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PhD Thesis

Optimising the progeny from primiparous sows

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Declaration of Authorship

This is to certify that:

1. This thesis comprises only my original work towards the degree of Doctor of Philosophy,
2. Proper acknowledgement has been made in the text to all other materials used,
3. This thesis is fewer than 100,000 words in length, exclusive of tables, figures and bibliographies.

Udani (Danni) Wijesiriwardana

November 2020

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Abstract

The progeny of primiparous sows (gilts) are recognized as a significant burden on the overall production efficiency of Australian pig herds. Gilt progeny (GP) grow slower and have higher rates of morbidity and mortality compared to the progeny of multiparous sows (sow progeny; SP). Previous research suggests that there is an underlying biological basis for differences between GP and SP. Therefore, the aims of this thesis were to clarify the timeframes of when these differences are in effect by investigating gastrointestinal (GI) development in the pre-weaning period and also to develop late gestational and lactational nutritional interventions to improve GP performance. In doing so, this thesis consists of a series of experiments in which three general hypotheses were tested: (1) GP display poorer production performance compared to SP; (2) GP consistently displayed reduced gastrointestinal (GI) tract development compared to SP and (3) GP production performance could be improved by targeting nutritional interventions towards late gestation and/or lactation.

Experiment 1 (Chapter 3) involved the quantification of GI functional development at 4 time points; birth (0h), 24 hours after birth (24h), 1-day pre-weaning (PrW) and 1-day post-weaning (PoW) of GI tissues (stomach, jejunum, ileum and colon). Transepithelial resistance (TER) was measured on all organs of the GI tract while the permeability of macromolecules fluorescein isothiocyanate (FD4) and tetramethyl rhodamine isothiocyanate (T150) labelled dextrans was measured in the mucosa of the jejunum and ileum. Additional GI function measurements were taken such as tight junction proteins and cytokine abundance in the jejunal and ileal tissues. The main findings were that GP showed early signs of lower GI tract function compared to SP at birth, particularly in terms of lower TER in the stomach tissues of the stomach, jejunum and ileum. However, differences between GP and SP were largely observed from PrW to PoW with GP exhibited poor GI barrier function at weaning reflected the reduced epithelial barrier integrity in response to weaning, mainly in the distal portions of the GI tract (ileum and colon). Differences in GI barrier function between the birth cohort and the weaning cohort suggests that the variation between GP and SP is established in the pre-weaning period. This indicates that the pre-weaning period is critical for optimal GI tract function development for GP and their reduced growth performance compared to SP is likely due to a lack of colostrum/milk consumption.

As the development in GI barrier function in the piglet is critical for survival and productive efficiency, we investigated the utility of quantifying intestinal fatty acid binding protein (I-FABP) as a biomarker of GI integrity (chapter 4). Fatty acid binding proteins

(FABP's) are ubiquitous intracellular transport proteins integral to fatty acid metabolism. However, organs of the GI tract have a specific intestinal isoform (I-FABP). The use of FABP's as biomarkers for organ damage have been proposed, but little is known about the utility of I-FABP as a biomarker in the pig. The outcomes of this experiment showed that plasma I-FABP concentrations were substantially lower in piglets than later in life and this led to significant challenges in detection using existing ELISA based methodologies. While it is interesting that I-FABP is expressed in such low concentrations early in life, to properly understand the utility of I-FABP as an intestinal biomarker will require more sensitive technologies such as mass spectroscopy, which will limit its utility as a biomarker.

In order to support growth of GP in the pre-weaning period, various nutritional strategies were investigated and implemented during either late gestation and/or lactation. In experiment 3 (chapter 4), the ketogenic substance 1,3-Butanediol (BD) was fed to gilts and sows from day 90 of gestation to farrowing in order to provide an alternative energy source to GP at farrowing. The main result was that birth litter weights were increased in GP from gilts fed the BD diet and this extended to day 21. Furthermore, the proportion of GP born less than 1.1kg were less from those gilts fed the BD diet, increasing their chances of survival to weaning.

Previous research suggests that gilts suffer from high levels of oxidative stress during gestation and possibly lactation compared to multiparous sows resulting in reduced GP growth. Experiment 4 (chapter 5) involved supplementing late gestation and lactation diets with a sugar-cane derived polyphenol mixture (Polygain) to improve GP growth during the pre-weaning period. Furthermore, due to the anti-inflammatory properties of Polygain, weaner diets were supplemented with this mixture to reduce post-weaning inflammation. Polygain was unable to increase GP growth in the pre-weaning period nor did it reduce inflammation measured by the circulation pro-inflammatory cytokine interleukin-1 beta (IL-1 β). Interestingly, results from this experiment do suggest that GP exhibit reduced immune function compared to SP reflected in their lower plasma IL-1 β concentrations overall compared to SP.

Lastly, experiment 5 (chapter 7) investigated the effects of feeding gilts and sows a diet supplemented with lucerne chaff or a diet formulated to have the same estimated fibre, amino acid and energy content as lucerne (SIMLUC) to improve GP performance. These diets were able to increase GP average weight and gilt litter weight at day 21 while a similar effect was not observed in SP. The improvement of D21 litter weights in gilts in response to the LUC and

SIMLUC diets fed in late gestation is an important finding which demonstrates the importance of targeted feeding strategies towards gilts to maximize GP performance.

In conclusion, results from this thesis indicate that GP are consistently born and weaned lighter and growth slower than SP. They display reduced GI tract development, with GP entering weaning with reduced GI integrity. Furthermore, GP display some signs of reduced immunocompetence compared with SP which may impede their ability to cope with the stressors of weaning. Therefore, nutritional interventions are most likely to work when they support higher milk yields in gilts and support growth of GP in the neonatal and pre-weaning period.

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Preface

This thesis contains the following original manuscripts by Udani Wijesiriwardana as the primary researcher and author:

Publications from thesis

- (1) Wijesiriwardana, U. J. R. Pluske, J.R. Craig, J.J. Cottrell, F.R. Dunshea (2019). "Dietary inclusion of 1, 3-butanediol increases dam circulating ketones and increases progeny birth weight." Animals **9**(8): 479
- (2) Wijesiriwardana, U. J. R. Pluske, J.R. Craig, J.J. Cottrell, F.R. Dunshea (2020). "Evaluation of sugarcane-derived polyphenols on the pre-weaning and post-weaning growth of gilt progeny." Animals **10**(6): 984

Conference proceedings

- (1) Wijesiriwardana, D. A., J. Craig, M. Ringuet, L. Fothergill, K. O'Halloran, D. Turpin, L. Munoz, C. Collins, J. B. Furness and J. Pluske (2019). "Characterisation of poor gastrointestinal tract development in gilt progeny." *Journal of Animal Science* **97**(no. Supplement_2): 113-113.
- (2) Wijesiriwardana, U. A., J. R. Pluske., J. R. Craig and F. R. Dunshea (2019). Dietary 1,3-Butanediol increases weight and decreases small for age progeny at birth. *Animal*.**10**(Supplement 1)

Chapter 1 General Introduction

Progeny born to primiparous sows (gilts) are generally characterised by their poorer production performance compared to those from multiparous sows (sow progeny; SP). Gilt progeny (GP) consistently grow slower, are born and weaned lighter (Miller, Collins et al. 2012, Carney-Hinkle, Tran et al. 2013, Craig, Collins et al. 2017), and have higher rates of post-weaning illness and mortality compared to SP (Craig, Collins et al. 2017, Holyoake 2006). Collectively, the poorer production performance exhibited by GP culminates in a longer time for them to reach optimal market weight and, ultimately, reduced revenue for the producer. While the reasons for poorer performance of GP are complex and multifaceted, they can largely be attributed to the gilt herself. Gilts are often mated at a young age that and this can have significant consequences on metabolism during gestation and lactation. For example, during gestation, gilts must partition dietary nutrients into key processes such as mammary gland growth, uterine and placental growth and foetal growth whilst also supporting their own body growth (Thomas, Goodband et al. 2018). During lactation, gilts preferentially partition energy into their own growth rather than milk production (Pluske, Williams et al. 1998), which in turn can also limit GP performance.

In order to improve GP performance, it is important to understand when the disparity in performance between GP and SP arise. In doing so, producers can target strategies such as nutritional interventions to improve GP performance. In order to determine when these differences occur, it is important to understand the biological basis for differences between GP and SP. This can be done by investigating the development of important organs such as the gastrointestinal (GI) tract. The GI tract plays important roles in animal production and health. Due to its roles in nutrient digestion and absorption as well as acting as a physical, immunological and microbial barrier against the luminal environment, it is important that the GI tract functions optimally. Therefore, the first aim of this thesis was to quantify aspects of GI barrier function of GP and compare it to that of SP at different time points, being birth, 24 hours after birth (a period in which they had suckled), one day before weaning, and one day after weaning. In order to investigate GI tract development, accurate methods must be developed in order to accurately quantify GI barrier function. Currently, methods that quantify barrier function are invasive and require harvesting of the specific organ. Development of less invasive methods such as plasma biomarkers are ideal as they can streamline investigations into GI function. Intestinal fatty acid binding protein (I-FABP) is an intracellular protein that is

said to leak into the blood stream in quantifiable amounts during conditions where intestinal cell damage occurs (Funaoka, Kanda et al. 2010). Therefore, the second aim of this thesis was to determine whether I-FABP could be a potential biomarker of intestinal damage at weaning. In order to improve GP performance, nutritional interventions that support growth of the piglet either during late gestation or lactation were investigated. For the first nutritional intervention, late gestation diets were supplemented with the ketogenic substance 1,3-Butanediol in order to increase foetal energy stores in preparation for the neonatal period. Secondly, late gestation and lactation diets were supplemented with the sugarcane-derived polyphenol mixture Polygain in attempts to improve GP performance. Furthermore, weaner diets of those piglets born to mothers supplemented with Polygain were also supplemented with the same polyphenol mixture in order to reduce inflammation that is commonly associated with post-weaning period. Finally, in attempts to support lactation performance and GP growth and metabolic parameters, lucerne hay or diets with similar nutritional profiles to lucerne hay were fed during late gestation and lactation due to their high levels of fibre and branched-chain amino acids (BCAA).

The general hypotheses examined in this thesis were that GP would display poorer production performance compared to SP and also have reduced GI tract development compared to SP, starting at birth, and that I-FABP would serve as an accurate biomarker for GI function at weaning. Furthermore, the hypothesis was examined that GP production performance would be improved by targeting nutritional interventions in the sow towards late gestation and lactation. Specifically, when late gestation diets were supplemented with 1,3-Butanediol, it was hypothesised that increased birth weights and reduced pre-weaning mortality would be observed in GP. It was also hypothesised that GP weights during the pre-weaning period would increase when late gestation and lactation diets were supplemented with Polygain and this effect would be extended after weaning when weaner diets were supplemented with Polygain. Furthermore, it was hypothesised that Polygain would reduce inflammation measured by the concentration of interleukin-1 beta (IL-1 β) over the weaning period, specifically 1 day prior to weaning to 7 and 14-days post-weaning. Lastly, it was hypothesised that providing a diet high in fibre with the addition of lucerne hay or a diet with a similar nutritional profile to lucerne hay during late gestation to early lactation would improve GP performance in the pre-weaning period.

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Chapter 2 Literature Review

2.1 Introduction

The success of the primiparous sow (gilt) greatly effects the productivity and profitability for the pig producer. Average sow replacement rates in Australia have been reported to be as high as 56% (Australian Pork Limited 2013), with a high proportion of replacement sows being gilts. Therefore, a high proportion of the growing herd on Australian commercial farms include gilt progeny (GP) (Holyoake 2006). Therefore, optimising gilt performance to produce viable progeny will ensure greater profitability and productivity for the producer. Viable progeny are those that survive, have an average body weight, consume enough colostrum and milk and have high growth rates. These factors lead to increased performance at weaning and shape the outcome of their performance. However, GP are known to have inferior performance compared to progeny of multiparous sows (sow progeny; SP) and are recognized as a significant burden on the efficiency of herds (Holyoake 2006, Craig, Collins et al. 2017). The reasons for this are complex but mainly stem from the physiological immaturity of the gilt compared to the multiparous sow at time of mating. This can ultimately result in poor prenatal and postnatal growth of their progeny. Due to their significant contribution to the Australian herd, improving the performance of GP is of vital importance when trying improving herd productivity.

Understanding the underlying biological basis for differences between GP and from SP is necessary for developing interventions to improve their performance. This review will aim to summarize the published literature that investigate differences between GP and SP. This includes the some of the biological differences between gilts and multiparous sows and the disparity in performance between GP and SP. Due to the importance of gastrointestinal tract development and function on animal performance, this review will also summarize the literature pertaining to differences in gastrointestinal tract features between GP and SP. Finally, interventions will be discussed that target late gestation and lactation sow feeding strategies to support the growth of the gilt and her progeny.

2.2 The primiparous sow/gilt

The gilt is mated at a physiologically young age to reduce non-productive days. Due to this, the gestating gilt partitions energy and nutrients differently to multiparous sows. The gestating gilt must support the growth and development of her own pregnancy as well as

partition energy and nutrients into her own growth. This can result in less nutrients available to support the key processes of gestation (Figure 2a). Furthermore, due to the gilts' need to partition nutrients towards her own growth, nutrients are partitioned away from supporting the metabolically demanding process of lactation resulting in reduced milk yield (Figure 2b). This section will aim to summarize the physiological demands placed on the gestating and lactating gilt in detail.

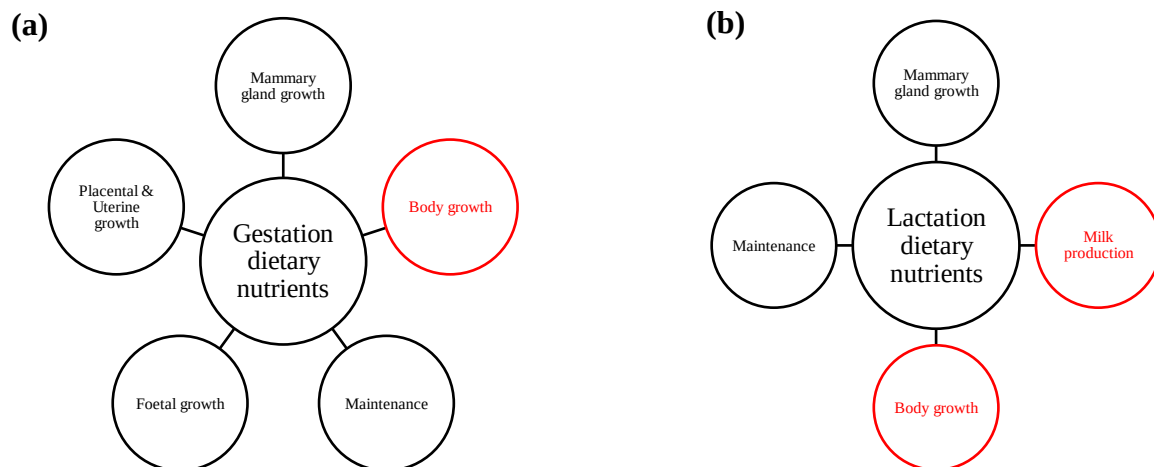


Figure 2.1 The partitioning of nutrients during **(a)** gestation and **(b)** lactation on key metabolic processes in the primiparous sow

2.2.1 The gestating gilt

The gestating gilt must partition energy and nutrients towards the major requirements of pregnancy such as maintenance, uterine and placental development and foetal requirements whilst also supporting her own growth (Whittemore 1996, Young, Tokach et al. 2005). Consequently, this nutrient partitioning may not always support foetal growth and result in the gilt partitioning energy towards her own growth rather than that of the conceptus. According to Thomas, Goodband et al. (2018a), gilts were reported to have increased their body weight in each period of gestation whereas the liveweight of dams of parities 2 and >3 remained static (Figure 2.2). Furthermore, it seems that the greatest portion of energy requirements are for maintenance and growth with gilts having greater proportional maintenance requirements from d 75 to 109 of gestation compared to multiparous sows (Thomas, Goodband et al. 2018a). In addition to this, foetal growth is said to be accelerated after day 69 in gilts (Ji, Wu et al. 2005). Collectively, this suggests that foetal growth in gilts maybe somewhat compromised during late gestation due to the preferential partitioning of nutrients and energy towards their own growth.

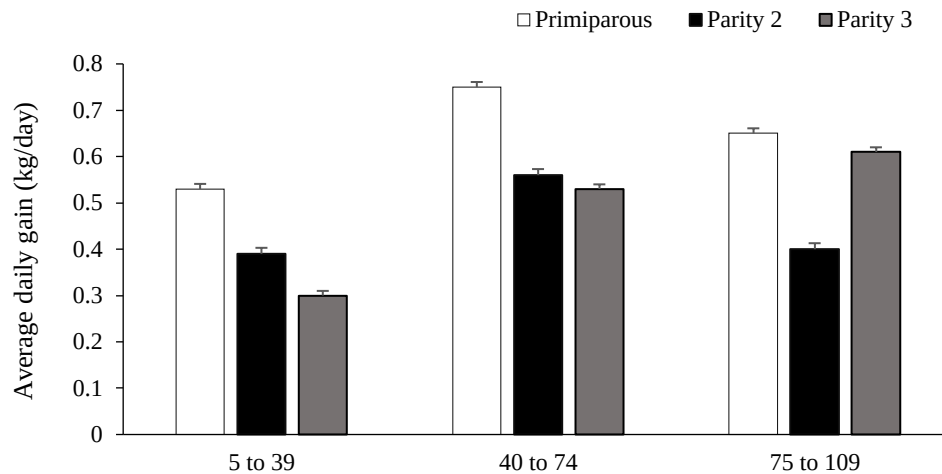


Figure 2.2 The average daily gain (ADG) of primiparous sows and sows of parities 2 and >3 during three periods of gestation. Adapted from Thomas, Goodband et al. (2018a).

2.2.1.1 Prenatal development of GP

A majority of foetal growth occurs during the last third of gestation (Dourmad, Étienne et al. 2008) reflected in increased weights of the conceptus (Thomas, Goodband et al. 2018a) and foetus (McPherson, Ji et al. 2004). However, in primiparous sows, the increase in conceptus weights of conceptus have been reported to be is lower compared to sows of higher parities (Thomas, Goodband et al. 2018a). It seems that this differentiation in foetal weights between primiparous and multiparous sows occurs sometime after day 50 of gestation (Gatford, Blasio et al. 2009) with increases in protein and fat accretions of the foetus increasing after day 69 of gestation, from 0.25 and 0.06 grams/day to 4.63 and 1.09 grams/day (McPherson, Ji et al. 2004). However, this increased growth during late gestation coupled with the reduced birth weights of GP indicate that gilts may have decreased uterine capacity. This has been suggested before by Foxcroft, Dixon et al. (2006) and offers a possible biological explanation for the reduced growth performance seen in GP.

Uterine capacity is correlated with foetal weights and refers to the number of fully formed foetuses that can be maintained by the uterus until farrowing (Wang, Feng et al. 2017). While there seems to be a scarcity of research in the uterine capacity of gilts, it seems that this could be an important factor in the poor growth performance observed in GP. Intrauterine growth restriction (IUGR) is defined by impaired growth and development of the foetus or its organs due to reduced blood flow, transfer of nutrients and oxygen from the mother to foetus

(Wu, Bazer et al. 2006). While there is a clear distinction between low birth weight (and by inference, GP) and IUGR piglets due to the ability for low birth weight piglets to recover given proper management (Edwards and Baxter 2014), GP do display some characteristics of IUGR beginning with asymmetric growth patterns. Asymmetric growth usually pertains to prioritization of organs of the nervous system such as the brain during early gestation and reduced weights of organs such as the liver, gastrointestinal (GI) tissue and muscle mass which develop later in gestation (Bauer, Walter et al. 1998). For example, McPherson, Ji et al. (2004) observed GI tract:foetal weight ratio increased as gestation progressed, which indicates that GI tract growth is accelerated towards the end of gestation. However, if uterine capacity is limited, this may restrict growth of organs that develop later during gestation. Town, Patterson et al. (2005) reported that parity affected brain:liver ratios with high ratios particularly seen in GP. Craig, Dunshea et al. (2019b) also found asymmetric growth patterns in GP reflected in increased brain:liver and brain:body weight found in GP compared to SP. Foetal piglets who experience retarded growth during the final trimester of gestation due to uterine restriction are more likely to exhibit low birth weights and slow growth throughout their lifetime (Lynegaard, Hansen et al. 2020). As GP are more likely to experience uterine restriction during late gestation they are more likely to have compromised development and lifetime growth rates.

2.2.1.2 Mammary gland development

Milk yield is a limiting factor for piglet growth and, therefore, optimal development and growth of the mammary gland is required to support lactation. Smaller mammary gland (MG) size and development in gilts is reported in the literature and is primarily attributed to their size (Balzani, Cordell et al. 2016). Growth of the MG in the gilt is slow during the first two thirds of gestation but increases during the last third of gestation, which is reflected in the increased DNA and tissue found during this stage (Sørensen, Sejrsen et al. 2002, Ji, Hurley et al. 2006). Ji, Hurley et al. (2006) also reported that this accelerated growth coincides with the increase of several mammogenic hormones such as oestrogen, relaxin and prolactin. A study by Farmer, Sorensen et al. (2000) demonstrated the importance of prolactin in MG development during the last third of gestation for gilts by inhibiting prolactin expression. They found a reduction in total DNA and total RNA in parenchymal tissue as a result. While administering prolactin during gestation to gilts may seem promising, King, Pettigrew et al. (1996) reported that administering exogenous prolactin to gilts during the end of gestation through lactation did not affect milk yield and was in fact reduced. Farmer, Sorensen et al.

(1999) suggested that this may be due to saturation of mammary receptors for prolactin and therefore, exogenous prolactin was unable to exert any positive effect.

Mammary gland development during the last third of gestation is responsive to nutritional changes, and therefore can be targeted with nutritional interventions. Most of these interventions emphasize that restricting feed can have detrimental impacts on MG development and that *ad libitum* feeding during late gestation can increase MG parenchymal weight (Farmer 2018). Farmer, Duarte et al. (2016) reported that underfeeding of pregnant gilts should be avoided as it can have negative impact on the mammary development of gilts, with an aim of 17 to 26 mm of backfat seeming to have no detrimental effects on MG development. Therefore, optimal feeding requirements that address the energetic and protein requirements of late gestating gilts may aid in increasing mammary secretions in gilts.

2.2.1 The lactating gilt

Lactation is a highly catabolic period where the dam mobilises body fat and protein reserves to support milk production. However, due to the continued selection for leanness, gilts often enter lactation with less reserves to support milk synthesis than older sows (Whittemore 1996). As a consequence, this reduces energy available for the piglet. Furthermore, milk yield is correlated to mammary gland size. Nielsen, Pedersen et al. (2001) reported that the amounts of mammary tissue and total DNA was higher in the multiparous sow than in the gilt after four weeks of lactation. Furthermore, they reported that the growth of piglets was positively correlated to the amount of tissue and DNA with multiparous sow progeny growing faster than GP, suggesting that lactation is a limiting factor for piglet growth.

2.2.2.1 Colostrum and Milk yield

Colostrum yield is also a limiting factor for piglet growth and survival in the neonatal period and is dictated by body reserves available for mobilisation, litter size, suckling intensity, nutrition and MG development (Mullan and Williams 1989, Quesnel, Farmer et al. 2014). Furthermore, milk yield is a limiting factor for piglet growth throughout the pre-weaning period with sow feed intake influencing milk yields in later lactation (Mullan and Williams 1989, Quesnel, Farmer et al. 2014). Low colostrum and milk yields in gilts have been previously reported throughout the literature (Speer and Cox 1984, Beyer, Jentsch et al. 2007, Devillers, Farmer et al. 2007, Strathe, Bruun et al. 2016, Pedersen, van Vliet et al. 2019). Milk production has been reported to increase from parity 1 to 2 (Etienne, Dourmad et al. 1998) and reaches its

maximum from parities 2 to 3 (Devillers, Farmer et al. 2007) and slowly decreases in subsequent parities. While it is important to consider that milk yield is also largely driven by litter size (Eissen, Kanis et al. 2000), Pedersen, van Vliet et al. (2019) reported that primiparous sows had lower colostrum yield of 5.2 kg compared to those of multiparous sows of 7.1 kg with no differences in litter size between the two parities at the time of sampling.

2.2.2.2 Colostrum and milk composition

Colostrum is the first secretion by the mammary gland. It is characterised by high immunoglobulins (IgG) concentrations and is one of the main determinants of piglet growth and survival (Rooke and Bland 2002). Colostral IgG provide passive immunity to the piglet during the neonatal period and is a key process in immunological development of the piglet. While colostral IgG concentrations between gilts and sows have been reported in the literature, their findings have often been contradictory. For example, Craig, Dunshea et al. (2019a) reported no differences in IgG concentrations between primiparous and multiparous sows whereas Klobasa (1987), Quesnel (2011) and Cabrera, Lin et al. (2012) reported that IgG concentrations were significantly lower in gilts. Craig, Dunshea et al. (2019a) suggested that these discrepancies are attributed to factors such as nutrition, genetics and sampling time. In comparison to milk, colostrum has higher concentrations of dry matter and crude protein concentrations with lower concentrations of lipids and lactose (Le Dividich, Rooke et al. 2005). In regard to differences between gilt and multiparous sow colostrum, total protein content is reportedly no different between gilts and multiparous sows (Declerck, Dewulf et al. 2015, Craig, Dunshea et al. 2019a) while fat and lactose content are variable between reports. Craig, Dunshea et al. (2019a) reported no differences in colostrum fat concentration while Szyndler-Nędza (2016), Quesnel, Farmer et al. (2012) and Declerck, Dewulf et al. (2015) reported that colostrum of gilts has a higher highest lipid content which generally decreases until parity 4. However, Beyer, Jentsch et al. (2007) reported the opposite where gilts had the lowest lipid content with concentrations increased until parity 4. Fat is the most variable macronutrient of colostrum and milk and is highly influenced by diet (Quesnel, Farmer et al. 2012) and probably by lipid reserves (Mahan, Cromwell et al. 1998), and this may explain the differences seen between these studies. Craig, Dunshea et al. (2019a) reported no difference in colostral lactose concentrations. However, Szyndler-Nędza (2016) reported lactose concentrations to be lower in colostrum of the gilt compared sows of parities 2 and 3. In addition to IgG and macronutrients, colostrum also contains high concentrations of hormones, growth factors and

immunogenic products that are imperative in intestinal development (Huerou-Luron and Ferret-Bernard 2014) and will be discussed later in this review.

