22	1.26	1.24	0.029	0.682
FCR d9-d22	**1.29**	**1.27**	**0.026**	**0.600**
FCR d22-36 †	1.60	1.59	0.016	0.879
FCR d36-exit †	1.69	1.79	0.025	0.010
FCR wean-exit †	1.62	1.66	0.015	0.088

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. **†**Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. *Required log10 transformation, actual means presented without s.e.m due to non-normal distribution.

The effect of pre-weaning creep consumption on ADG reduced over time (*P* =0.015, antedependence order 1 with additional uniform correlations within subject; Figure 69)

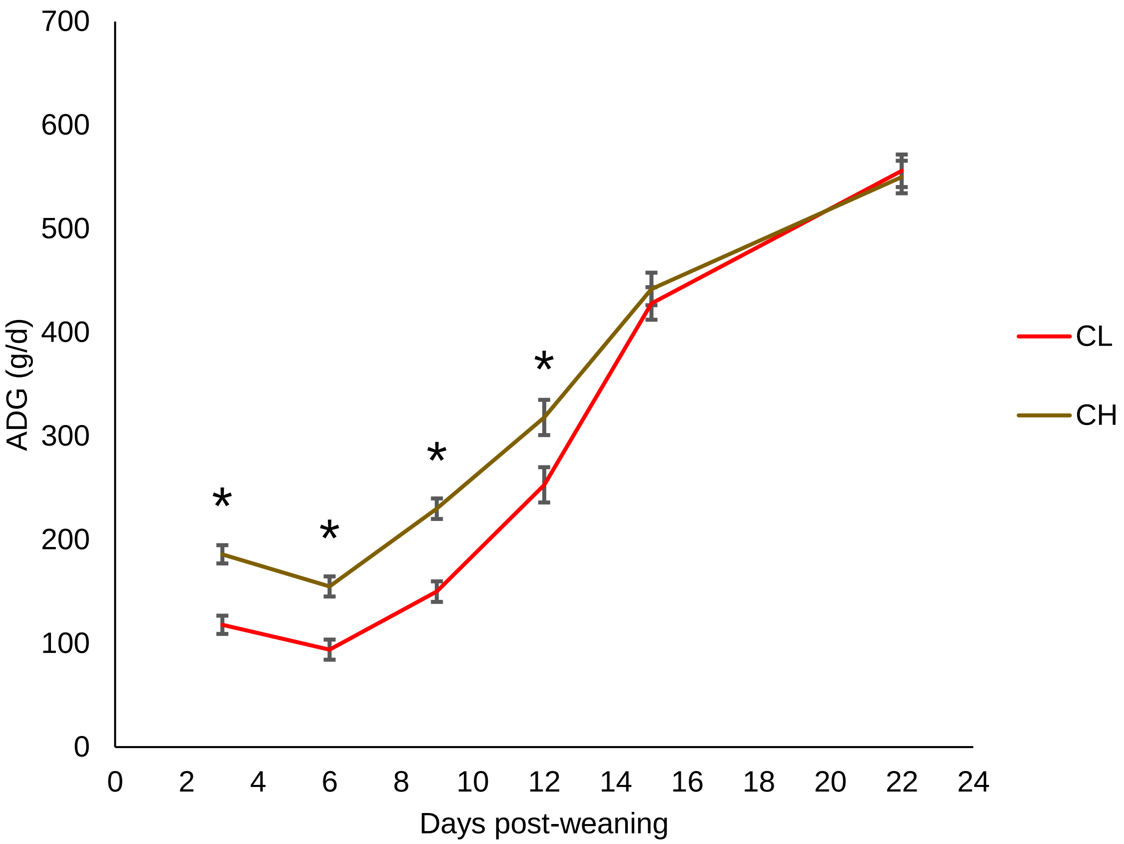

Figure 69: Change in effect of low (L) or high (H) pre-weaning creep consumption (C) on pig ADG from weaning to d22 post-weaning. Error bars represent s.e.m. * Denotes significance within day at *P* <0.05.

Feed conversion ratio was lower in CH pigs than CL, from weaning to d9 (*P* <0.05). Day 6 post-weaning showed a large increase in FCR, but the magnitude increase was lower in CH than CL pigs. The effect of pre-weaning creep consumption on FCR reduced over time (P =0.041, antedependence order 1; Figure 70). From d36-exit FCR was higher in CH pigs than CL pigs (*P* =0.010), and this led to a tendency for higher FCR overall (*P* =0.088).

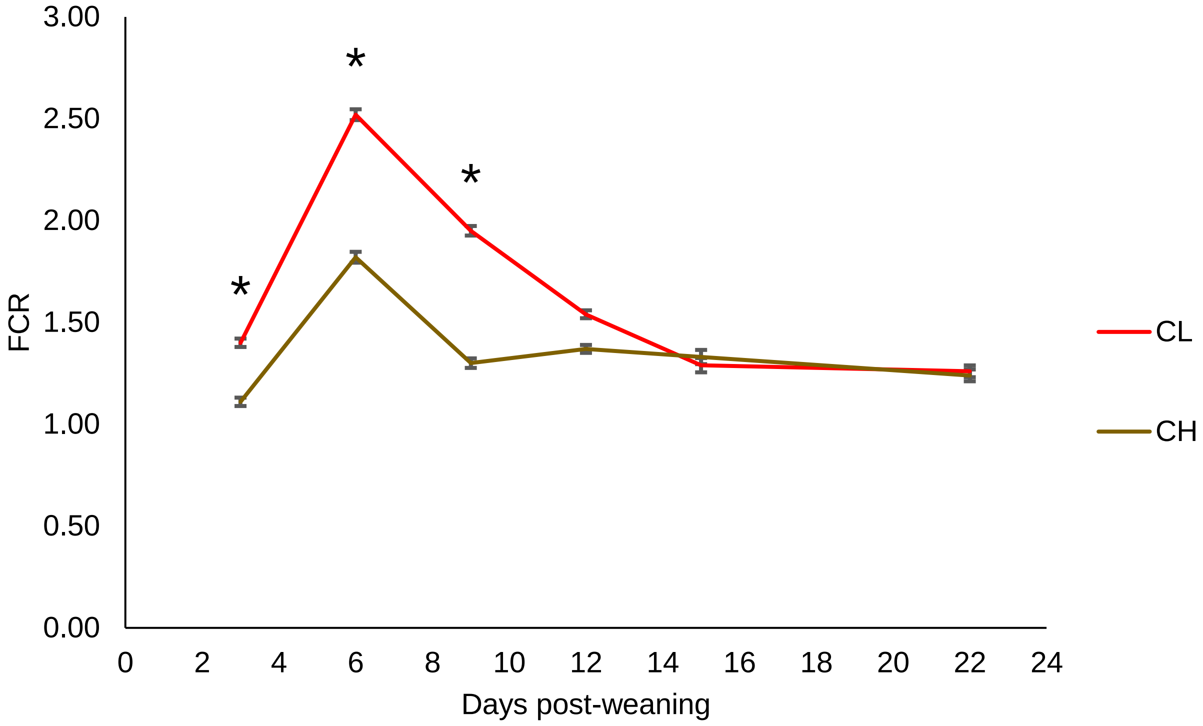

Figure 70: Change in effect of low (L) or high (H) pre-weaning creep consumption (C) on pig FCR from weaning to d22 post-weaning. Error bars represent s.e.m. * Denotes significance within day at *P* <0.05.

6.3.4.5.2. Post-weaning diet specification

Although there was a trend for SH being heavier than SL at weaning (*P* =0.052; Table 75), the magnitude of the difference was small (0.3 %). When weaning weight was included in the analysis as a co-variate, the adjusted means were similar to the actual means, and the co-variate had a significant effect (*P* <0.05) at d3, 9, 12 and 15 only (see footnote). Pigs fed SH had higher ADFI from weaning-d3 (*P* =0.005), but after this point there was no effect of diet on ADFI (*P* >0.05).

Table 75: Effect of low (L) or high (H) post-weaning diet specification (S) on pig average weight and ADFI from weaning to d54 post-weaning.

	SL	SH	s.e.m	*P*-value S
n (pens)	*32*	*32*		
Average creep score (s/kg/d)	*10662*	*10864*		
Weight at weaning (d0; kg)	9.10	9.13	0.011	0.052
Weight d3 (kg)*	9.51	9.64	0.026	<0.001
Weight d6 (kg)	9.86	10.04	0.037	0.001
Weight d9 (kg) α	10.40	10.66	0.053	0.001
Weight d12 (kg)¥	11.17	11.62	0.082	<0.001
Weight d15 (kg) ¥	12.43	12.97	0.107	<0.001
Weight d22 (kg)	16.21	16.93	0.173	0.004
Weight d36 (kg) †	25.7	26.9	0.27	0.003
Weight exit (d54; kg) †	42.5	44.1	0.38	0.006
ADFI wean-d3 (kg/d)	0.16	0.18	0.004	0.005
ADFI d3-d6 (kg/d)	0.23	0.24	0.008	0.812
ADFI d6-d9 (kg/d)	0.26	0.24	0.006	0.199
ADFI wean-d9 (kg/d)	**0.22**	**0.23**	**0.004**	**0.100**
ADFI d9-12 (kg/d)	0.37	0.39	0.010	0.243
ADFI d12-15 (kg/d)	0.56	0.56	0.009	0.829
ADFI d15-22 (kg/d)	0.68	0.68	0.011	0.766
ADFI d9-d22 (kg/d)	**0.58**	**0.58**	**0.009**	**0.891**
ADFI d22-36 (kg/d) †	1.08	1.11	0.015	0.106
ADFI d36-exit (kg/d) †	1.59	1.58	0.028	0.858
ADFI wean-exit (kg/d) †	0.98	0.98	0.013	0.757

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. **†**Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. *Covariate effect of weaning weight, but treatment means unchanged. αCovariate effect of weaning weight: SL mean reduced and SH mean increased by 0.01 kg. ¥Covariate effect of weaning weight: SL mean reduced and SH mean increased by 0.02 kg. Non-adjusted means are presented in Table 75 for all timepoints.

Pigs fed SH diets were heavier throughout than those fed SL diets (*P* <0.05; Figure 71), with the difference in weight tending to increase throughout the period that the diets were fed (*P* =0.051; antedepence order 1)

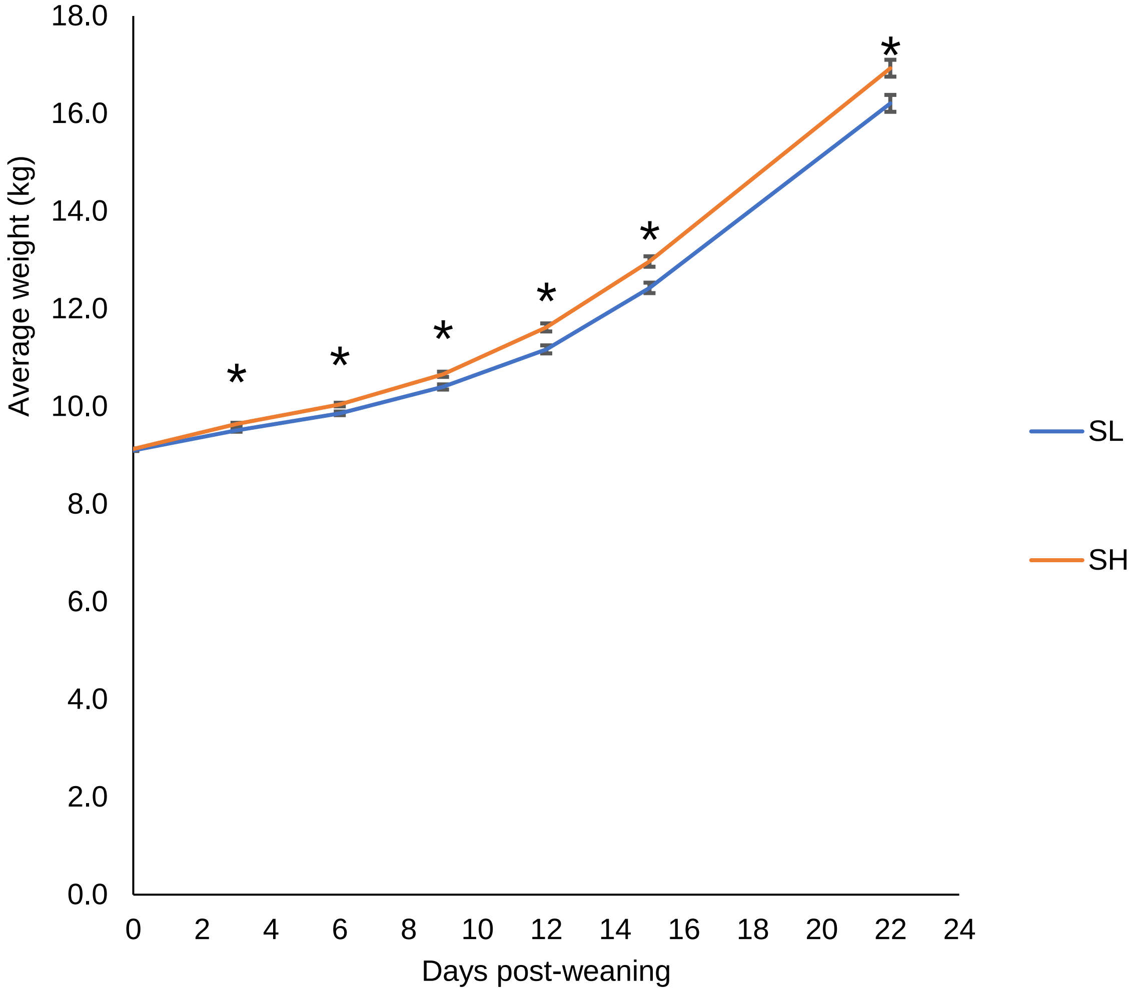

Figure 71: Change in effect of low (L) or high (H) post-weaning diet specification (S) on pig average weight from weaning to d22 post-weaning**.** Error bars represent s.e.m. * Denotes significance within day at *P* <0.05.

Pigs fed SH had higher ADG from weaning-d3, weaning-d9, and overall from weaning-exit (*P* <0.05; Table 76), with a trend for similar results at d9-12, d9-22 and d22-36 (*P* <0.10). Although there was no effect of diet specification on FCR at each weighing point (*P* >0.05), the combined FCR from weaning-d9 (stage 1 diet) and d9-22 (stage 2 diet) were both lower for pigs fed SH than SL (*P* <0.05), resulting in FCR from weaning-exit tending to be lower for pigs fed SH than those fed SL (*P* =0.055).

Table 76: Effect of low (L) or high (H) post-weaning diet specification (S) on pig ADG and FCR from weaning to d54 post-weaning.

	SL	SH	s.e.m	*P*-value S
n (pens)	*32*	*32*		
Average creep score (s/kg/d)	*10662*	*10864*		
ADG wean-d3 (g/d)	136	169	8.8	0.009
ADG d3-d6 (g/d)	117	132	9.7	0.288
ADG d6-d9 (g/d)	180	200	9.9	0.179
ADG wean-d9 (g/d)	**144**	**170**	**6.0**	**0.004**
ADG d9-12 (g/d)	263	307	17.0	0.073
ADG d12-15 (g/d)	420	450	15.7	0.183
ADG d15-22 (g/d)	539	567	15.7	0.226
ADG d9-d22 (g/d)	**448**	**480**	**12.3**	**0.073**
ADG d22-36 (g/d) †	675	703	11.6	0.086
ADG d36-exit (d54; g/d) †	910	932	12.1	0.221
ADG wean-exit (g/d) †	590	615	7.3	0.018
FCR wean-d3*	1.31	1.20		0.294
FCR d3-d6*	2.26	2.08		0.164
FCR d6-d9*	1.66	1.59		0.561
FCR wean-d9*	**1.66**	**1.45**		**0.027**
FCR d9-12*	1.51	1.40		0.218
FCR d12-15	1.35	1.27	0.035	0.103
FCR d15-22	1.28	1.22	0.029	0.114
FCR d9-d22	**1.32**	**1.24**	**0.026**	**0.025**
FCR d22-36 †	1.60	1.59	0.016	0.582
FCR d36-exit †	1.74	1.73	0.025	0.805
FCR wean-exit †	1.66	1.62	0.015	0.055

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. †Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. *Required log10 transformation, actual means presented without s.e.m due to non-normal distribution.

6.3.4.5.3. Post-weaning diet quality

At each timepoint from d3-exit, pigs fed QH were lighter than those fed QL ($P < 0.05$; Table 77). From weaning-d3, d6-d9 and d9-d12, ADFI was lowest in pigs fed QH compared to QL ($P < 0.05$).

Table 77: Effect of low (L) or high (H) post-weaning diet quality (Q) on pig average weight and ADFI from weaning to d54 post-weaning.

	QL	QH	s.e.m	*P*-value Q
n (pens)	*32*	*32*		
Average creep score (s/kg/d)	*10820*	*10736*		
Weight at weaning (d0; kg)	9.11	9.12	0.011	0.693
Weight d3 (kg)	9.61	9.53	0.026	0.036
Weight d6 (kg)	10.04	9.86	0.037	<0.001
Weight d9 (kg)	10.68	10.38	0.053	<0.001
Weight d12 (kg)	11.58	11.20	0.082	0.002
Weight d15 (kg)	12.96	12.43	0.107	<0.001
Weight d22 (kg)	16.86	16.28	0.173	0.019
Weight d36 (kg) †	26.7	25.8	0.27	0.017
Weight exit (d54; kg) †	44.1	42.5	0.38	0.004
ADFI wean-d3 (kg/d)	0.18	0.16	0.004	0.032
ADFI d3-d6 (kg/d)	0.24	0.23	0.008	0.192
ADFI d6-d9 (kg/d)	0.28	0.25	0.006	0.007
ADFI wean-d9 (kg/d)	**0.23**	**0.21**	**0.004**	**0.008**
ADFI d9-12 (kg/d)	0.40	0.37	0.010	0.028
ADFI d12-15 (kg/d)	0.57	0.55	0.009	0.269
ADFI d15-22 (kg/d)	0.69	0.67	0.011	0.435
ADFI d9-d22 (kg/d)	**0.59**	**0.58**	**0.009**	**0.173**
ADFI d22-36 (kg/d) †	1.10	1.08	0.015	0.334
ADFI d36-exit (kg/d) †	1.59	1.57	0.028	0.568
ADFI wean-exit (kg/d) †	0.99	0.97	0.013	0.259

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. **†**Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold.