The transient stage of lactation is generally between day 2 and day 4 of lactation and is marked by the increase in lipid concentration (Hurley 2014). From day 10 onwards, the composition of milk tends to be relatively stable with little to no change occurring in composition (Beyer, Jentsch et al. 2007, Hurley 2014). From day 2 of lactation, lactose concentrations increase and are essentially stable from day 10 onwards (Klobasa 1987, Theil, Nielsen et al. 2012, Craig, Dunshea et al. 2019a). Reports of lactose concentrations between parities are varied. Beyer, Jentsch et al. (2007) reported that lactose concentrations were lower overall in milk of gilts. Craig, Dunshea et al. (2019a) reported no differences overall in lactose concentrations between gilts and sows. However, both Szyndler-Nędza (2016) and Craig, Dunshea et al. (2019a) observed lower concentrations observed towards the end of lactation with the latter attributing this to the inability of gilts to keep up with the demand of milk production. The reporting of lipid content of milk between parities has also been varied. Beyer, Jentsch et al. (2007) reported that fat was lowest in milk produced from gilts in comparison to multiparous sows. However, Szyndler-Nędza (2016) and Klobasa (1987) reported higher concentrations of fat in milk from gilts. Craig, Dunshea et al. (2019a) reported no overall differences in total fat concentrations of milk. However, fat content was higher at day 3 of lactation in multiparous sows compared to gilts. Unlike fat and lactose, protein concentrations of mature milk is reportedly no different between parities and tends to decrease over the course of lactation (Klobasa 1987, Craig, Dunshea et al. 2019c)

2.3 Growth performance of gilt progeny

The reduced growth performance of GP in comparison to SP is widely acknowledged in the literature and reflected in commercial practice. This poor performance is initially observed in the pre-weaning period and persists after weaning. The physiological differences between gilts and sows during gestation and lactation are primarily responsible for the large variation between growth performance of these progenies. This section of the review will aim to summarize the reported literature on the growth and development of GP in comparison to SP in the pre-weaning and post-weaning phases.

2.3.1 Pre-weaning growth

Pre-weaning growth is largely determined by birth weight of the piglet (Dunshea, Kerton et al. 2003). However, the selection for larger litters in recent years has resulted in an indirect selection for lower average piglet weights and reduced rearing capacities for larger litters (Ward, Kirkwood et al. 2020).

2.3.1.1 Birth weight

Gilt progeny are consistently reported as being born lighter than their SP counterparts (Gourley, Sanson et al. 2020, Liu Zhang et al. 2020, Pedersen, van Vilet et al. 2020). However, the actual differences in reported birth weights vary between studies with the largest differences in parities being from parity 1 compared to parities 2-4. When birth weights from parities higher than 4 are included in the analysis, differences in average birth weights between GP and SP are smaller. For example, Mahan (1991) reported a difference of 120 grams between individual GP and SP from parity 2 with similar reports from van Wettere, Willson et al. (2016) who reported a difference of 140 grams. Carney-Hinkle, Tran et al. (2013) reported differences of up to 240 grams between GP and SP from parity 4. This average difference decreases again when higher parities are included in the analysis such as when Craig, Collins et al. (2017) reported differences of 60 grams between GP and SP from parities 2-8. Regardless of this variation, the low birth weight (LBW) of GP is widely agreed upon and is consistently linked with their poor growth.

Low birth weight is likely attributed to the immaturity of the gilt due to her size, nutrient partitioning and oxidative stress during gestation (Roy, Lavoie et al. 2016). Birth weight is regarded as an important predictor of performance with Dunshea, Kerton et al. (2003) reporting that a 0.37 kg difference at birth increased to 1.9 kg by 2 weeks of age and ultimately 13 kg over 20 weeks. Similarly, Quiniou, Dagorn et al. (2002) reported that it took 3 additional weeks for piglets that weighed less than 600 grams to reach 25 kg than piglets of who were born heavier. Other than poor growth performance in the pre-weaning period, piglets of LBW have increased live-born mortality and latency to first suckle (Baxter, Jarvis et al. 2008), lower immunity and neonatal growth (Devillers, Le Dividich et al. 2011). Furthermore, Ferrari, Sbardella et al. (2014), showed that while GP cross-fostered to multiparous sows grew better than those not cross-fostered, LBW was still had a greater effect on growth and survival.

2.3.1.2 The neonatal period

Newborn piglets are at high risk of mortality during the first 3 days after birth (Tuchscherer, Puppe et al. 2000). The neonatal period is marked by an abrupt physiological shift for the piglet from intrauterine to extrauterine life. This is associated with a sudden exposure to reduced temperatures (Le Dividich and Noblet 1981) and a transplacental to oral supply of nutrients in the form of colostrum (Siggers, Sangild et al. 2011). The active pursuit of colostrum whilst also having to thermoregulate places a high energy demand on the newborn piglet (Noblet, Dourmad et al. 1997). These energy requirements are met by the mobilisation of glycogen reserves deposited *in utero* and serve as the primary source of energy prior to the consumption of colostrum (Mellor and Cockburn 1986, Devillers, Le Dividich et al. 2011, Theil, Cordero et al. 2011). These energy stores are deposited in the foetal liver and muscle during the last month of gestation and are rapidly depleted within 16 hours of life in the perinatal period with depletion occurring at a faster rate in cold conditions to enhance thermogenesis (Dividich and Noblet 1983, Theil, Lauridsen et al. 2014). If energy reserves are inadequate and colostrum consumption or availability is scarce, piglets have an increased risk of starvation or crushing by the sow (Theil, Lauridsen et al. 2014). Low birth weight piglets are more vulnerable to starvation and crushing, in part due to reduced glycogen reserves. According to Vanden Hole, Ayuso et al. (2019), LBW piglets (with a mean weight of 0.79kg) showed lower muscle and liver glycogen in hindleg stores and 50% lower glycogen stores in the liver. These reduced glycogen stores in low birth weight piglets which can result in reduced vigour, standing and taking the teat and have been linked to reduced parity piglets (Roldan Santiago, Martínez Burnes et al. 2019). From the literature, it can be concluded that low birth weight piglets, by inference, gilt progeny, have lower glycogen reserves to metabolise and therefore are at high risk of starvation and mortality in this pre-weaning period. The low milk yield of gilts (Beyer, Jentsch et al. 2007, Devillers, Farmer et al. 2007) should also be taken into account when considering GP vigour and vitality in this neonatal period. Therefore, increasing survival of GP in this neonatal period should be of particular focus in order to increase pre-weaning survival.

2.3.1.3 Growth and variation in the pre-weaning period

Gilt progeny are known to grow slower than SP in the pre-weaning period (van Wettere, Willson et al. 2016, Craig, Muller et al. 2019b). This has not only been reported several times but is also predicted by their lower birth weights (Dunshea, Kerton et al. 2003). Milk is the main determinant of piglet growth in established lactation, and therefore milk output must be

sufficient to support this growth. Craig, Dunshea et al. (2019a) showed no differences in overall composition of colostrum and milk between gilts and multiparous sows, demonstrating that yield is one of the most influential factors effecting GP growth in the pre-weaning period. Indeed, lower average daily gain has been reported in the pre-weaning period in GP (Terry, Kind et al. 2015, Craig, Collins et al. 2017). Large within-litter variations are mostly reported at weaning and can result in lower weaning weights (Milligan, Fraser et al. 2002). This may be attributed to intrauterine crowding in the prenatal period, which can reduce number of muscle fibres during foetal development (Rehfeldt and Kuhn 2006) and the number of secondary muscle fibres in GP result in uneven growth of the litter up to weaning (Town, Putman et al. 2004, da Silva, Dalto et al. 2013).

2.3.2 Weaning and the post-weaning period

Due to their lower pre-weaning growth rates and that piglets are typically weaned at a common age, GP are typically smaller and lighter at weaning than SP and continue to exhibit poorer growth after weaning (Terry, Kind et al. 2015, Craig, Collins et al. 2017, Vallet and Miles 2017, Craig, Muller et al. 2019b). However, average weaning weights of GP in comparison to SP are difficult to determine from the literature due to the varied age of weaning in these studies. This is reflected in published ranges for weaning weights from 200 g (Pettigrew, Cornelius et al. 1986) to approximately 2 kg lighter than SP (Terry, Kind et al. 2015, Craig, Collins et al. 2017, Vallet and Miles 2017, Craig, Muller et al. 2019b). The variation for in these weights can be attributed to not only weaning age but genetics and management practices.

The lower post-weaning growth rates in GP were quantified by Craig, Collins et al. (2017), who reported that GP were 920 g lighter at weaning and 2.6 kg lighter at 10 weeks of age. In a separate study by Miller, Collins et al. (2012), GP were 1.11 kg lighter than SP at week 10. In using a stochastic model Schinckel, Einstein et al. (2010) predicted that GP were 4.9 to 5.7 kg lighter at 150 days of age compared to SP. Zotti, Resmini et al. (2017) reported differences of up to 0.79 kg at approximately 14 weeks of age between GP and progeny from sows of parities 2 and 3. Furthermore, GP are reported to have reduced average daily feed intake after weaning (Miller, Collins et al. 2012, Edwards, Campbell et al. 2013). Interestingly, Gondret, Lefaucheur et al. (2006) reported no differences in feed intake from week 1 onwards for low weight piglets and suggested that undernutrition did not affect the appetite of theses pigs but rather inefficient digestion and/or nutrient absorption. This was further indicated by

the lower gain: feed (G: F) ratio which has also been reported in GP (Miller, Collins et al. 2012). This suggests that GP may suffer from a more severe post-weaning growth check in comparison to SP. Overall, these seems to be unequivocal evidence that GP have lower weaning weights and poorer post-weaning growth performance resulting in longer times to reach market weight and fewer of these pigs reaching market (Schinckel, Einstein et al. 2010).

There is limited information on differences in carcass characteristics of GP in comparison to SP. Gilt progeny have been reported to have lighter hot carcass weights and a lower dressing percentage than SP (Craig, Collins et al. 2017, Zotti, Resmini et al. 2017). Craig, Collins et al. (2017) reported no differences in body composition in terms of carcass muscling and fat levels. However, Gondret, Lefaucheur et al. (2006) reported higher carcass fat levels in LBW piglets at market weight with increased lipid contents in backfat although parity was not reported. Similarly, Collins (2007) found LBW pigs were fatter at any liveweight over the finishing phase than heavy birth weight pigs.

2.4 Morbidity and mortality of gilt progeny

2.4.1 Before weaning

Reported GP mortalities seem to vary in the literature. Piglets of LBW have often been reported as having high mortalities. Quiniou, Dagorn et al. (2002) reported a correlation between a higher risk of mortality and birth weight below 1.60 kg, with piglets weighing below 0.8 kg having a 29% chance of dying within the first 24 hours and a 52% chance of dying at weaning. From studies such as this, it seems that GP would also be expected to be at high risk of mortality, especially within this pre-weaning period. However, Carney-Hinkle, Tran et al. (2013) and Milligan, Fraser et al. (2002) found no link between parity and pre-weaning mortality. Edwards, Campbell et al. (2013) and Holyoake (2006) reported that GP show higher rates of mortalities before weaning. Contrary to this, Miller, Collins et al. (2013), Baxter, Jarvis et al. (2008) and Muns, Manteca et al. (2015) reported higher rates of mortality in SP. The reason for this inconsistency is unclear. However, in regard to increased mortalities in SP in this period, Muns, Nuntapaitoon et al. (2016) suggested that this may be due to increases in litter size accompanying parity (Roehe and Kalm 2000) resulting in more piglets with lower birth weights causing a larger within-litter variation. As LBW is associated with increased mortality, this is perhaps where the absence of relationship between parity and mortality is seen.

There are a variety of factors that are said to influence pre-weaning mortality in GP. Low birth weight seems to be a primary factor for survival overall in piglets during the pre-weaning period (Fix, Cassady et al. 2010). Colostrum yield and consumption is also a major factor that contributed to pre-weaning mortality. Devillers, Farmer et al. (2007) showed that higher parities tended to have greater colostrum yields in comparison to gilts. According to Ferrari, Sbardella et al. (2014), GP consumed less colostrum than SP and therefore, immune protection of SP maybe greater compared to GP. Colostrum and its ability to improve immune protection in the piglet will be discussed in subsequent sections of this review.

2.4.2 After weaning

Post-weaning mortalities of GP are relatively low in number with the exception of a few studies. Edwards, Campbell et al. (2013) found a higher prevalence of respiratory-related mortality in GP in the nursery period with Craig, Collins et al. (2017) also reporting this issue but for the grower-finisher period. Miller, Collins et al. (2012) suggested that GP are more susceptible to illness than SP due to their LBW and reduced humoral immune responsiveness due to lower colostrum consumption. Furthermore, increased rates of medication are also reported in GP in this period after weaning (Holyoake 2006, Craig, Collins et al. 2017). Miller, Collins et al. (2012) and Holyoake (2006) also reported that GP were more likely die after weaning. Collectively, this further validates that the stressors of weaning are particularly exacerbated in GP compared to SP.

2.5 The GI tract and techniques for measuring barrier function

Optimal growth of the intestine is important to animal production due to the role it plays in metabolism, physiology, disease status and performance of pigs at each stage of growth (Pluske, Turpin et al. 2018). Understanding the background of intestinal epithelial structure and common research techniques used to assess barrier function are important to assess health and performance of pigs (Wijtten, Meulen et al. 2011). The following section aims to provide a brief overview this.

2.5.1 Gastrointestinal tract

2.5.1.1 Organisation of GI tract

The main organs of the GI tract are the stomach, small intestine and colon in which a majority of the digestion occurs. Their structure and function are complex and multifaceted. They serve as a site of digestion and nutrient absorption while also acting as a barrier against the luminal environment. The whole GI tract is lined by a mucosal barrier that consists of a mucous layer and a single layer of epithelial columnar cells. The mucous layer is predominantly composed of glycoproteins secreted by goblet cells and is the initial component of the GI barrier that contents from the lumen come into contact with.

The intestine is where a majority of nutrient digestion and absorption takes place and is comprised of a single layer of columnar cells organised into crypts and villi (Peterson and Artis 2014). These villi are responsible for increasing the mucosal surface area for digestion and absorption. Furthermore, these villi are lined with microvilli which are microscopic finger-like projections which form the brush border of the intestine. Digestive enzymes are localised in the brush border of intestinal epithelial cells (IECs) and are responsible for luminal digestion of complex dietary carbohydrates into absorbable monosaccharides (Van Beers, Büller et al. 1995). The IECs are diverse in their nature, with specialized lineages for absorption of nutrients (Peterson and Artis 2014) and secretory cells such as enteroendocrine cells, goblet cells and Paneth cells (Goto and Ivanov 2013). The primary role of enteroendocrine cells is to act as the link between the central and neuroendocrine systems by secreting various hormone regulators responsible for digestive functions (Peterson and Artis 2014). Goblet cells are responsible for mucus secretion (Burkey, Skjolaas et al. 2009) and Paneth cells secrete antimicrobial peptides (Sang and Blecha 2009). These cells are differentiated from intestinal stem cells found in the crypts which travel to the luminal pole of the crypt-villus axis (Potten, Booth et al. 1997).

The intestinal epithelium regulates the transport of molecules such as dietary nutrients, electrolytes and water from the lumen via two major routes transcellular and paracellular pathways (Groschwitz and Hogan 2009). Transcellular transport requires a polarized channel which allow the movement of solutes or water through the IEC and paracellular transport is associated with the movement of ions and solutes between IECs, which are regulated by tight junctions (Capaldo and Nusrat 2015). Furthermore, paracellular pathways can be divided into “pore” and “leak” pathways. Leak pathways allow large molecules to pass the intestinal barrier via a non-selective manner while pore pathways are low capacity, ion charged and size selective (Capaldo and Nusrat 2015)

2.5.1.2 Tight junctions

The integrity of the intestinal epithelial barrier is highly dependent on tight junction complexes which allow for the paracellular transport of solutes across the intestinal barrier. Tight junction complexes are a meshwork of various strand-like proteins anchored to the membrane of the epithelial cells that span across the paracellular space (Barmeyer, Fromm et al. 2017). The proteins found in these complexes laterally associate with adjacent IECs to form a paired strand and reduce the intercellular distance between the cells (Saitou, Furuse et al. 2000). Tight junction complexes are attached to the actin cytoskeleton of the through specialised domain proteins via the zonulins proteins (ZO-1,2,3) or protease-activated receptor adaptor proteins (Henderson, van Limbergen et al. 2011).

Within the tight junction complex, there are three major transmembrane proteins; occludin, claudins and junction adhesion molecule (JAM) proteins with each protein having a specific functionality. Occludin is said to play a key role in barrier structure and function (Cummins 2012) and can be used as a structural marker for barrier function (Kimura, Shiozaki et al. 1997). The importance of occludin has been previously shown by Saitou, Furuse et al. (2000) where occludin knockout mice showed several histological abnormalities such as chronic inflammation and poor TJ integrity in various epithelial tissues. In this TJ complex, claudins determine the integrity of the intestinal epithelial barrier by helping to establish its polarity (Barmeyer, Fromm et al. 2017).

Originally, claudin and tight junction structures were believed to be rigid and static. However, more recent research has demonstrated that tight junctions including claudins, undergo continuous molecular remodelling (Capaldo and Nusrat 2015). Claudins can be classified as either “sealing” (claudins 1, 3, 5, 11, 14 and 19) or “leaky” (claudins 2, 10, 15, and 17) (Krug, Schulzke et al. 2014, Capaldo and Nusrat 2015). According to the literature, claudins can be upregulated or downregulated in response to disease states. For example, claudin-2 expression is increased in response to diseases such as Crohn’s disease and ulcerative colitis and can cause a reduction in transepithelial electrical resistance (TER) (Luettig, Rosenthal et al. 2015). Under these conditions, this is mainly due to the increase in pro-inflammatory cytokines and the ability of these cytokines to modulate certain molecular mechanisms of TJ function (Capaldo and Nusrat 2009). Furthermore, claudin-2 has been shown to increase in response to hormones such as corticotropin releasing factor (CRF) (Yu, Liu et al. 2013), which is upregulated when the hypothalamic-pituitary-adrenal (HPA) axis is

activated in times of stress. Finally, JAM proteins are said to regulate barrier function and play a role in immune cell trafficking across the epithelium (Moeser, Klok et al. 2007b, Vetrano, Rescigno et al. 2008, Ma, Nighot et al. 2018).

2.5.1.3 Electrophysiological measurements of intestinal barrier integrity

Epithelial barrier integrity is frequently assessed using electrophysiological techniques by quantifying the TER and macromolecular flux (Figure 3). Transepithelial resistance is a quantitative technique that measures the integrity of the tight junction complexes by applying a direct current voltage to the single cell layer and measuring the resulting current (Srinivasan, Kolli et al. 2015). The current is carried by the ion flux across the membrane with TER being a direct measurement of this flux (Srinivasan, Kolli et al. 2015). High TER can reflect either an increase in ion flux due to tight junction disruption or an increase in the opening of pore-forming channels (Varadarajan, Stephenson et al. 2019) with interpretation of results relying on the context of the experiment. Another method used to quantify epithelial barrier integrity is by measuring the movement of a labelled probe such as a fluorescent dextran that traverses the epithelium via the paracellular space (Blikslager 2010). Various sizes of fluorescent dextrans can be used to measure the selectivity and permeability of the epithelial barrier. Well-formed tight junctions are characterised by high TER and low solute permeability. The permeability of TJs can be determined by measuring the paracellular fluxes of ions and small molecules (Lu, Ding et al. 2013).

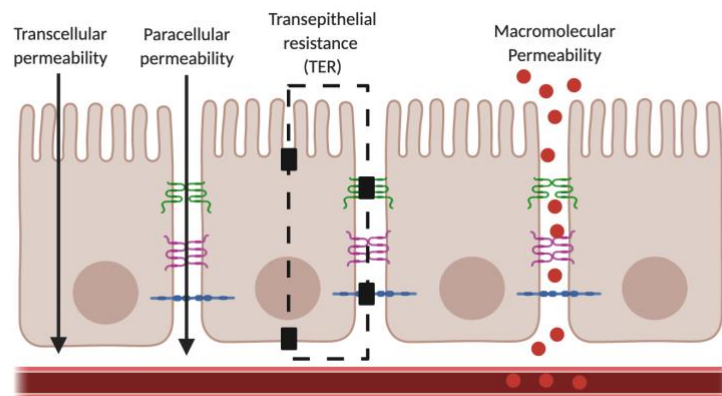


Figure 2.3 Pathways of molecular movement across the intestinal barrier and methods of measuring barrier integrity

2.5.2 Biomarkers of intestinal injury – intestinal fatty acid binding proteins (I-FABP)

The use of biomarkers in assessing intestinal injury can help streamline investigations into the prediction, cause, diagnosis, and progression or outcome of a treatment (Mayeux 2004). Intestinal fatty acid binding protein (I-FABP) has received much attention in this context. Fatty acid binding proteins (FABP) are cytoplasmic proteins that are involved in cytosolic fatty acid transport (Richieri, Ogata et al. 1992). These proteins can be organ specific with expression of FABPs found in such organs as the liver (L-FABP), heart (H-FABP) and brain (B-FABP) for example. However, their expression profiles are not exclusive to that specific organ, for example, while L-FABP is expressed in the liver it can also be found in the intestine, pancreas, kidney, lung and stomach (Smathers and Petersen 2011).

The reason why I-FABP is of particular interest as a biomarker for intestinal injury is that during periods of intestinal injury, I-FABP is released into the circulation to and possibly be detected at elevated amounts (Guthmann, Borchers et al. 2002) (Figure 4). Niewold, Meinen et al. (2004) first suggested plasma I-FABP as a biomarker as a marker of mild intestinal damage to the intestinal mucosa in pigs. This was based on the significant increase in I-FABP plasma concentrations 15-30 minutes after ischemia was induced in the small intestine of the pig. From these results, numerous studies have investigated the use of plasma I-FABP as a biomarker for intestinal injury with equivocal results.

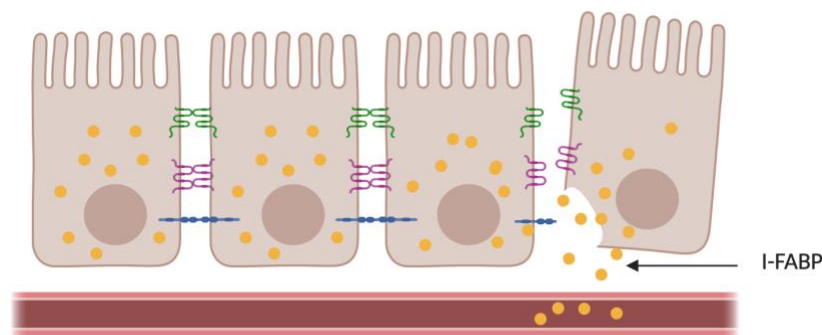


Figure 2.4. Intestinal fatty acid binding protein (I-FABP) is leaked into the bloodstream in quantifiable amounts under conditions of intestinal cell damage.

Various models have been used to test I-FABP in pigs. These models mainly have used weaning in pigs as a condition of intestinal injury due to the intense structural and functional transformations that occur in the intestine at this time. In a study by Zong, Huang et al. (2018), dietary sulphur amino acids (SAA) were fed to weaned piglets in order to reduce intestinal dysfunction in piglets. They found that SAA was able to improve growth performance and

intestinal digestive and absorptive functions of weaning piglets. Furthermore, they found that in piglets fed SAA, I-FABP concentrations in jejunal mucosa were increased compared to those control pigs. This indicates that those weaned piglets who had increased intestinal injury in response to weaning had reduced I-FABP in intestinal cells that may have been released into circulation. However, this study did not measure circulating I-FABP.

Berkeveld, Langendijk et al. (2007) assessed the use of plasma I-FABP and citrulline, the product of glutamine metabolism exclusively found in intestinal enterocytes and shown to also be a good biomarker of absorptive function (Crenn, Coudray–Lucas et al. 2000). While citrulline and mannitol absorption were decreased in weaned piglets, plasma I-FABP concentrations showed great variation within treatments and no differences were observed. This large variation has been reported in a number of studies. Niewold, Meinen et al. (2004) reported high variation baseline concentrations prior to necrotising enterocolitis (NEC) of the small intestine (352 ± 170 pg/mL, mean $\pm$ SD). They attributed this variation to a potential unknown cross-reacting factor in the plasma. However, their results were said to be unaffected by this. In another study by Van der Meulen, Koopmans et al. (2010), weaning of piglets did not result in increased plasma I-FABP concentrations, which was corroborated by Bomba, Minuti et al. (2014). However, in a study by López Colom, Yu et al. (2019), weaned pigs orally challenged with *Salmonella* Typhimurium and enterotoxigenic *E. coli* F4 showed increased I-FABP concentrations in response to mild-to-severe diarrhoea after the challenge. Furthermore, villous:crypt ratio showed a strong negative correlation with increased I-FABP. In this study, I-FABP was also positively correlated to TNF- α which increased in response to pathogenic challenge.