The difference in weight between QL and QH increased over time (*P* =0.005, antedependence order 1; Figure 72).

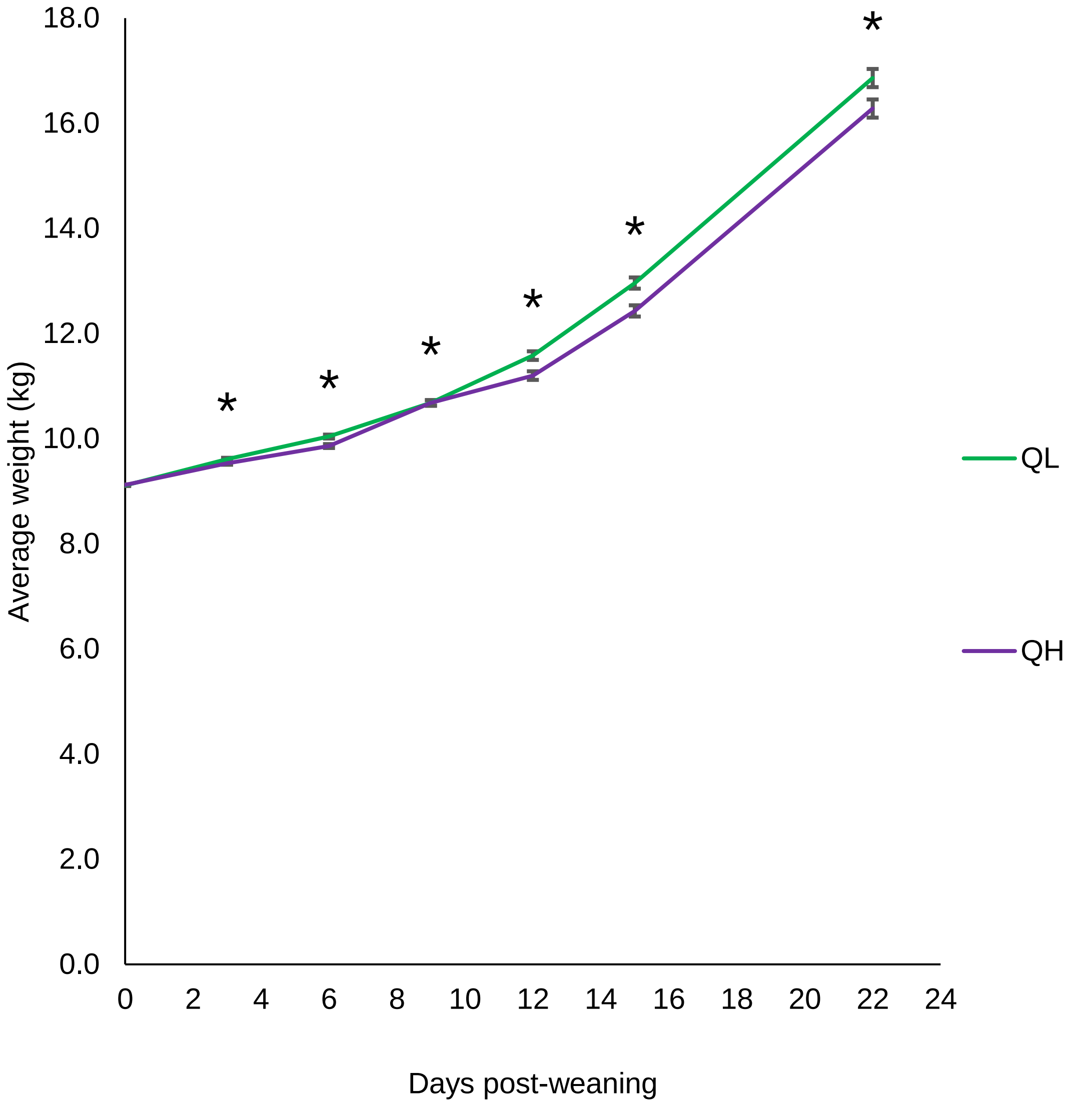

Figure 72: Change in effect of low (L) of high (H) post-weaning diet quality (Q) on pig average weight from weaning to d22 post-weaning. Error bars represent s.e.m. * Denotes significance within day at *P* <0.05.

Pigs fed QL had higher ADG than those fed QH from weaning-d3, d3-d6, d6-d9, d12-d15 and from weaning to exit combined (P <0.05; Table 78). Pigs fed QL also had a lower FCR than those fed QH at every timepoint from weaning-d9, and from d12-15 (*P* <0.05), but not from weaning-exit (*P* >0.05).

Table 78: Effect of low (L) or high (H) post-weaning diet quality (Q) on pig ADG and FCR from weaning to d54 post-weaning.

	QL	QH	s.e.m	*P*-value Q
n (pens)	*32*	*32*		
Average creep score (s/kg/d)	*10820*	*10736*		
ADG wean-d3 (g/d)	167	138	8.8	0.025
ADG d3-d6 (g/d)	142	107	9.7	0.013
ADG d6-d9 (g/d)	214	166	9.9	0.001
ADG wean-d9 (g/d)	**174**	**137**	**6.3**	**<0.001**
ADG d9-12 (g/d)	300	271	17.0	0.228
ADG d12-15 (g/d)	460	410	15.7	0.028
ADG d15-22 (g/d)	557	549	15.7	0.703
ADG d9-d22 (g/d)	**475**	**453**	**12.3**	**0.193**
ADG d22-36 (g/d) †	699	679	11.6	0.243
ADG d36-exit (d54; g/d) †	929	897	16.0	0.160
ADG wean-exit (g/d) †	617	588	7.3	0.007
FCR wean-d3*	1.15	1.36		0.018
FCR d3-d6*	2.00	2.34		0.043
FCR d6-d9*	1.36	1.89		0.002
FCR wean-d9*	**1.40**	**1.71**		**0.001**
FCR d9-12*	1.42	1.48		0.519
FCR d12-15	1.26	1.36	0.035	0.030
FCR d15-22	1.25	1.26	0.029	0.801
FCR d9-d22	**1.26**	**1.30**	**0.026**	**0.290**
FCR d22-36 †	1.59	1.60	0.016	0.655
FCR d36-exit †	1.72	1.76	0.025	0.316
FCR wean-exit †	1.62	1.65	0.015	0.180

Average creep score: Creep score calculated as duration of visits to the creep feeder (s)/ADG (g/d) for the final week pre-weaning. †Experimental diets fed from weaning-d22, thereafter a common grower diet was fed to all pigs to d54. Performance for each experimental dietary stage is in bold. *Required log10 transformation, actual means presented without s.e.m due to non-normal distribution.

6.3.5. Finisher pen performance

Statistical analysis was not performed on the creep consumption*diet specification*diet quality interaction as there were only two replicates per treatment. All pens followed the same dietary regime in the finishing phase. There was a 5.5 kg range in weight at first draw, with CLSLQL lightest and CHSHQH heaviest, despite being equal in weight at entry (Table 79).

Table 79: Effect of low (L) or high (H) pre-weaning creep consumption level (C), post-weaning diet specification (S) and post-weaning diet quality (Q) on pig finisher performance from d54 post-weaning to 19 weeks old.

	CL				CH			
	SL		SH		SL		SH	
	QL	QH	QL	QH	QL	QH	QL	QH
Weight at entry (kg)	43.5	41.2	44.6	43.4	43.5	42.0	44.3	43.5
Weight at 19 weeks old (kg)	103.6	105.2	107.1	108.3	105.9	105.8	107.8	109.1
ADFI entry-19 weeks old (kg/d)	2.60	2.68	2.70	2.74	2.70	2.67	2.63	2.72
ADG entry-19 weeks old (g/d)	1015	1091	1053	1107	1060	1100	1067	1116
FCR entry-19 weeks	2.56	2.46	2.57	2.45	2.54	2.43	2.47	2.43

Statistical analysis was not performed at the two-way interaction level due to low (4) replicates per treatment. Therefore, only main effects are presented in the following tables.

6.3.5.1. Pre-weaning creep consumption

There was no effect of pre-weaning creep consumption level on physical performance in the finisher phase (*P* >0.05; Table 80).

Table 80: Effect of low (L) or high (H) pre-weaning creep consumption level (C) on pig finisher performance from d54 post-weaning to 19 weeks old.

	CL	CH	s.e.m	*P*-value
n *(pens) (pens)*	*8*	*8*		
Weight at entry (kg)	43.2	43.3	0.70	0.898
Weight at 19 weeks old (kg)	106.1	107.1	1.32	0.572
ADFI entry-19 weeks old (kg/d)	2.68	2.68	0.034	0.982
ADG entry-19 weeks old (g/d)	1066	1086	16.1	0.413
FCR entry-19 weeks old	2.51	2.41	0.019	0.128

6.3.5.2. Diet specification

There was no effect of post-weaning diet specification on physical performance in the finisher phase (*P* >0.05; Table 81).

Table 81: Effect of low (L) or high (H) post-weaning diet specification (S) on pig finisher performance from d54 post-weaning to 19 weeks old.

	SL	SH	s.e.m	*P*-value
n *(pens)*	8	8		
Weight at entry (kg)	42.6	44.0	0.70	0.183
Weight at 19 weeks old (kg)	105.1	108.1	1.32	0.138
ADFI entry-19 weeks old (kg/d)	2.66	2.70	0.034	0.501
ADG entry-19 weeks old (g/d)	1066	1083	16.1	0.414
FCR entry-19 weeks old	2.50	2.48	0.019	0.555

6.3.5.3. Diet quality

Pigs that received QH had higher ADG and lower FCR during the finisher phase than those that received QL (*P* <0.05; Table 82).

Table 82: Effect of low (L) or high (H) post-weaning diet quality (Q) on pig finisher performance from d54 post-weaning to 19 weeks old.

	QL	QH	s.e.m	*P*-value
n (pens)	8	8		
Weight at entry (kg)	44.0	42.5	0.70	0.159
Weight at 19 weeks old (kg)	106.1	107.1	1.32	0.610
ADFI entry-19 weeks old (kg/d)	2.66	2.70	0.034	0.406
ADG entry-19 weeks old (g/d)	1049	1103	16.1	0.034
FCR entry-19 weeks old	2.54	2.44	0.019	0.005

6.3.6. Economic assessment

Pigs fed SLQL had the lowest feed cost/kg gain to d22 post-weaning (Table 83), but higher cost/kg gain in the finisher period. The lowest cost/kg gain for CL pigs was SHQH, but for CH pigs it was SHQL. Pigs with high pre-weaning creep consumption had lower cost/kg gain for diet 1, finisher diet and overall than CL pigs.

Table 83: Economic effect of feeding pigs with low (L) or high (H) pre-weaning creep consumption level (C), post-weaning diets to d22 post-weaning with low (L) or high (H) specification (S) and quality (Q).

	CL				CH			
	SL		SH		SL		SH	
	QL	QH	QL	QH	QL	QH	QL	QH
n (pens)	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*
Diet 1 cost difference vs SLQH (£/T)	*-135*	*0*	*62*	*72*	*-135*	*0*	*62*	*72*
Diet 2 cost difference vs SLQH (£/T)	*-80*	*0*	*3*	*80*	*-80*	*0*	*3*	*80*
Weaning weight (kg)	9.26	9.25	9.28	9.28	8.94	8.96	8.98	8.99
Weight d9 (kg)	10.43	10.12	10.74	10.21	10.64	10.41	10.91	10.76
Weight d22 (kg)	16.54	15.41	16.95	16.35	16.91	15.97	17.07	17.37
Weight d54 (kg)	43.5	41.2	44.6	43.4	43.5	42.0	44.3	43.5
Weight 19 week (kg)	103.6	105.2	107.1	108.3	105.9	105.8	107.8	109.1
Cost difference diet 1/kg gain (p/kg)	-23	0	-9	17	-18	0	-8	9
Cost difference diet 2/kg gain (p/kg)	-10	0	0	10	-10	0	0	10
Cost difference grower diet/kg gain (p/kg)	0	0	-1	-1	-3	0	-3	-1
Cost difference finisher diet/kg gain (p/kg)	3	0	3	0	4	0	1	0
Actual cost grower diet/kg gain (p/kg)	58	58	57	57	58	61	58	60
Actual cost finisher diet/kg gain (p/kg)	80	77	80	77	80	76	77	76
Relative weighted cost/kg gain to 105 kg (p/kg)	61.0	61.0	61.2	60.4	60.4	60.8	59.6	60.3

The cost of the trial diets was confidential (diet 1 and 2, wean-d22), but the difference in cost compared to the SLQH regime was available. The grower diet cost £347/T, and finisher diet cost £313/T. Relative weighted cost/kg gain = $(\Sigma(\text{cost difference*weight gain})_{\text{diet }1,2})$+ $(\Sigma(\text{actual cost*weight gain})_{\text{grower, finisher}}))$ /105.

6.3.7. Duodenal morphology

The villi and crypts measured for morphological parameters were different to the ones on which goblet cell (GC) density was calculated, as a different stain was used and so correlations of GC density with morphological parameters were not performed. Although acid mucins were identified within Brunners glands, there were no acid-mucin secreting GC identified in the crypt or villus epithelium, and so GC refer to those producing neutral mucin.

6.3.7.1. Pre-weaning

Slides from three CL piglets were unable to be analysed, as there were insufficient well-orientated villi. This was due to errors in sample collection and preparation rather than a treatment effect. There were no differences in morphological measurements between piglets classified as CL or CH on the day prior to weaning (*P* >0.05; Table 84).

Table 84: Effect of low (L) or high (H) pre-weaning creep consumption (C) on pig duodenal morphology at weaning

	CL	CH	s.e.m	P-value
n (pigs)	*5*	*8*		
Average villus height (µm)*	726	739		0.464
Average crypt depth (µm)	231	222	14.8	0.634
Villus height:crypt depth	3.29	3.57	0.328	0.509
Villus GC density (cell/mm^2)†	334	310		0.902
Crypt GC density (cell/mm^2)	1665	1481	135	0.350
CV villus length (%)	17.9	17.3	2.33	0.864
CV crypt depth (%)	25.3	21.3	3.10	0.335
CV vh:cd (%)	28.8	24.4	3.91	0.388
CV villus GC density (%)	41.7	45.6	5.23	0.607
CV crypt GC density (%)	19.9	25.6	2.48	0.125

*Kruskal-Wallis test performed. †Required square-root transformation, actual means presented without s.e.m due to non-normal distribution.

There was no relationship between ADG from d12-weaning and morphological parameters measured (*P* >0.05). There was a positive relationship between crypt GC density and ADG d19-weaning for CL pigs (*P* =0.003; Table 85).

Table 85: Relationships between ADG from d19-weaning and duodenal crypt goblet cell density in pigs with low (L) or high (H) pre-weaning creep (C) intake.

Parameter	Equation	r^2	s.e	*P*-value
Crypt goblet cell density (cell/mm^2)		0.485	274	0.007
CL	5.22*adg+114	0.777		0.003
CH	5.22*adg-339	0.196		0.116

There were no other relationships between morphological measurements and ADG d19-weaning (*P* >0.05). There were no correlations between creep score and morphological parameters for CL or CH piglets (*P* >0.05).

6.3.7.2. Comparison of pig duodenal morphology pre- and post-weaning

There was a visibly-apparent difference in pre- and post-weaning morphology, with villi appearing stunted, deeper crypts and larger luminal space in the post-weaning samples (Figure 73).

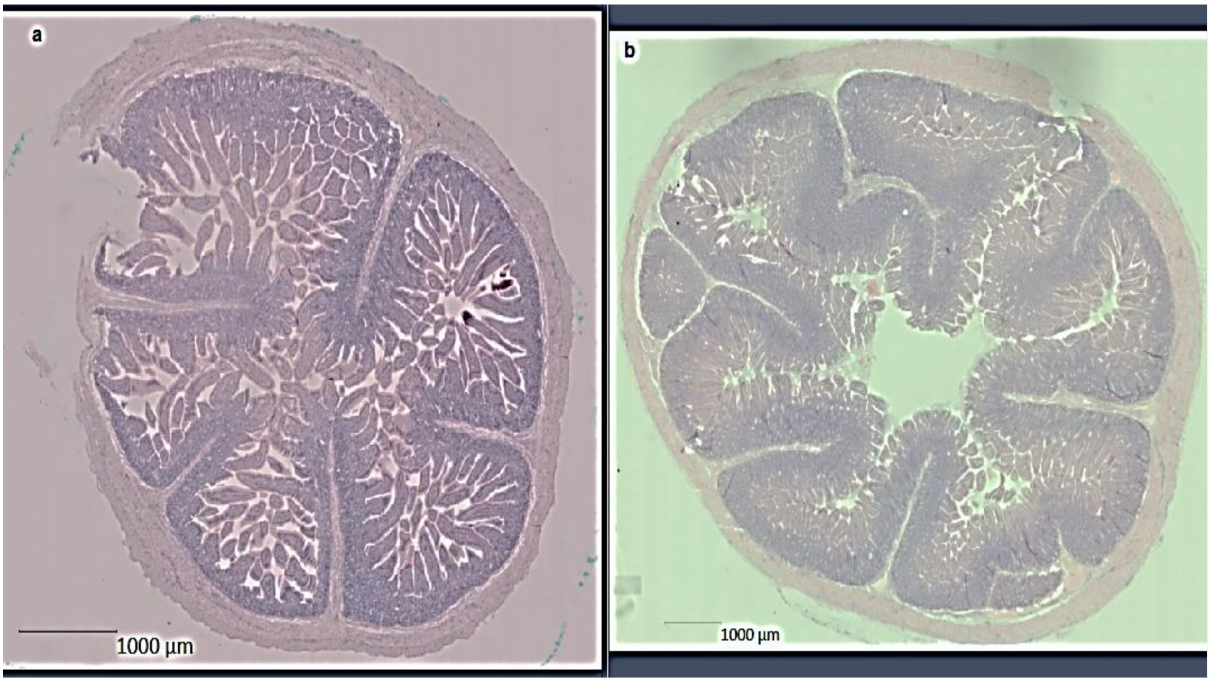

Figure 73: Visual comparison between piglet duodenal morphology at weaning (a) and d9 post-weaning (b)

This was evident in the statistical analysis: At d9 post-weaning, villi were shorter, and crypts were deeper than observed in piglets pre-weaning (*P* <0.001; Table 86). This resulted in lower villus height:crypt depth ratio piglets post-weaning compared to pre-weaning. Goblet cell density was lower post-weaning in both the villi (*P* =0.005) and crypts (*P* <0.001).