Huygelen, De Vos et al. (2015) measured I-FABP in LBW and normal birth weight piglets at birth with no detectable amounts of I-FABP observed, a finding that was corroborated by Cheung, Gill et al. (2012) and Zamora, Stoll et al. (2015). However, Zamora, Stoll et al. (2015) were able to detect plasma I-FABP at 48 hours after enteral feeding in piglets who suffered from NEC. From these studies, it seems that I-FABP may only be an informative biomarker during certain types of intestinal stress and perhaps only in older pigs. However, the low concentrations of I-FABP in piglet plasma as well as the high variation between samples often leads to inconclusive and inconsistent results. Clearly more work is required in this area.

2.6 Intestinal growth and development of the piglet

Understanding the growth and developmental process of the GI tract may help to elucidate some of the underlying biological differences between GP and SP. Literature on the characterisation of intestinal development in GP compared to SP is scarce. However, parallels can be drawn between GP and piglets who display poorer production performance such as those of low birth weights or those that suffer from IUGR.

The following sections will present literature on barrier integrity and function in the context of two major events of the piglet's life; birth and weaning. Birth is marked by establishment of the GI epithelial barrier which is supported by the ingestion of colostrum. In contrast to birth, weaning induces a breakdown of GI barrier function due to a variety of stressors. Pineiro, Pineiro et al. (2008) suggested that parity and poor colostrum play a major role in weaning performance of GP with this data indicating that events very early on in the life of the new-born piglet from gilts impact on whole-of-life performance.

2.5.1 Early intestinal growth and development

During the weeks prior to parturition, the intestine of the foetal piglet grows rapidly in comparison to other organs of its body. Sangild, Fowden et al. (2000) reported that the intestine of the foetal piglet can increase up to 70-80% in size over the last three weeks prior to parturition. Similar findings were reported by McPherson, Ji et al. (2004) who found that GI tract:foetal weight increased in fetuses from gilts at the end of gestation suggesting that growth of the GI tract is accelerated during this period. However, if uterine capacity is restricted or foetal growth during late gestation is limited, it can have deleterious impacts on the growth and development of the SI during late gestation as maybe the case for GP.

The neonatal period is a window of intense GI tract growth and development. This occurs in response to a shift from a continuous supply of parental nutrients, via the placenta, to the consumption of colostrum and milk via the GI tract (Jensen, Elnif et al. 2001). To accommodate these changes, the GI tract displays a high degree of plasticity during this window which allows the intestine to grow and develop accordingly. Intestinal growth and development are primarily influenced by colostrum consumption in two ways. The first is through colostral bioactive compounds including various types of growth factors such as epidermal growth factor, insulin-like growth factor-1 (IGF-1), IGF-2 and transforming growth

factor- β (Xu, Wang et al. 2000) and their ability to promote epithelial cell proliferation and maturation (Skrzypek, Kazimierczak et al. 2018). The activity of these growth factors is responsible for increases in tissue growth and functional changes (Widdowson and Crabb 1976), increases in mucosal RNA content and protein content (Simmen, Cera et al. 1990), and increases in jejunal and ileal protein synthesis (Burrin, Shulman et al. 1992). This ultimately results in increased intestinal mucosa and increased intestinal weights (Wang and Xu 1996, Zhang, Malo et al. 1997, Jensen, Elnif et al. 2001). The other means of uptake of colostral immunoglobulins and other proteins, electrolytes and water that fills the intestinal enterocytes and increases mucosal weights (Barszcz and Skomiał 2011, Skrzypek, Kazimierczak et al. 2018). Colostrum consumption is the key determinant of intestinal growth in the neonatal period. However, based on their lower birth weights and poorer growth during the pre-weaning period, it seems that GP do not consume optimal amounts of colostrum (Craig, Dunshea et al. 2019a). This may result in delayed growth of the GI tract and explain some of the underlying differences between GP and SP.

2.5.2 Early immune development

2.5.2.1 Passive immunity

One of the distinct features of the neonatal piglet intestine is its ability to absorb colostral immunoglobulins, namely IgG. The acquisition of passive immunity via colostrum consumption is imperative to the development of early immunity due to the immunological naivety of the newborn piglet (Rooke and Bland 2002) and is reported widely as it is a critical factor in determining piglet survival (Varley, Maitland et al. 1985, Cabrera, Lin et al. 2012). In regards to GP, plasma or serum IgG concentrations are reported to be lower in GP compared to SP during the first 24 hours of life (Klobasa, Butler et al. 1986, Carney-Hinkle, Tran et al. 2013, Craig, Dunshea et al. 2019c). The transfer of intact IgGs across the GI tract occurs within a short window of time in the neonatal intestine with some reports of this window being 24 hours post-parturition (Speer, Brown et al. 1959), while others report this window to be between the first 18-36 hours of life (Westrom, Svendsen et al. 1984). It seems that the cessation of IgG absorption greatly decreases past this point and is commonly referred to as ‘gut closure’. According to Lecce and Morgan (1962), gut closure occurs sooner when piglets consumed colostrum compared to starved piglets and is considered a key process in intestinal development (Rooke and Bland 2002). While no reports on the timing of gut closure in GP seem to be available, this may be a contributing factor to IgG absorption in GP. Furthermore,

colostrum profiles between gilts and sows have been reported to be relatively similar including concentrations of IgG (Craig, Dunshea et al. 2019a). Therefore, the reduced IgG concentrations found in GP may be attributed to colostrum yield and timing of gut closure. The timing of gut closure in GP requires further investigation as it may explain the the increased morbidity and mortality seen in GP. If GP have increased permeability following birth, vulnerability to bacterial infection and invasion via the lumen in the period prior to weaning may explain the disease rates seen in GP (Miller, Collins et al. 2012).

Passive immunity also encompasses IgM and IgA, maternal leucocytes, milk glycans, anti-inflammatory cytokines and peptides that act to neutralize intestinal microbes (Nguyen, Yuan et al. 2007, Pohl, Medland et al. 2015). Since passive immunity is derived solely from the dam, the immunocompetence of the dam is imperative. Dams gain immunity by encountering different pathogens (Friendship and O’Sullivan 2014) with younger gilts most likely having less exposure to pathogens than multiparous sows due to their age (Carney-Hinkle, Tran et al. 2013). This may effect the maternal transfer of immunogenic products to the piglet and in turn reduce maternal immunity transmission to GP after birth. A lower immunocompetence of GP may not only contribute to the increased morbidity and mortality observed in GP but also reduce their growth performance by partitioning nutrients away from growth towards support of the immune system (Johnson 2012). Therefore, Friendship and O’Sullivan (2014) suggest that vaccination to increase immunocompetence of the gilt and improve passive immunity in colostrum and thus, to the piglet. For example, in current production systems, sows are routinely immunized with vaccines against bacterial and viral pathogens such as *E. coli*, *Bordetella bronchiseptica*, rotavirus, parvovirus, pseudorabies virus, swine influenza and circovirus to increase the production of antibodies which can be transferred to the piglet (Salmon, Berri et al. 2009).

While passive immunity is required to maintain a low inflammatory environment in the neonate, the neonatal immune system has additional mechanisms to circumvent this. The neonatal piglet is born with few lymphocytes. Furthermore, the neonatal immune system is biased towards a T helper 2 (Th2) immune response which promotes a low inflammatory environment and suppresses T helper 1 (Th1) cytokines which promote a pro-inflammatory environment (Adkins, Leclerc et al. 2004). This low inflammatory mechanism is imperative to the neonate as it provides temporary immune support to the piglet until their active immune response is developed.

2.5.2.2 Active immunity

The active immune system is mediated through interactions between the innate and adaptive immune system (Pluske, Kim et al. 2018). Adaptive immunity relies on immunological memory formed through contact between immune cells and antigens. However, this type of immune response takes 2 to 3 weeks to establish as the newborn has very few lymphocytes in its IEC and lamina propria (Šinkora and Butler 2009) with age playing a significant role in lymphocyte subsets in mucosal tissue (Solano-Aguilar, Vengroski et al. 2001). Therefore, the neonatal piglet is highly reliant on its innate immune system. Innate immunity traditionally refers to the part of the immune system that does not require previous exposure to a pathogen (Ygberg and Nilsson 2012). The innate immune system in the GI tract refers to the mucosal barrier that itself produces antimicrobial peptides, enzymes and cytokines that restrict the movement of bacterial products across the epithelial barrier and/or reduce the inflammatory effects of lipopolysaccharides (Huerou-Luron and Ferret-Bernard 2014). Colostrum supports the immune response by promoting intestinal development. Without sufficient colostrum consumption, piglets may have delayed/reduced intestinal immune functions, an issue that may affect GP due to their lower colostrum intake.

Long-term immune development in GP has not been extensively reported. However, impaired or attenuated immune responses have been demonstrated using IUGR models due to their reduced and/or compromised intestinal growth and development. Dong, Zhong et al. (2014) reported a reduced number of goblet cells and lymphocytes in IECs in the SI of IUGR neonates along with reduced gene expression of cytokines, while D'Inca, Che et al. (2010) saw a reduction in IL-6. Furthermore, Zhong, Li et al. (2012) showed that IUGR piglets have reduced proliferation of peripheral blood lymphocytes, lower concentrations of cytokines and increased expression of HSP70, which downregulates the activity of NF- κ B in the intestine of neonatal piglets. Furthermore, it seems that when these IUGR piglets did not consume adequate colostrum and milk, they displayed lower circulating leucocyte and lymphocyte counts (Hu, Liu et al. 2015) despite IUGR piglets requiring more electrolytes and/or colostrum (Amdt, Krogh et al. 2013). The compromised intestinal immune development of the IUGR and/or the LBW piglet can impact the ability for the piglet to mount an optimal immune response in the long term. This pre-weaning development of the immune response is imperative in preparation for the piglet for the intense intestinal changes it experiences at weaning.

2.5.3 Weaning and intestinal function

The changes that occur in the intestinal tract in response to weaning are heavily reported on in the literature as a result of the significant impact these changes have on production. Under commercial settings, weaning occurs at an earlier stage than in wild piglets and this results in environmental, physiological and social stressors on the piglet. Weaning stressors are attributed to the abrupt maternal separation, shift from easily digestible milk to a complex plant-based feed, mixing with new pigs and changes in temperature (Pluske 2016). Furthermore, these stressors are reported to activate the hypothalamic-pituitary-adrenal (HPA) axis resulting in elevated cortisol and corticotropin-releasing factor (CRF) (Moeser, Klok et al. 2007b). These hormones have an essential role in regulating intestinal permeability of the weaned piglet with weaning consistently associated with reduced intestinal barrier function, high inflammation and degeneration of intestinal structure (Overman, Rivier et al. 2012).

2.5.3.1 Structural changes after weaning

When the piglet is weaned there is a precipitous decrease in villous height and a concomitant loss of surface area, with Hampson (1986) reporting a 50% reduction within 24 hours. Villous height along the small intestine (SI) remains decreased for up to 7 days after weaning (Cera, Mahan et al. 1988, Pluske, Williams et al. 1996). Reduced villous height and increased crypt depth also contributes to reduced growth after weaning as shown by Pluske, Williams et al. (1996). Furthermore, alterations in intestinal structure can cause a decrease in digestive and absorptive capacity in the SI (Pluske, Hampson et al. 1997) and reduced intestinal barrier function (Moeser, Pohl et al. 2017). Spreeuwenberg, Verdonk et al. (2001) further showed that reduced voluntary intake is the major contributor to changes in the structure and function of the SI rather than the change in type of nutrients. In this context, GP have been shown to have lower feed intake after weaning than SP (Edwards, Campbell et al. 2013) and this may impact SI structure. However, little research has been published on the structural differences in terms of epithelial cell organisation, function and development of the SI between GP and SP after weaning and more clarification in these areas is needed.

2.5.3.2 Intestinal barrier function and immune status

Weaning has long been associated with a susceptibility to infections (Hopewood and Hampson 2003). This is mainly attributed to the reduced intestinal barrier function in response to the stressors of weaning (Hu, Xiao et al. 2013) and the translocation of pathogenic molecules from the lumen to circulation (Wijtten, Meulen et al. 2011). This reduced barrier function is

said to be in response to elevated CRF concentrations and the interplay between CRF and mast cells (Moeser, Ryan et al. 2007a). Mast cells have an important regulatory role in the inflammatory response and excessive inflammation can result in reduced barrier function (Moeser, Pohl et al. 2017). Excessive inflammation can cause anorexia, fever and increased muscle proteolysis and reduced animal performance (Pluske, Kim et al. 2018). In addition to this, when luminal LPS enters circulation and stimulates an immune response, this can cause further damage to the intestinal epithelium (Lambert 2009).

There is not one optimal immune response but rather an appropriate immune response for specific circumstances (Black and Pluske 2011). It seems that while excessive inflammation can be harmful to the piglet, improper intestinal immune development that leads to an attenuated immune response can be just as damaging to the GI tract. Events that can cause improper intestinal immune development can be stressors such as early weaning, mixing or even inadequate milk intake which can result in reduced immune responses later on in life. Hohenshell, Cunnick et al. (2000) reported dysregulation of the HPA axis between the pituitary and adrenal glands in early weaned pigs that was linked to a reduced immunological response. For example, McLamb, Gibson et al. (2013) reported that when pigs were weaned early (15 to 16 days of age), they exhibited suppressed IL-6, IL-8 and neutrophil responses to F4 *E. coli* challenge compared with pigs who were weaned later. Furthermore, Davis, Sears et al. (2006) reported lower concentrations of leucocytes of GP in response to *Streptococcus suis* infection, a disease that would usually raise leucocyte concentrations. Pigs were also reported to be less immunologically responsive to the stress of mixing, a finding that is reported in the literature (Gimsa, Tuchscherer et al. 2018). Lessard, Blais et al. (2018) showed that piglets that experience slower growth in the pre-weaning period had reduced mucosal and systemic immune responses reflected by impaired cell proliferation of immune cell populations after weaning, reduced populations of antigen presenting cells, and reduced B cells. Ultimately, it seems that while inflammation may increase after weaning in pigs with developed immunity, poorer development of the immune system in GP could result in attenuated immune responses, and this may partly explain poorer growth and higher post-weaning mortalities (Craig, Collins et al. 2017). Further work in the characterisation of immune responses in GP is required.

2.7 Feeding during late gestation and early lactation to improve gilt progeny performance

This review has addressed the main issues that seem to impact GP performance such as restricted growth during the late stages of gestation, lower birth weights and reduced growth during lactation. Dietary interventions in the sow targeted towards late gestation and/or early lactation have the potential to directly affect piglet pre-weaning growth and survival and therefore, may be beneficial for improving GP performance. The following section will discuss the main nutrient requirements of gestation and lactating gilts using two of the major constituents of gestation and lactation diets; energy and lysine. Furthermore, it will also discuss feeding strategies that have been previously investigated to increase GP birth and weaning weights, and finally will suggest possible dietary interventions that may help improve GP growth and performance.

2.7.1 The transition period

For the purposes of this review, late gestation (defined from day 90 to farrowing) and early lactation (from farrowing to day 10 of lactation) will be referred to as the transition period. The transition period is particularly important due to the various metabolic and physiological events that take place which can potentially be affected by sow nutrition (Theil 2014). These events include sow maintenance, colostrum/milk production, mammary growth, foetal growth, uterine components and additional heat loss (Figure 2.5), and therefore diets must be formulated meet these requirements. During late gestation, nutrient requirements increase exponentially to support mammary gland development, foetal growth and sow maintenance. This is especially important for primiparous sows due to their greater energy and lysine requirements for growth and maturity in comparison to older sows where body weight may simply be maintained (Heo, Yang et al. 2008, Goodband, Tokach et al. 2013). Lactation diets are traditionally fed to support milk production which is a determining factor of piglet growth. It seems imperative to not only determine nutrient requirements for this period but also define a feeding strategy as late gestation and lactation have two different goals: feed restriction and increased feed intake, respectively. Two major nutritional requirements that determine the reproductive success lie in the content of utilisable energy (characterised below as metabolizable energy; ME) and amino acid (AA) content with these requirements varying the most during the transition period compared to early gestation and late lactation (Figure 2.5). During late gestation, energy and AA requirements can be divided into five different tissue

pools; maintenance, growth (maternal protein and fat deposition), foetus, mammary tissue, uterus and placenta and fluids (Goodband, Tokach et al. 2013). During early lactation, energy and AA requirements can be divided into four different pools; maintenance, growth (maternal protein and fat deposition), mammary growth and milk production (Feyera and Theil 2017). Due to the varying requirements throughout the transition period, it seems that it may be unfavourable to feed a single diet during transition and lactation and that phase feeding is more optimal (Feyera and Theil 2017).

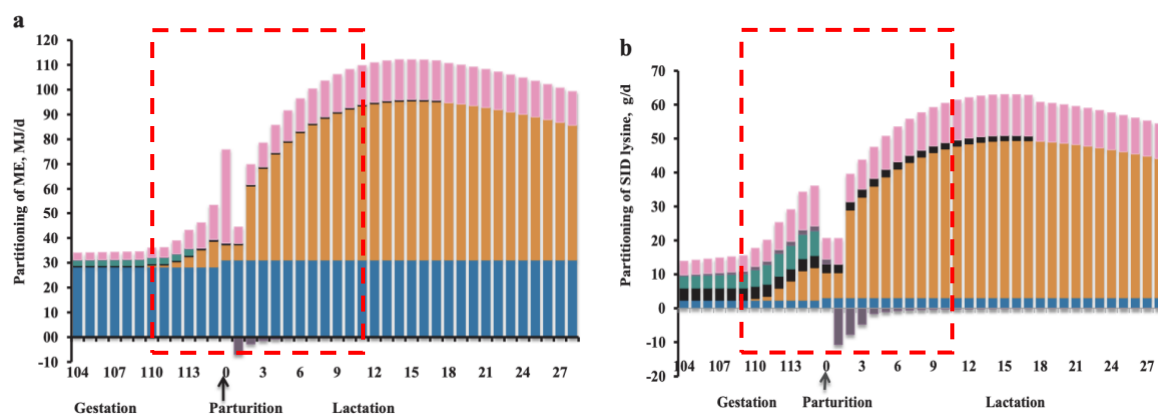


Figure 2.5. The partitioning of **(a)** metabolizable energy ME and **(b)** the standard ileal digestible lysine for maintenance (blue), colostrum/milk production (orange), mammary growth (black), foetal growth (green), uterine components (purple) and additional heat loss (pink) during the transition period (red dashed line) for sows. Adapted from (Feyera and Theil 2017).

According to Theil (2014), the transition period is important because it can potentially effect:

1. Neonatal piglet mortality and neonatal energy supply
2. Foetal growth, piglet birth weight and litter size
3. Glycogen depots in newborn piglets
4. Placenta, uterus, fluids and membranes
5. Mammary growth
6. Colostrum synthesis
7. Time of the onset of lactation
8. Milk synthesis
9. Heat production of transition sows
10. Body condition and body mobilisation.

2.7.2 Feeding strategies during late gestation

Feeding strategies that support the exponential growth of the foetus during late gestation while also supporting the growth of the gilt may provide a possible method of improving GP birth and weaning weights. Nutritional interventions that increase energy and/or lysine intake during late gestation have been investigated extensively in the gilt. These studies involve either feeding more of a ration with a set energy and AA content or providing two diets fed at differing levels of energy and/or lysine content. The issue with providing late gestation diets with greater energy content is that heavy gilts and those with higher back fat thickness (>19 mm) at farrowing can have issues with farrowing, reduced feed intake and increased body mobilisation during lactation (Dourmad 1991). The smaller body size of the gilt and therefore lower body reserves (Revell, Williams et al. 1998, Amdi, Giblin et al. 2013, Kim, Yang et al. 2015) leads to impaired milk yield and therefore reduced GP growth. Despite this, the effects of increasing energy and/or lysine intake during late gestation on GP birth and weaning weights has been controversial. It seems that increasing energy levels are able to increase birth weights in some studies (Cromwell, Hall et al. 1989, Shelton, Neill et al. 2009, Gonçalves, Gourley et al. 2016). However, a majority of the studies report no effects of energy level on piglet birth weight (Heo, Yang et al. 2008, Smits, Henman et al. 2012, Mallmann, Betiolo et al. 2018, Mallmann, Camilotti et al. 2019) with most of these studies using energy inclusion levels in the range of 12.6 MJ ME/kg (Liu, Zhang et al. 2020) to up to 28.9 MJ ME/kg (Estienne, Harper et al. 2003). To support the studies that report no effects of increased energy levels of GP performance, these studies along with others have observed reduced feed intake during lactation and therefore, reduced GP performance during lactation when GP weaning weights are reported. Furthermore, in a study by Liu, Zhang et al. (2020), increasing energy levels from 35.1 MJ/d ME to 47.7 MJ/d ME during late gestation caused greater oxidative stress at parturition, which further supports the deleterious effect of increased energy supplementation during late gestation. The differences between studies that successfully increased birth weights in GP compared to those that did not may lie in a variety of reasons, such as differences in the number of days in which the diet was initially implemented or the confounding effect of differences in amino acid inclusion in each of these studies (Goodband, Tokach et al. 2013). However, it seems that the evidence for reduced feed intake during lactation may prevent the implementation of high energy diets during late gestation in a commercial setting. Clearly, further work is required in this area in order to determine whether this type of feeding strategy is beneficial to GP. This work must evaluate whether the additional cost of feed/gilt can offset the additional sow weight gain and possible reduction in feed intake.

While a majority of studies have investigated the effects of increased energy intake to increase piglet birth weight to no avail, increasing SID lysine intake has yielded more beneficial results on individual and total litter weights of GP. The requirements for lysine and other AA increases over the course of gestation. However, the provision of a single diet over the course of gestation may not accommodate for this increase and therefore could be either above the requirements of early gestation and below the requirements of late gestation (Goodband, Tokach et al. 2013). Furthermore, primiparous sows require higher amounts of lysine:energy during late gestation compared to multiparous sows and therefore, may be below their requirement during late gestation (Figure 2.6). In a recent study by Gaillard, Gauthier et al. (2019), concentrations of SID Lys for primiparous sows was 5.0 grams/kilogram while multiparous sows required 4.5 grams/kilogram. In a study by Heo, Yang et al. (2008), gilts consumed 24.6 grams/day SID lysine from day 80 of gestation and had heavier litters at birth compared to gilts fed 17.7 grams of SID lysine (15.3 vs 13.2 kg). Furthermore, this study showed that increasing energy levels did not affect litter weights of gilts, rather only lysine levels did. According to Soto, Greiner et al. (2011), when gilts and sows consumed 15 and 20 g/d of total lysine, birth weights increased in gilts but not in sows. These data demonstrated that increasing SID Lysine inclusion may be a key component in increasing birth weight. The use of increasing SID lysine along with other AA in late gestation diets for gilts may be a promising nutritional intervention due to the support in mammary and body growth of the gilt during gestation.

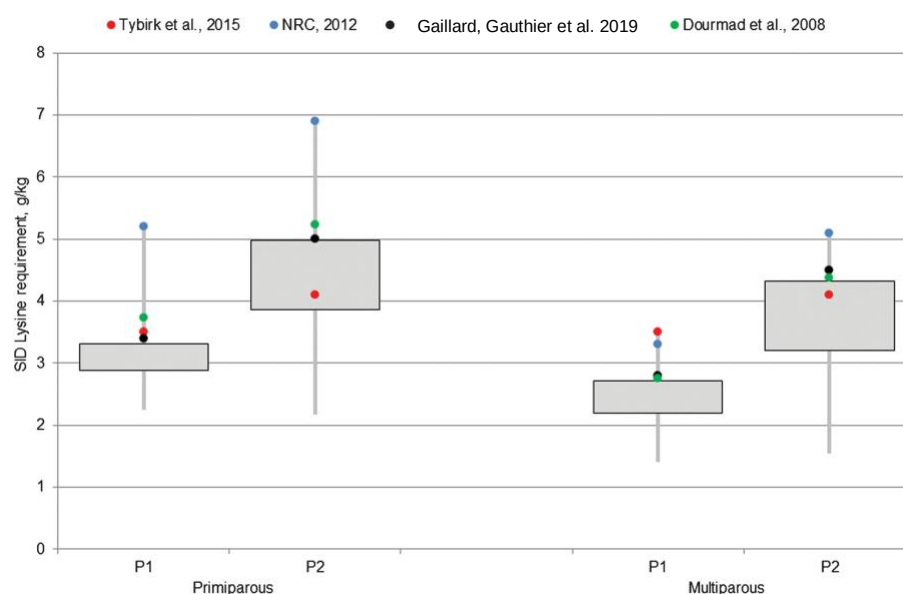


Figure 2.6 A boxplot of SID lysine requirements per kg of feed for gestating primiparous and multiparous sows in early gestation (P1) and late gestation (P2) for four different studies. (Gaillard, Gauthier et al. 2019)

2.7.3 Feeding strategies during early lactation

In order to support the demands of lactation, dams are traditionally consume feed *ad libitum*. Lactating gilts rarely eat enough to support the demands of lactation such as maintenance, milk production and body growth (Noblet, Dourmad et al. 1990), with gilts commonly reported to consume less than multiparous sows (Mahan 1994, Mahan 1998, Gatford, Smits et al. 2010, Mallmann, Betiolo et al. 2018). Increasing milk yield and therefore piglet growth is challenging in the gilt due to the requirements of their own growth as well as lactation. In a study by Pluske, Williams et al. (1998), when made anabolic, gilts partitioned additional nutrients to their own growth rather than to milk production. Furthermore, gilts are susceptible to losing significantly more backfat and body weight during lactation compared to multiparous sows (Moeller, Goodwin et al. 2004, Mallmann, Betiolo et al. 2018). This is an issue due to the fact that the smaller gilt has less body reserves to mobilise to support lactation (Whittemore 1996) which contributes to the reduced growth seen in GP. In addition, their size contributes to their lower GI tract capacity compared to those of multiparous sows. Sinclair, Edwards et al. (1996) reported that feed intake of gilts and parity 3 sows were similar during the first two weeks of lactation. However, gilts had lower feed intake from 3 to 5 weeks during lactation. This increase may be a result of greater energy requirements associated with the higher body weights of multiparous sows (Eissen, Kanis et al. 2000). Furthermore, due to their larger and longer GIT, mature multiparous sows have increased gastrointestinal capacity and increased time of digesta remaining the intestine compared to the growing gilt (Goff, Milgen et al. 2002) and may allow for increased digestion and absorption of nutrients.