Table 86: Differences in pig duodenal morphology at weaning and d9 post-weaning

	Weaning	d9 post-weaning	s.e.m	*P*-value
n (pigs)	*13*	*32*		
Average villus height (µm)	733	363	22.7	<0.001
Average crypt depth (µm)	226	356	15.6	<0.001
Villus height:crypt depth	3.44	1.18	0.125	<0.001
Villus GC density (cell/mm^2)	322	223	19.3	0.005
Crypt GC cell density (cell/mm^2)	1573	1184	77.2	<0.001
CV villus height (%)	17.6	24.5	1.59	<0.001
CV crypt depth (%)	23.2	34.2	2.12	<0.001
CV vh:cd (%)	26.5	44.2	3.54	<0.001
CV villus GC density (%)	43.6	48.1	4.55	0.429
CV crypt GC density (%)	22.8	27.7	2.47	0.105

Measurements of villus length, crypt depth, villus height:crypt depth, villus height:villus width, and crypt depth:crypt width were more variable (had higher CV %) post-weaning than pre-weaning (*P* <0.05). These differences were unaffected by creep intake (*P* >0.05; Table 87).

Table 87: Effect of weaning and pre-weaning low (L) or high (H) creep (C) consumption on pig duodenal morphology

	Weaning		d9 post-weaning			
	CL	CH	CL	CH	s.e.m	*P*-value
n (pigs)	*5*	*8*	*16*	*16*		
Average villus height (µm)	726	739	357	357	36.1	0.954
Average crypt depth (µm)	231	222	367	347	24.8	0.759
Villus height:crypt depth	3.29	3.57	1.11	1.24	0.198	0.622
Villus GC density (cell/mm^2)	334	310	202	244	38.6	0.336
Crypt GC density (cell/mm^2)	1665	1481	1128	1240	76.0	0.118
CV villus height (%)	17.9	17.3	24.8	24.1	2.53	0.958
CV crypt depth (%)	25.3	21.3	31.1	36.8	3.38	0.063
CV vh:cd (%)	28.8	24.4	43.8	44.5	5.63	0.541
CV villus GC density (%)	41.7	45.6	50	46.2	6.48	0.498
CV crypt GC density (%)	19.9	25.6	28.7	26.8	3.46	0.208

There was also a trend (*P* =0.063) for crypt depth to be more variable post-weaning in CH piglets than CL, whereas pre-weaning CV was similar between the creep consumption classes.

6.3.7.3. Post-weaning

6.3.7.3.1. Three-way interaction

There was no three-way interaction of creep *diet specification*diet quality on morphological parameters at day 9 post-weaning (*P* >0.05; Table 88). There were only 4 replicates per treatment, and with the high variability observed (high CVs), it is likely there was insufficient power. Using power analysis (Genstat) to calculate required replication at 80 % power and *P* <0.05, with the variance observed in this Study, seven replicates per treatment at the three-way interaction level would have been required to detect a difference in crypt depth post-weaning. Ten replicates per treatment would have been required to detect a difference in villus height, and vh:cd.

Table 88: Effect of low (L) or high (H) pre-weaning creep consumption (C), post-weaning diet specification (S) and diet quality (Q) on pig duodenal morphology at day 9 post-weaning

	CL				CH					
	SL		SH		SL		SH			
	QL	QH	QL	QH	QL	QH	QL	QH	s.e.m	*P*-value
n (pigs)	*4*	*4*	*4*	*4*	*4*	*4*	*4*			
Average villus height (µm)	314	356	371	388	384	347	398	340	33.1	0.981
Average crypt depth (µm)	329	405	371	365	353	357	308	316	22.2	0.182
Villus height:crypt depth	1.12	0.97	1.15	1.17	1.07	1.12	1.5	1.28	0.128	0.224
Average mucosal thickness (µm)	702	773	759	753	792	733	799	793	49.4	0.359
Villus GC density (cell/mm^2)	192	181	217	218	294	245	218	218	37.2	0.729
Crypt GC density (cell/mm^2)	1032	1141	1165	1174	1249	1369	1209	1133	139	0.808
CV villus height (%)	24	23.2	25.2	26.9	19.8	23.7	26.4	26.6	3.01	0.483
CV crypt depth (%)	36.1	27.7	29.1	31.6	33.6	36.2	38.7	38.8	4.05	0.257
CV vh:cd (%)	53.1	40.6	39.7	41.9	37.5	45.5	46.1	49.0	7.13	0.337
CV mucosal thickness (%)	9.8	9.9	9.2	11.7	11.6	17.4	10.5	17.7	3.75	0.927
CV villus GC density (%)	40.2	52.4	54.8	52.5	52.2	43.2	44.8	44.6	10.70	0.449
CV crypt GC density (%)	37.4	18	36.1	23.4	20.8	29.8	29.6	26.9	4.82	0.188

6.3.7.3.2. Two-way interaction

There were no creep consumption*diet specification interactions on duodenal morphological parameters at day 9 (*P* >0.05; Table 89).

Table 89: Effect of low (L) or high (H) pre-weaning creep consumption (C) and post-weaning diet specification (S) on pig duodenal morphology at day 9 post-weaning

	CL		CH			
	SL	SH	SL	SH	s.e.m	*P*-value
n (pigs)	*8*	*8*	*8*	*8*		
Average villus height (µm)	335	380	365	369	22.9	0.377
Average crypt depth (µm)	367	368	353	312	16.0	0.207
Villus height:crypt depth	1.05	1.16	1.10	1.39	0.092	0.344
Average mucosal thickness (µm)	738	756	762	796	34.8	0.828
Villus GC density (cell/mm^2)	187	217	269	218	25.8	0.125
Crypt GC density (cell/mm^2)	1087	1170	1309	1171	96.4	0.264
CV villus height (%)	23.6	26.0	21.7	26.5	2.10	0.576
CV crypt depth (%)	31.9	30.3	34.9	38.8	2.88	0.356
CV vh:cd (%)	46.9	40.8	41.5	47.6	5.04	0.240
CV mucosal thickness (%)	9.8	10.4	14.5	14.1	2.60	0.849
CV villus GC density (%)	46.3	53.7	47.7	44.7	7.51	0.496
CV crypt GC density (%)	27.7	29.8	25.3	28.3	3.46	0.900

There were no creep consumption*diet quality interactions on duodenal morphological parameters at day 9 (*P* >0.05; Table 90), but variation of crypt GC density was higher in CLQL pigs than CLQH, with both CH treatments intermediate (*P* =0.010).

Table 90: Effect of low (L) or high (H) pre-weaning creep consumption (C) and post-weaning diet quality (Q) on pig duodenal morphology at day 9 post-weaning

	CL		CH		s.e.m	*P*-value
	QL	QH	QL	QH		
n (pigs)	*8*	*8*	*8*	*8*		
Average villus height (µm)	343	372	391	343	22.9	0.106
Average crypt depth (µm)	350	385	329	336	16.0	0.403
Villus height:crypt depth	1.14	1.07	1.29	1.20	0.092	0.908
Average mucosal thickness (µm)	731	763	795	763	34.8	0.363
Villus GC density (cell/mm^2)	205	199	256	232	25.8	0.720
Crypt GC density (cell/mm^2)	1099	1158	1229	1251	96.4	0.849
CV villus height (%)	24.6	25.1	23.1	25.1	2.10	0.713
CV crypt depth (%)	32.6	29.6	36.1	37.5	2.88	0.453
CV vh:cd (%)	46.4	41.2	41.8	47.3	5.04	0.301
CV mucosal thickness (%)	9.5	10.8	11.1	17.5	2.60	0.332
CV villus GC density (%)	47.5	52.4	48.5	43.9	7.51	0.529
CV crypt GC density (%)	36.7^{a}	20.7^{b}	25.2^{ab}	28.4^{ab}	3.46	0.010

Superscripts denote significance at P <0.05.

There were no diet specification*diet quality interactions on duodenal morphological parameters at day 9 (*P* >0.05; Table 91) or on variation in morphological parameters (*P* >0.05). Analysing the diets separately (non-factorial) did not affect these results (*P* >0.05 for all), and so this analysis is not shown.

Table 91: Effect of low (L) or high (H) post-weaning diet specification (S) and post-weaning diet quality (Q) on pig duodenal morphology at day 9 post-weaning

	SL	SH

	QL	QH	QL	QH	s.e.m	*P*-value
n (pigs)	*8*	*8*	*8*	*8*		
Average villus height (µm)	349	351	385	364	22.9	0.616
Average crypt depth (µm)	339	381	339	340	16.0	0.218
Villus height:crypt depth	1.10	1.04	1.33	1.22	0.092	0.784
Average mucosal thickness (µm)	747	753	779	773	34.8	0.861
Villus GC density (cell/mm^2)	243	213	218	218	25.8	0.553
Crypt GC density (cell/mm^2)	1141	1255	1187	1154	96.4	0.451
CV villus height (%)	21.9	23.5	25.8	26.8	2.10	0.888
CV crypt depth (%)	34.9	32.0	33.9	35.2	2.88	0.472
CV vh:cd (%)	45.3	43.1	42.9	45.5	5.04	0.636
CV mucosal thickness (%)	23.14	14.2	18.56	13.64	3.91	0.474
CV villus GC density (%)	46.2	47.8	49.8	48.6	7.51	0.853
CV crypt GC density (%)	29.1	239	32.8	25.2	3.46	0.721

6.3.7.3.3. Main effects

At d9 post-weaning, piglets with high pre-weaning creep consumption had lower crypt depth than those with low pre-weaning creep consumption (*P* =0.040; Table 92). There was a trend (*P* <0.100) for CH piglets to have higher variation in crypt depth than CL piglets.

Table 92: Effect of low (L) or high (H) piglet pre-weaning creep consumption (C) on duodenal morphology measurements and variation at day 9 post-weaning.

	CL	CH	s.e.m	*P*-value
n (pigs)	*16*	*16*		
Average villus height (µm)	357	367	16.4	0.677
Average crypt depth (µm)	367	332	11.5	0.040
Villus height:crypt depth	1.11	1.24	0.063	0.131
Average mucosal thickness (µm)	747	779	23.7	0.350
Villus GC density (cell/mm^2)	202	244	18.3	0.117
Crypt GC density (cell/mm^2)	1128	1240	66.9	0.247
CV villus height (%)	24.8	24.1	1.42	0.722
CV crypt depth (%)	31.1	36.8	2.00	0.054
CV vh:cd (%)	43.8	44.5	3.55	0.887
CV mucosal thickness (%)	10.1	14.3	1.78	0.109
CV villus GC density (%)	50.0	46.2	5.11	0.603
CV crypt GC density (%)	28.7	26.8	2.65	0.607

The non-significant longer villi and shallower crypts associated with SH compared to SL pig combined to result in the ratio of villus height:crypt depth being higher in pigs fed high specification diets than those fed low specification diets (*P* =0.028; Table 93). Variation in villus height tended to be higher in SH piglets than SL piglets (*P* =0.084).

Table 93: Effect of low (L) or high (H) post-weaning diet specification (S) on pig duodenal morphology measurements and variation at day 9 post-weaning.

	SL	SH	s.e.m	*P*-value
n (pigs)	*16*	*16*		
Average villus height (µm)	350	374	16.4	0.307
Average crypt depth (µm)	360	340	11.5	0.238
Villus height:crypt depth	1.07	1.28	0.063	0.028
Average mucosal thickness (µm)	750	776	23.7	0.449
Villus GC density (cell/mm^2)	228	218	18.3	0.700
Crypt GC density (cell/mm^2)	1198	1171	66.9	0.776
CV villus height (%)	22.7	26.3	1.42	0.084
CV crypt depth (%)	33.4	34.5	2.00	0.693
CV vh:cd (%)	44.2	44.2	3.55	0.997
CV mucosal thickness (%)	12.2	12.3	1.78	0.972
CV villus GC density (%)	47.0	49.2	5.11	0.762
CV crypt GC density (%)	26.5	29	2.65	0.506

There were no effects of post-weaning diet quality on morphological parameters measured (*P* >0.05; Table 94). Variation in crypt GC density tended (*P* =0.099) to be higher in pigs fed QL.

Table 94: Effect of low (L) or high (H) post-weaning diet quality (Q) on pig duodenal morphology measurements and variation at day 9 post-weaning.

	QL	QH	s.e.m	*P*-value
n (pigs)	16	16		
Average villus height (µm)	367	358	16.4	0.695
Average crypt depth (µm)	339	361	11.5	0.192
Villus height:crypt depth	1.21	1.13	0.063	0.379
Average mucosal thickness (µm)	763	763	23.7	0.994
Villus GC density (cell/mm^2)	230	215	18.3	0.567
Crypt GC density (cell/mm^2)	1164	1204	66.9	0.671
CV villus height (%)	23.8	25.1	1.42	0.535
CV crypt depth (%)	34.4	33.6	2.00	0.774
CV vh:cd (%)	44.1	44.3	3.55	0.975
CV mucosal thickness (%)	10.3	14.1	1.78	0.134
CV villus GC density (%)	48.0	48.2	5.11	0.982
CV crypt GC density (%)	30.9	24.5	2.65	0.099

6.3.7.3.4. Relationships between measured parameters

Only significant relationships (*P* <0.05) and trends (*P* <0.10) are reported, for all others *P* >0.10. There were no significant results from including post-weaning diet quality as a grouping factor. There were no significant results from multiple regression combining ADG, creep score and treatment grouping factors.

There was a weak positive correlation between villus GC density (cells/mm^2) and ADG (g/d) from d3-6 (*P* =0.040; s.e =70.2, r^2 =0.077), although these were not strong relationships as evidenced by low r^2:

$$Villus\ GC\ density = 0.237 * adg3 - 6 + 183.2$$

There was also a tendency towards a weak positive correlation (r^2 =0.065, s.e =89.5) between creep score and mucosal thickness (*P* =0.085).

Villus height was positively correlated with ADG from d3-6 for CH piglets, but there was no relationship for CL piglets (*P* <0.001; Table 95).

Table 95: Relationship between ADG from d3-d6 post-weaning, low (L) or high (H) pre-weaning creep consumption (C) pig duodenal villus height at d9 post-weaning.

Parameter	Equation	r^2	s.e	*P*-value
Villus height (µm)		0.418	48.9	<0.001
CL	372.1-0.100*adg	0.052		0.359*
CH	219.5+0.784*adg	0.668		<0.001*

**P*-value of ADG*creep consumption

Average daily gain at all other points showed no correlation with morphological parameters (*P* >0.05).

When post-weaning diet specification was included as a grouping factor, ADG from d3-d6 post-weaning tended (*P* <0.010) to correlate with crypt depth.

There were significant positive correlations between ADG d3-6 and villus height:crypt depth, and mucosal thickness (*P* <0.05; Table 96).

Table 96: Relationship between ADG from d3-6 post-weaning, low (L) or high (H) post-weaning diet specification (S) and pig duodenal morphological parameters at d9 post-weaning

Parameter	Equation	r^2	s.e	*P*-value
Crypt depth (µm)		0.138	57.1	0.068
SL	314.1+0.287*adg	0.194		0.056¥
SH	363.8-0.190*adg	0.005		0.292¥
Villus height:crypt depth		0.174	0.247	0.024
ADG d3-6				0.093†
SL	0.896+0.000841*adg	0.073		<0.001*
SH	1.171+0.000841*adg	0.127		<0.001*
Mucosal thickness (µm)		0.147	85.4	0.038
ADG d3-6				0.015†
SL	660.3+0.431*adg	0.112		<0.001*
SH	721.5+0.431*adg	0.333		<0.001*

**P*-value of post-weaning diet specification. † *P*-value of ADG d3-6. ¥*P*-value of ADG*post-weaning diet specification

Average daily gain at all other points showed no correlation with morphological parameters when post-weaning diet specification was included as a grouping factor (*P* >0.05).

Creep score correlated with crypt depth (*P* =0.045; Table 97) and villus height:crypt depth (*P* =0.023) when diet specification was included as a grouping factor. There was a tendency for a positive correlation between creep score and cd for pigs fed SL (*P* =0.096), and a negative correlation between creep score and cd for pigs fed SH (*P* =0.082). There was no correlation between villus height:crypt depth and creep score for SL piglets *P* =0.638), but creep score was positively correlated with villus height:crypt depth for SH piglets (*P* =0.032).

Table 97: Relationship between creep score (duration of visits to the creep feeder/ADG in the final week pre-weaning), low (L) or high (H) post-weaning diet specification (S) and pig duodenal morphological parameters at d9 post-weaning

Parameter	Equation	r^2	s.e	*P*-value
Crypt depth (µm)		0.166	56.2	0.045
SL	312.2+0.00804*creep score	0.141		0.096*
SH	393.8-0.00742*creep score	0.248		0.082*
Villus height:crypt depth		0.207	0.242	0.023
SL	$1.15-9.6\text{x}10^{-5}$*creep score	0.023		0.638*
SH	$0.99+4.0\text{x}10^{-4}$*creep score	0.214		0.032*

**P*-value of creep score*diet specification

6.4. Discussion

6.4.1. Pre-weaning

Between the three studies, pre-weaning performance was at a similar level, and equivalent to UK top 10 % (AHDB, 2024). The lower pre-weaning mortality in Study 3 was due to improved colostrum management, as evidenced by the lower proportion of deaths due to being considered not viable, and lower mortality of lightest birthweight piglets: colostrum was collected from sows during parturition and piglets that were chilled, splayed or <1 kg birthweight were bottle-fed for the first 24 hours after birth. Thereafter, they were encouraged to use the SM bowl if they hadn’t obtained a teat to suckle. This was in addition to litters with >14 piglets at birth being split-suckled, whereby the heaviest half of the litter were removed from the farrowing pen for two hours in the morning and afternoon until fostering occurred.