2.7.4 Feeding ketogenic substances during late gestation

In order to increase foetal energy stores in preparation for the neonatal period, additives fed in late gestation have been investigated. In order to directly be deposited in the foetus, the sow must metabolise these additives into components that can pass the placenta. One such metabolite that can easily pass transplacentally is glucose. This is mainly due to the deposition of glucose into foetal muscle and liver in the form of glycogen which then can be used as a primary energy source for the neonatal piglet. Ketone bodies (KB) also offer an alternative primary energy source for the neonatal piglet as they readily pass through the placenta and can

be deposited in utero (Figure 6). Furthermore, KB are able to inhibit glycolysis (Yellen 2008) and thus decreases available glucose. This may lead to an accumulation of glucose which could then be synthesized into glycogen storage. Furthermore, exogenous KB have been shown to have anti-lipolytic properties (Taggart, Kero et al. 2005). High circulating KB can be used by brain and muscle which may spare glucose and amino acids. It is well established that small-for-age pigs, and by inference GP, have lower glycogen and fat reserves than those of heavier pigs and are more susceptible to hypothermia and crushing in the early neonatal period. Furthermore, pigs are susceptible to hypoglycaemia when undernourished due to inability to access the teat or reduced colostrum yield in the dam. Increasing liver glycogen may reduce this risk or providing an alternative energy source during this time may mitigate some of this.

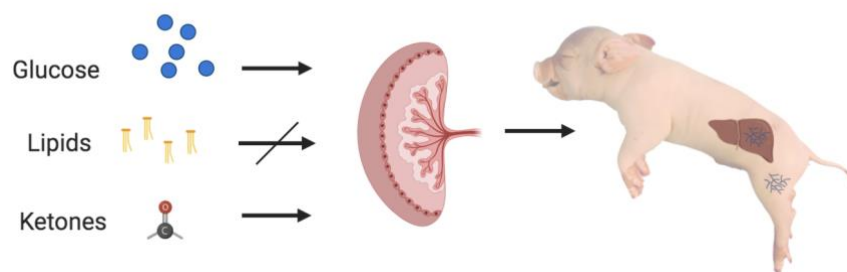


Figure 2.7. Glucose and ketones can pass transplacentally to the foetal piglet.

The use of the additive 1,3-butanediol (BD), a synthetic carbohydrate, in late gestation diets have been previously investigated in regard to increasing survival. According to Tate, Mehlman et al. (1971), BD is catabolized into beta-hydroxybutyrate (β -OHB) in the liver of the rat via a liver alcohol dehydrogenase pathway. Assuming the same occurs in the gestating dam, early studies by Stahly, Cromwell et al. (1985) supplemented gestation diets with BD at 20% of their daily (ME) intake 9 days prior to farrowing and reported increased pigs weaned per litter and improved survival rates of pigs from birth to weaning by 5.7% compared to those born to dams fed isocaloric additions of starch. In another study by Stahly, Cromwell et al. (1986), which also fed BD during late gestation at 20% of their daily ME intake, similar effects whereby piglets weaned per litter were increased by 0.45 and survival rate was improved from birth to weaning by 4.3%. This increase in survival is most likely due to the increased glycogen concentrations found in the liver of the neonatal piglet, which was found in a study that supplemented late gestation diets with BD at 15% ME (Spence, Boyd et al. 1985). Supplementation of gestation diets also seems promising for LBW piglets due to their reduced

glycogen reserves (Vanden Hole, Ayuso et al. 2019). Stahly, Raff et al. (2014) showed that not only feeding BD at a lower concentration (8% of ME) reduced pre-weaning mortality but specifically could reduce it in LBW piglets. Feeding at this level was reported to be the more economically viable option at the time of the study. While this result was seen in piglets from dams of a parity average of 2.48, this could be a promising additive to increase LBW found in GP and improving their survival.

The shift of primary energy source from glucose to ketones may raise concerns due to issues such as neonatal hyperketonemia which normally caused by the change to a lipid-based metabolism after birth. However, this does not seem to be the case for pigs. Adams and Odle (1993) showed that neonatal piglets have poor capacity for ketogenesis with fasted new-born pigs showing plasma β -OHB below the lower limit of detection and can be partially explained by the limited lipid stores available for mobilisation in the neonatal piglet. Furthermore, they showed that the use of KB by extrahepatic tissue in the foetal and neonatal pig is controlled by circulating KB rather than the formation of KB themselves. This is most likely due to the limited stores of lipids available as ketogenic substrates (Dividich and Noblet 1983).

2.7.5 Polyphenols

Plant polyphenols have been shown to exert a variety of health benefits due to their anti-oxidative and anti-inflammatory properties. The use of plant polyphenols in livestock has been growing in popularity due to the conditions of oxidative stress and inflammation that occur within intensive systems (Gessner, Ringseis et al. 2017). Increased oxidative stress during gestating and lactation has been previously reported with dams not fully recovering until weaning (Berchieri-Ronchi, Kim et al. 2011) due to the increased demand for energy which increases with oxygen uptake and contributes to oxidative stress (Agarwal, Saleh et al. 2003). A similar finding was also reported by (Tan, Wei et al. 2015) where dams exhibited increased reactive oxygen species during late gestation and early lactation. Lipiński, Antoszkiewicz et al. (2019) showed that both gilts and sows showed oxidative stress at 1 day after parturition and on day 28 of lactation, which contributed to reduced litter weights at weaning. However, the supplementation of gestation and lactation diets with the plant polyphenols, anthocyanidin, flavonol, catechin and procyanidin, increased litter weights at birth and weaning. Wang, Jiang et al. (2019) showed that grape seed polyphenols were able to improve pre-weaning survival in litters and also found greater IgM and IgG content in colostrum. While the supplementation

of gilt diets with polyphenols has not been studied, given the LBW and low pre-weaning survival in GP, feeding plant polyphenols to gilts may have benefits from improving pre-weaning GP performance.

2.7.6 Fibre

Feeding fibre to sows in late gestation has been shown to have many beneficial effects in the literature. The use of high fibre diets during late gestation is said to be useful due to their ability to decrease stereotypical activities associated with restricted feeding during late gestation (Meunier Salaün, Edwards et al. 2001) and increase voluntary feed intake during lactation (Matte, Robert et al. 1994, Farmer, Robert et al. 1996, Quesnel, Meunier-Salaün et al. 2008). This increased feed intake has been reported to be anywhere from 5% (Matte, Robert et al. 1994) to 15% (Quesnel, Meunier-Salaün et al. 2008), with the variation most likely attributed to the source and inclusion level of the fibre. Factors such as housing and feeding conditions as well as age of the sow can also contribute to the response of the sow to dietary fibre inclusion. Furthermore, numerous different methods are used to measure and describe fibre making it difficult to compare the effect to fibre between studies. For example, some studies use the term “crude fibre” which describes a laboratory estimation of organic residue left after treating feed with petroleum ether hot sulphuric acid, boiling water and alkali (Meunier-Salaün and Bolhuis 2014). Alternatively, neutral detergent fibre (NDF) refers to the cellulose, hemicellulose and lignin as the major components while acid detergent fibre (ADF) provides an estimate of just lignin and cellulose (Van Soest, Robertson et al. 1991). Dietary fibre includes a wide range of sources such as non-starch polysaccharides such as pectins, cellulose, hemicelluloses, β -glucans and fructans along with oligosaccharides and starches and can be categorised as either soluble or insoluble fibres (Jarrett and Ashworth 2018). Furthermore, dietary fibres play different roles in swine diets. For example, soluble fibres which are usually fermented in the colon produce gases and physiologically active by-products while insoluble fibres are not metabolised easily and are often used to provide bulk to the diet (Jarrett and Ashworth 2018).

Generally, beneficial effects of fibre have been reported and are primarily as a result of the bulking effect of fibre due to (1) the indigestibility/insolubility of cellulose and lignin and their gut filling properties and (2) its water binding capacity (Meunier-Salaün and Bolhuis 2014). This allows the sow to be satiated without the provision of extra energy during gestation.

In fact, according to EU welfare legislation, all pregnant, dry sows and gilts must be provided with sufficient amounts of high fibre feed to alleviate welfare issues during gestation and prepare them for *ad libitum* feed intake during lactation (Guillemet, Hamard et al. 2007). Primiparous sows generally have lower gut capacity compared to those of multiparous sows which may limit their feed intake during lactation (Theil, Nielsen et al. 2012). In order to improve feed intake during lactation, diets high in soluble fibre may improve the feed intake of primiparous sows which may promote feed intake and increase milk production resulting in growth of GP. Furthermore, Priester, Visscher et al. (2020) reported that a diet rich in fibre (primarily consisting of sugar-beet pulp and alfalfa/lucerne) was able to improve feed intake and weaning weight of litters in primiparous sows, while the provision of lucerne as enrichment was able to improve sow welfare during farrowing and lactation and reduce the number of still born piglets among various parities (Edwards, Plush et al. 2019). Lucerne is a silage high in branched-chain amino acids (BCAA; Homolka, Koukolová et al. 2008), which also have been shown to have various beneficial effects when included in gestation and lactation diets.

2.7.7 Branched chain amino acid (BCAA)

With milk being the sole source of nutrients during the neonatal period for the piglet, interventions to improve milk yield and mammary gland synthesis in the gilt are a potential option to improve neonatal growth and survival for GP. Amino acid requirements are increased during late gestation and lactation to support mammary gland growth and development and milk production. Of these amino acids, BCAA, which include leucine, isoleucine and valine, have shown promising results in improving piglet growth. Most of these studies were conducted using multiparous sows during lactation rather than in late gestation. The BCAA have been reported to be extensively catabolised by lactating mammary tissue (Rezaei, Wu et al. 2016). Dunshea, Bauman et al. (2005) reported that supplementation with BCAA improved milk protein content and improved piglet growth in multiparous sows. The uptake of BCAA from plasma by the lactating mammary gland exceeds BCAA concentration in the milk (Trottier, Shipley et al. 1997, Li, Knabe et al. 2006) demonstrating that BCAA are metabolised in the mammary gland. The mammary gland is said to synthesize glutamine from BCAA with Li, Knabe et al. (2006) showing that supplementing BCAA increased glutamine in the milk. Glutamine is a major substrate for intestine synthesis of citrulline and arginine, two amino acids involved in neonatal growth (Wu, Knabe et al. 2004).

Isoleucine and leucine are involved in protein synthesis in the mammary gland (Lei, Feng et al. 2012). However, among the three BCAA, the role of valine in milk synthesis has been most reported with numerous studies demonstrating the link between increased dietary valine and improved milk concentrations. Moser, Tokach et al. (2000) reported improved litter weaning weights by of 2 kg in piglets from both gilts and multiparous sows when dietary valine was increased from 0.8 to 1.2%, and this effect was independent of either increased leucine or isoleucine. However, they did not report differences in milk composition. Strathe, Bruun et al. (2016) also found that increasing dietary valine-to-lysine ratio had no effect on milk production or composition. Contrary to this, Richert, Goodband et al. (1997) found increased protein content of milk in response to increased dietary valine.

Many of these studies have been performed using multiparous sows and the information on the effects of dietary BCAA on gilts and their progeny is difficult to find. However, in a comprehensive study by Che, Xu et al. (2019), valine supplementation during late gestation was able to increase protein and some amino acid content in the colostrum. This study also reported increased total fat in colostrum. Furthermore, their findings seem to indicate that valine enhanced mammary gland development in late gestation and protein synthesis was increased in colostrum via the mTOR signalling pathway. Taken together, it seems that the use of BCAA supplemented diets have the potential to increase mammary development in the gilt and either improve milk and/or milk protein content. This may be useful for increasing survival and improving neonatal growth of GP.

2.8 Conclusions

Gilt progeny represent a significant loss for the pig industry. The reasons for their underperformance are complex and sometimes unclear. This literature review has outlined some of the possible reasons that may contribute to their poor performance compared to progeny from multiparous sows such as reduced colostrum and milk consumption and restricted prenatal growth, particularly in late gestation. However, investigating underlying biological differences between GP and SP may provide more clarity and solutions to reduce the disparity between GP and SP. The aims of this thesis will be to investigate differences between GP and SP by studying early growth and development of the gastrointestinal tract and due to its important role in nutrient absorption and digestion and barrier function. In line with this, it also aims to investigate biomarkers for barrier function such as I-FABP. Furthermore,

it will also aim to investigate nutritional interventions targeted at late gestation and lactation to improve GP growth and survival.

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Chapter 3 Characterisation of impaired development of the gastrointestinal tract in progeny born to primiparous sows

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3.1 Abstract

Progeny born to primiparous sows (gilt progeny; GP) are characterised by their low birth and weaning weights in relation to sow progeny (SP), which results in poorer lifetime growth performance. This study aimed to quantify some of the underlying biological bases for the differences in performance between GP and SP by investigating the effect of parity on aspects of gastrointestinal tract (GIT) barrier function at birth and weaning. This was performed by quantifying differences in GIT development at birth ("0h", 6 GP/9 SP), 24 h after birth ("24 h", 8 GP/7 SP), pre-weaning ("PrW", 29 d of age, 10 GP/10 SP) and 24 hours post-weaning ("PoW", 10 GP/10 SP). Due to heterogeneity of measures from piglets at birth and weaning the results were analysed in two cohorts, birth (0 vs. 24 h) and weaning (PrW vs. PoW). All analyses were performed according to a 2x2 factorial design in each cohort for the main effects of progeny (GP vs SP) and cohort (0 vs 24 h or Pre-W vs PoW), and their interaction. Organs of the GIT (stomach, jejunum, ileum and colon) were excised after euthanasia and barrier integrity was determined. Transepithelial resistance (TER) was measured in all organs of the GIT using mucosa. The permeability of macromolecules fluorescein isothiocyanate (FD4) and

tetramethyl rhodamine isothiocyanate (T150)-labelled dextrans was measured in the mucosa of the jejunum and ileum. Furthermore, tight junction proteins claudin-2 and -3 and inflammatory markers interleukin-8 (IL-8), IL-1 β and tumour necrosis factor-alpha (TNF- α) were also assessed in jejunal and ileal mucosa. Jejunal and ileal TER was higher at 24 h postnatally ($p = 0.004$ and 0.002 , respectively) in the birth cohort while stomach and colon TER was lower at 24 hours postnatally ($p = 0.048$ and 0.004 , respectively). In response to weaning, stomach and jejunal TER decreased ($p = 0.014$ and 0.003 , respectively) while ileal TER tended to decrease ($p = 0.066$). However, an interaction between parity and time was observed such that colon TER reduced in response to weaning only in GP while no changes were observed in SP over the weaning period. Permeability towards FD4 and T150 was higher at 24 h only in jejunal tissue ($p = 0.003$ and $p = 0.001$, respectively), with no main or interactive effects observed in ileal tissue. In the weaning cohort, jejunal and ileal permeability to FD4 ($p = 0.05$ and 0.022 , respectively) was higher in GP while permeability towards T150 was only observed in the ileum ($p = 0.032$). The GP tended to exhibit lower claudin-3 expression in the jejunum compared to SP ($p = 0.058$) in the birth cohort, with no other main or interactive effects observed in claudin expression in the jejunum or ileum. At weaning, claudin-3 expression was lower after weaning in both GP and SP ($p = 0.03$). Furthermore, while claudin-2 expression tended to be higher in the ileum of SP overall ($p = 0.063$), claudin-2 expression in GP was specifically upregulated in response to weaning while no changes were observed between the two timepoints in SP ($p = 0.043$). Finally, concentrations of IL-8 were higher in the jejunum of GP ($p = 0.011$) and tended to be higher in the ileum ($p = 0.063$) in the birth cohort. However, IL-8 was lower at 24 h in the jejunum ($p = 0.002$) and ileum ($p = 0.003$). Both IL-1 β and TNF- α concentrations were higher at 24 h than at birth in the jejunum ($p = 0.017$ and $p < 0.001$, respectively), while only ileal TNF- α was higher at 24 h ($p = 0.008$). Other than higher TNF- α observed in the jejunum of SP ($p = 0.016$), no other main or interactive effects were observed in inflammatory markers in the weaning cohort. Collectively, results from this study indicate that while there are early signs of variation in GIT development in the first 24 hours between GP and SP, this difference increases throughout the pre-weaning period and GP enter the weaning period with poorer GIT development and function.

3.2 Introduction

The progeny of primiparous sows (gilt progeny; GP) are characterised by their poorer lifetime growth performance in comparison to those from multiparous sow progeny (sow

progeny; SP) (Holyoake 2006, Miller, Collins et al. 2013, Craig, Collins et al. 2017). The reasons for their relative underperformance are complex but can in part be attributed to reduced development of the gastrointestinal (GI) tract present at birth. In a previous experiment we compared differences in the growth and development of the GI tract between GP and SP in two different cohorts, the first being 24 hours after birth and the second being the periweaning period (Craig, Dunshea et al. 2019a). Birth and weaning periods are two major events in a piglet's life which can dictate their long-term health with significant growth and development occurring between these two timepoints. Furthermore, these two events have major impacts on the gastrointestinal (GI) health, required for nutrient digestion and metabolism, disease status and growth and development of the piglet (Pluske, Turpin et al. 2018), all of which are imperative for long-term success of the piglet.

In our previous study, while GP were born significantly smaller than SP, organs of the GI tract such as the stomach were also significantly smaller in GP when normalised to weight while no differences were observed in small intestinal weight and length. However, by weaning, GP had lower stomach weights, small intestinal weight to length ratios and also had lower jejunal and ileal protein per gram of mucosa compared to SP. Collectively, this suggests that a reduction in uptake of colostral proteins may compromise GI tract development and the disparity in GI growth between GP and SP is largely established during the pre-weaning period.

Given the relative underperformance of lighter piglets that have a lesser developed GI tract at weaning (Pluske, Kerton et al. 2003), it is likely that GP experience delayed or under-development of the GIT. This reduced GI tract development may be exacerbated by the stressors of weaning and result in poorer performance of GP. In order to further quantify functional differences of the GI tract, factors affecting epithelial barrier integrity were investigated such as electrophysiological measurements, inflammatory marker concentrations and tight junction protein concentration were measured in tissues of the GI tract. The aims of this experiment were to quantify aspects of the development of epithelial barrier function of the GIT in GP compared to SP at birth, and how this translates to barrier function immediately before and after weaning.

3.3 Materials and Methods

All experimental procedures were approved by the Rivalea (Australia) Animal Care and Ethics Committee (protocol number 16P014) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

3.3.1 Animals and Experimental Design

Tissue samples of the stomach, jejunum, ileum and colon were taken from 73 opportunistically selected piglets (36 male and 37 female) born to 42 Large White dams (Primegro genetics, New South Wales, Australia), as previously described in detail by Craig, Dunshea et al. (2019a). Briefly, 36 piglets born to primiparous sows (gilts; parity 1) and 37 born to multiparous sows (sows; parities 3 and 4) were euthanised and GI tract samples harvested at birth (0h), 24 hours after birth (24h), one day prior to weaning at approximately 29 days of age (Pre-weaning; PrW), and at 24 hours after weaning (Post-weaning; PoW) (Table 1). Piglets were divided into either the “birth cohort” (0h and 24h) or the “weaning cohort” (Pre-weaning and Post-Weaning) for further analysis (Table 1). Blood samples were taken prior to euthanasia as described in detail by Craig, Dunshea et al. (2019a).

Table 3.1 Number of piglets euthanised within each parity (gilt progeny, GP; or sow progeny, SP) and sex (male, M; or female, F) at each time point

Dam Parity	GP		SP	
Sex	M	F	M	F
0 h	2	4	4	5
24 h	2	5	5	4
PrW	5	5	5	5
PoW	5	5	5	5

Piglets were sedated with an intraperitoneal injection of 17 mg/kg of body weight of xylazine hydrochloride (Ilium Xylazil-100; Troy Laboratories Pty Ltd, Australia) and then administered an intraperitoneal anaesthetic dose of 0.1 mL/kg of sodium pentobarbitone (Lethabarb; Virbac, Australia), and then finally a lethal dose of 0.5 mL/kg intra-cardiac sodium pentobarbitone prior to dissection. Fresh stomach, jejunum, ileum and colon samples (approximately 5 cm in length) were excised and immediately placed into chilled 0.01M PBS (pH 7.2) for later quantification of intestinal barrier function. Sections of the jejunum and ileum

were also harvested and cleaned using PBS. Sections were subsequently snap-frozen in liquid nitrogen and stored at -80 °C until further analysis.

3.3.2 Ussing chambers

Mucosal transepithelial resistance (TER) was quantified from the stomach, jejunum, ileum and colon and macromolecule permeability was quantified in the jejunum and ileum as described by Cottrell, Furness et al. (2020). Fresh tissue sections were immediately placed in PBS and opened along the mesenteric border by blunt dissection, and muscle layers placed in Krebs solution (11.1 mM glucose, 118 mM NaCl, 4.8 mM KCl, 1.0 mM NaH₂PO₄ 1.2 mM MgSO₄, 25 mM NaHCO₃, 2.5 mM CaCl₂, pH 7.4), continuously bubbled with carbogen (95% CO₂, 5% O₂, BOC, Australia). The mucosa was then mounted on a round slider (0.72 cm²) and placed vertically into a two-part Ussing chamber (EasyMount diffusion Chambers, Physiologic Instruments) with 5 mL of modified Krebs solution added to mucosal and serosal chambers, where the glucose was replaced with mannitol to prevent active transport of glucose as an energy substrate. A multichannel voltage-current clamp (VCC MC6, Physiologic Instruments) was linked to dual voltage sensing electrodes and dual current passing electrodes. Each electrode was submerged in agarose (3% agarose/ 3 M KCl) and installed on opposite sides of the tissue. The tissue was clamped at a voltage of 0 mV and electrical resistance was determined by administering two-second, 2 mV pulses. Voltage and *I*_{sc} readings were acquired using a PowerLab amplifier and recorded using LabChart®5 (AdInstruments, Australia). The TER was calculated by Ohm's law from recordings immediately after a 10-minute equilibration period. Macromolecule permeability was assessed using fluorescein isothiocyanate (FITC)-labelled 4 kDa dextran (FD4, 78331, Sigma, USA) and tetramethyl rhodamine isothiocyanate (TRITC)-labelled 150 kDa dextran (T150, T10337, Sigma, USA). Two hundred mL of 50 mg/mL each of FD4 and T150 was added to the mucosal side of the Ussing chamber. Then, 200 mL of solution was collected from each side of the chamber at 1, 60 and 120 min and frozen at -20 °C until analysis. All tissues were measured for TER and apparent permeability (*P*_{app}) in triplicate. The *P*_{app} of FD4 and T150 were quantified by the following equation:

$$\text{Apparent permeability } (P_{\text{app}}) = dQ / (dt \times A \times C_o)$$

Equation 3.1 where *dQ* is the transport rate in mg/mL, corresponding to the linear slope of the timepoints measured. This was multiplied by the initial concentration in the donor chamber (*C*_o) and the area of the slider (Tomita, Ohkubo et al. 2004).

3.3.3 Inflammatory marker analysis

Approximately 100 µg of snap-frozen whole jejunum and ileum tissue was weighed and homogenised in lysis buffer as previously described (Helm, Curry et al. 2019) with borosilicate 2 mm beads (Cat no. Z273627, Sigma, USA) using a mini bead beater (Cat. No. 607, BioSpec, USA). Samples were then centrifuged at 15,000 x *g* for 10 minutes at 4 °C and the supernatant was collected. The supernatant from each sample was analysed by ELISA for interleukin-8 (IL-8), tumour necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) (catalogue numbers DY535, DY690B and DY681, respectively; R&D Systems, USA). Samples were assayed as per the manufacturer's guidelines with the exception that tissue supernatant was used rather than plasma as outlined by Pearce, Mani et al. (2013). Assays were performed in duplicate and 100 µg/well of protein was used, with protein concentrations determined using a Bicinchoninic acid (BCA) assay (Cat no. 23225, Thermo Fisher Scientific, USA). The intra-assay and inter-assay CV for IL-8 were 8-19% and 9% respectively, for TNF-α, 3-7% and 15% respectively, and for IL-1β, 4-6% and 5%, respectively.