The more rapid increase and overall higher creep consumption in the week prior to weaning observed in Study 3 compared to Study 2 is assumed to be due to the removal of SM on day 18, in accordance with findings from the pilot study at the end of Chapter 5, but consumption was still lower than in the studies of Bruininx *et al.* (2002a), Wattanakul *et al.* (2005) and Muns and Magowan (2018). Creep consumption was also lower than observed in the pilot study, which may implicate feeder design as causative: a commercially-available multi-space feeder was utilised in the pilot study as individual intake was not of interest, and allowed social feeding. This represents a limitation of the method of recording creep consumption in the current study.

The greater difference in creep score between CL and CH observed in Study 3 (CH score 124 % higher than CL) compared to Study 2 (CH score 59 % higher than CL) is a result of selecting piglets from the first and third quartiles for pre-weaning creep consumption, as opposed to the top and bottom half, as the mid-range consumers were not included.

The effect of creep consumption classification on pre-weaning performance was similar to Study 2, although the difference in weaning weight between CL and CH was higher in Study 2 (610 g) than Study 3 (300 g). This is probably because non-sucklers were not selected in Study 3 for post-weaning work and so were eliminated from defining CL and CH; in Study 2 these piglets were CH but had very light weaning weights. In future work these piglets could be included as a separate treatment, as they have the most potential to improve as a result of optimisation of pre- and post-weaning nutrition.

Birthweight group 1 appeared to compensate most for SM withdrawal when considering creep consumption, with du/d increasing more for group 1 and 4 than group 2 and 3. In Study 2, piglets from birthweight group 1 had the lowest creep consumption, and group 4 was highest. This is logical as piglets from birthweight group 1 had the highest SM consumption relative to ADG in Study 2, and so had a greater need for additional nutrition once SM provision was removed. For the same reason, non-sucklers had a dramatic increase in creep consumption once SM was removed.

Average weaning weight (9.12 kg) was heavier than four-week weaned pigs reported by Collins *et al.* (2017) at 7.40 kg, but lighter than Muns and Magowan (2018) at 9.41 kg. However, the number of piglets weaned was higher in the current study than in that of Muns and Magowan (2018), with 14.7 piglets weaned compared to 10, which resulted in a higher litter weaning weight.

In Study 2, there was no effect of creep consumption level on pre-weaning ADG, which was also previously observed by Sulabo *et al.* (2010a), Huting *et al.* (2017) and Muns and Magowan (2018), but in the current study ADG from birth-d11, d11-18 and overall from birth-weaning was lower in CH pigs compared to CL. This is probably an indication of lower milk intake from the sow, as there was no difference in birthweight. Although creep consumption was higher for CH piglets than CL from d11-18, it was insufficient to compensate for this low milk intake, but from d19-weaning, the difference in creep consumption was greater, enabling CH piglets to grow at a similar rate as CL piglets. The higher creep intake of the CH piglets prepared them for weaning, as the reduction in ADG for the first three days post-weaning was lower than for CL piglets (36 % *vs* 60 % reduction in ADG respectively).

6.4.2. Post-weaning

The stage 1 diets post-weaning were all lower CP and lysine than the creep diet fed pre-weaning (220 g/kg CP, 17 g/kg total lysine). The closest diet to the pre-weaning creep in terms of CP and lysine was SHQH stage 1.

6.4.2.1. Performance

The lack of a creep consumption*diet specification*diet quality interaction indicated that the effect of diet specification*diet quality on post-weaning performance did not differ between pigs with low or high pre-weaning creep consumption. There is little published literature investigating optimisation of post-weaning feeding regime for pigs with differing pre-weaning creep consumption, presumably due to the difficulty of measuring creep consumption on an individual basis, and none appear to have examined the effect of both diet quality and diet specification. It is possible that low replication at the three-way interaction was responsible for the lack of significance of performance parameters in the current study, as particularly in the first nine days post-weaning performance figures were highly variable. Numerically, from d6-9 the difference in FCR between the postweaning diets was larger in the CL pigs, and this was significant in the analysis of the creep consumption*diet quality interaction. From d6-9, FCR was similar between pigs fed CHQL and CHQH, whereas FCR for pigs fed CLQH was greatly in excess of CLQL (which was similar to both CH treatments). This interaction has not been reported previously, but previous studies have weighed pigs less frequently than the three-day periods in the current study, and so effects of diet in the immediate post-weaning period may have been overlooked.Callesen *et al.* (2007) reported no effect of creep consumption*diet quality, but pigs were not weighed until 14 days post-weaning. The only period where there was an effect of creep consumption*diet quality was from d6-9 in the current study, and the first week post-weaning was identified by Skinner *et al.* (2014) as being the period where ADG was most adversely affected by feeding low-quality diets (ADG reduced by 30 % compared to pigs fed high-quality diets). Muns and Magowan (2018) compared feeding a low *vs* high level of high-quality starter to pigs identified as moderate or high eaters (using chromic oxide marker), or pigs not offered creep pre-weaning. They reported no creep provision*diet quality interaction. In that study, diet quality was differentiated by varying starter feed allocation rather than ingredients, with all pigs receiving at least 2 kg/pig of the first stage. From their ADFI figures (169-210 g/d for the first week), this allocation would have lasted for approximately 10 days, and so the acute phase of weaning had passed by the time dietary treatment was applied.

The results of the current study with regards to diet quality contradict most previous research, which has demonstrated that higher quality diets result in higher ADG, ADFI and lower FCR (Mavromichalis *et al.*, 2001b; Skinner *et al.*, 2014; Muns and Magowan, 2018) or at least have no adverse effects on performance (Leliveld *et al.*, 2013). Skinner *et al.* (2014) concluded the positive effect of high-quality diets was due to lower anti-nutritive factors in the high-quality diets, because of lower soyabean meal inclusion, or that the protein in the low-quality diets was insufficiently digestible. The high-quality diets fed in the current study had higher cooked cereals and milk products, and lower soya content than the low-quality diets, which are common distinctions in the literature. The lower ADG for pigs fed QH was not expected, and was a consequence of both lower ADFI and higher FCR. The repeated measures analysis of weight and ADG revealed that the poor performance of QH was mainly due to that of pigs fed SLQH, as at all points, pigs fed SHQH had similar ADG and weight to pigs fed both QL diets. The poor performance of pigs fed SLQH to d22 post-weaning could indicate that the higher digestibility of ingredients of the high-quality diet could not compensate for the lower SID lysine (and other essential amino acids, as they were formulated as a proportion of SID lysine), and DE content of the low specification diet. It is possible that digestibility of raw materials could have been over-estimated, but this would have also affected the pigs fed SHQH. Engelsmann *et al*. (2022) proposed that feed ingredients are not as digestible as predicted to newly-weaned pigs, as coefficients of digestibility are commonly assessed on 40 kg pigs with a mature digestive tract. Conversely, it is also possible that the QH diet over-supplied amino acids by the protein by being too highly-digestible, with the absorptive capacity of the small intestine being insufficient. This would lead to excess amino acids being fermented, and resulting in the higher FCR observed as the amino acids were not utilised for growth (Berrocoso *et al.*, 2012). This would have been exacerbated in SHQH, as the CP and lysine content was the highest in this diet, and may be the case in the first six days post-weaning, as SHQH had the highest FCR, but not thereafter. A limitation of this study is that faecal scores were not recorded, or faecal samples taken for DM and NH_3-N analysis, which would have indicated incidence of diarrhoea and fermentation of amino acids respectively. Blood samples to assess plasma urea nitrogen (PUN) levels could also have been taken, as an increase in PUN indicates amino acid fermentation or catabolism (Heo *et al.*, 2009). These physiological measurements may have enabled a more thorough understanding of the interactions between diet specification and quality as they would have indicated nitrogen usage efficiency.

After d6 post-weaning (and potentially from weaning), it is likely that low essential amino acid and energy intake was the cause of poor performance: Total daily requirement of essential amino acids are not published for pigs immediately post-weaning by Whittemore *et al.* (2003), with the standard being applicable to pigs from 10-30 kg, but diets were formulated to be equal to or in excess of SID essential amino acid requirements relative to SID lysine, per kg of feed. The Standard (Whittemore *et al.*, 2003) suggests a 0.55 kg/d feed intake for an average pig at 10 kg weight; in the current study, pigs were approximately 10 kg on d9 post-weaning, but with a feed intake of 0.37-0.41 kg/d. This resulted in daily essential amino acid intake being lower than the requirement for a 10 kg pig until d12-15 for most amino acids. Pigs fed SLQH consistently had numerically lowest ADFI, resulting in lowest amino acid consumption, which would directly negatively affect protein deposition in the muscles, and adversely impact small intestinal function. For pigs fed SLQH, recommended DE intake for a 10 kg pig consuming 0.55 kg/d of 8.53 MJ DE/d was not attained until d15-22, when pigs were 12.20-15.69 kg and consuming 0.65 kg of feed, and so energy was limiting in this diet, in addition to amino acid supply. This was also the case for pigs fed SLQL, and so indicated that DE supplied by the lower specification diets was insufficient. When dietary energy supply through fat and carbohydrate is insufficient, amino acids will be catabolised through the gluconeogenesis pathway, further reducing availability for growth.

It is debatable how long high-quality diets may be required, and may depend on ingredients. Leliveld *et al.* (2013) reported no benefit to feeding a high allocation (4 kg/pig) of high-quality diet compared to a low allocation (1 kg/pig), concluding that the lower allocation was sufficient to aid in adaptation to the post-weaning diet. Muns and Magowan (2018) however found that ADG was higher and FCR was lower from weaning-21d and 42d post-weaning in pigs fed a high allocation (6 kg/pig compared to 2 kg/pig) of starter diet, and this was unaffected by pre-weaning creep consumption (eater/not offered). Muns and Magowan (2018) speculated that the effect of competition at the feeder may influence the effectiveness of high-quality diets, as in their study pigs were housed in large groups (assumed to be high competition and so benefitted from a longer period of higher quality diet), but in Leliveld *et al.* (2013), pigs were housed in pairs. In the current study, pigs were housed in pens of six (five after d9 sampling), with a three-space feeder, and so competition was minimised.

Most studies commence with a first stage diet for one week-10 days post-weaning, characterised by a large difference in the proportions of animal- and plant-derived ingredients between low- and high-quality diets. The subsequent diets are then more similar in ingredients (with lower animal and higher plant-based ingredients) to allow pigs fed high-quality first stage to adapt to plant-derived protein sources (Skinner *et al.*, 2014; Sulabo *et al.*, 2010b; Mavromichalis *et al.*, 2001b). This similarity in later diets results in lower differences in performance parameters typically observed after the initial period post-weaning, and explains the lack of effect of the second stage diets on performance in the current study, as ADFI was unaffected after d12, and ADG and FCR were only significantly affected by diet quality until d15. Previous studies have reported a continuation in higher ADG and ADFI associated with high quality diets in the second stage, but not FCR (Mavromichalis *et al.*, 2001b; Sulabo *et al.*, 2010b; Skinner *et al.*, 2014). However, the specification*quality interaction on ADG d15-22 showed that although the averages for QL and QH were similar, this masked a large difference in ADG between SLQH and SHQH, whereas SLQL and SHQL were similar. This was reflected in the repeated measures analysis as after d12, SHQH pigs exhibited an ability to equal the growth rate of the pigs fed both QL diets.

Commonly, any beneficial effects of high-quality diets are only noticed while they are fed, with Mavromichalis *et al.* (2001b) and Skinner *et al.* (2014) reporting no effect of previous dietary regime on performance in the grower-finisher phase once common diets were fed. Conversely in the current study, pigs fed QH had higher ADG and lower FCR in the grower-finisher phase from 80-140 d old. This suggests that feeding high quality diets better adapted the pig to consuming cereal- and soya-based diets, but the reason for this is unknown, as there were no differences in duodenal morphology at the time of sampling.

The contradiction of results regarding diet quality in the current study to most published literature may be due to the SID lysine:CP ratio of the diets. A meta-analysis by Rocha *et al.* (2023) suggested that the minimum CP level for nursery pigs that maintains performance, if lysine and other amino acids are maintained to recommended levels is 183 g/kg, which all the diets utilised in the current study exceeded. They also proposed that SID lysine:CP was an important predictor of performance, and that it should be a maximum of 6.6, after which most studies showed a reduction in ADG and gain:feed. There were however several studies included in the analysis of Rocha *et al.* (2023) whereby there was a positive relationship of ADG and gain:feed with the ratio of SID lysine:CP, within the range of ~6.0-8.0. Results of the current study, when performance was analysed by diets separately rather than a factorial would agree with these, with higher ADG and lower FCR associated with the higher SID lysine:CP for pigs fed SHQL compared to SLQH. The maximum of 6.6 proposed by Rocha *et al.* (2023) was more applicable to low CP diets, whereby the supplementation of synthetic amino acids could not be a substitute for very low levels of CP providing insufficient substrate for biosynthesis of non-essential amino acids. As diets in the current study were above the 183 g/kg CP threshold suggested, it appears that a higher level of lysine (and consequently other essential amino acids) was beneficial to performance, and that this had more of an effect on performance than the predicted higher digestibility of high-quality ingredients.

To the best of my knowledge, there have been no previous studies investigating the effect of pre-weaning creep consumption*diet specification on post-weaning performance. In the current study, the sole interaction was observed when all pigs were consuming a common grower diet, with CHSH having lower ADG than CLSH. The reason for this is unknown, but it did not preclude a consistent reduction in ADG, as pigs fed CHSH were heaviest at 19 weeks old.

The difference in CP content of the diets (10 g/kg) in the current study is smaller than those in previous studies, as these diets were formulated to inform commercial practice rather than investigate the effects on performance associated with more extreme reductions in CP level. The 10 % difference in SID lysine is greater than most other studies, which have maintained lysine level. The lower ADG for pigs fed SL from weaning-d3 and overall to nursery exit was expected due to the consequent lowering of amino acid intake, although ADFI was similar at all points except for weaning-d3. The combination of lower ADFI and 10 % lower dietary lysine level (g/kg) compounded into a 20 % reduction in lysine intake for the first three days post-weaning for pigs fed SL compared to SH. This agrees with Nyachoti *et al.* (2006) who found that ADG, ADFI and gain:feed decreased as dietary CP levels decreased during the 21 days post-weaning feeding period, and Wellock *et al.* (2008), who reported lower ADFI in pigs fed lower CP (130 g/kg) and lysine (8.1 g/kg) diets for the first three days post-weaning, but not thereafter. Nyachoti *et al.* (2006) maintained lysine levels at 14.0 g/kg, but were unable to balance valine levels as at that point in time synthetic valine and isoleucine were unavailable (Wellock *et al.* 2008). In addition to the higher ADFI, Wellock *et al.* (2008) reported higher feed efficiency accounting for higher ADG from weaning-d14 post-weaning for pigs fed high CP (230 g/kg) and lysine (14.3 g/kg) diets. After dietary treatments ceased at d14 post-weaning their study continued to d42 post-weaning but there was no longer-term effect of dietary CP level on performance. In the current study, the overall lower ADG during the nursery period observed for SL compared to SH was due to small but non-significant increases in FCR at each weigh point up to d22 when the common diet was introduced, that resulted in overall FCR to exit tending to be higher for pigs fed SL.

Previous studies investigating the effects of low protein diets have indicated that performance may be maintained if the lysine level and essential amino acid balance relative to lysine is maintained. Heo *et al.* (2009) fed post-weaning diets with 180 and 240 g/kg CP (maintaining SID lysine at 11.0 g/kg), with valine and isoleucine added to ensure the lower CP diet met recommended standards. In this way, there was no difference in ADG, ADFI or gain:feed from weaning to d14, when the trial diets finished, and to d28. However, they did not weigh pigs more regularly, and in the current study, and that of Wellock *et al.* (2008), the greatest effect of CP level on performance was in the period immediately post-weaning. A similar approach to Heo *et al.* (2009) was taken by Zhang *et al.* (2013), and although they also did not weigh pigs frequently (only d14 and 28), they demonstrated that by supplementing low CP (179 g/kg) diets with isoleucine and valine to match the total as-fed level in the high CP (209 g/kg) diet, ADFI, ADG and FCR were similar. These performance parameters were superior to that of pigs fed low CP diets without branched chain amino acid (BCAA; valine, leucine, isoleucine) supplementation. In the current study the addition of synthetic amino acids was not investigated, but levels relative to SID lysine were maintained between dietary treatments. As a consequence, the SL diets were lower in all essential amino acids than SH diets. It remains to be ascertained whether it is financially viable to reduce CP level but maintain performance by the addition of synthetic amino acids. The minimum CP level to maintain growth also remains to be established, as the addition of synthetic amino acids cannot compensate fully for CP being too low, and addition of one amino acid may lead to a different one becoming limiting (Opapeju *et al.*, 2008). Lynegaard *et al.* (2022) investigated the effects of an extra-low CP diet (155 g/kg) that was supplemented with synthetic Ile, Leu, His, Phe, Tyr, in addition to the Lys, Met, Thr, Trp, Val added to the high CP (188 g/kg) diet, with SID lysine level maintained at 12.0 g/kg. They found that growth and relative weight of the small intestine of pigs fed the extra-low protein diet was lower, but didn’t measure feed intake. Gene expression associated with protein synthesis and immune response was also reduced in pigs fed the extra-low protein diet. They concluded that N availability was too low to allow biosynthesis of non-essential amino acids in pigs fed the extra-low CP diet, probably due to reduced amino acid transporter gene expression, which had previously been shown to be lower in pigs fed a low CP diet.