3.3.4 Western blotting

Snap-frozen whole jejunum and ileum samples were pulverised then homogenised with radioimmunoprecipitation (RIPA) lysis buffer with protease and phosphatase inhibitors. Samples were homogenised with a bead beater for 30 seconds and centrifuged at 15,000 x *g* for 10 minutes at 4 °C, before the supernatant was collected and stored at -20 °C until analysis. The BCA assay was used to standardise protein and separated by electrophoresis using 10-20% Tricine Protein gels (Cat no. EC66255BOX, Thermo Fisher Scientific, USA) under denaturing conditions and each sample was randomly selected for inclusion on each gel. Following this, the gels were then soaked in MilliQ water for 5 minutes and transferred to a 0.2 µM nitrocellulose membrane, iBlot 2 NC stacks (Cat no. IB23001, Thermo Fisher Scientific, USA) using the iBlot 2 system (Cat no. IB21001, Thermo Fisher Scientific, USA). Following transfer, membranes were briefly soaked in PBS and then immediately stained using LiCor REVERT total protein stain kit (Cat no. 11010, LiCor, USA) and imaged using the Odyssey ® Imagine System at 700 nm. Membranes were washed with MilliQ water and then blocked in 5% non-fat dry milk (NFDM) in TBST (1 x Tris Buffered Saline, 1 % Tween-20) for 2 hours. Membranes were then incubated with primary antibodies claudin-2 and claudin-3 (Cat no. 51-6100 and PA5-16867, Thermo Fisher Scientific, USA) with 5% NFDM in TBST overnight

with dilutions 1:250 and 1:750, respectively. Following this, the membrane was washed three times with TBST for 5 minutes and then incubated with IRDye 800CW secondary antibodies (Cat no. 32213, Licor, USA) in 5% NFDM in TBST for one hour. After incubation steps, the membrane was washed three times with TBST for 5 minutes. Following final washing, the membrane was imaged using infrared fluorescence of the Odyssey® Imagine System (Licor, USA) at 800 nm. Both images of the total protein stain and antibody stain were analysed using Licor Image Studio (Licor, USA). A loading control of pooled jejunum homogenate was used on each blot in order to normalize all blots to each other.

3.3.5 Statistical analyses

Data were analysed using a linear mixed model (Genstat, 18th edition; England, UK). Data from the birth and weaning cohorts were analysed separately. Parity (gilt vs sow) and time within cohorts (birth; 0h vs 24h and weaning; PrW vs PoW) were analysed as the main and interactive effects when analysing intestinal integrity and inflammatory markers. Where main or interactive effects were found to be statistically significant, post-hoc analysis using Fisher's LSD test was performed. Data was transformed when skewed to restore normality using a Log_e transformation and analysed as above. Data are presented as the pooled means for the main effects of parity, time, or the interaction, $\pm$ standard error of the difference (SED). Means were considered significantly different at $p < 0.05$ and $p < 0.10$ was considered a trend.

3.3 Results

3.3.1 Birth cohort

3.3.1.1 Mucosal TER and permeability

The TER declined over the first 24 h after birth in the stomach (98.5 vs. 76.1 $\Omega \cdot \text{cm}^2$, $p = 0.048$) and colon (130 vs 100 $\text{cm} \cdot \Omega^2$, $p = 0.004$), but increased in the jejunum (24.4 vs 34.5 $\text{cm} \cdot \Omega^2$, $p = 0.004$) and ileum (26.5 vs. 38.4 $\text{cm} \cdot \Omega^2$, $p = 0.002$). Stomach TER was lower in GP than in SP overall (72.9 vs 101 $\text{cm} \cdot \Omega^2$, $p = 0.015$). An increase in jejunum FD4 (470 vs 1451 respectively, $p = 0.003$) and T150 (68.6 vs 364 respectively, $p = 0.001$) permeability was observed between 0 and 24 h and no other main or interactive effects were observed in the jejunum and no effects of parity or time were observed in the ileum (Table 3.2).

Table 3.2 The effects of parity (P; GP vs SP) and time (T; 0h vs 24h) on transepithelial resistance (TER, cm.Ω²) and FD4 and T150 apparent permeability of the jejunum and ileum.

Parity (P)	GP		SP		SED	p-value		
	0h	24h	0h	24h		P	T	P*T
Time (T)								
TER (cm.Ω²)								
Stomach	76.8	69.1	120	83.2	16.96	0.015	0.048	0.23
Jejunum	23.7	35.2	25.0	33.9	4.51	0.78	0.004	0.68
Ileum	27.6	35.3	25.5	41.6	5.29	0.73	0.002	0.27
Colon	133	102	127	99.4	13.30	0.94	0.004	0.84
P_{app} (x 10⁻⁴ cm·sec⁻¹)								
Jejunum								
FD4 ⁺								
Log _e	6.28	7.07	6.02	7.20	0.478	0.59	0.003	0.33
(x 10 ⁻⁴ cm·sec ⁻¹)	(533)	(1176)	(412)	(1808)				
T150 ⁺								
Log _e	4.59	5.69	3.87	6.10	0.644	0.23	0.001	0.23
(x 10 ⁻⁴ cm·sec ⁻¹)	(98.5)	(296)	(47.9)	(446)				
Ileum								
FD4	241	393	434	294	145	0.66	0.96	0.17
T150	86.3	72.1	89.2	120	34.9	0.95	0.98	0.30

⁺ Due to sample heterogeneity a Log_e-transformation was performed. Values were then back transformed and presented in parentheses for comparison purposes.

3.3.1.2 Tight junction protein expression

No main or interactive effects were observed on claudin-2 expression in the jejunum (Figure 3.1a). However, claudin-3 concentrations in the jejunum tended to be lower in GP compared to SP (2.51 vs 4.36 AU, $p = 0.058$; Figure 3.1b). No main or interactive effects were observed on claudin-2 and -3 expression in the ileum (Figure 3.1c, d).

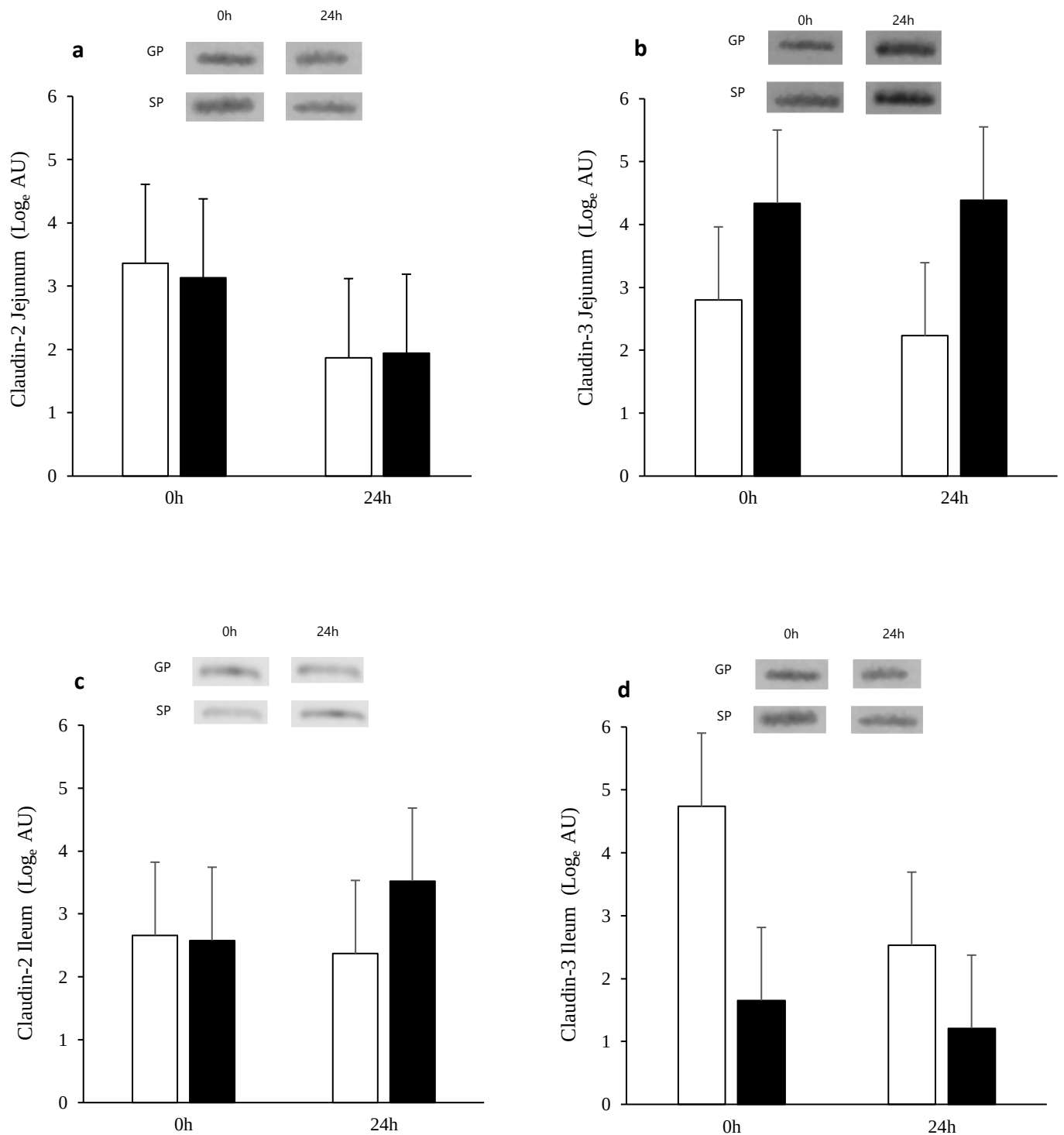


Figure 3.1 The effect of parity (GP vs SP) and time (0 h vs. 24 h) on jejunum **(a)** claudin-2 and **(b)** claudin-3 expression and ileum **(c)** claudin-2 and **(d)** claudin-3 expression. Data in figures are presented as Log_e -transformed means and standard error of the transformed mean for the P×T interaction. The p – values for the main and interactive effects of parity and time were **(a)** 0.81, 0.15 and 0.87 **(b)** 0.058, 0.79 and 0.75 **(c)** 0.60, 0.79 and 0.51 **(d)** 0.18, 0.36 and 0.51.

3.3.1.3 Cytokine abundance

The IL-8 concentrations in the jejunum were higher in GP compared to SP (5.67 vs 4.14 pg/ μ g, $p = 0.011$) and declined from 0 h to 24 h (5.69 vs 4.12 pg/ μ g, $p = 0.002$). A main effect of time was present for IL-1 β such that its concentration increased from 0 h to 24 h (0.78 vs 1.20 pg/ μ g, $p = 0.017$). Jejunal TNF- α concentrations tended to be lower in GP compared to SP (0.12 vs 2.72 pg/ μ g, $p = 0.087$) and was greater at 24 h (0.12 vs 2.72 pg/ μ g, $p < 0.001$). No other main or interactive effects were observed in jejunal cytokine abundance (Table 3.3).

The IL-8 concentrations in the ileum tended to be higher in GP compared to SP (4.09 vs 2.71 pg/ μ g, $p = 0.063$) and were higher at 0 h compared to 24 h (4.26 vs 2.54 pg/ μ g, $p = 0.003$). The TNF- α concentrations increased from 0 h to 24 h (0.66 vs 1.12 pg/ μ g, $p = 0.008$). No other main or interactive effects were observed in ileal cytokine abundance (Table 3.3).

Table 3.3 The effects of parity (P; GP vs SP) and time (T; 0 h vs 24 h) on expression of interleukin-8 (IL-8), interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α) in the jejunum and ileum.

Parity (P)	GP		SP		SED	p-value		
Time (T)	0h	24h	0h	24h		P	T	P*T
<i>Jejunum (pg/μg)</i>								
IL-8	6.60	4.74	4.78	3.49	0.654	0.011	0.002	0.54
IL-1β ⁺								
Log _e	-1.53	0.06	-1.02	0.30	0.793	0.68	0.017	0.82
(pg/mg)	(0.22)	(1.06)	(0.36)	(1.35)				
TNF-α	0.12	2.02	0.12	3.41	0.881	0.087	<0.001	0.28
<i>Ileum (pg/μg)</i>								
IL-8	5.35 ^a	2.83 ^b	3.17 ^b	2.25 ^b	0.701	0.063	0.003	0.12
IL-1β	0.74	0.98	0.59	1.27	0.398	0.86	0.13	0.44
TNF-α	0.04	1.12	0.03	3.05	1.326	0.16	0.008	0.34

⁺ Due to sample heterogeneity a Log_e transformation was performed. Values were then back-transformed and presented in parentheses for comparison purposes.

3.3.2 Weaning cohort

3.3.2.1 Mucosal TER and permeability

The TER of the stomach (102 vs 82 Ω .cm², $p = 0.014$) and jejunum (51.0 vs 36.4 Ω .cm², $p = 0.003$) declined from PrW to PoW. The GP tended to have lower TER in the ileum compared to SP (67.9 vs 76.9 Ω .cm², $p = 0.099$). Furthermore, TER of the ileum tended to decline from PrW to PoW (77.8 vs 67.0 Ω .cm², $p = 0.066$). An interaction between parity and time was observed such that colon TER was higher at PrW than PoW in GP, while no effect was observed in SP ($p = 0.002$) (Table 3.4).

Gilt progeny had higher jejunal permeability to FD4 compared to SP (699 vs. 320 x 10⁻⁴ cm·sec⁻¹, $p = 0.05$). The permeability of the ileum towards FD4 was higher in GP compared to SP (414 vs 207 Ω ·cm², $p = 0.022$) with a similar effect observed for T150 (127 vs 65.2 Ω ·cm², $p = 0.032$). No other main or interactive effects were observed (Table 3.4).

Table 3.4 The effects of parity (GP vs SP) and time (PrW vs PoW) on transepithelial resistance (TER, cm. Ω^2).

Parity (P)	GP		SP		SED	<i>p</i> -value		
Time (T)	PrW	PoW	PrW	PoW		P	T	P*T
<i>TER (cm.Ω²)</i>								
Stomach	104	87.0	100	77.0	11.0	0.36	0.014	0.69
Jejunum	47.8	33.1	54.2	39.6	6.59	0.17	0.003	0.99
Ileum	68.8	67.1	86.9	66.9	7.91	0.099	0.066	0.11
Colon	82.7 ^a	57.1 ^b	58.5 ^b	69.2 ^{a,b}	7.52	0.14	0.17	0.002
<i>P_{app} (x 10⁻⁴ cm sec⁻¹)</i>								
Jejunum								
FD4 ⁺								
Log _e	6.50	6.60	5.86	5.67	0.540	0.050	0.90	0.71
(x 10 ⁻⁴ cm·sec ⁻¹)	(665)	(735)	(351)	(290)				
T150 ⁺ (x 10 ⁻⁴ cm·sec ⁻¹)	111	67.1	72.2	72.2	0.476	0.54	0.47	0.47
Ileum								
FD4	379	449	130	284	120.5	0.022	0.20	0.62
T150	105	149	63.7	66.6	38.38	0.032	0.40	0.46

⁺ Due to sample heterogeneity a Log_e-transformation was performed. Values were then back-transformed and presented in parentheses for comparison purposes.

3.3.2.2 Tight junction protein expression

No main or interactive effects were observed on jejunal claudin-2 expression (Figure 3.2a). In fact, no claudin-2 was detected in GP in the PrW group. There were no parity effects on claudin-3 expression ($p = 0.97$) (Figure 3.2b). However, claudin-3 was lower after weaning (3.45 vs 1.35 AU, $p = 0.03$) (Figure 3.2b). No interactive effects were observed in jejunal claudin-3 expression ($p = 0.41$) (Figure 3.2b). Gilt progeny tended to have lower ileal claudin-2 expression compared to SP (2.26 vs 2.94 AU, $p = 0.069$) (Figure 3.2c), while no main effect of time was observed ($p = 0.11$) (Figure 3.2c). An interaction between parity and time was observed such that claudin-2 expression in the ileum was lower in GP than SP before weaning but not after weaning ($p = 0.043$) (Figure 3.2c). No main or interactive effects were observed on ileum claudin-3 expression (Figure 3.2d).

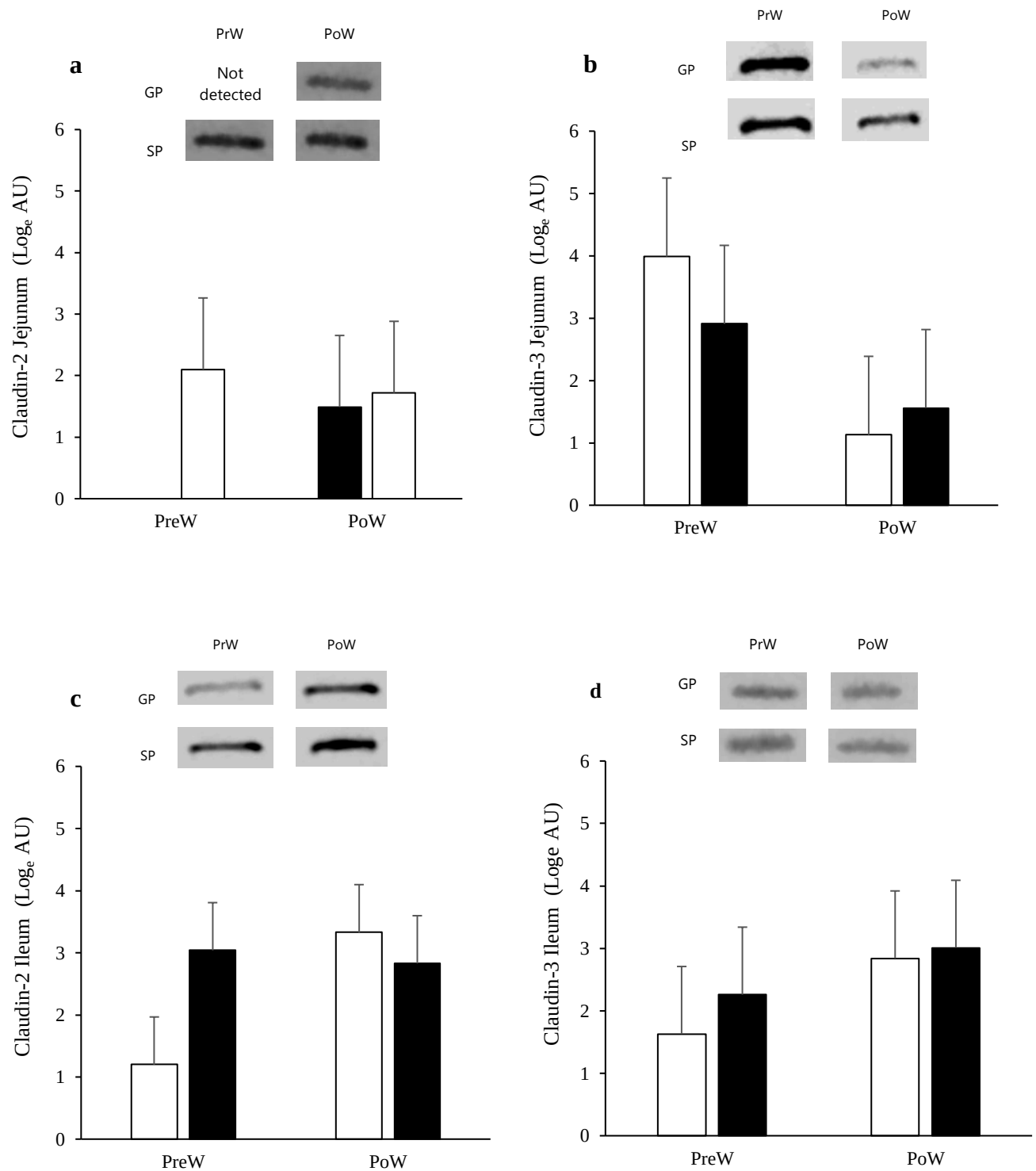


Figure 3.2 The effect of parity (GP vs SP) and time (PrW vs. PoW) on jejunum **(a)** claudin-2 and **(b)** claudin-3 expression and ileum **(c)** claudin-2 and **(d)** claudin-3 expression. Data in figures are presented as log_e-transformed means and standard error of the transformed mean for the Parity (P) × Time (T) interaction. The p – values for the main and interactive effects of P and T were **(a)** 0.18, 0.39 and 0.18 **(b)** 0.97, 0.03 and 0.41 **(c)** 0.069, 0.11 and 0.043 **(d)** 0.40, 0.23 and 0.77.

3.3.2.3 Cytokine abundance

Jejunal TNF- α concentrations were lower in GP than in SP (0.21 vs 0.34, $p = 0.016$) while no other main or interactive effects of parity or time were observed in the jejunum. Furthermore, no main or interactive effects were observed in IL-8, IL1 β and TNF- α concentrations in the ileum (Table 3.5).

Table 3.5 The effects of parity (P; GP vs SP) and time (T; PrW vs PoW) on expression of interleukin-8 (IL-8), interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α) in the jejunum and ileum.

Parity (P)	GP		SP		SED	p-value		
Time (T)	PrW	PoW	PrW	PoW		P	T	P*T
Jejunum (pg/ μ g)								
IL-8	8.97	10.8	8.53	9.54	1.490	0.37	0.18	0.69
IL-1 β	0.27	0.20	0.14	0.12	0.105	0.19	0.77	0.50
TNF- α	0.22	0.20	0.31	0.38	0.078	0.016	0.73	0.42
Ileum (pg/ μ g)								
IL-8	8.82	11.20	10.5	8.52	2.133	0.67	0.85	0.16
IL-1 β	0.48	0.57	0.54	0.33	0.149	0.35	0.52	0.17
TNF- α	0.17	0.15	0.17	0.33	0.117	0.21	0.45	0.31

3.4 Discussion

Gilt progeny are more likely to exhibit lower birth weights and poorer lifetime performance compared to SP (Craig, Collins et al. 2017), but the development of their GI tract remains poorly characterised. The results from the current study confirm that the variation in GI tract growth and development between GP and SP occurs mainly throughout the pre-weaning period. Consequently, GP enter weaning with reduced GI tract barrier function compared to SP. The poorer GIT development exhibited by GP during the pre-weaning period may contribute to their poor growth performance and higher mortality after weaning (Craig, Collins et al. 2017)

3.4.1 Early growth and development of the GI tract in gilt vs. sow progeny

The lower birth weights of GP suggest that these piglets experience a form of restricted growth during gestation. Results from previous studies conducted under the same commercial conditions suggest that this restriction occurs mainly during late gestation, based on the asymmetric growth patterns observed in GP (Craig, Dunshea et al. 2019a). Previously we had observed that the stomach weight in GP was the same as SP (Craig, Dunshea et al. 2019a), but the current study found that GP exhibited lower TER than SP. As the TER was measured on

stripped mucosa, this result may indicate that the mucosal thickness was less in GP than SP. The small intestine tended to be proportionally lighter in new-born GP (Craig, Dunshea et al. 2019a) and by 24 h the SP tended to have elevated jejunal claudin-3 expression than GP. Expression of claudin-3 is important for the integrity of the mucosal barrier, serving to reduce paracellular permeability (Milatz, Krug et al. 2010) and as the GIT undergoes substantial remodelling early in life following the consumption of colostrum (Simmen, Cera et al. 1990, Burrin, Shulman et al. 1992) the reduced expression of claudin-3 could be indicative of reduced mucosal development in the GP jejunum. It may be that the GP GI tract is less responsive to growth stimulus, but perhaps it reflects lesser colostrum intake. This could be due to lower colostrum production by the gilt, or potentially poorer maternal skills leading to less suckling opportunities for the progeny. Recently we characterised that the composition of milk is similar between sows and gilts so it is unlikely that the composition of the milk is a contributing factor (Craig, Dunshea et al. 2019b).

During the first 24 hours of life, the GI tract experiences significant increases in growth, development and function, all of which are stimulated by the composition and amount of colostrum ingested. In agreement, increased small intestinal development was also observed in both GP and SP in the current study where TER in the jejunum and ileum was higher at 24 hours postnatally, confirming that colostrum consumption is a key trigger for intestinal development. Furthermore, an increase in small intestine weight and length was also observed in piglets of this study in response to colostrum consumption (Craig, Dunshea et al. 2019a).

Previous findings have shown that the early neonatal period is marked by increased intestinal permeability, measured by the movement of macromolecules such as fluorescent dextrans, BSA and IgG, from one side of the epithelium to the other (Weström, Svendsen et al. 1984, Jensen, Elnif et al. 2001, Varadarajan, Stephenson et al. 2019). Furthermore, colostrum consumption has been reported to stimulate a reduction in permeability approximately 24-36 hours postnatally in a process referred to as “gut closure” (Speer, Brown et al. 1959, Lecce and Morgan 1962, Westrom, Svendsen et al. 1984). This is considered a key process in the development of the intestine and further supports the important role of colostrum in GI tract development (Lecce and Morgan 1962, Burton and Smith 1977). In this study, jejunal permeability was assessed with 4 and 150 kDa labelled dextrans. This dual marker approach assessed the permeability of different sized macromolecules to assess the formation of gut closure, with T150 selected due to its similar size to IgG. The results showed that permeability

to both markers increased from 0 to 24 h, indicating that permeability increased rather than decreased as expected with gut closure. Westrom, Svendsen et al. (1984) found that intestinal permeability to FITC-dextran started to decline from 18 to 36 hours after birth while Lecce and Morgan (1962) reported that nursing piglets lost their capacity to absorb polyvinylpyrrolidone (PVP) approximately 24 to 36 hours. Furthermore, Speer, Brown et al. (1957) first noted that intestinal closure to IgG takes place as early as 24 h of age in suckling piglets. Although intestinal absorption of macromolecules of this nature are generally used to assess intestinal permeability, this does not necessarily mean that the epithelium allows for transfer of IgG due to the different transport mechanisms used for specific molecules (Sangild 2003). However, these previous studies (Westrom, Svendsen et al. 1984, Jensen, Elnif et al. 2001) show that intestinal permeability to IgG and these macromolecules do exhibit degrees of similarity in the timing of their movement across the epithelium in this neonatal window.