The higher ADG and lower FCR observed demonstrate that lysine was not oversupplied in SH diets at 15.0 g/kg (SID lysine). It should be noted that these were pigs with no prior history of enteric illness, and there was a comprehensive cleaning and disinfection protocol that resulted in PWD being rare on the research unit. It is possible that in disease-challenged pigs the higher lysine levels may induce bacterial fermentation, and affect performance as observed by Wellock *et al.* (2008), who also concluded that the magnitude of response would be proportional to the challenge encountered.

Classifying pigs according to their pre-weaning creep consumption by quartiles rather than halves (as in Study 2) resulted in a greater difference in both creep score and performance between CL and CH. Weighing more frequently in the current study enabled a more detailed elucidation of the effect of pre-weaning creep consumption level on early post-weaning performance. Average daily feed intake between weaning and d15 was higher for CH pigs in the current study, whereas this was not affected by pre-weaning creep consumption in Study 2. Higher feed intake for pigs with high pre-weaning creep consumption has previously been reported by Bruininx *et al.* (2002a), Sulabo *et al.* (2010a) and Muns and Magowan (2018), who concluded that this was due to familiarity with solid feed and consequently lower stress. In the study of Muns and Magowan 2018), higher ADFI did not result in higher ADG, which the authors attributed to the difference in ADFI being too small. In the current study, the higher ADG of CH between weaning and d12 resulted in CH pigs equalling the weight of CL by d6, whereas this did not occur until d29 in Study 2. In common with Study 2, FCR was lower for CH pigs for the first nine days post-weaning. The reason for the higher FCR of CH pigs from d36-54 is unknown, as all pigs were fed a common diet at this point, but it resulted in lower ADG for CH than CL for this period. Day six post-weaning was the point where ADG was lowest and FCR was highest, although ADFI was higher between d3-6 than weaning-d3. This negative effect of weaning on ADG and FCR was less extreme in CH than CL, indicating that CH pigs were better adapted to weaning physiologically (lower FCR) and psychologically (higher ADFI).

6.4.2.2. Post-weaning feed cost and finisher performance

There are few studies that continued recording pigs to slaughter after the trial feeding phase, or that publish any financial assessment. The results for diet quality in the current study disagrees with Skinner *et al.* (2014) and Collins *et al.* (2017), who reported that nursery diet quality had no effect on ADFI, ADG or FCR in the finisher phase. In the current study, pigs fed QH were 1 kg heavier at 19 weeks old (weights not significantly different) than those fed QL despite being 1.5 kg lighter at nursery exit, due to higher ADG and lower FCR. Although there were no significant effects of pre-weaning creep consumption level or diet specification on finisher performance, the lowest relative weighted cost/kg gain was for pigs fed SH, independent of creep consumption classification, and CH pigs had the lowest relative weighted cost/kg gain for all diets. Collins *et al.* (2017) calculated that the additional feed cost to slaughter for feeding high quality weaner diets was economically beneficial for light pigs at weaning, as cost/kg gained was AU$0.02 /kg lower. For medium and heavy pigs, the cost/kg gained was higher (AU$0.03 /kg and AU$0.01 /kg respectively). In the current study, pigs were not grouped by weaning weight, but feeding SHQH was the optimal regime economically for CL pigs, and SHQL was the optimal regime for CH pigs. An assumption can be made that pigs that were better adapted to weaning (CH pigs or medium/heavy pigs in Collins *et al.*, 2017), did not require high quality diets, but these were beneficial for pigs with poorer adaptation. Christensen and Huber (2021) didn’t continue recording performance to slaughter, but did publish an economic assessment for the end of the nursery phase at 59 d post-weaning. They found that feeding high-quality diets cost CAN$0.10/kg gained more than feeding low quality diets, but the high-quality fed pigs were 3 kg heavier. The authors stated that the improvement in weight was not proportional to the extra cost of feed, but a 3 kg difference in weight at 59 d post-weaning could make a large difference to slaughter weight, and so increase the value of the slaughter pig. In the current study and that of Collins *et al.* (2017), pigs fed high-quality diets post-weaning also had the highest cost/kg gained at the end of the nursery period, due to more expensive feed. In the current study, this was exacerbated by the lighter weight at nursery exit. The optimum regime financially depends on whether pigs are sold as stores after the nursery phase, or kept to finish. At the end of the nursery phase pigs fed SLQL had the most negative difference in cost/kg gain (comparing all diets to SLQH) and so this option would suit producers selling stores. This regime had the highest cost/kg gain in the finishing period (+3 p/kg for CL and +4 p/kg for CH), which due to the volume of feed consumed and the weight gain experienced had a greater effect on overall relative weighted cost/kg gain than the lower nursery feed cost. For CL pigs, the optimum regime was SHQH, indicating that this diet prepared the pig for transition to the cereal-based grower and finisher diets most efficiently. For CH pigs, the optimum diet was SHQL, suggesting that their higher pre-weaning creep intake had initiated adaptation to solid feed, and so the high digestibility ingredients of the QH diets were unnecessary. For both creep consumption classifications, a higher lysine level was associated with the optimum regime, indicating that lysine was not oversupplied.

6.4.2.3. Duodenal morphology

6.4.2.3.1. Pre-weaning

The effect of level of pre-weaning creep consumption on pig vh, cd and vh:cd at weaning agrees with the previous research of Bruininx *et al.* (2004) and Muns and Magowan (2018), who also reported no effect of creep intake on these parameters. In the current study, vh was higher than that reported by Bruininx *et al* (2004) and Muns and Magowan (2018), at an average of 734 µm compared to 453 µm and 485 µm respectively, but cd was similar (current study averaged 225 µm compared to 226 µm and 222 µm respectively). The lack of effect of pre-weaning creep consumption on most of the parameters measured probably reflects that pre-weaning nutrient intake was sufficient, and so there was no imbalance between enterocyte apoptosis and proliferation. The amount of creep consumed was relatively small in comparison to sows milk consumption, and so had little effect on maturation of the gut, which would have resulted in deeper crypts for higher consumers (Bruininx *et al.*, 2004). For pigs classified as CL and CH, vh:cd was above the threshold of 3, suggested by Bekebrede *et al.* (2020) as the ideal ratio.

6.4.2.3.2. Post-weaning

The adverse effect of weaning on intestinal histomorphology has been well-described, with the shortening of villi, deepening of crypts and reduction in the vh:cd ratio observed in the current study agreeing with previous investigations (For example Montagne *et al*., 2007; King *et al.*, 2008; Engelsmann *et al.*, 2022). This change in morphology was also described by Makkink *et al*. (1994), who reported that on d6 post-weaning all villi were stunted (tongue- and leaf-like) and there were no finger-like villi. In support of the visual assessment, variation in vh, cd and vh:cd was also much higher post-weaning. Reduction in villus height (villus atrophy) post-weaning is due to epithelial cells being lost from the tip of the villus at a faster rate than they proliferate and migrate from the crypts, caused by lack of nutrients directly available to support the cells (Pluske *et al*., 1997). This was demonstrated by Bruininx *et al.* (2002b), who reported that pigs with a higher rate of increase in feed intake post-weaning had longer villi than pigs with a lower rate of increase in feed intake. Low feed intake causes a reduction in stimulation of the intestinal stem cells for proliferation, via GLP-2 signalling (Burrin *et al.*, 2003; Baldassano and Amato, 2014). Secretion of GLP-2 is initiated by nutrient intake (Burrin *et al.*, 2003), and activates the PI 3-kinase-dependant AKT-mTORC1 pathway, activating mTOR which regulates phosphorylation of ribosomal protein kinase p70S6K1 and binding protein 4E-BP1 (Nojima *et al.*, 2003; Ahmed *et al.* 2019). Phosphorylation of p70S6K1 occurs at threonine residue 389, which causes folding of the protein to activate it (Ahmed *et al.*, 2019). These proteins increase ribosomal mRNA translation and thus protein synthesis, resulting in cell proliferation (Shi *et al.*, 2011; Deng *et al.*, 2023). Wang M *et al.* (2022) reported that the number of duodenal cells expressing the ki67 marker that indicates cell proliferation was lowest on d1 and d3 post-weaning, but had recovered to pre-weaning levels by d7 post-weaning, agreeing with Kim and Kim (2017) who observed that epithelial cells are renewed every 4-5 days. Although it was not possible to measure individual feed intake in the current study, there were positive correlations between morphological measurements and ADG. These could be due to pigs with higher ADG having higher feed intake, and so lessening the negative effect of reduced post-weaning feed intake on villus atrophy. Alternatively, pigs with less post-weaning damage to the duodenal structure would be able to absorb digested nutrients more efficiently and consequently have higher ADG. Given that the correlation with ADG was for d3-6, and the epithelium is renewed every 4-5 days (Kim and Kim, 2017) it suggests that the (presumed) higher feed intake indicated by higher ADG initiated a higher rate of cell proliferation, that was then evident in the morphology on d9.

Villus atrophy results in reduced enzyme activity, as the brush border enzymes are located in the microvilli of mature enterocytes that are lost from the tip (Engelsmann *et al.*, 2022). Lower vh and vh:cd results in reduced efficiency of digestion and absorption due to lower absorptive surface area (Nabuurs *et al.*, 1993; Ma *et al*., 2019). Combined with reduced pancreatic enzyme activity, this accounts for a reduction in digestibility of CP and amino acids immediately post-weaning (Engelsmann *et al.*, 2022). This effect was apparent in the current study by the reduction in ADG from weaning to d12 post-weaning, and by FCR being higher from weaning-d9 than from d9-22.

The lower goblet cell (GC) density in both crypts and villi observed post-weaning indicates a reduction in the barrier function of the intestine due to reduced mucin secretion, which increases the risk of disease from pathogenic bacteria (Lee *et al.*, 2016). It was not associated with post-weaning diarrhoea, presumably due to the high health of the pigs utilised for this study. Lower GC density post-weaning was also reported by Bruininx *et al.* (2002b), and was proposed to be due to poor feed intake reducing mTOR signalling of cell proliferation by Wang M *et al.* (2022). It could also be due to reduced differentiation of epithelial cells, as reported by Yang *et al*. (2016) in the jejunum of pigs post-weaning. Wang M *et al.* (2022) reported that gene expression for differentiation of secretory cells (goblet cells, Paneth cells and enteroendochrine cells) was lowest between weaning and d7 post-weaning, with the lowest levels of expression of mucin 2 (MUC2) and atonal homolog 1 (Atoh1), which code for the secretory cells. Goblet cell density in the villi and crypts of the duodenum and jejeunum were also found to be lowered by infection with enterotoxigenic *E. coli* (ETEC) in the studies of Kwon *et al*. (2014) and Lee *et al.* (2016). There was no noted incidence of diarrhoea in the current study, and so the reduced ADFI immediately post-weaning is the most likely cause.

The degree of replication (n=4) for the creep consumption*diet specification*diet quality analysis was insufficient to allow conclusions to be drawn. This degree of replication was chosen due to the practicalities of sampling that number of pigs on a single day. Given that there was no difference in performance parameters, it is likely that this result would remain if replication was increased to n=7 (crypt depth) or n=10 (villus height and vh:cd), which were the required levels identified by power analysis.The benefit of increasing replication should be considered against the number of pigs to be euthanised given this likelihood, and that an additional source of variation would be introduced by the sampling being performed across two days. At the level of the two-way interactions, there is a lack of published research with which to compare these results. Due to the only significant creep consumption*diet specification effects on performance being after these samples were taken (d36-exit), it is unsurprising that there was no effect on duodenal morphology. However, when considering the creep consumption*diet quality interaction, for CLQH pigs, from d6-9 post-weaning ADG was lower and FCR higher than CHQH, and so a lower villus height or vh:cd for CLQH pigs could have been expected if the reason for the poorer FCR was a reduction in absorptive capacity of the duodenum. A similar effect could also have been expected for the diet specification*diet quality interaction, as FCR from d3-6 was lower in pigs fed SHQL that

SHQH, but there was no difference in any of the parameters measured. There were significant correlations between morphological measurements and ADG d3-6, so it is possible that the timing of sampling was incorrect for effects of diet quality on duodenal morphology.

The lack of creep consumption*diet quality effect on morphology agrees with Muns and Magowan (2018), at one-week post-weaning and three-weeks post-weaning. However, in their study, they fed a common starter diet in low and high allocations, and by the time of the one-week sampling, all pigs would still have been consuming the first stage diet, and so they would not have expected to see a diet quality effect. By the time of the three-week sampling, it is unlikely there would be any residual benefit from creep feeding on morphology, especially as they did not see an improvement in post-weaning ADG due to creep intake.

The main effects of creep consumption, diet specification and diet quality on intestinal morphology have been more thoroughly investigated. Muns and Magowan (2018) reported that level of creep consumption had no effect on post-weaning intestinal morphology at one- or three-weeks post-weaning, and so the higher ADFI associated with eaters of creep did not compensate for villus atrophy, and this was reflected in the lack of effect of creep consumption on post-weaning ADG. The only effect of pre-weaning creep consumption on average morphological parameters in the current study was that cd was lower in CH pigs, and this is contradictory to Bruininx *et al.* (2004), who found that moderate and good eaters had deeper crypts than non-eaters at d5 post-weaning. They concluded that this may be because the intestine was starting to recover due to higher feed intake of good and moderate creep eaters, as an increase in cd indicates more crypt cells that can then differentiate and migrate to the villus epithelium. This maturation of the intestine (Boston *et al.*, 2022) is stimulated by the withdrawal of sow's milk, and may be influenced by sex, with gilts having higher vh than boars (Pluske *et al.*, 2003). This indicates a limitation in the current study, as both boars and gilts were sampled, adding a potential sex effect.

A higher vh:cd is generally considered to be favourable (Nabuurs *et al*, 1993), and in the current study ADG from d3-6 post-weaning was positively associated with vh, but only in CH pigs. *In vitro* work by Kuller *et al.* (2007) found that nutrient absorption at d4 post-weaning was higher in pigs that consumed creep pre-weaning compared to non-consumers. It is possible that the sampling in the current study was too late to capture any effect of creep consumption on villus height or vh:cd, as the intestinal structure may begin to recover from d4 post-weaning (Bruininx *et al.* 2004) with cell proliferation back to pre-weaning levels by d22 (Wang M *et al.*, 2022).However, an extreme reduction in CP level can have lasting adverse effects of intestinal morphology. Yu *et al.* (2019) compared pigs fed diets of 140 g/kg CP and 200 g/kg CP, and found that even 45 d post-weaning vh and vh:cd in the duodenum and jejunum were lower in the 140 g/kg CP diet. They concluded that this was due to there being insufficient protein to maintain the epithelium, particularly BCAA (leucine, isoleucine, and valine) as these were not supplemented and were lower than recommended levels, whereas lysine, methionine, threonine, and tryptophan levels were maintained at recommended levels between treatments.

Villus atrophy is more severe in the duodenum as it relies on luminal nutrition, compared to the ileum, which receives mainly circulatory nutrition that may be supplied by catabolism of tissue during anorexia (van Beers-Schreurs *et al*., 1998), but the duodenum recovers more quickly (Modina *et al.*, 2021). The sampling day in the current study was chosen to allow for the detection of any effect of post-weaning diet on morphology, and to avoid the unpredictability of the acute weaning phase. The period in which performance was worst affected by weaning was from d3-6 post-weaning, and this was the only period to show correlations of ADG with morphological measurements, particularly for diet specification.

The higher vh:cd observed for SH pigs indicates that absorptive capacity of the duodenum was higher in these pigs, and evidence for this is provided in the form of the lower FCR recorded compared to SL, leading to higher ADG from weaning-d9 post-weaning. As noted by Larsen *et al.* (2021), previous research is inconclusive when considering the effect of CP level on intestinal villus height and crypt depth. There are variations in CP levels investigated, amino acid levels, area of intestine sampled, and day of sampling post-weaning. Weaning age is an important consideration, as four-week weaned pigs will be better adapted to weaning than younger pigs, and so may be less sensitive to a reduction in CP (Opapeju *et* al., 2008). Some studies have an acclimatisation period where pigs were fed a common diet for a period post-weaning (Opapeju *et al.*, 2008; Yu *et al.*, 2019), and this means that any opportunity for diet specification to influence intestinal morphology in the acute post-weaning phase may be missed. There may also be genetic differences, both between different lines of pigs and in genetic progress over time (Muns and Magowan, 2018). Engelsmann *et al.* (2023b) reported no effect of CP level (241 *vs* 203 g/kg) on intestinal morphology, or diarrhoea incidence. They concluded that the time of sampling was too long after weaning (28 days) to observe any effect of protein level on intestinal morphology, and this is likely as several studies have identified that morphology recovers to pre-weaning levels by d14 post-weaning at the latest (Hedemann *et al*., 2003; Degroote *et al.*, 2020; Engelsmann *et al.*, 2022). Nyachoti *et al.* (2006) reported that CP level (170, 190, 210, 230 g/kg CP) had no effect on duodenal morphology, but they maintained SID lysine level between treatments, indicating that negative effects of low CP diet on intestinal morphology may be ameliorated by maintaining lysine level, and including synthetic amino acids to maintain the essential amino acid balance relative to lysine. As shown in the current study, daily essential amino acid intake was considerably lower for pigs fed SL compared to SH, as they were formulated based on the recommended level relative to SID lysine (Whittemore *et al.*, 2003), which was 1.5 g/kg lower for SL than SH. It is possible that this reduction in lysine was too high to maintain essential amino acid supply required for maintenance of duodenal morphology in SL pigs. Zhang *et al.* (2013) reported that a low CP diet with a 0.7 g/kg reduction in lysine level had an adverse effect on vh; this effect was reduced by the addition of BCAA, but not eliminated. In addition to protein synthesis, essential amino acids have specific functions in the intestine, with reductions in dietary leucine and threonine likely to be particularly damaging. Branched chain amino acids (leucine, isoleucine, and valine) and lysine regulate expression of intestinal amino acid transporters (Zhang *et al.*, 2013), and leucine also activates the mTOR pathway to stimulate tissue growth, with threonine residue 389 being the key binding site on the ribosomal protein kinase S6K1 (Ahmed *et al.*, 2019). Threonine is a crucial component of mucosal proteins and mucin (Święch *et al.*, 2019).

When CP level is reduced, there may also be deficiency of non-essential amino acids. Yue and Qiao (2008) maintained amino acid balance between diets containing 172 g/kg and 231 g/kg CP, but reported lower vh in pigs fed the lower CP diet. They suggested that glutamine levels in the lowest CP pigs were insufficient to maintain gut structure, as glutamine becomes a limiting amino acid under stressful conditions. Glutamine helps regulate enterocyte proliferation by stimulating growth factors (IGF-1, EGF, TGF-α) and mitogen-activated protein kinases (MAPKs), and reduces oxidative apoptosis as a substrate for the production of glutathione (Kim and Kim, 2017). Glutamine is also a precursor to arginine, which increases expression of vascular endothelial growth factor (VEGF), thereby enhancing vascular development of the small intestine (Yao *et al.*, 2011). Glutamine, along with glutamate and aspartate are the main sources of energy for intestinal cells (Deng *et al.*, 2023). This reflects the conclusion of Opapeju *et al.* (2008), that supplementation of essential amino acids leads to others becoming limited, and if nitrogen generally is deficient there is insufficient capability for biosynthesis. Therefore, synthetic amino acids cannot be a substitute for dramatic reductions in CP. Yu *et al.* (2019) suggested that CP levels should not be reduced by more than 30 g/kg to ensure sufficient protein for maintenance of intestinal structure.

In consideration of these studies, it appears that the low SID lysine level of the SL diet is responsible for the lower vh:cd observed, as all other amino acids were maintained at recommended levels relative to SID lysine and so will have suffered a concomitant decrease in supply. The reduction in CP was relatively low, so nitrogen availability for biosynthesis of non-essential amino acids should not have been limiting. The higher vh:cd observed in SH pigs infers that lysine and other essential amino acids were not over-supplied in the high specification diet, as the resultant dysbiosis is associated with a reduction in vh:cd (Li *et al.*, 2024).

The lack of effect of diet quality on post-weaning duodenal morphology was unexpected, given the adverse effect of the high-quality diet on performance from weaning-d9. It is possible that development of the small intestine was prioritised over muscle protein deposition, as the gastrointestinal tract of the piglet has a high rate of growth post-weaning in response to the change in diet (Le Dividich and Sève, 2000), with both length and weight increasing (Engelsmann *et al.*, 2022). The enterocytes of the duodenum rely on luminal nutrition, and so many essential amino acids will be highly utilised directly by the duodenum without entering circulation (Montagne *et al.*, 2007; Modina *et al.*, 2021). Similar to dietary CP level, there are conflicting results regarding the effects of high-quality diets on pig intestinal morphology. Levesque *et al.* (2012) sampled the jejunum and ileum from pigs fed low- and high-quality diets, and reported that vh tended to be lower in pigs fed low-quality diets at two weeks post-weaning than at four- or eight-weeks post-weaning, but there was no difference in pigs fed the high-quality diet. This implies that the high-quality diet supported villus growth through the first two weeks post-weaning through improved digestibility of nutrients. Muns and Magowan (2018) observed that pigs fed a high level of starter feed had longer villi but deeper crypts in the duodenum at three weeks post-weaning, resulting in no difference in vh:cd. They concluded that higher starter feed allowance didn’t compensate for villus atrophy occurring due to weaning, and that there was a lag between treatment and effect, as there was no difference in duodenal, jejunal or ileal morphology at one-week post-weaning. However, due to the quantity of starter diet fed (2 kg/pig for the low allocation) and based on their published ADFI of <200 g/day, both treatments would have consumed the same diet until the second week post-weaning. Most studies, in common with the present one, have reported no effect of diet quality on intestinal morphological parameters post-weaning. Engelsmann *et al.* (2022) reported no effect of protein source (casein, wheat, soyabean meal or rapeseed meal) on jejunal morphology on d7, 14, 21 or 28 post-weaning, but these diets also contained differing CP levels and amino acid profiles. Montagne *et al.* (2007) found no difference in small intestinal structure when wheat replaced whey powder and maltodextrin. Makkink *et al*. (1994) observed there was no effect of protein source (skimmed milk powder, soyabean protein concentrate, soyabean meal, fishmeal) on vh or cd in the proximal or distal small intestine on d6 post-weaning, but reported a positive relationship of vh (proximal and distal) and cd (distal only) with feed intake. Koo *et al.* (2020c) reported no effect of diet quality on vh, cd, vh:cd or number of goblet cells (GCs) in the jejunum, however they found that pigs fed high-quality diets had lower plasma IL-10 (day 7) and IL-6 (day 14), indicating a reduced inflammatory response, probably attributable to lower soya content. In response to IL-6, GCs increase mucin production to protect the epithelium (Deplancke and Gaskins, 2001), and Claus *et al*. (2001) identified that pigs with diarrhoea had almost twice as many duodenal GCs as healthy pigs, concluding this was due to an increased secretion of EGF in response to damage to the epithelium. There was no evidence of increased goblet cells in pigs fed low quality diets in the current study, and mucin secretion was not measured. Engelsmann *et al.* (2023a) found that the number of GCs producing acid mucin at d28 increased with feed intake, but there was no effect of this on neutral mucin-producing GCs, which were the only ones identified in the current study. However, Faba *et al.* (2024) reported that higher ADFI was associated with a lower goblet cell density, and Bruininx *et al* (2002b) found no effect of latency to start feeding, or rate of increase in feed intake on GC density at d5 post-weaning, so it appears that the effect of post-weaning diet and the associated small changes in feed intake on GC density is yet to be determined. Święch *et al.* (2019) concluded that inconsistent effects of CP level (and particularly threonine level, which comprises 30 % of the amino acid content of mucin) on GC density were due to the differences between treatments being too small to elucidate an effect, which may be the case in the current study. Although individual feed intake was unable to be recorded in the current study, there was a weak positive correlation of ADG from d3-6 with villus GC density on d9 post-weaning, but this was not related to diet quality. Higher GC density should be beneficial to gut health (and by extension, pig performance) due to improved barrier function of the intestine from increased mucin secretion (Święch *et al.*, 2019).

In the current study, performance parameters from d3-6 were associated with morphological changes at d9, but with no effect of level of pre-weaning creep consumption or diet quality. It appears that the timing of sampling was correct for comparing the effect of low- and high specification diets post-weaning on the recovery of the duodenum from the acute phase of weaning; there were significant correlations of duodenal morphology at d9 (vh, cd, vh:cd, GC density) with ADG during the period where performance was worst affected by weaning (d3-d6). For interventions applied pre-weaning in an attempt to prepare the intestine for weaning (level of creep consumption), it is likely that sampling was too late to notice any effect, as Wang M *et al.* (2022) reported that cell proliferation returned to pre-weaning levels by d7 post-weaning.

6.5. Conclusion

Pigs with high pre-weaning creep consumption had higher feed intake and ADG, and lower FCR for the first 12 days post-weaning, indicating better adaptation to solid feed. However, there was no improvement in duodenal morphology at d9 attributable to higher pre-weaning creep consumption. Pigs fed a high specification diet were heavier at nursery exit with higher ADG and lower FCR, indicating they were able to absorb the higher nutrient level, due to higher villus height:crypt depth ratio as a result of sufficient dietary amino acid supply. Pigs fed a high-quality diet were lighter at nursery exit, but the SID lysine:CP level was lower in these diets, indicating lysine level may have been limiting. The regime with the lowest adjusted feed cost/kg gained for the nursery period was low specification*low quality, independant of pre-weaning creep intake. In the finishing stage, both low- and high-creep consumers benefitted from having consumed high lysine from weaning-d22, but for CL pigs the higher quality (digestibility) regime resulted in the lowest cost/kg gained, whereas for CH pigs high quality ingredients post-weaning were unnecessary. The mechanism for this difference was unable to be identified from examining duodenal morphology.

7. General discussion and conclusion

7.1. Introduction

This project aimed to assess the effect of supplementary milk and creep consumption on performance, behaviour, and physiology of piglets in sow-suckled litters. Pre-weaning nutrition of piglets is increasingly of interest due to higher litter sizes, with supernumerary piglets compared to functional teats becoming more common. There is also a focus on pre-weaning mortality from a welfare and efficiency point of view. The increase in popularity of batch farrowing as a strategy to maintain health by enabling an all-in, all-out system results in less opportunity for rearing extra piglets, as shunt-fostering is not possible, and keeping nurse sows risks transferring any disease between batches. Piglets from larger litter sizes are typically at higher risk of mortality due to low birthweight or starvation. Previous research regarding the timing of introduction of SM found that it needed to be offered from birth to reduce mortality, with no benefit observed from introduction at seven days old, as piglets start to starve from birth without sufficient nutrition (Stewart *et al.*, 2010). Previously there have been conflicting results with regards to the effect of SM on performance, due to the lack of facility to determine which piglets were consuming SM. The effect of creep provision on performance and physiology has also been inconsistent, with limited ability to distinguish between low and high consumers. In this project a novel EID and antenna system was employed to identify piglets consuming SM and creep. After initial development work to refine the design of the equipment and improve functionality so that visits to the SM and creep feeders were confidently recorded, the project commenced with focussing on SM provision and characterising which piglets were high consumers of SM in Study 1 (by birthweight and suckling position), and the effect of SM consumption on pre- and post-weaning performance. In Study 2, creep consumption was investigated, using the same characterisation categories as Study 1. The aim was to assess the combination of SM and creep, and the effects of low and high consumption levels on post-weaning performance and the plasma metabolome to determine if there was any physiological adaptation connected to level of supplementary nutrition consumption. This revealed that creep consumption had a lifetime effect on performance, and so a short pilot study was performed to determine the effect of early SM withdrawal on creep consumption, informing the design of Study 3. This final study withdrew SM provision one week prior to weaning to increase creep consumption. The aim of Study 3 was to examine the effects of low and high pre-weaning creep consumption on optimal post-weaning dietary regime, with regards to lysine level (specification) and diet digestibility (quality of ingredients), and the combined effect of creep consumption and dietary regime on duodenal morphology nine days post-weaning.

7.2. Contribution to knowledge, and limitations of the project

7.2.1. Novel methodology

A unique method for quantifying pre-weaning supplementary nutrition consumption of piglets was employed. The antenna system with EID and frequent weighing enabled litter SM and creep consumption to be estimated from daily duration of visits and piglet weight. Using the calibration equations revealed daily DMI patterns of both SM and creep, without needing to weigh either.

This system enabled the quantification of individual consumption of SM and creep with a higher degree of discrimination than traditional methods of utilising in-feed markers, which are subject to dilution from high SM or water intake, and typically only allow characterisation at the “eater”/”non-eater” level. It allowed continuous recording of consumption of SM and creep, eliminating observer fatigue and any temporal bias associated with diurnal variation in activity during direct observation. The calibration equation for SM allowed estimation of litter consumption from duration of visits and piglet weights, with r^2 0.84. By incorporating piglet weight, a single equation could be used for the entire suckling period. Duration of visits was also a significant term in the creep calibration equations, although these didn’t have such high r^2 as those of SM. The final week pre-weaning had the highest variance accounted for, at 0.684 when data from Study 2 and 3 were combined.

Continuous recording of SM and creep consumption allowed multiple regression equations to be calculated to relate pre-weaning duration of visits and post-weaning pig weight to post-weaning performance, revealing that level of creep consumption had a positive association with ADG until slaughter, but that the effect of SM was short-lived when both SM and creep were offered concurrently. This knowledge prompted a pilot trial, withdrawing SM one week early, as increasing creep consumption would have a greater positive effect on lifetime performance than increasing SM consumption, and creep is less expensive on a DM basis.

The quantification of SM and creep consumption for individual piglets and frequent weighing allowed an assessment of the relative importance of each nutrition source to the piglet’s overall nutrition to be made (the milk or creep score: duration/ADG). Using this score was also an indirect method of assessing sow milk consumption, as a piglet with a low score would have high ADG compared to the amount of SM/creep consumed, indicating that a higher proportion of nutrition came from the sow. Conversely, a piglet with a high score would have low ADG compared to the amount of SM/creep consumed, indicating a lower proportion of nutrition came from the sow. This was thought to be more relevant biologically than just estimating total consumption, as a heavy pig suckling a productive teat may consume more SM or creep than a light pig suckling a less productive teat, or not suckling, but the contribution of the energy and protein from the supplementary sources to the heavy pigs' overall nutritional balance will be lower, and so require less physiological adaptation. The feed efficiency relating to the nutrition sources was assumed to not vary between piglets, which for healthy piglets consuming sows' milk is probably a justified assumption. This method was used to assess the effects of both SM and creep consumption on post-weaning performance and the metabolome (Study 2) and the effect of creep consumption on post-weaning performance and duodenal morphology (Study 3). This project identified an improvement in FCR immediately post-weaning for piglets with high pre-weaning creep intake in both Study 2 and 3 (creep was not provided in Study 1), whereas most previous research has found that creep improved ADFI but not FCR. It is probably this consideration of the biological significance of level of creep consumption, combined with improved accuracy of recording that indicated that pre-weaning creep intake has an effect on physiological development of piglets. In previous studies, the classification of eaters may have included both low and high consumers of creep, thus physiologically there could be a smaller difference compared to non-eaters.

The use of metabolomics is an emerging technique, and the metabolomic analysis performed in Study 2 is believed to be the first time that the plasma metabolomic profile of suckled piglets has been analysed where SM and creep were offered simultaneously, and that a relative level of consumption of both was included.

To the best of my knowledge this project is the first to assess the optimum feeding regime post-weaning for pigs with differing creep consumption, considering both diet specification (lysine level) and quality (ingredient digestibility).

7.2.2. Key findings and limitations

7.2.2.1. Pre-weaning

This project demonstrated that a large litter size does not necessarily compromise performance or increase mortality, and that supernumerary piglets compared to functional teats can be successfully reared using SM, without being removed from their sow and littermates, reducing social stress. The vast majority of litter growth came from the sow, with SM and creep playing a supporting role, and this project aimed to maximise milk yield by feeding a high protein and energy ration to the lactating sows, with as close to *ad libitum* feeding in the final two weeks of lactation as the housing and management system would allow. Although it was not the main focus of this project, sow performance in the subsequent parity was unaffected by rearing supernumerary piglets, with no difference between IS (insufficient teats) and S (sufficient teats) sows in wean-service interval (WSI), number born alive, or litter weight born alive. This was probably due to the similarity in sow weight change between S and IS sows, as Thaker and Bilkei (2005) observed a quadratic relationship between lactational weight loss and WSI, farrowing rate and total number born in the subsequent parity. They showed that there was little difference in these parameters when lactational weight loss was <10%. Of the 43 sows included in this Study, only three (two IS and one S) had an adjusted weight loss of >10 %. The low degree of weight loss observed overall in this Study was assumed to be due to the high feed intake of the sows. The similarity in sow weight change between S and IS sows was probably due to higher SM consumption in IS litters limiting excess demand from the piglets, as sow feed intake was similar. This demonstrates that rearing a large litter does not necessarily equate to poor lifetime performance of the sow, although sows were followed only to the subsequent parity and not thereafter, with no assessment of longevity.

The SM and creep calibration equations obtained in Studies 1 and 2 respectively enabled an estimation of daily SM and creep DMI from the recorded duration of visits and piglet weights. The highest SM and creep DMI was in the final week prior to weaning for all three studies, which is generally accepted to be the period when sow milk yield is thought to limit piglet growth (Hughes and Varley, 1980). To support piglet growth observed in the final week pre-weaning in Study 2 of 302 g/d for 14.3 piglets, a sow would have to yield 17.3 kg/d milk, working on the assumption of 1kg sow milk for 250 g piglet growth (Whittemore *et al.*, 2003). The estimated supplementary DMI provided by SM and creep for this period was 65.8 g/piglet per day, or 59.2 g/piglet per day by removing the 25 % over estimation identified from the weighed creep intake compared to calibration equations. This provided 846 g litter growth per day, equivalent to 3.4 kg/d sow milk. As shown in Figure 74, sows' contribution to litter growth increased from d4-11 to d12-18, but then reduced during d19-weaning, with piglet supplementary nutrition providing the shortfall. Supplementary milk consumption during Study 3 was not recorded, and so only the final period where SM was unavailable is presented.