Using piglets from the same study, Craig, Dunshea et al. (2019a) found higher serum IgG at 24 hours in SP compared to GP despite the lack of any differences observed in intestinal macromolecular permeability measurement in the current study. As sow and gilt colostrum have similar IgG concentrations and nutrient profiles at this timepoint (Craig, Dunshea et al. 2019b) this suggests that SP ingest more colostrum than GP in this neonatal period, which has also been suggested by Ferrari, Sbardella et al. (2014). A reduction in IgG intake can result in reduced mortality and immunocompetence since piglets rely on IgG as their primary immune defence until developing their own antibodies. This may explain the higher mortalities observed in GP compared to SP (Holyoake 2006, Edwards, Campbell et al. 2013, Craig, Dunshea et al. 2019c).

3.4.2 Cytokine abundance in the jejunum and ileum in the birth cohort

Colostrum is a rich source of cytokines which help elicit an immune response during the neonatal period as the GIT is typically a low inflammatory environment (Adkins, Leclerc et al. 2004, Pluske 2016). In the current study, higher concentrations of jejunal IL-1 β and TNF- α and ileal TNF- α were observed at 24 h postnatally. The higher concentration of these cytokines in these tissues can most likely be attributed to the transfer of these cytokines via colostrum (Nguyen, Yuan et al. 2007) and in small part in response to tolerogenic induction (Pohl, Medland et al. 2015). In this light, TNF- α may serve as a marker of increased colostrum consumption explaining why higher concentrations of this cytokine were found in the jejunum

of SP. Unlike the current study, Nguyen, Yuan et al. (2007) reported undetectable concentrations of serum TNF- α after consuming colostrum. The difference between the findings could be attributed to the short half-life of this cytokine effecting its presence in serum compared to direct absorption by the intestine. Furthermore, the low inflammatory environment of the neonate may have downregulated the presence of TNF- α in piglet serum. To this authors' knowledge, the concentrations of IL-1 β in sow colostrum has yet to be elucidated but results from this study indicate a similar transfer of IL-1 β . Therefore, further work in characterising lactogenic cytokines in sow and gilt colostrum is needed.

Gilt progeny in this study had higher concentrations of IL-8 in the jejunum and ileum compared to SP. Interleukin-8 is a pro-inflammatory cytokine due to its neutrophil chemoattractant property, responsible for recruiting neutrophils to sites of injury or infection. However, IL-8 exerts other effects in the foetal GIT such as stimulating maturation without the influx of neutrophils (Maheshwari, Lu et al. 2002). As GP exhibit less small intestinal development compared to SP, the specific role of IL-8 in this instance may not exclusively involve stimulating maturation of the SI of GP. Higher IL-8 concentrations in foetal blood have been associated with intra-uterine growth restriction (IUGR) in humans (Spurlock 1997). Furthermore, the exposure of intestinal tissues to excessive concentrations of pro-inflammatory cytokines can predispose the foetus to abnormal immune development and maladaptive immune responses later in life (Pohl, Medland et al. 2015), a finding that has been reported in IUGR piglets (Dong, Zhong et al. 2014). Piglets that display IUGR have also been reported to suffer from reduced immunodeficiency, reduced barrier integrity and increased vulnerability to infectious diseases later in life (Hu, Peng et al. 2017). While GP are not IUGR piglets, as they are overrepresented in underweight cohorts, they share some characteristics of IUGR (Craig, Dunshea et al. 2019b), allowing inferences to be made between the two. Using the comparison with IUGR piglets the high intestinal IL-8 concentrations in GP may represent early signs of abnormal immune development, but this observation will require validation.

3.4.3 The effect of weaning on GI tract function in GP vs. SP

Weaning represents a significant challenge to the piglet under commercial conditions (Pluske 2016). This challenge arises due to the various stressors the piglet encounters during this period such as an abrupt separation from the dam, psychosocial stressors from mixing and moving, sudden changes in diet, and changes in temperature (Wijtten, Meulen et al. 2011).

These stressors can activate the hypothalamic-pituitary-adrenal (HPA) axis causing an elevation of stress hormones such as corticotropin factor (CRF) and cortisol which can trigger breakdown of the intestinal mucosal barrier (Moeser, Ryan et al. 2007) and impair immune functions (Gimsa, Tuchscherer et al. 2018). In line with these studies, the current study demonstrated that compartments of the GIT (stomach, jejunum and ileum) also exhibited breakdown of intestinal barrier integrity reflected in the lower TER after weaning. Furthermore, the reduced barrier function after weaning is also reflected in the overall reduction claudin-3 protein expression in the jejunum in the current study.

The TER did not differ between GP and SP after weaning in the proximal components of the GI tract (stomach and jejunum). However, the effects of weaning were particularly evident in the distal parts of the GIT in GP (ileum and colon), with GP exhibiting lower ileal TER overall at weaning and lower colon TER post-wean than pre-wean. Interestingly, while claudin-2 expression was higher in SP overall, claudin-2 expression in GP was higher than SP in response to weaning while there was no change in SP from the PrW to PoW timepoints. Claudin-2 is a tight junction protein that facilitates ion secretion and mediates water transport (Amasheh, Meiri et al. 2002), which are critical for proper functioning of the GI tract. However, an upregulation of claudin-2 expression specifically in response to stress or low-grade inflammation can result in reduced epithelial integrity (Ishimoto, Oshima et al. 2017). Liu, Yang et al. (2013) reported that CRF can cause increased expression of claudin-2 in human cell lines which reduced TER in epithelial monolayers. Low-grade inflammation and increased concentrations of CRF reflect the conditions of weaning and, therefore, may explain the increased claudin-2 expression seen in this study in GP in response to weaning. While higher claudin-2 expression was seen in SP overall, this could be attributed to overall greater intestinal function development in SP. Furthermore, the higher claudin-2 expression of SP did not seem to negatively affect intestinal barrier function of SP to the same extent as GP at weaning (i.e. poor intestinal function and growth after weaning). However, since CRF was not measured in this study and claudin-2 expression in response to CRF has mainly been analysed in cultured epithelial monolayers, this is speculative, and more research is required in this area.

Gilt progeny also exhibited highly permeable ileal tissue to both FD4 and T150 in addition to their low ileal TER at weaning. Furthermore, their lower colonic TER in response to weaning indicates a higher susceptibility towards leakiness in the colon in response to weaning, which has previously been reported (Moeser, Klok et al. 2007). This reduction in

barrier integrity can contribute to a higher susceptibility to luminal antigens entering the circulation and trigger an influx of inflammatory cytokines, while also promoting a higher incidence of post-weaning diarrhoea (Johnson 1997, Nabuurs 1998, Moeser and Blikslager 2007c, Pluske, Turpin et al. 2018). Furthermore, this reduction in barrier integrity most likely contributes to the reduced growth observed in GP after weaning (Craig, Collins et al. 2017, Wjesiriwardana, Pluske et al. 2020).

Results from this study demonstrated that while there are early signs of variation in GI tract functional development between GP and SP, this variation increases during the pre-weaning period with GP entering weaning with an underdeveloped GI tract barrier function. Therefore, results from this study suggest that milk consumption is a limiting factor of GP GIT development during the pre-weaning period and due to this, they enter the weaning period with less intestinal barrier integrity compared to those of SP. It is not surprising that gilts would produce less milk due to their low feed intake (Pedersen, van Vliet et al. 2019), smaller litters (Gonçalves, Gourley et al. 2016) and smaller reserves to support milk synthesis (Whittemore 1996). Low milk yields can result in suboptimal growth and development which have long-term impacts. For example, Lessard, Blais et al. (2018) reported that slow growing piglets have reduced immunocompetence. Cytokine abundance analysis in the current study also suggests this lower jejunal concentrations of TNF- α were observed in GP compared to SP at weaning. Furthermore, colostrum and milk has been shown to contain bioactive molecules that promote intestinal maturation, development of immune system and modulation of intestinal microbiota with faster growing pigs exhibiting more beneficial bacteria compared to slow growing pigs (Morissette, Talbot et al. 2018). Reducing variation between GP and SP during the pre-weaning period can support GP GI tract development prior to weaning and aid in post-weaning health and growth rates. Therefore, management strategies that focus on supporting higher milk yields in gilts may be more useful in improving GIT growth and development and GP performance.

3.5 Conclusion

In conclusion, the findings from this study help to clarify the timeframes of GI tract development in both GP and SP in the pre-weaning period. Gilt progeny exhibited early signs of reduced GI tract development during the first 24 hours of life. However, these differences are particularly evident at weaning, indicating that the disparity between GP and SP GI tract development increases throughout the pre-weaning period. This results in GP entering the

weaning period with poorer GI tract integrity compared to SP. Furthermore, the effects of weaning on the GI tract are more prominent in GP compared to SP due to their poorer development during this period. This most likely contributes to their poor growth and higher mortality rates after weaning. Interventions that target late gestation and lactation may help support the GI tract development during the pre-weaning period and ultimately improve long-term performance of GP.

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Chapter 4 The use of I-FABP as an accurate biomarker of intestinal damage

4.1 Abstract

There is a lack of clinical biomarkers to assess the health and integrity of the GIT under production settings. Fatty acid binding proteins (FABP) have been suggested as a biomarker for gut health. FABPs are ubiquitous proteins involved in fatty acid metabolism and due to their small molecular weight their appearance in plasma in clinical states FABP's have been viewed as a potential biomarker of tissue injury. There is an intestinal specific isoform of FABP (I-FABP) which may prove useful in diagnosing disruption to the small intestine. Therefore, the aim of the study was to quantify the changes in plasma I-FABP in pigs after weaning or during heat stress (HS), which are known to cause disruption of the intestinal mucosa. Three commercially available I-FABP ELISA kits were used, FineTest porcine I-FABP ELISA kit (FineTest kit), R&D Systems human FABP2/I-FABP ELISA kit (R&D kit) and Hycult human I-FABP ELISA kit (Hycult kit). The results showed that I-FABP from weanling pigs was low ($n = 62$), with 45% of the samples not below the lower detection limit of the assay (17.8 pg/mL), with the average and median concentrations being 117 and 20.1 pg/mL. For the HS pigs' pregnant gilts were measured ($n=15$), which were approximately 18 m.o. and averaged 1543 pg/mL I-FABP. No effect of HS was observed on ileal TER or plasma I-FABP. When comparing kits from different manufacturers the R&D and Hycult kits, which were raised against human I-FABP epitopes had superior spike recovery to the FineTest porcine specific I-FABP (92.5, 91 and <32% respectively). Together these experiments showed that the concentrations of I-FABP in piglets at weaning is >10 fold lower than later in life and close to the limit of quantitation of commercially available I-FABP ELISA kits. Therefore, to properly evaluate the utility of I-FABP in weanling pigs more sensitive methodologies are required.

4.2 Introduction

Fatty acid binding proteins are low molecular weight proteins that play important roles in the transportation and metabolism of fatty acids (Funaoka, Kanda et al. 2010). There are a number of fatty acid binding protein (FABP) isoforms expressed, with most tissues expressing more than one isoform. Importantly there is an FABP isoform that is specific to the small intestine, and colon (I-FABP). It is expressed from the FABP2 gene, which is not expressed outside of the gastrointestinal tract (GIT) (Pelsers, Namiot et al. 2003). The appearance of

FABP's in plasma following injury have led to investigations about their utility as biomarkers. For example, plasma FABP has been observed to increase ~3 h after acute myocardial infarction and returning to baseline by approximately 24 h (Glatz et al 1997). As I-FABP is specific to the small intestine it may be a useful biomarker to quantify intestinal injury and in studies investigating intestinal ischemia (Kanda, Fujii et al. 1995) and necrotising enterocolitis (Guthmann, Borchers et al. 2002) have observed increased plasma I-FABP concentrations. Therefore, the role of I-FABP as a biomarker in plasma can play an imperative role in the prediction, cause, diagnosis, progression or outcome of treatment (Mayeux 2004) in relation to intestinal injury rather than the use of invasive techniques.

The GIT of pigs are challenged at different times on farm, but remain difficult to diagnose in part due to the lack of clinical biomarkers. Examples include post-weaning, which can be associated with significant disruption to the GIT, as evidenced by mucosal and villous atrophy accompanying a generalised growth check (Pluske, Turpin et al. 2018). Preceding weaning the early neonatal period is marked with substantial remodelling as the GIT adapts to milk feeding. Furthermore, the neonatal intestine is initially highly permeable to allow the paracellular absorption of immunoglobulins, but then experiences “gut closure” as mucosal barrier function is established (Lecce and Morgan 1962, Rooke and Bland 2002). The effects of climate change also present a challenge to the GIT mucosa as heat stress (HS) in pigs redistributes blood flow away from the GIT, resulting in oxidative stress and elevated intestinal permeability (Pearce, Mani et al. 2013, Liu, Cottrell et al. 2016). Validation of biomarkers such as I-FABP could streamline the identification of compromised gut health and be used as a tool to improve productivity. Therefore, the objective of this study was to quantify I-FABP in newborn and weanling piglets and HS sows.

4.2 Methods

4.2.1 Ethics statement

This experiment utilised previously harvested plasma samples which were collected under the following approvals:

1. Study 1 and 2 sample collection was approved by the Rivalea (Australia) Animal Care and Ethics Committee (protocol 16P014) where samples were collected from a commercial piggery in New South Wales, Australia (Rivalea Australia, Pty Ltd., Corowa, NSW, Australia)

2. Study 3 sample collection was approved by the University of Melbourne Faculty of Veterinary and Agricultural Sciences Animal Ethics Committee (protocol number 1513462.1) and was collected from an animal facility on campus.

4.2.2 Study 1 — I-FABP ELISA kits and validation

Three different commercially acquired kits were validated for analysis using pig plasma samples. All validations were performed by a single operator and where feasible, the same samples were used the validation of each kit. Samples were all assayed in duplicate.

4.2.2.1 R&D Systems human FABP2/I-FABP (R&D kit)

The assay was a quantitative sandwich ELISA with antibodies raised to human FABP2/I-FABP (R&D Systems Cat. No. DY3078, R&D Systems, MN, USA). The assay was performed as per manufacturers recommendations. Briefly 50 mL of plasma diluted 1:5 in calibrator diluent or standard (0, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 pg/mL) were pipetted into provided microtitre plate wells in duplicate and incubated for 2 h at room temperature. Where low plasma I-FABP concentrations were encountered a 1:2 dilution or neat plasma was also assayed in a subset of samples. The sample was then aspirated and the well washed 4 times with wash buffer and incubated with 200 mL I-FABP conjugate for 2 h at room temperature. The wells were then aspirated and washed as previously described, then incubated with 200 mL of substrate solution for 30 minutes. Fifty mL of stop solution was then added and A_{450} quantified. The concentrations of samples were interpolated from a standard curve of a four-parameter logistic (4-PL) curve-fit generated using Graphpad Prism 8 (Version 8.4.3). This is an “s” shaped curve often used to analyse ELISAs in order to measure concentrations outside a linear range. According to the manufacturer the assay is compatible with both EDTA and heparin anticoagulants.

4.2.2.2 Hycult human I-FABP (Hycult kit)

The assay was performed as per the manufacturer’s recommendations (Cat. No. HK406-02 Hycult, The Netherlands). Briefly the plasma was diluted 1:2 in the supplied sample dilution buffer and 100 mL of either diluted sample or standard (0, 46.9, 93.8, 188, 375, 750, 1500, 3000 pg/mL) were pipetted into provided microtitre plate wells and incubated for 1 h at room temperature. However, due to low endogenous I-FABP in plasma samples observed for this assay, neat (undiluted) plasma samples were also assayed. The sample was then aspirated and washed 4 times with wash buffer and incubated with 100 mL biotinylated tracer antibody

for 1 h at room temperature. The wells were then aspirated and washed as previously described, then incubated with 100 mL of streptavidin peroxidase conjugate for 60 minutes. The wells were then aspirated and washed as previously described with 100 mL of TMB solution added and incubated for 30 min then 100 mL of stop solution added and the A_{450} was then quantified. Graphpad Prism 8 (Version 8.4.3) was used to generate a standard curve where mean absorbance was plotted on the Y-axis and corresponding concentrations on a logarithmic X-axis and sample concentrations were interpolated from the curve. According to the manufacturer the assay best used with heparin anticoagulants. Three different samples were assayed over two plates.

4.2.2.3 *FineTest porcine I-FABP (FineTest kit)*

The assay was a quantitative sandwich ELISA with antibodies raised to porcine FABP2/I-FABP (Cat. No. EP0072 FineTest, Wuhan China) and was performed as per the manufacturer's recommendations. Briefly the plasma was pre-processed with the acid alcohol extracting solutions included in the kit according to the manufacturer's instructions which resulted in a 1:12 dilution. The sample was diluted 1:2 in the supplied sample dilution buffer and 100 μ L of either diluted sample or standard (0, 78.1, 156, 312, 625, 1250, 2500 and 5000 pg/mL) were pipetted into provided microtitre plate wells in duplicate then incubated for 90 min at 37°C. Samples were assayed at both 1:12 and 1:24 dilutions. The sample was then aspirated and washed 3 times with wash buffer and incubated with 100 mL biotin-labelled antibody and incubated for 1 h at 37°C. The wells were then aspirated and washed as previously described, then incubated with 100 mL of horseradish peroxidase-streptavidin conjugate for 37 min at 37°C. The wells were then aspirated and washed as previously described, 90 mL of TMB solution added and incubated in the dark at 37°C for 30 min then 50 mL of stop solution added. The A_{450} was then quantified and concentration of samples calculated vs. standards using four parameters logistic regression (4PL). According to the manufacturer the assay is compatible with both EDTA and heparin anticoagulants. The same two samples were used for each validation.

4.2.2.4 Partial validation of ELISA kits

I-FABP ELISAs were validated using the intra-assay variation, precision, recovery, parallelism and minimum detectable dose as outlined by Andreasson, Perret-Liaudet et al. (2015) and Steel, Athorn et al. (2018). For the intra-assay variation, a control sample was assayed in duplicate with each run (4 separate wells) and the covariation percentage (CV %) calculated of the two duplicates for each plate. For precision, the CV% was calculated between assays (inter-assay variation). The acceptance criteria was < 15% for both parameters. For recovery, plasma was spiked with a known concentration of I-FABP standard and recovery calculated against the expected concentration of the neat (non-spike) sample and concentration of I-FABP added with the acceptance criteria being between 80-120%. Minimum detectable dose was assessed by assaying 10 blank samples and calculating the value two standard deviations away from the mean of the blanks.

4.2.3 Study 2 — The effect of parity and weaning on I-FABP concentrations

Stored plasma samples collected from Chapter 3 were used for analysis of plasma analysis of I-FABP concentrations. Plasma was collected from gilt- and sow progeny (GP and SP respectively) pre- and post-weaning (PreW and PoW respectively). The number of samples analysed for each treatment group was GP PreW (8), GP PoW (10), SP PreW (9) and SP PoW (7) and the R&D Human FABP2/I-FABP Quantikine ELISA (R&D Human I-FABP) kit was used for the analysis.

4.2.4 Study 3 – The effect of heat stress on I-FABP and intestinal integrity

4.2.4.1 Gilts and sample collection

Twenty pregnant gilts were acclimatised to the animal facility for 7 days, then exposed to cyclic heat stress (HS) conditions (33°C 9am-5pm/ 28°C rest of the day) for 18 days. Plasma samples were collected via the auricular vein and jugular vein with a 21G needle and syringe while the jugular blood sample was collected using a 21G needle and 10mL lithium and heparin Vacutainer (BD Vacutainer North Ryde, NSW, Australia) and inverted 4 times to ensure anticoagulant was distributed throughout the blood. Tubes were then placed on ice until centrifugation at 4 °C for 10 min. Plasma was collected and stored at - 20 °C until analysis. Intestinal tissue samples were collected and either snap frozen in liquid nitrogen and stored at -80°C for further analysis or placed in chilled phosphate buffered saline (PBS) for quantification of intestinal barrier function.

4.2.4.2 Intestinal barrier function

Measurements for intestinal barrier function have been previously described in Chapter 3. Intestinal permeability was quantified by the ileal apparent permeability coefficient (P_{app}) to 4 kDa fluorescein isothiocyanate (FITC, Cat No. 78331, Sigma, St Louis, MO, USA).

4.2.4.3 Western blotting

Western blotting was performed on plasma and ileum homogenates to determine if I-FABP was present in these samples. Snap frozen whole ileum tissues were homogenised with Radioimmunoprecipitation (RIPA) lysis buffer with protease and phosphatase inhibitors (Cat No. 87786 and 78420 respectively, Thermo Scientific, USA). Samples were homogenised with a bead beater (Bead beater 8, Biospec Products, Bartlesville OK) for 30 seconds and centrifuged at 15,000 g for 10 minutes at 4°C. Supernatant was collected and stored at 20°C until analysis. Bicinchoninic acid (BCA) assay (Thermo Scientific, USA), was used to standardise protein supernatants and plasma as well as a recombinant human I-FABP protein (Abcam, USA, Catalogue No. ab84197) used as a positive control. Supernatant and plasma were diluted to 4 $\mu\text{g/mL}$ while the positive control was diluted to 0.2 $\mu\text{g/mL}$. Standardized samples and positive control were separated by electrophoresis with Tricine Protein Gels (10-20%, Catalogue No. EC66255BOX, Thermo Scientific, USA). The gel was then removed and soaked in sterilised water for 5 minutes prior to transfer. Transfer was performed using the iBlot 2 Dry Blotting System (Catalogue No. IB21001, Thermo Scientific, USA) onto 0.2 μm nitrocellulose membrane (catalogue No. IB23001, Thermo Scientific, USA) for 7 minutes at 20 volts. Membranes were then blocked for 2 hours in 5 % non-fat dry milk in TBST (1 x Tris-buffered saline, 0.1% Tween20). Membranes were then incubated overnight with 1:500 I-FABP primary antibody (goat polyclonal, Catalogue No. ab60272, Abcam, USA). Membranes were then washed three times in TBST and incubated with 1:1000 anti-goat secondary antibody for one hour. The membrane was then washed as previously described and bands visualised using infrared fluorescence with the excitation at 800 nm (Odyssey, LiCor, USA).

4.2.7 Statistical analysis

Means for Study 1 were compared using a Mann-Whitney rank sum test using Sigmaplot (v14, Systat Software, San Jose CA). All analysis was performed using Minitab (v19). For study 1, plasma I-FABP concentrations were determined using a general linear model with parity (gilt vs. sow) and time (pre-weaning vs post-weaning) as main and

interactive effects. A Spearman's rank correlation test was used to determine the correlation between plasma I-FABP and ileal P_{app} . For study 2, plasma I-FABP concentrations and ileal TER were analysed using a one-way ANOVA with temperature (TN vs. HS) designated as the main effect. A Spearman's test correlation test was used to determine the correlation between intestinal integrity measurements and plasma I-FABP concentrations. A value of $p < 0.01$ was used to indicate statistical significance and a value of $p < 0.10$ was considered a statistical trend.

4.3 Results

4.3.1 Study 1— Partial validation of I-FABP ELISAs

4.3.1.1 Precision (%) and inter-assay CV (%)

All kits met the acceptability criteria of <15% Precision for the R&D kit yielded values of 5 and 3% for validations 1 and 2 respectively while the inter-assay CV was 7 (%) (Table 4.1). The HyCult kit yielded precision values of 5 and 4% for validations 1 and 2 respectively with an inter-assay CV of 6%. The FineTest kit yielded precision values of 7, 7 and 3% for validations 1, 2 and 3 respectively with an inter-assay CV of 5%.

Table 4.1. The precision (%) and inter-assay CV (%) of the R&D Human FABP2/I-FABP Quantikine ELISA kit (R&D systems), the Hycult Human IFABP ELISA kit (Hycult) and FineTest Porcine IFABP/FABP2 kit (FineTest)

Kit	Replicate	Precision (%)	Inter-assay CV (%)
R&D Systems	1	5	8
	2	3	
Hycult	1	5	6
	2	4	
FineTest	1	7	5
	2	7	

4.3.1.2 Recovery

The R&D kit yielded recovery values above the acceptable range for samples A and B when assayed neat (200 and 128%) (Table 4.2). Sample C was assayed using a 1:2 dilution and yielded an acceptable recovery value (117 %) (Table 4.2). However, when sample D was assayed using a 1:2 dilution, a recovery value above the acceptable range was observed (160 %) (Table 4.2). When samples A and B were assayed at a 1:5 dilution, they both yielded

acceptable recovery values (84 and 101 % respectively) (Table 4.2). The HyCult kit yielded recovery values below the acceptable range for neat plasma for samples 1 and 2 (70 and 70% respectively). However, when samples were run at a 1:2 dilution, both samples 1 and 2 yielded acceptable recovery values of 92 and 90% respectively. The FineTest kit yielded recovery values below the acceptable range for the recommended 1:12 dilution (32 and 29% respectively).

Table 4.2 Spike-recovery percentages for the R&D Human FABP2/I-FABP Quantikine ELISA kit (R&D systems), the Hycult Human IFABP ELISA kit (Hycult) and FineTest Porcine IFABP/FABP2 kit (FineTest) when samples were assayed at various dilutions.