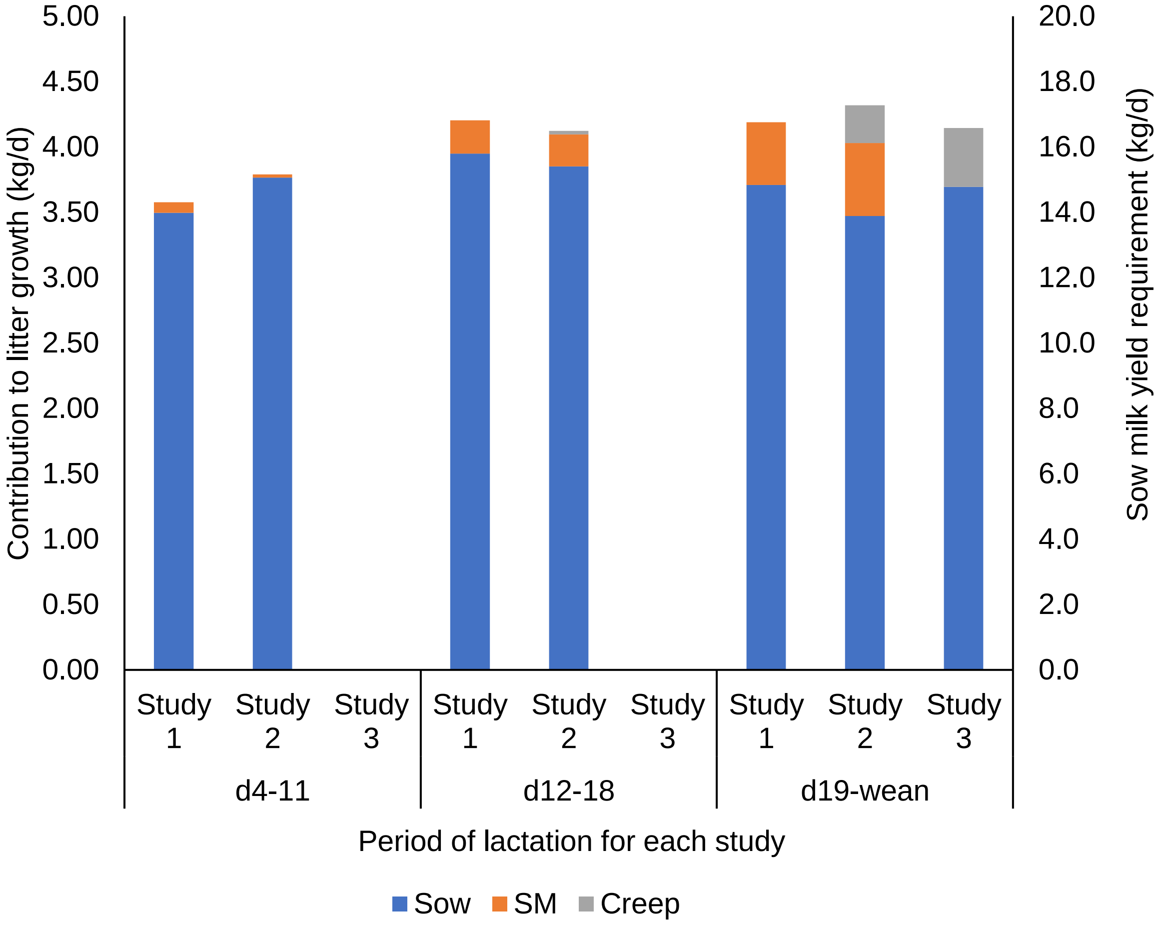

Figure 74: Contribution of sow and supplementary piglet nutrition to litter growth during lactation. Combined height of the column represents sow milk yield requirements if no supplementary piglet nutrition is provided, based on 1 kg sow milk:250 g piglet growth (Whittemore *et al.*, 2003).

Without supplementary nutrition the reduction in milk yield would have limited piglet growth. It is unknown whether the sow initiated the reduction in milk production, but this seems unlikely as there was no reduction in feed intake, or whether it was a response to reduced demand from the piglets, due to their increasing consumption of SM and/or creep. It is possible that the high feed allocation and lower milk yield during the final week of lactation prevented excessive sow weight loss, and maintained performance into the following parity, as sow weight loss after taking account of litter birthweight was lower than in other published studies (for example Pustal *et al.*, 2015; Kobek-Kjeldager *et al.*, 2020a).

Studies 1 and 2 demonstrated that the main benefit of SM was in rearing piglets without a teat, but suckled piglets that were heavy at birth also had relatively high SM intake in the final week of suckling. When creep was provided, the lightest birthweight piglets preferred to continue consuming SM, whereas the heavier birthweight piglets had higher creep consumption in the final week pre-weaning. However, when SM was not available in Study 3, the lightest birthweight piglets had similar creep consumption to the heaviest birthweight piglets. This was probably driven by non-sucklers rapidly transferring to consuming creep once SM was withdrawn on d19 in Study 3. Non-sucklers had higher ADG during this week than from d12-19, potentially due to the higher DM content of the creep diet compared to SM. This rapid switch in nutrition is similar to that which occurs at weaning, and so by the day of weaning, the non-suckled piglets were probably better adapted than suckled piglets. Non-sucklers were not recorded post weaning in Study 3, but in Study 1 and 2 when SM was provided to weaning, they had similar ADG to suckled piglets for the week post-weaning despite having lighter weaning weight, and so it could be anticipated that the higher creep intake observed in Study 3 would improve this. It is possible that the stress of weaning is lower in non-suckled piglets as they have not been relying on the sow as a source of nutrition, and are thus more self-sufficient than suckled piglets. Study 1 identified that supernumerary piglets compared to functional teats could be reared, but these piglets were likely to be light at weaning, due to the lower fat content of SM, and Study 2 revealed that creep provision did not compensate for this. Due to their low weaning weight, piglets without a teat to suckle were largely ineligible for characterisation of the metabolome, and were not included for investigation of duodenal morphology to simplify the experimental design as the 2*2*2 factorial already resulted in eight treatments. This is a limitation of this study, as these piglets would be likely to have the greatest difference compared to suckled piglets with low SM consumption, and also had the highest creep intake when SM was removed. As previously stated, non-suckling piglets are those with the highest potential to improve. Identifying differences in the metabolome between suckled and non-suckled piglets may enhance understanding of pre-weaning nutritional requirements, and lead to improved milk replacer formulation.

Motivations for consumption of supplementary nutrition vary from survival (for non-sucklers) to matching appetite (suckled pigs). For non-sucklers this is a necessity in response to being unable to secure a teat to suckle due to high competition from more vigorous littermates. Suckled piglets made use of SM from the beginning of the recording period at d4, and consumption is assumed to occur between suckling bouts. Whether it reflects genuine need for extra nutrition at this point, or is a result of exploratory behaviour and finding something that tastes good is unknown. The SM provided was not warmed, apart from at initial mixing, so it is unlikely that suckled pigs would wean themselves from the sow in order to consume SM in preference, especially as the sow also produces pig-appeasing pheromone (PAP) from the udder to attract the piglets to suckle (Temple *et al.*, 2016). It is possible that piglets are hungry between suckling bouts, as udder massage between suckling bouts has been documented (Torrey and Widowski, 2007; Ocepek *et al.*, 2017), with a negative correlation with growth rate (Torrey and Widowski, 2007); whether this is reduced when SM is provided is unknown. It is energetically costly to the pig to engage in udder massage (Torrey and Widowski, 2007), and so consumption of SM would be favourable. In this case it seems counterintuitive that the posterior-suckled piglets did not consume more SM. They were very similar in birthweight to the centre-suckled piglets, and so would be expected to have a similar weaning weight, if nutrition was not limiting. Lower milk yield from posterior teats has been widely observed, and so it could be anticipated that the posterior-suckled piglets would increase their SM consumption to compensate, but this was not in evidence in either Study 1 or 2, or for creep intake in study 2, and they were considerably lighter (750 and 800 g Study 1 and 2 respectively) than centre-suckled piglets at weaning. The ADG of posterior-suckled piglets was lower from d4 onwards, suggesting that early nutrition was deficient. It is possible that lower sows' milk intake from early in the suckling period limited appetite, at a time when the piglets had not fully discovered SM. Sows milk contains growth factors that stimulate gut development and so it is possible that the gastrointestinal tract was less-developed and limited appetite of the posterior-suckled piglets.

All piglets consumed very little creep during the first week of provision, preferring to consume SM. This is probably due to familiarity with the diet, as it was provided from birth, is in a similar physical form to the sows' milk and is easily digestible. The more rapid increase in creep consumption observed in Study 3 (when SM was withdrawn early) compared to Study 2 (SM provided to weaning) demonstrated that when SM was available piglets preferred to consume that, but when it was removed they still required supplementary nutrition. The non-suckling piglets had the greatest degree of increase in creep consumption, and their ADG from d19-weaning was higher from d11-19 when both SM and creep were available. The creep intake of the suckled piglets could not compensate for removal of SM, and they had lower ADG in the final week prior to weaning. It is unknown why the suckled piglets did not increase their creep consumption to the same degree as the non-sucklers; if they had replaced their SM volume with creep they would have consumed approximately six times as much dry matter compared to SM DM, and would probably not have suffered a reduction in growth. This is where the difference in motivation is most apparent-the non-sucklers had to increase their creep intake or they would die, whereas it was not so crucial for the suckled piglets.

7.2.2.2. Post-weaning

Study 1 showed that SM consumption had a positive association with weight at nursery exit (d54 post-weaning) and ADG to nursery exit, but in Study 2 this effect ceased after d15 post-weaning, whereas creep consumption had a positive relationship with ADG to slaughter. This may indicate that some form of supplementary nutrition is beneficial pre-weaning, but when both SM and creep are provided, creep has the most sustained effect, as it prepares the piglet for the post-weaning diet. Piglets that are high consumers of supplementary nutrition are likely to be more self-sufficient, as they have not been relying on the sow as much. They may be quicker to seek out alternative sources of nutrition (as observed previously with piglets classified as eaters of creep: Bruininx *et al.*, 2002a), leading to higher ADFI (Sulabo *et al.*, 2010a; Muns and Magowan *et al.*, 2018), and suffer lower separation stress. There was no consistent indication of a physiological advantage of higher SM consumption pre-weaning: FCR was lowest in the very high SM consumers in the immediate post-weaning period, but it was not different to the low and high consumers, only the medium consumers, and there was a trend for ADFI to be higher in the very high- compared to medium consumers. This lack of physiological adaptation was shown in the similarity of the metabolome between low and high SM consumers in Study 2, and was the reason for not continuing with SM quantification for Study 3. This project has shown that SM provision is a useful strategy to rear extra piglets, but increasing creep consumption has a longer-lasting positive effect on post-weaning performance. This allowed the prioritisation of creep provision demonstrated in the pilot study; whether SM provision could be withdrawn any earlier without adversely affecting mortality is currently unknown, but it may be unlikely as consumption of creep did not start to increase significantly until the final week pre-weaning when both SM and creep were offered concurrently. Earlier introduction of creep may increase intake, and has been shown to increase the number of eaters (Sulabo *et al.*, 2010a), but in the current study all piglets consumed some creep. Studies investigating the effect of early weaning typically wean at 14-18 days so it may be possible to remove SM provision earlier, but it should be considered that the piglets who are mostly relying on SM have a limited alternate source of nutrition, and are the weakest piglets, so are most likely to be adversely affected by early removal of SM. There is little economic (or ethical) benefit to removing SM early to increase creep consumption of suckling piglets, but as a consequence increase mortality of non-suckling piglets.

In Studies 2 and 3, FCR of high creep consumers was lower in the immediate period post-weaning, resulting in higher ADG. This is rarely reported, with most studies observing an increase in ADFI post-weaning (Sulabo *et al.*, 2010a; Muns and Magowan *et al.*, 2018), and indicates physiological adaptation to weaning associated with higher creep consumption. At weaning, only the glycine, serine, threonine metabolism pathway was enriched by high pre-weaning creep consumption, influenced by plasma threonine concentration in CH pigs. This was originally believed to be an indication of CH pigs having improved duodenal structure, as up to 71 % of threonine is utilised directly in the small intestine (Schaart *et al.*, 2005). Absorptive capacity would be increased and so reduce FCR, due to the involvement of threonine in mucin production (Luise *et al.* 2020) and enterocyte proliferation (Koo *et al.*, 2020b), with additional threonine then absorbed into the circulation for use in growth, but there was no evidence for this in Study 3. There was no difference in villus height, vh:cd or goblet cell (GC) density at weaning or d9 post-weaning relating to pre-weaning creep consumption level. It is possible that the day of sampling was incorrect for determining the effect of pre-weaning creep consumption on duodenal morphology, as renewal of enterocytes occurs every 4-5 days (Kim and Kim, 2017), and so acute effects of weaning were probably missed. Thus, it remains unclear how enrichment of the glycine, serine, threonine pathway identified in Study 2 affects physical performance of pigs, or what the mechanism is for the lower FCR observed in CH pigs compared to CL pigs immediately post-weaning. As shown in the enriched pathway network generated in Study 2 (Figure 51), elevated levels of threonine in the plasma may have been metabolised into glycine, which is a precursor to the antioxidant glutathione, and the redox status of the CH pigs may have been improved post-weaning. However, enrichment of the glutathione metabolism pathway was not enriched in CH pigs post-weaning. It was expected that there would be differences in fat and carbohydrate metabolism due to level of creep consumption, given the differing energy sources in sows' milk and creep, but this was not observed. As anticipated, differences in fat and carbohydrate metabolism were observed in plasma post-weaning compared to pre-weaning, and so the analytical methodology was sound. It is possible that the distinction between creep consumption levels was too small for further effects of creep consumption on the metabolome to be determined, as most nutrition came from the sow, and this is a limitation of this study.

Enrichment of amino acid pathways post-weaning in Study 2 demonstrated the importance of having the correct balance and level of supply to maximise growth. The low specification diets fed in Study 3 were not only low in lysine content, but as the level of other essential amino acids in the diet were formulated relative to SID lysine, all essential amino acids were deficient. Energy was also deficient as the SID lysine:DE ratio was similar between diets. Pigs fed low specification diets had an extended period of insufficient essential amino acid intake compared to those fed high specification diets, and this had a negative effect on intestinal morphology with lower vh:cd at d9 post-weaning. This would have reduced absorptive surface area and resulted in the observed higher FCR and lower ADG, compared to pigs fed SH. Proliferation of enterocytes is particularly influenced by dietary leucine level, as this amino acid activates the mTOR pathway (Wessels *et al.*, 2016). As the pigs utilised in this study were high-performing and high-health, the lower essential amino acid intake from

SL diets had a negative effect on efficiency of growth and total growth that was greater than any benefit to the microbiome (and by extension, pig health) conferred by a lower proportion of CP passing undigested to the distal intestine. A limitation of Study 3 is that there was no physiological measure of nitrogen-use efficiency, such as plasma urea-N level or faecal NH_3-N level, which may have indicated whether there was any over-supply in the high specification diets, and allowed further optimisation of post-weaning feeding regime.

In Study 3, for piglets from both creep consumption categories, the diet with the lowest cost/kg gain to slaughter was high specification, ie high lysine and CP level. This appears to be contradictory to the more recent trend for reducing CP level of weaner diets, but is probably due to the health status of the pigs. The heavy weaning weights achieved with such large litters (121 kg, 124 kg and 130 kg litter weaning weights at 27 days old for Study 1, 2, 3 respectively) are testimony to the high health status of the weaned piglets that was maintained throughout the duration of this project; scour was rarely observed, and pharmaceutical levels of ZnO were not used. This means that the majority of pigs did not lose weight post-weaning, but it also necessitates that the results reported here are considered in this context. There are many units that have a lower health status, with nursery/finishing units bringing piglets from other farms being particularly challenging, and so optimal nutrition revealed here may not be a universal solution. Combining the data from Studies 1, 2 and 3, piglets that lost weight in the first week post-weaning (3.6 %) grew 59.3 g/d slower to d54 post-weaning than those that gained weight in the first week post-weaning (*P*<0.001). They were similar in weight at weaning (9.03 kg compared to 8.85 kg for loss and gain respectively), but piglets that lost weight were nearly 3 kg lighter at d54 post-weaning (41.04 kg *vs* 43.98 kg). The relationship between ADG from weaning-d6 and from weaning-d54 was linear. There was no indication that these pigs were unhealthy, but it demonstrates how important the initial post-weaning growth is. Pathogenic challenge at this point will result in lower growth, and the aim of lower protein diets is to reduce the amount of protein passing undigested to be fermented by microbes in the distal intestine. Study 3 showed that reducing lysine by 10 % and reducing CP was ineffective as a method to improve post-weaning performance in healthy pigs being weaned into a clean environment, and adversely affected performance. It is possible that the benefits of high creep consumption may be strengthened for piglets in a challenged situation, as they have already begun the process of adaptation, and are likely to suffer less stress. Stress has an adverse effect on the immune system: the secretion of corticotrophin-releasing hormone (CRH) activates mast cells, which release cytokines and stimulate the immune system. Proteases and tumour necrosis factor α (TNF-α) increase permeability of the intestinal epithelium (Moeser *et al.*, 2007), increasing the risk of toxins and pathogenic bacteria accessing the paracellular space (Spreeuwenberg *et al.*, 2001). In Study 2 and 3 there was a positive linear relationship between creep DM consumption from d19-weaning and ADG from weaning-d6 (*P* <0.001, s.e =105, r^2=0.059; Figure 75). The r^2 was low, and there were not many pigs that lost weight, but it did indicate that every pig that consumed at least 170 g DM gained weight post-weaning.

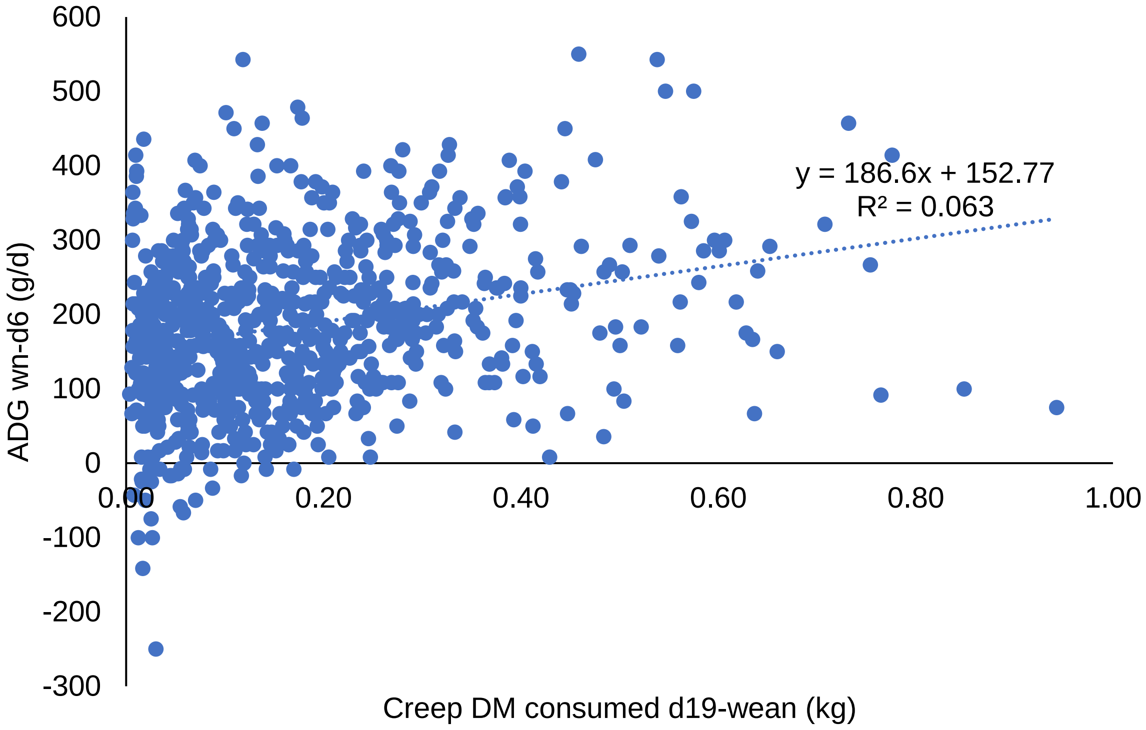

Figure 75: Relationship between piglet creep DM consumed d19-weaning and ADG wean-d6.