Kit	Dilution	Nonspiked (pg/mL)	Spiked (pg/mL)	Spike Conc (pg/mL)	Recovery (%)
R&D	Neat	27.1	427	200	200
	Neat	272	1553	1000	128
	1:2	264	498	200	117
	1:2	36.4	356	200	160
	1:5	0	168	200	84
	1:5	117	1124	1000	101
HyCult	Neat	23.4	66.0	61.2	70
	Neat	21.4	63.9	61.2	70
	1:2	10.8	67.3	61.2	92
	1:2	13.9	68.9	61.2	90
FineTest	1:12	119	281	500	32
	1:12	168	312	500	29

4.3.1.3 Minimum detectable dose

The minimum detectable dose for the R&D kit was 17.8 pg/mL, the Hycult kit has a minimum detectable dose of 15.5 pg/mL and the FineTest kit has a minimum detectable dose of 36.7 pg/mL (Table 4.3).

Table 4.3. The minimum detectable dose (pg/mL) of the validation of 3 commercial kits: R&D Systems Human FABP2/I-FABP Quantikine ELISA kit (R&D Systems), HyCult Human I-FABP ELISA kit (Hycult) and FineTest porcine FABP2/I-FABP ELISA kit (FineTest).

Kit	Minimum detectable dose (pg/mL)
R&D Systems	17.8
Hycult	15.5
FineTest	36.7

4.3.2 Study 2 — Gilt progeny vs. sow progeny at weaning

A total of 62 samples were analysed, with a range between 0 and 929 pg/mL I-FABP. The average and median were 117 and 20.1 pg/mL I-FABP respectively and 28 of the samples (45%) were below the minimum detectable dose of 17.8 pg/mL. When means were compared between gilt progeny vs sow progeny and pre-weaning and post-weaning, no differences were observed between groups (Figure 4.1a). Furthermore, no correlation between plasma and I-FABP and ileal permeability was observed ($\rho = -0.096$, $p = 0.60$) (Figure 4.1b)

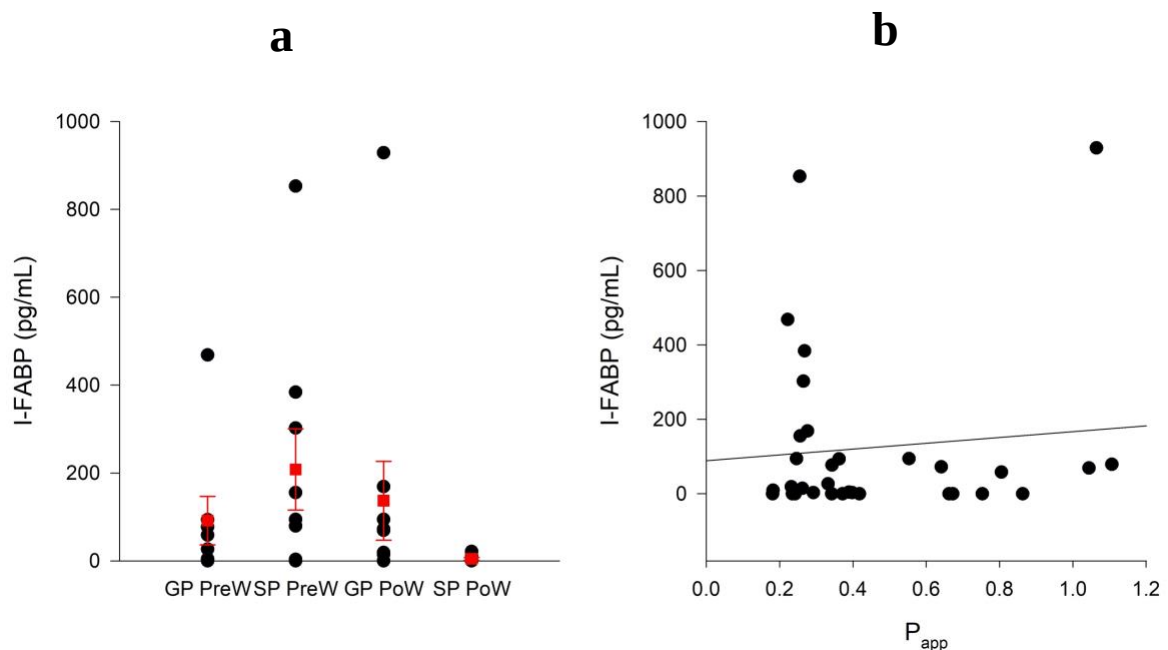


Figure 4.1 I-FABP concentrations from gilt progeny (GP) and sow progeny (SP) pre- and post-weaning (PreW and PoW respectively) from R&D Human I-FABP (a) Vertical scatter plot of treatment groups with individual results presented in black circles and group mean in red squares (■) $\pm$ standard error of the mean (b) Scatterplot of I-FABP concentrations vs ileal apparent permeability (P_{app}) to 4 kDa FITC dextran.

4.3.3 Study 3 — I-FABP analysis and intestinal barrier function in HS gilts

A total of 11 samples were analysed using the FineTest kit with a measurable range between 1224 and 1863 pg/mL I-FABP, with an average of 1543 pg/mL. No correlation between TER and I-FABP was observed ($\rho = -0.27$, $p = 0.75$, Figure 4.2).

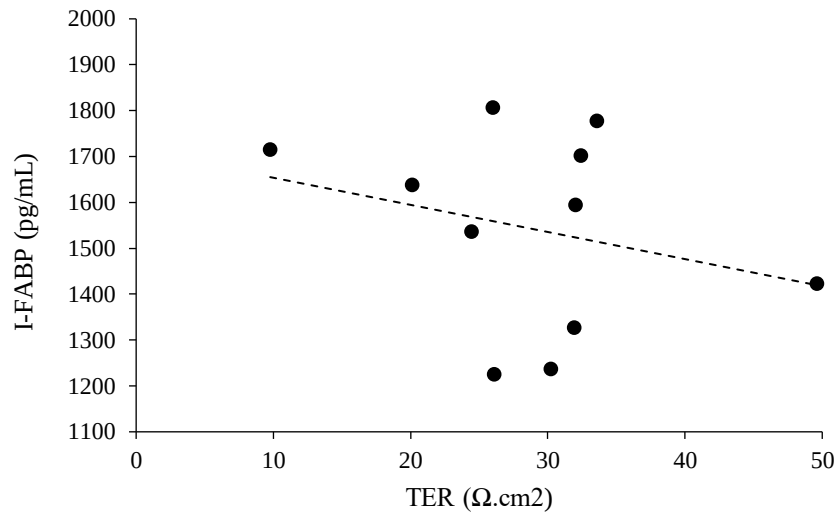


Figure 4.2. Scatterplot of ileal TER vs plasma I-FABP (FineTest kit) for pregnant gilts exposed to 28 days of elevated temperatures (30°C).

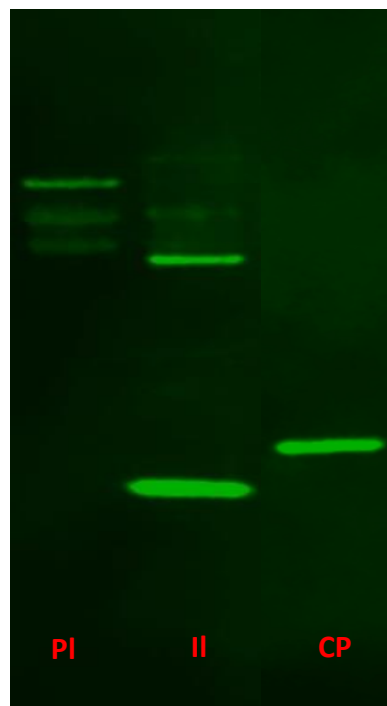


Figure 4.3. Western blot of plasma (PI), ileum homogenate (II) and I-FABP control protein (CP). The PI sample contained 1770 pg/mL I-FABP as determined by the FineTest porcine FABP2/I-FABP ELISA kit assay.

4.4 Discussion

The main findings from this study were that plasma I-FABP concentrations in newborn and weanling pigs overall are low compared to older pigs (sows). Nearly half of the samples analysed in newborn and weanling pigs was not detectable. To be properly validated as a biomarker more sensitive methodologies will be required, but also it will be important to verify how I-FABP expression changes with age. From the results of this experiment, it is quite likely that I-FABP is expressed at low levels at least until weaning and therefore will not be suitable for use as a biomarker of gut health in this age cohort.

Other studies have shown that I-FABP expression increases with gestational age between premature and term lambs and humans (Reisinger, Elst et al. 2014). Although the I-FABP concentrations in lambs and humans was substantially higher than in pigs, there may be a similar pattern in pigs where I-FABP expression is very low in neonatal pigs. There have been investigations into circulating I-FABP at weaning and they have either shown low concentrations between 2-8 ng/mL (Berkeveld, Langendijk et al. 2008) or at 4-7 weeks of age were ~300-800 pg/mL (Van der Meulen, Koopmans et al. 2010). Collectively the results display a large natural variation in I-FABP in neonatal pigs and at weaning. Studies in older pigs are limited, but Niewold, Meinen et al. (2004) quantified I-FABP concentrations in 20-25 kg pigs (approximately 12 wk. age) ranged between 100 and 500 pg/mL.

Due to the low concentrations in the neonatal and weaning piglets we evaluated assays of neat plasma or lesser dilutions, which appeared to result in matrix suppression and low sample recovery. The assay utilised in studies by Niewold, Meinen et al. (2004), Berkeveld, Langendijk et al. (2007), Van der Meulen, Koopmans et al. (2010) and Reisinger, Elst et al. 2014, has near identical precision, spike recovery and minimum detectable dose to the assay used in Study 1 and are both human specific I-FABP, but clearly cross react with pig I-FABP. For Study 2 we evaluated a porcine specific I-FABP ELISA and those results showed a far greater I-FABP concentration in sow plasma. However, spike recovery experiments for the FineTest kit used study 2 yielded recovery values of approximately 30%. Therefore, results obtained from this particular kit should not be considered due to its inaccuracy. In order to further investigate this, the relative abundance of I-FABP in both ileal tissue and plasma were compared by Western blot. While an abundance of I-FABP was found in ileal tissue, this same finding could not be confirmed in plasma. Although, this was a different anti-body than used

in the ELISA assays, it may be possible that non-specific binding could lead to erroneous results when assaying I-FABP by ELISA. Therefore, western blotting may be considered a more accurate measurement of intestinal damage rather than plasma if I-FABP was to be used as a marker. However, this would obviously be a highly invasive technique and inaccessible as an on-site method to assess intestinal damage.

In Study 1 due to the low concentrations of I-FABP we evaluated assaying neat or a lesser dilution of plasma than specified by the manufacturer and found that this resulted in interference and an elevated spike-recovery, indicating reactivity with the matrix. Therefore, we recommend that future research into I-FABP investigate either mass spectrometry or plasma protein removal methodologies to minimise plasma matrix effects. Some investigations have quantified I-FABP in combination with faecal calprotectin (Tian et al., 2016) and a ratiometric approach may reduce the variability between experiments. Furthermore, investigations into the levels of I-FABP tissue expression in developing pigs would also be highly informative to underpin periods of production where I-FABP may serve as a useful biomarker.

4.5 Conclusion

The main findings from this study are that the I-FABP concentrations in pig plasma seem to be either too low or variable to quantify accurately by ELISA. While plasma I-FABP may be an acceptable biomarker for intestinal ischemia, it does not seem to accurately reflect the damage experienced by the pig intestine at weaning. Furthermore, available commercial kits may not be sensitive enough to reliably quantify plasma I-FABP in pigs.

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Chapter 5 Dietary inclusion of 1,3-Butanediol improves weight at 24-hours and pre-weaning survival in gilt and sow progeny

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5.1 Simple Summary

Gilt progeny are born and weaned lighter and have poorer life-time performance than sow progeny. Low birth weights and pre-weaning mortality are highly associated and are often a result of reduced milk consumption and vigour. Glycogen stores are deposited *in utero* and are relied on heavily within the first hours of life. Because of rapid depletion of these stores, piglets must consume enough milk immediately *post-partum* for survival. Similar to glucose, ketone bodies have the ability to readily pass the placenta for the piglet to use in the neonatal period. Supplementing late gestation diets with ketogenic substances, an alternative source of

energy can potentially be used by the piglet, reducing the rapid depletion of glycogen stores. This study supplemented late gestation diets of both gilts and sows with the ketogenic substance 1,3-Butanediol and found that 24-hour individual weights and total litter weights were increased in both gilt and sow progeny. While Butanediol can potentially increase birthweight and growth performance in the post-parturition period, a strong parity effect was still evident throughout the study with sow progeny outperforming gilt progeny.

5.2 Abstract

1,3- Butanediol (BD) is a ketogenic substance that can improve piglet growth and survival and potentially increase performance in gilt progeny when provided as a dietary supplement during late gestation. Gilts (n=77, parity 1) and sows (n=74, parities 2 and 3) were fed either a standard commercial gestation diet or a diet supplemented with 4% BD from day 90 of gestation until farrowing. Dams fed diets supplemented with BD had higher plasma beta-hydroxybutyrate ($p = 0.01$) and lower non-esterified fatty acid concentrations ($p < 0.001$). The percentage of progeny that were light-for-age (<1.1 kg) at 24 h of age was decreased by BD (18.2 vs 13.5%, $p < 0.006$), particularly in gilts (24.0 vs 18.3%, $p < 0.034$). Twenty-four-hour individual and litter weights tended to be increased by the BD diet ($p = 0.085$ and 0.078 , respectively) although these effects were not maintained to weaning. Pre-weaning mortality was greater in gilt than in sow progeny and was not altered by dietary BD. Feeding BD in late gestation can improve birth weight, but further work is needed to see if these effects are carried through subsequent stages of growth, particularly in gilt progeny.

Keywords: gilt progeny, parity, birthweight, ketones, pre-weaning mortality

5.3 Introduction

Gilt progeny (GP) present a significant burden on pig producers since they exhibit lower birth and weaning weights, a higher risk of disease and mortality and poorer whole-of-life performance compared to sow progeny (SP) (Craig, Collins et al. 2017). Low birth weight (LBW) or light-for-age piglets, those between 0.8 and 1.1kg (Gondret, Lefaucheur et al. 2005), and pre-weaning mortality are strongly linked since smaller piglets are born with lower body energy reserves in the form of glycogen, and show reduced thermoregulatory capacity (Dividich and Noblet 1983). This in turn results in more time taken to reach the udder and consequently, these piglets have reduced colostrum and milk consumption (Noblet, Dourmad

et al. 1997, Herpin, Damon et al. 2002). Furthermore, glycogen stores are rapidly depleted in the first 16 hours of life and therefore, energy supplied from colostrum is imperative to piglet survival (Theil, Cordero et al. 2011).

Neonatal piglets are born devoid of body fat due to an inability of lipids to pass transplacentally from the sow to the foetus. Therefore, piglets rely on glycogen stores accumulated from glucose passed from mother to foetus as a primary source of energy in the neonatal period, prior to colostrum consumption (Mellor and Cockburn 1986, Devillers, Le Dividich et al. 2011). Ketone bodies are also readily able to pass through the placenta from mother to foetus, hence exogenous ketones offer a novel source of energy to the piglet and spares metabolism of glycogen stores deposited in utero in times of fasting or reduced nutrient intake. The neonatal period of life is a critical time for a piglet and can be a major cause of pre-weaning loss (Tuchscherer, Puppe et al. 2000, Quesnel, Farmer et al. 2012). Therefore, formulation of gestation diets that can increase neonatal survival and reduce pre-weaning mortality are imperative for optimizing GP growth performance.

In this study, a late gestation diet supplemented with 1,3-Butanediol (BD), a synthetic carbohydrate which is metabolized to beta-hydroxybutyrate (β -OHB), was fed to gilts and sows. The aim of this study was to evaluate whether BD would increase birth weights and decrease pre-weaning mortality in GP by increasing circulating ketones (i.e. β -OHB) in gestating gilts and sows. The hypothesis examined was that progeny from dams fed BD would have piglets with higher 24-hour weights, fewer light-for-age piglets and decreased pre-weaning mortality with the responses being greater for progeny from gilts than from sows.

5.4 Materials and Methods

5.4.1 Ethics Statement

Experimental procedures were approved by the Rivalea (Australia) Pty Ltd Animal Care and Ethics Committee (protocol number 16P054C) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

5.4.2 Animals, Diet and Experimental Design

The experiment was conducted under commercial conditions at a piggery in New South Wales, Australia (Rivalea Australia, Pty Ltd; Corowa, NSW, Australia), from June 2016 to February 2017. The experiment was a 2 x 2 factorial design (parity x diet) with a total of 77 gilts (parity 1) and 24 multiparous sows (parities 1, 2 and 3) being used across a total of five replicates. Gilts and sows were randomly allotted to their control or BD diet. From day 90 (90 $\pm$ 0.2 days; mean $\pm$ SE) gilts and sows were fed a common gestation diet (13.8 MJ DE/kg, 14.3% CP, 0.4% SID Lys; as-fed basis) with experimental diets containing 4% BD replacing wheat (Table 5.1) and tallow was added. Feed was delivered once daily at 09:00 with gilts and sows receiving 2.5 kg/d respectively from d90 until moving to the farrowing house on day 110. Once in the farrowing house, both gilts and sows were fed 2.5kg/d of their allotted diet until parturition, with all feed refusals recorded. Once farrowed, administration of the treatment diet ceased, and farrowed sows were placed on the commercial lactation diet at 2.5kg on the day of farrowing, 3kg one day post farrowing and up to 4kg from the second day having *ad libitum* access till weaning.

Table 5.1 Composition of late gestation diets.

Ingredient (%)	Control	Butanediol (BD)
Wheat	49.8	48.3
Barley	35.0	35.0
Canola meal 38%	6.0	6.0
Meat meal 58%	4.0	4.0
Tallow	2.5	
1,3 Butylene Glycol		4.0
Limestone	2.0	2.0
Threonine	0.05	0.05
Mineral blend	0.18	0.18
Vitamin blend	0.13	0.13
Calculated composition (DM basis)		
Energy (MJ DE/kg)	13.8	13.8
Crude protein (%)	14.3	14.2
Crude fat (%)	4.30	3.48
Crude fibre (%)	3.88	3.84
Ash (%)	5.28	5.25
Available lysine (%)	0.71	0.71

¹ 1,3 Butylene Glycol (Consolidated Chemical Co., Dandenong South, Australia). ² Supplied per kg of diet: Fe 80 mg; Zn 60 mg; Mn 30 mg; Cu 8 mg, as organic trace elements. ³ Supplied per kg of diet: vitamin A, 15,000 IU; vitamin D3, 3125 IU; vitamin E, 75 IU; vitamin K, 1 mg; vitamin B1, 1.5 mg; vitamin B2, 5 mg; vitamin B6, 3 mg; vitamin B12, 70 g; niacin, 20 mg; pantothenic acid, 15 mg; folic acid, 10 mg; vitamin C, 50 mg

Gilts and sows were weighed and P2 backfat measured on day 90 of gestation and they were subsequently housed in groups of 7 depending on parity and diet from day 90 to day 110. Prior to farrowing room entry on day 110, gilts and sows were weighed again, and blood samples were taken. From day 110, gilts and sows were housed in the same farrowing room in alternating farrowing crates fitted with the nipple drinkers for mothers and piglets, and a heat lamp in creep area. Piglets from each litter were individually weighed and tagged within 24 hours of birth. This time was chosen for the initial weight so that there was no interference in piglets obtaining colostrum. Two focal piglets were selected for each litter for plasma samples within 24 hours. Following this, cross-fostering was carried out following commercial procedures. Piglets were fostered within parity and diets when possible. Number of total piglets, number of piglets born alive, number of still births, number of mummified foetuses were recorded for each litter. Piglets were individually weighed again at 21 days of age. A total of 1,822 piglets were used for a 24-hour measurement and 1,518 piglets were used for 21-day weights. A total of 241 piglets were used for plasma metabolite analysis.

Blood from gilts, sows and piglets were collected via jugular venipuncture into lithium and heparin Vacutainers (BD™ Vacutainer™) and inverted 4 times to ensure anticoagulant was distributed throughout the blood. Tubes were then placed on ice until centrifugation at 4 °C for 10 min. Plasma was collected and stored at -20° C until analysis.

5.4.3 Metabolite and Hormone Analysis

Sow, gilt and piglet plasma was assayed for beta-hydroxybutyrate (β -OHB), non-esterified fatty acids (NEFA), glucose and insulin was assayed for gilts and sows only. Plasma β -OHB (Cayman Chemical, Ann Arbor, Michigan, USA 0.7-16.2% intra-assay CV and 13.9% inter-assay CV), NEFA (NEFA-C, Wako Chemical Industries, Osaka, Japan; 0.5-10.7% intra-assay CV, 11% inter-assay CV), glucose (Thermo Fisher Scientific, Waltham, Massachusetts, USA 1.3-7% intra-assay CV, 7.9% inter-assay CV), and insulin (Mercodia, Uppsala, Sweden; 0.5%-5.6% intra-assay CV, 10% inter-assay CV) concentrations were determined by following manufacturer's procedures.

5.4.4 Statistical Analysis

Live weight, P2 backfat, metabolite data and hormone data were analyzed by linear mixed model analysis using GENSTAT (16th edition). Parity and diet were analyzed as the

main and interactive factors for all parameters. Replicate was designated as the random term for all production and metabolite analyses with sow ID designated as the random term for individual piglet growth and metabolite analysis. Piglet and litter were used as the experimental unit. The percentage of light-for-age at 24 hours (<1.1 kg) and pre-weaning mortality in piglets were analyzed using a Chi-Squared (χ^2) test. Data are presented as a $\pm$ standard error of the differences (SED). A value of $p < 0.05$ was used to indicate statistical significance and a value of $p < 0.10$ was considered a statistical trend.

5.5 Results

5.5.1 Farrowing performance

There was no effect of dietary BD on dam live weight just prior to farrowing (d110) ($p = 0.11$) or at weaning ($p = 0.43$) (Table 5.2). There was a tendency for dams on the control diet to have less P2 backfat compared to those on the BD diet (19.3 vs 20.5 mm; respectively; $p = 0.082$) (Table 5.2). As expected, gilts were lighter just prior to farrowing (209 vs 256 kg, $p < 0.001$) and at weaning (187 vs 230 kg, $p < 0.001$). Sows tended to lose more live weight compared to gilts (-12.7 vs -8.1 kg, $p = 0.09$) and lost more backfat (-3.3 vs -2.0 mm, $p = 0.019$). There were no main or interactive effects of parity or dietary BD on total born alive, still born or mummified piglets (Table 5.2).

Table 5.2 Farrowing performance of gilts and sows fed a control or a BD diet, and their litter characteristics

Parity (P)	Gilt		Sow		s.e.d.	<i>p</i> -value		
Diet (D)	Control	BD	Control	BD		P	D	PxD
Dam performance								
<i>n</i>	38	37	37	39				
d90 liveweight (kg)	195	195	239	249	5.0	<0.001	0.15	0.16
d110 liveweight (kg)	208	209	252	260	4.9	<0.001	0.21	0.32
Wean liveweight (kg)	186	186	227	232	5.6	<0.001	0.43	0.50
Δ liveweight d90 - Wean (kg)	-8.1	-8.1	-11.2	-14.1	3.7	0.096	0.58	0.59
Wean P2 backfat (mm)	19.2	19.9	19.4	21.2	1.00	0.36	0.082	0.46
Δ P2 backfat d90 - Wean (mm)	-1.3	-2.9	-3.2	-3.4	0.76	0.025	0.11	0.20
Litter characteristics at birth								
Born alive	11.8	12.4	12.4	12.1	0.43	0.57	0.67	0.17
Still born	0.43	0.30	0.51	0.42	0.23	0.53	0.48	0.90
Mummified	0.08	0.22	0.17	0.12	0.10	0.97	0.53	0.18

5.5.2 Piglet performance

Piglets born to dams supplemented with BD tended to be 50 g heavier at 24 hours compared to control dams (1.48 vs 1.43 kg, $p = 0.085$), and these differences extended to total

litter weights (17.9 vs. 17.0 kg, $p = 0.078$) (Table 5.3). Gilt progeny were 170 g lighter than sow progeny (1.37 vs. 1.54 kg, $p < 0.001$) at 24 hours, and these differences also extended to total litter weight (16.3 vs 18.6 kg, $p < 0.001$) (Table 5.3). Addition of BD tended to reduce individual piglet weights at 21 days (5.60 vs 5.56 kg, $p = 0.064$) but this effect did not extend to total litter weights (56.8 vs 56.7 kg, $p = 0.83$). However, there was an indication of an interaction ($p = 0.10$) that suggested that BD increased weaning litterweight of GP whereas it was reduced in SP (Table 5.3). Gilt progeny were lighter at 21 days than SP (5.00 vs 6.11 kg, $p < 0.001$), and these differences were evident in total litter weight (48.9 vs 64.6 kg, $p < 0.001$) (Table 5.3). There were no effects of either BD or dam parity on the intra-litter coefficient of variation (CV) of 24-hour or 21-day weights (Table 5.3).

Table 5.3 Growth performance parameters of gilt and sow progeny fed a control or BD diet at 24 hours and 21-days of age.