7.3. Practical applications/implications

7.3.1. Use of SM and creep

The only additional cost to rearing non-suckling piglets is the cost of milk powder, and the initial capital outlay, which for a modern system that mixes SM automatically *ad libitum* is around £230/crate. Using an *ad libitum* system, the labour requirement is low; for the system used in this project it was approximately 15 minutes/day to empty the tank and refresh the milk, plus two hours at the end of the batch to clean and disinfect. Modern systems do all this automatically and so require even less labour input. In Study 1, litters were fostered to have sufficient (S) or insufficient (IS) functional teats for the number of piglets, and IS sows weaned one extra piglet compared to the number of functional teats at farrowing, and two piglets more than S sows. The IS litters consumed 52 kg more SM than litters with sufficient teats over lactation; if you assume that each piglet with a teat from the IS litters consumed the same amount of SM as each piglet in the S litters (3.78 kg/piglet), there was 47.7 kg remaining for the one extra pig (assuming that the sows functional teats at farrowing remain functioning to weaning). At a mixing rate of 150 g/L and cost of milk powder of £3000/T, the additional piglets cost £21.46 to rear to weaning. With a 7 kg weaner being worth approximately £50 (AHDB, 2024), there is still a margin to be made on these piglets, even accounting for them being lighter at weaning, and potentially needing extra time and feeding to attain a suitable transport weight. If they are remaining on their birth-farm they may be lighter at slaughter, but there was no additional effect of suckling position (anterior, centre, posterior or none) on ADG post-weaning, so non-suckling pigs have the same ADG relative to weight as suckled piglets. From the above calculation, the capital payback can be as little as 10 litters/crate if an extra piglet is weaned from each litter. Although there was no interaction between teat supply and suckling position, piglets from IS litters at all suckling positions had higher du/d than those in S litters (*P* <0.001; Figure 76), and so the extra cost of milk powder cannot solely be attributed to the non-sucklers.

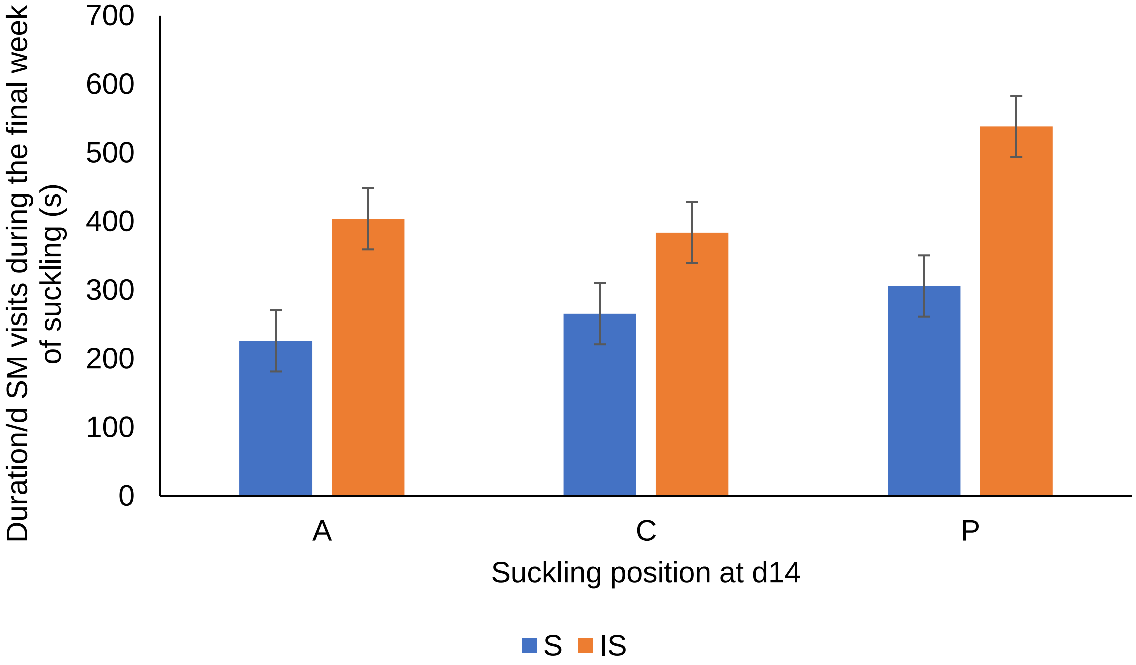

Figure 76: Duration of visits to the SM bowl during the final week of suckling for piglets in litters with sufficient (S) or insufficient (IS) teats.

Rearing additional piglets per sow by utilising SM improves efficiency of production:

- The sow has to be fed during gestation independent of how many piglets she rears.
- There were no “lost” days due to nurse sows suckling for an extra three weeks (the unit operated a three-week batch system).
- Lactational feed intake was unaffected by number of piglets weaned.

- Sow weight or P2 loss during lactation and performance in the subsequent parity was not adversely affected by rearing a larger litter.
- There was no additional space required for rearing extra piglets.
- The labour input was minimal (compared to Rescue Decks for example) as piglets did not need to be moved, and most found the SM unassisted.

When creep is also provided from two weeks prior to weaning, the cost of rearing all piglets can be reduced by withdrawing SM provision one week prior to weaning, with no effect on mortality. The pilot study between Studies 2 and 3 demonstrated that for the final week pre-weaning, the cost of supplementary feeding could be reduced by almost 50 %. In addition, the higher level of creep consumption resulting from this strategy improves post-weaning performance, by reducing FCR and so further reduces cost of production. One of the most effective methods of reducing carbon footprint on an indoor pig unit is to improve efficiency by increasing the number of piglets reared/sow/year and reducing post-weaning FCR.

7.3.2. Optimising post-weaning feeding regime

Study 3 demonstrated that there could be a benefit to differential feeding of low and high creep consumers, but there needs to be an easy method to identify which piglets are likely to fall in the low and high categories, as the antenna system used in this project is expensive and time-consuming to set-up and analyse data, and so is not an on-farm solution. Realistically, having two different regimes is likely to be the maximum that is practically achievable, given that each regime is likely to comprise of at least two stages. Combining Study 2 and 3, 87 % of non-sucklers were in the top 50 % for creep consumption, and these are easily identified during the mid-point of lactation. As reported in Study 1, before this time the teat order may not be established, and in the final week pre-weaning the high degree of competition makes determining suckling success difficult, and would be likely to require multiple observations of each litter to ensure accuracy. Non-sucklers could be identified by inserting a tag into their ear. Determining which sucklers had high creep consumption is not as simple: high creep consumers had lower than average ADG to weaning for their birthweight classification, but this requires individual weighing and recording of piglets, and doesn’t account for any effect of health on ADG. Piglets reared in litters where there were insufficient teats were more likely to be high creep consumers, with 63 % of piglets in litters with two additional piglets compared to functional teats being high consumers, and 56 % in litters where there was one extra piglet. This was largely due to creep consumption of piglets in birthweight groups 1 and 2 (Figure 77).

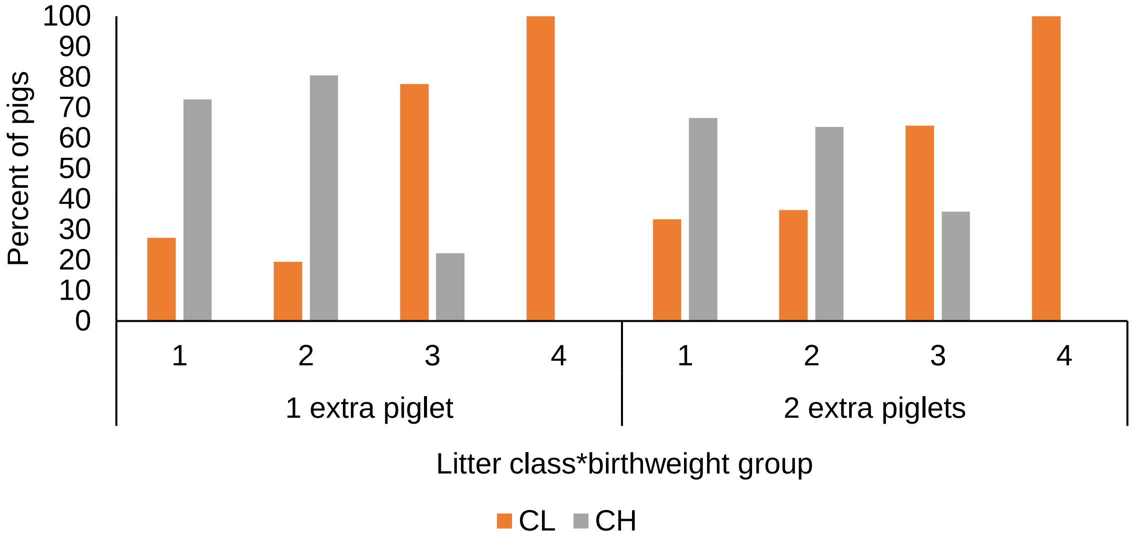

Figure 77: Percent of pigs in each birthweight group in litters with one or two extra piglets compared to functional teats with low (CL) or high (CH) pre-weaning creep consumption.

There were no CH piglets from birthweight group 4 in these litters, but this cannot be relied upon as a selection method as there only four birthweight group 4 piglets in this population. This was a result of the minimal fostering protocol, as litters with insufficient teats were those with a high number born, and so were less likely to contain heavy birthweight piglets.

From the combined data of Study 2 and 3, it is likely that piglets with high creep consumption will be non-sucklers from any litter, or sucklers from the lightest 50 % birthweight piglets in litters with insufficient teats. This however only accounts for approximately 10 % of the top 50 % of creep consumers, with litters where there are sufficient teats being harder to classify. If ADG is utilised as a selection method for the remainder of the pigs (as high consumers had lower ADG than would be expected according to their birthweight), this requires significant labour input, and as mentioned does not account for poor growth due to health problems (which would not necessarily result in increased creep consumption). Differential feeding of piglets according to creep consumption is likely to appeal to farmers with an interest in precision technology, and the use of EID and individual piglet data capture would facilitate this. Electronic ID has reduced in cost, and technology now exists that enables data to be coded to the piglets' individual tag, and revealed once scanned. This means that suckling could be observed and any non-sucklers tags recorded, and when the tag was scanned at weaning it would reveal the suckling status. Weighing piglets individually at birth is relatively simple, as they will be handled to perform standard management routines of iron injection at least. The aspect that requires commitment is weighing at weaning to determine the average ADG for that group. If the on-farm average is known this could be used, but there may be considerable variation between batches due to health, environment, and sow performance. Obtaining an accurate ADG that can then be used to classify pigs requires weighing the day prior to weaning, which is a time-consuming job, then piglets can be sorted on the day of weaning. If piglets were to receive pre-weaning vaccination, weighing them at that point to calculate ADG would be less additional work. It is possible that differential feeding does not need to be split 50/50 for creep consumption; this project did not identify the creep consumption threshold at which a high specification, low quality diet became the optimal regime.

Without a reliable method of identifying high creep consumers on-farm, the SHQH regime would have been optimal in Study 3: it had the lowest relative weighted cost/kg gain for CL pigs, and second-lowest relative weighted cost/kg gain for CH pigs.

7.4. Recommendations for future research

This project emphasises the importance of feeding the hyperprolific sow a high quantity of high specification diet, to enable her to rear the litter for which she has potential, as milk yields were above those utilised in Whittemore *et al.* (2003) nutritional requirements. Optimising feeding of the hyperprolific sow is an area that warrants further research, and will need to include provision of supplementary piglet nutrition. Given that milk yield appeared to reduce in the final week of lactation in response to increased piglet creep intake, can sow feed allocation be reduced, or does this risk adversely affecting performance in the subsequent parity?

The poor performance of non-sucklers presents an opportunity to make a large improvement in overall performance. The reason for their lower pre-weaning ADG needs to be determined: Is it due to the lower fat content of SM compared to sows' milk, or is there some genetic effect or behavioural reason? Non-sucklers were omitted from the physiological investigations in this project to avoid adding extra variation, and so comparison of their metabolome, intestinal morphology, enzyme activity and microbiome is of interest.

Reducing weight variation within the batch: Given that non-sucklers had a high proportion of lower birthweight piglets, and were therefore the weaker pigs born, is it better to make litters of light birthweight piglets with sufficient teats, and then overload the other sows, or does this sacrifice performance of heavier birthweight piglets at the expense of piglets that may not grow much more anyway? Do heavier birthweight piglets perform better as non-sucklers, or does it impart too much of a negative effect on their growth? How do we maximise batch weaning weight?

Why didn't SM consumption of posterior-suckled piglets increase in response to their lower sow milk intake? Do they have less-developed digestive systems and appetite due to early feed restriction? Is it affected by birth order and colostrum intake? Does the quality of sows' milk change from anterior-posterior?

Supplementation of amino acids: pathways enriched in Study 2 post-weaning were mostly amino acid-based. Previous work has identified the importance of additional valine and isoleucine when CP is reduced and the valine, leucine, isoleucine biosynthesis and degradation pathways were significantly affected post-weaning. Also, the glycine, serine, threonine metabolism pathway was enriched by creep consumption level. The enriched pathways can highlight which amino acids should be investigated. Over-supply of any synthetic amino acids is not recommended, as there will be the same problem with bacterial fermentation as is observed with CP oversupply. There is also the risk that non-essential amino acids will become limiting if SID lysine:CP ratio is too high, as there is insufficient N for biosynthesis. This would not be economically viable. The analysis of SID lysine:CP showed that performance improved as the proportion of lysine in CP increased, but it is possible the threshold was not reached. For CH pigs the lowest cost/kg gain was highest SID lysine:CP, CL pigs was second highest, so did CH pigs have capacity to use more lysine?

Metabolomic analysis with just two creep consumption level groupings (split into halves) indicated differences in the metabolome at weaning. The EID and antenna system utilised for this project would allow piglets to be grouped by creep consumption classification either in more discreet groupings (such as the quartiles employed in Study 3) or to group along a more graduated scale, for example 10 percentiles, which would enable investigation of correlations between supplementary nutrition and metabolome, and comparisons between more extreme groupings. The quartiles employed in Study 3 ensured separation between the high and low consumption groups, and this resulted in a more defined performance difference, and the regression analysis based on individual duration of visits rather than groupings was more sensitive in identifying performance differences due to creep intake. Now that metabolomic analysis has been performed successfully and there is an indication of a physiological difference due to level of pre-weaning creep consumption, the mild severity of taking blood samples is more ethically justifiable and so could be performed on a higher number of pigs with a refined grouping technique. Some form of grouping would still be required as heatmaps and pathway analysis cannot currently be generated from continuous data. Blood sampling earlier post-weaning may reveal differences in metabolism during the acute phase of weaning. The period in which FCR was most adversely affected by weaning was from d3-6, and there was a large difference between the creep consumption classifications, which may indicate that this period is where the metabolome is most affected by creep consumption.

Knowledge of pathways enriched by creep consumption could assist in design of creep and starter diets for piglets to minimise the disruption at weaning. In Study 3, the optimum post-weaning dietary regime was affected by creep intake. If differences in the metabolome allowed further refinement and more specialised diets to be formulated for high and low creep consumers this is associated with the problem of how to easily recognise these pigs on-farm. If a more graduated grouping structure was utilised, it may identify the threshold at which post-weaning nutritional requirements change. This would assist in determining strategies for on-farm recognition of the different categories of creep consumers. For both creep consumption classifications, a high specification diet was optimal. With this knowledge it may be easier to adjust diet quality but maintain CP and lysine level.

The duodenal morphology showed no effect of creep consumption, so does enzyme activity explain physiological adaptation to weaning for CH pigs? Or was the lack of effect due to timing? The optimal dietary regime for CH pigs was SHQL (rather than SHQH for CL pigs), so by weaning have they already developed sufficient maltase and sucrase activity to digest cereal-based diets, and sufficient protease activity to digest lower-digestibility protein? Or does the intestinal microbiome have an effect?

To further assist in determining a piglet creep consumption classification model to use on-farm, the random forest technique could be utilised, but this may require more data collection, particularly considering the low number of heavy birthweight piglets in litters with insufficient functional teats in this project. In Study 3, the quartiles used allowed separation of the categories and identified that there was a performance and economic benefit to differential feeding, but the threshold will lie somewhere between 25 and 75 percentile. Further work with narrower groups (for example 10 percentiles) is required to determine the threshold, and combining this with increased pre-weaning data collection may allow a strategy to be formulated for on-farm identification of piglets that would benefit from a high- or low-creep-consumer regime.

7.5. Conclusion

Supplementary milk facilitates rearing of supernumerary piglets compared to functional teats in sow-suckled litters, and is an economically viable method of increasing production efficiency (pigs weaned/sow/year). It also supports litter growth, particularly of heavy birthweight piglets in the final week of lactation. There is little benefit of SM consumption per-weaning to post-weaning performance, and no effects on the plasma metabolome were observed. Creep feeding supports litter growth in the final week of lactation, particularly when SM is withdrawn, and has a lasting positive relationship with ADG. The glycine, serine, threonine metabolism pathway was enriched at weaning in high creep consumers, but duodenal morphology was unaffected by creep consumption level. All pigs benefitted from being fed high specification (lysine) diets post-weaning, but lowest cost/kg gain to slaughter for low creep consumers was achieved with a high-quality post-weaning diet, whereas the improved adaptation of high creep consumers to cereal-based diets resulted in lowest cost/kg gain from lower quality diets.

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