Parity (P) Diet (D)	Gilt		Sow		s.e.d.	<i>p</i> -value		
	Control	BD	Control	BD		P	D	PxD
24-hour liveweight (<i>n</i>)	428	468	455	471				
Individual liveweight (kg)	1.34	1.39	1.51	1.56	0.02	<0.001	0.085	0.98
Total litter (kg)	15.5	17.1	18.5	18.7	0.69	<0.001	0.078	0.15
Intra-litter CV (%)	17.2	17.5	18.4	16.6	0.01	0.89	0.39	0.21
21-day liveweight (<i>n</i>)	327	385	395	411				
Individual (kg)	5.02	4.98	6.18	6.03	0.01	<0.001	0.064	0.43
Total litter (kg)	47.1	50.6	66.4	62.8	3.10	<0.001	0.83	0.10
Intra-litter CV (%)	20.4	18.5	20.7	21.3	0.17	0.17	0.58	0.30

The percentage of progeny that were light-for-age (<1.1 kg) at 24 h of age was decreased by BD (18.2 vs 13.5%, $p = 0.006$), particularly in GP (24.0 vs 18.3%, $p = 0.034$) (Figure 5.1a). Overall, SP had a lower percentage of progeny that were light-for-age at 24 h of age than GP (20.9 vs 10.8%, $p < 0.001$) (Figure 5.1a). Pre-weaning mortality was lower in SP than in GP (8.83 vs 13.7%, $p < 0.001$) but there was no effect of BD (11.6 vs 10.9%, $p = 0.64$) (Figure 5.1b). Even within GP there was no significant effect of BD on mortality rate (13.0 vs 11.2%, $p = 0.39$).

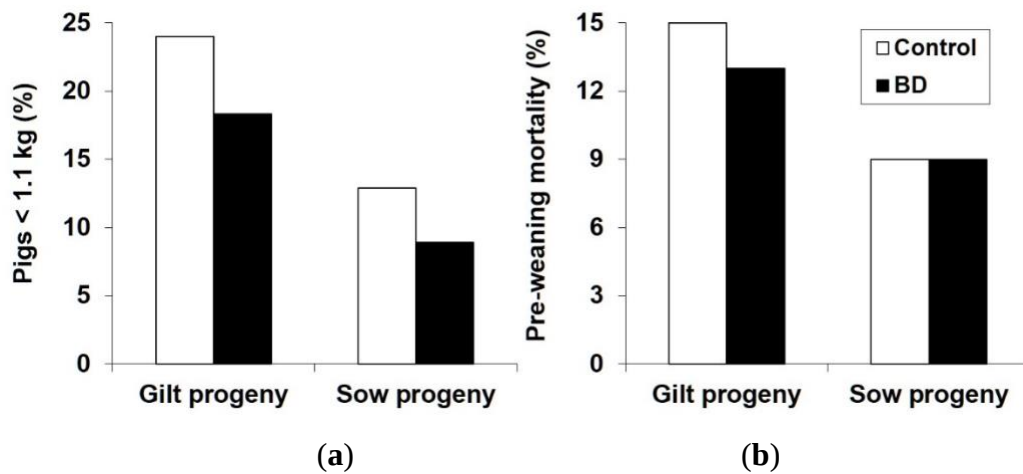


Figure 5.1 Effect of parity and diet (control vs 1,3-Butanediol (BD) on: (a) Percentage of pigs that are small-for-age at 24 hours (<1.1 kg); (b) Pre-weaning mortality. See text for statistical analysis.

5.5.3 Plasma Metabolite Concentrations

On day 110 on gestation plasma β -OHB concentrations were higher in dams fed the BD-supplemented diet compared with those on the control diet (0.43 vs 0.52 μ M, respectively, $p = 0.01$) (Table 5.4). Plasma glucose concentration tended to be higher in dams fed the control diet compared with those fed the BD diet (7.72 vs 7.50 mM, $p = 0.09$) (Table 5.4). Plasma NEFA concentrations were lower in dams fed the BD diet (327 vs 249 μ M, respectively, $p < 0.001$) (Table 5.4). There were no main or interactive effects of parity or diet on plasma insulin concentrations (Table 5.4). There were no main or interactive effects of parity or diet on plasma β -OHB, NEFA or glucose concentrations in dam progeny (Table 5.4).

Table 5.4 Plasma metabolites of gilts and sows and their progeny for the Control and BD diets.

Parity (P)	Gilt		Sow		s.e.d.	<i>p</i> -value		
Diet (D)	Control	BD	Control	BD		P	D	PxD
Dam (day 110 of gestation)								
β-OHB (mM)	0.49	0.54	0.45	0.49	0.040	0.96	0.01	0.12
Glucose (mM)	7.72	7.63	7.71	7.36	0.181	0.27	0.09	0.32
Insulin (pM)	409	404	435	433	63.6	0.54	0.94	0.97
NEFA (μM) ⁺	5.75 (314)	5.48 (241)	5.83 (340)	5.54 (256)	0.089	0.28	<0.001	0.86
Progeny (24 h after birth)								
β-OHB (mM)	0.42	0.42	0.43	0.36	0.050	0.49	0.35	0.28
Glucose (mM)	8.28	8.47	8.64	8.50	0.209	0.21	0.68	0.27
NEFA (μM) ⁺	6.51 (799)	6.46 (891)	6.32 (662)	6.49 (780)	0.110	0.30	0.53	0.17

⁺ Data were log_e transformed before analyses due to heterogeneity of variances. Values in parentheses are back-transformed means

5.6 Discussion

The major finding from this study was that dietary BD supplementation to both gilts and sows from d90 of gestation to parturition reduced the percentage of piglets born light-for-age and tended to increase individual piglet and total litter weights. Where responses occurred they appeared to be greater in GP. Dietary BD supplementation increased dam plasma β-OHB concentrations while decreasing fat mobilisation as indicated by reduced plasma NEFA in late gestation. The present study also confirmed the established differences in growth performance between GP and SP.

The higher maternal plasma β-OHB concentrations found in dams supplemented with BD were expected due to the ketogenic nature of metabolised BD. Elevated plasma β-OHB concentrations indicate the potential for increased energy sparing in the dam, thus allowing more energy to be diverted towards late gestational growth and energy deposition in the fetus, or in the case of gilts, growth of the dam. Plasma NEFA concentrations were reduced in dams supplemented with BD indicating reduced fat mobilisation since plasma NEFA concentrations are directly related to NEFA turn over and fat mobilization (Taggart, Kero et al. 2005). Furthermore, at weaning P2 backfat tended to be increased in dams that consumed the BD diet. Although no P2 measurements were taken at d110, there seems to be this effect of reduced fat mobilisation throughout the pre-weaning period stemming from d110 reflected in increased P2 backfat at weaning. A reduction in plasma NEFA concentrations due to exogenous ketone

supplementation has previously been reported in humans and is most likely attributed to a homeostatic negative feedback mechanism via the PUMA-G receptor (Taggart, Kero et al. 2005, Stubbs, Cox et al. 2017). This unique co-existence of low NEFA and high β -OHB is likely due to the high affinity of β -OHB to the PUMA-G receptor in adipose tissue with suppresses lipolysis, reducing the plasma NEFA concentrations (Stubbs, Cox et al. 2017). Moreover, plasma glucose concentrations tended to be lower in dams supplemented with BD. This effect has been previously associated with exogenous ketone supplementation and was attributed to the ability of ketones to reduce blood glucose by limited hepatic gluconeogenesis and increasing peripheral glucose uptake (Mikkelsen, Seifert et al. 2015, Stubbs, Cox et al. 2017). Dietary BD supplementation of the dam had no effect on β -OHB concentrations in the progeny.

Piglets born to dams on the BD diet were heavier at 24 hours of age compared to their control counterparts. There were also less piglets that were light-for-age and therefore at high risk of morbidity and mortality. Low body weight indicates poor productivity and higher chances of pre-weaning mortality in the piglet (Fix, Cassady et al. 2010, Magnabosco, Bernardi et al. 2016, Craig, Collins et al. 2017, Lanferdini, Andretta et al. 2018). Therefore, based on the 24-hour weights, BD has the potential to increase pre-weaning survival and productivity. Elevated plasma β -OHB concentrations in the BD-supplemented dams, coupled with increased 24-hour weights, suggest that ketone bodies effectively passed through the placenta and provided an energetic substrate for the neonatal piglet. Although these piglets did not have increased plasma β -OHB concentrations at 24 hours, this may have been due to its rapid metabolism within the first 24-hour period. Such metabolism could provide enough energy for the piglet to rapidly reach the udder and obtain colostrum. Increased NEFA concentrations in piglets have been previously reported as approximately twenty-fold higher than generally observed in growing pigs (Taggart, Kero et al. 2005) and is most likely due to increased colostrum consumption. Colostrum has a high triglyceride content, particularly in gilts (Quesnel, Farmer et al. 2012, Decaluwé, Maes et al. 2013, Szyndler-Nędza 2016) and hydrolysis of circulating triglycerides can contribute to elevated NEFA when pigs consume a high fat diet (Liong, Dunshea et al. 2007). If glycogen stores were rapidly metabolised in the first 16 hours of life, a switch to ketone metabolism may provide supplementary energy for the piglet to rapidly reach the udder and obtain colostrum in piglets born to dams fed the BD diet. While there were no main or interactive effects, BD did not have a significant effect on

individual or litter weight at 21 days of age. Therefore, further work is needed to determine whether the effect of BD on birthweight is carried through to weaning and subsequent market weight.

There were differences in mortality within treatment groups in the present study although the predominant effect was that mortality was greater in GP than in SP. While there was no effect of BD on mortality in the present study, a reduction in mortality of piglets born to dams supplemented with BD has been previously reported (Spence, Boyd et al. 1985, Stahly, Raff et al. 2014). For example, two studies reported an increase in fetal hepatic glycogen stores in the piglet from sows fed diets supplemented with BD (Spence, Boyd et al. 1985, Stahly, Raff et al. 2014). The effect is largely attributed to the glucose sparing mechanism of ketones which provide an alternative substrate for oxidation in peripheral tissues in the neonatal pig. However, the present study did not investigate fetal hepatic glycogen levels. With BD supplementation in diets, the provision of an alternative energetic substrate and increased glycogen stores could increase thermoregulatory capacity which is strongly related to piglet survival. Increased thermoregulatory capacity also decreases time to reach the udder and in turn, increases colostrum and milk consumption. The increased 24-hour weights seen in piglets of the BD diet suggest this mechanism.

5.7 Conclusions

The supplementation of late gestation diets with BD reduced the percentage of small-for-age piglets for both GP and SP and increase birthweight as indicated by their 24-hour weights. Dietary BD also increased dam plasma β -OHB concentrations and decreased plasma NEFA and glucose concentrations indicating an energy sparing effect in the dams. However, the effects of BD were not evident at weaning. Further work is required to determine whether the effect of BD on birthweight is carried through subsequent stages of growth, particularly in GP.

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Chapter 6 Evaluation of Sugarcane-Derived Polyphenols on the Pre-Weaning and Post-Weaning Growth of Gilt Progeny

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6.1 Simple Summary

Gilt progeny are characterised by their poor lifetime growth performance compared with sow progeny. Various feeding strategies that employ the use of additives may be used to improve their growth. Gilts are said to be in increased oxidative stress throughout lactation, which may contribute to the reduced growth performance seen in their progeny. Furthermore, weaning is associated with increased inflammation, which can reduce growth after weaning. In this study, both late gestation/lactation diets and weaner diets were supplemented with a sugarcane extract rich in polyphenols owing to their anti-oxidative and anti-inflammatory properties to collectively improve the growth of gilt progeny. However, no improvements of growth performance of gilt progeny in both the pre-weaning and post-weaning periods were observed in response to polyphenol supplementation and sow progeny continuously outperformed gilt progeny. Furthermore, when inflammation was measured using the inflammatory cytokine interleukin-1 β , no differences were found between the control and polyphenol supplemented group. However, gilt progeny exhibited reduced circulating interleukin-1 β overall. In summary, gilt progeny experience persistent underperformance that may be coupled in part to poorer immune development and polyphenol supplementation did not overcome the persistent underperformance.

6.2 Abstract

Gilt progeny (GP) exhibit poorer growth compared with sow progeny (SP), particularly in the pre-weaning and post-weaning period. Late gestation/lactation sow diets and weaner diets were supplemented with 0.5% Polygain (POL), a sugarcane extract rich in polyphenols, to collectively improve GP growth in these periods. Gilts ($n = 60$) and sows ($n = 68$, parities 2 and 3) were fed a control or POL diet. Weaned GP ($n = 79$) and SP ($n = 92$) born to these dams were also fed either a CON or POL diet. Gilts litters weighed less than sow litters at birth and 21 days ($p < 0.001$ for both) and were not improved by POL ($p = 0.80$ and 0.54 , respectively). GP were lighter than SP at day 7 and day 14 post-weaning ($p < 0.001$ for both) and were not improved by the POL diet at these timepoints ($p = 0.61$ and 0.97 , respectively). Plasma interleukin-1 β (IL-1 β) was increased at weaning despite POL supplementation ($p = 0.022$) and GP had reduced IL-1 β overall ($p = 0.021$). Overall, POL was unable to improve growth in GP and the attenuated immune response seen in GP could be contributing to their poor growth performance.

Keywords: gilt progeny; sugarcane; polyphenol

6.3 Introduction

Gilt progeny (GP) are characterised by low birth weights, slow growth, higher medication rates, and greater rates of post-natal mortality (Miller., Collins. et al. 2012, Carney-Hinkle, Tran et al. 2013, Craig, Collins et al. 2017), resulting in significant losses for pork producers. Gilt replacement exceeds 50% of the Australian reproductive herd (Limited 2013); therefore, the success of their progeny is critical for pig production. Gilts are mated at a physiologically young age to reduce non-productive days, and so the gestating gilt must support her own continued growth and development throughout pregnancy. However, this often compromises the growth and development of her progeny and, as a result, GP consistently perform more poorly compared with multiparous sow progeny (SP) (Craig, Collins et al. 2017, Craig, Muller et al. 2019b). Gestation and lactation are energetically demanding periods for the dam that are associated with systemic oxidative stress (Berchieri-Ronchi, Kim et al. 2011). This is more acute in the gilt than in the sow (Roy, Lavoie et al. 2016) and is linked to impaired milk production in the pre-weaning period (Berchieri-Ronchi, Kim et al. 2011). The comparatively poorer growth of GP during the pre-weaning period (Craig, Collins et al. 2017) can partially be attributed to the lower milk yield of the gilt (Beyer, Jentsch et al. 2007, Craig, Dunshea et al. 2019a), meaning that reducing oxidative

stress in lactating gilts may be a useful management strategy for increasing productivity in GP. This was demonstrated by Lipiński, Antoszkiewicz et al. (2019), who observed antioxidant polyphenol supplementation improved litter and body weights at weaning. Polyphenols are secondary plant metabolites that exhibit anti-oxidative effects (Gessner, Ringseis et al. 2017). In addition to their antioxidative properties, polyphenols also exhibit anti-inflammatory properties (Zhang and Tsao 2016).

Increased inflammation is often associated with weaning (Pié, Lallès et al. 2004). This is usually in response to the functional and structural changes of the gastrointestinal tract (Pluske, Williams et al. 1996, Dunshea 2003) caused by the abrupt shift of nutrients from highly digestible milk solids to complex cereal feed. Furthermore, this immediate post-weaning period is often characterised by reduced growth rates, which is particularly observed in GP (Craig, Collins et al. 2017, Craig, Muller et al. 2019b). Whether increased inflammation contributes specifically to the inferior post-weaning growth performance seen in GP is yet to be fully elucidated. However, supplementing weaner diets with plant polyphenols may reduce weaning associated inflammation (Gessner, Ringseis et al. 2012, Chen, Xie et al. 2018) and potentially improve GP growth. Polygain (POL) is a sugarcane molasses-derived extract that is rich in polyphenols including chlorogenic acid, caffeic acid, sinapic acid, syringic acid, vanillin, homoorientin, orientin, vitexin, swertisin, diosmin, apigenin, tricin, and diosmetin, as well as a number of apigenin-, methoxyluteolin-, and tricin-O-glycosides (Gessner, Ringseis et al. 2012). Dietary POL has been shown to alleviate oxidative stress and increase growth rate of broiler chickens suffering heat stress (Shakeri 2019).

This study aimed to evaluate the effects of POL supplementation on the growth of GP during the lactation and early post-weaning period. It was hypothesized that supplementing late gestation/lactation and weaner diets with POL would collectively improve pre-weaning and weaning performance in GP.

6.4 Materials and Methods

6.4.1 Ethics Statement

Experimental procedures were approved by the Rivalea (Australia) Pty Ltd. Animal Care and Ethics Committee (protocol number 18N006C) in accordance with the Australian

Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

6.4.2 Animals, Experimental Design, and Diets

The experiment was conducted under commercial conditions at a piggery in New South Wales, Australia (Rivalea Australia, Pty Ltd., Corowa, NSW, Australia), from March to April 2018.

6.4.3 Gestation and Lactation Phases

The study consisted of a 2×2 factorial design (parity $\times$ diet) with a total of 60 gilts (parity 1) and 66 multiparous sows (parities 2 and 3; Large White $\times$ Landrace, Primegro™ Genetics, Corowa, Australia) (2 ± 0.1 parities; mean $\pm$ SE) over two replicates. Gilts and sows were weighed and P2 backfat measurements were taken at day 110 upon entry to farrowing house and at weaning. Gilts and sows were then randomly allocated to a wheat- and canola meal-based diet (CON) (14.8 MJ digestible energy (DE)/kg, 16.5% crude protein (CP), 8.3% crude fat, 4.3% crude fibre, and 0.9% standardized ileal digestible (SID) Lys; as-fed basis) or the CON diet with 0.5% POL, with the POL replacing wheat. Diets were fed from day 110 (110 ± 0.2 days; mean $\pm$ SE) of gestation and throughout lactation (24.6 ± 0.1 days; mean $\pm$ SE) until weaning. Whole diets were analysed for total polyphenol content as per methods described by Deseo, Elkins et al. (2020) and the addition of POL resulted in an 11% increase in total polyphenol content of the diet (2.71 vs. 2.44 mg/g). Gestating gilts and sows were fed 3.5 kg/day of their allotted diet at 09:00 h each day after entry to the farrowing house. Once farrowed, gilts and sows were fed 3.5 kg on the day of farrowing, 5 kg one day after farrowing, and ad libitum from the second day until weaning. Feed intake and refusals were recorded daily in this period. Gilts and sows were housed in the same farrowing room in alternating farrowing crates fitted with nipple drinkers for both dams and piglets, with piglets having access to a heat lamp in the creep area. There was no creep feed provided. All live piglets were individually weighed and tagged within 24 h of birth. The two median weight male focal piglets were selected from each litter for blood samples to be taken at 1 day prior to weaning. The number of born alive piglets, still born, and mummified foetuses were recorded for each litter before any cross fostering, which was carried out as per commercial procedures with piglets fostered within parity and diet where possible. At 21 days, piglets were individually weighed again. A total of 25 GP and 30 SP were used for inflammatory marker analysis for the pre-weaning period.

6.4.4 Post-Weaning Phase

Piglets were weaned at approximately 24.6 (± 0.1 days) days of age. The male focal piglets of each litter in lactation were relocated to individual pens in the weaner unit of the farm, while non-focal piglets continued on to regular commercial production. Weaner pigs were offered a common starter wheat, dehulled oats, and soybean meal-based diet (14.7 MJ DE/kg, 20.3% CP, 4.08% crude fat, 2.6% crude fibre, and 1.3% SID Lys; as-fed basis), with experimental diets containing 0.5% POL replacing wheat. The addition of POL resulted in an 8% increase in total polyphenol content of the diet (2.23 mg/g vs. 2.05 mg/g). Weaned pigs from dams that had been fed the control diet were also fed the control diet, whereas those that were from dams fed POL were also fed the POL diet. After weaning, weights were not taken upon entry to the weaner pens owing to resource constraints associated with the commercial batch weaning system. However, piglets were individually weighed at 7 and 14 days after weaning. Feed intake was recorded from weaning to 14 days, after which all focal piglets were returned to regular commercial production.

6.4.5 Inflammatory Marker Analysis

Blood samples were taken the day before and at 3 and 14 days after weaning. Pigs were mildly restrained in dorsal recumbency by a trained operator, and blood was then collected via jugular venepuncture using a 21-gauge needle for new-borns, and a 20-gauge needle for piglets aged 21 to 40 days, into lithium heparin vacutainer tubes (BD™ Vacutainer™, North Ryde, NSW, Australia). Each tube was inverted 3–4 times to ensure adequate mixing of the sample and anticoagulant. Tubes were then placed on ice until centrifugation for 10 min at 4 °C. Plasma was collected and stored at –20 °C until analysis. Piglet plasma was assayed for interleukin-1 beta (IL-1 β) (DuoSet® Porcine IL-1 β /IL-1F2, catalogue number DY681, R&D systems, Minneapolis, MN, USA; 2–5% intra-assay covariation (CV) and 5% inter-assay CV), as per the manufacturer's instructions.

6.4.6 Statistical Analysis

Liveweight, P2 backfat, litter weights, weaned piglet weights, feed intake, and circulating IL-1 β were analysed by linear mixed models using GENSTAT (16th edition, Hemel Hempstead, UK). Dam parity (gilt vs. sow) and diet (control vs. POL) were the main and interactive factors for all analysis except circulating IL-1 β . Circulating IL-1 β was analysed using dam parity, diet, and time (pre-weaning, 3 days post-weaning, and 14 days post-weaning) as main and interactive factors. Replicate was designated as the random model. The sow was

designated as the random model for post-weaning growth performance. Piglet age at weaning was designated as a covariate in the random model when analysing day 7 and day 14 post-weaning weights. Sow ID and Piglet ID were designated in the random model for IL-1 β analysis. When interactive effects were found to be significant in IL-1 β analysis, a post-hoc Fisher's Least Significant Difference (LSD) test was performed for multiple pairwise comparisons. Chi-squared (χ^2) tests were used to analyse pre-weaning mortality between parities and between diets. Data are presented as mean $\pm$ standard error of the differences (SED). A value of $p < 0.05$ was used to indicate statistical significance.

6.5 Results

6.5.1 Farrowing Performance

Gilts were lighter (202 vs. 276 kg, $p < 0.001$) and had lower P2 backfat (19.0 vs. 21.1 mm, $p = 0.009$) than sows at entry into the farrowing house at d110 of gestation (data not shown). Similarly, gilts were lighter (190 vs. 257 kg, $p < 0.001$) and had lower P2 backfat (19.0 vs. 22.0 mm, $p < 0.001$) than sows at weaning. There was no effect of dietary POL on either dam liveweight ($p = 0.26$) or P2 back fat ($p = 0.55$) at weaning. Dietary POL supplementation decreased lactation feed intake (6.61 vs. 7.15 kg/day, $p = 0.001$), while gilts had lower lactation feed intake than sows (7.18 vs. 7.84 kg/day, $p = 0.002$). Sows had more piglets born alive compared with gilts (12.2 vs. 11.2 pigs, $p = 0.021$) with no effect of diet ($p = 0.61$). There were no parity ($p = 0.49$) or dietary ($p = 0.92$) effects on the number of stillborn piglets. Similarly, there were no parity ($p = 0.73$) or dietary ($p = 0.71$) effects on the number of mummified piglets (Table 6.1).

6.5.2 Pre-Weaning Performance

Gilts had a lighter total litter weight (13.7 vs. 18.3 kg, $p < 0.001$) and individual piglet weight (1.25 vs. 1.50 kg, $p < 0.001$) at birth compared with sows. Supplementation with POL had no effect on total litter weight ($p = 0.80$) at birth but resulted in lower individual birth weight (1.34 vs. 1.41 kg, $p = 0.029$). There were no parity ($p = 0.25$) or dietary effects ($p = 0.28$) on intra-litter CV in birth weight. Gilts showed a lighter total litter weight (41.4 vs. 61.8 kg, $p < 0.001$) and individual piglet weight (4.68 vs. 6.31 kg, $p < 0.001$) at 21 days of age than multiparous sows. There were no effects of dietary POL on total litter weight ($p = 0.25$) or individual piglet weight ($p = 0.30$) at 21 days. Gilt litters had lower intra-litter weight CV at 21 days (19.7 vs. 23.2%, $p = 0.006$), but this was not affected by dietary POL ($p = 0.68$). Total

weight gain was lower in litters from gilts (1.30 vs. 2.11 kg/day, $p < 0.001$), but was not altered by dietary POL ($p = 0.48$) (Table 6.1). There were no effects of dietary POL (11.2 vs. 11.6%, $\chi^2 = 0.07$, $p = 0.79$) or between GP and SP (11.8 vs. 11.0%, $\chi^2 = 0.26$, $p = 0.61$) on pre-weaning mortality.

Table 6.1 The effect of parity (gilt vs. sow) and diet (control, CON vs. 0.5% dietary Polygain, POL) on gestation and lactation performance of dams and their progeny. SED, standard error of the differences.

Parity (P) Diet (D)	GILT		SOW		SED	<i>p</i> -value		
	CON	POL	CON	POL		P	D	PxD
(<i>n</i>)	29	31	34	33				
Dam weaning liveweight (kg)	189	190	259	255	6.4	<0.001	0.26	0.58
Dam weaning P2 backfat (mm)	20.0	18.0	21.5	22.4	1.33	0.001	0.55	0.14
Dam lactation feed intake (kg/day)	6.93	6.19	7.38	7.04	0.236	<0.001	0.002	0.24
Piglets born alive (#/litter)	10.9	11.3	12.1	12.3	0.66	0.020	0.61	0.82
Piglets still born (#/litter)	0.92	1.06	1.23	1.17	0.429	0.49	0.92	0.74
Mummified piglets (#/litter)	0.18	0.30	0.31	0.12	0.128	0.73	0.71	0.09
Birth litter weight (kg)	13.8	13.6	18.4	18.3	0.86	<0.001	0.80	0.89
Birth piglet weight (kg)	1.29	1.20	1.55	1.49	0.049	<0.001	0.029	0.64
Birth weight intra-litter CV (%)	17.8	18.1	18.3	20.3	1.63	0.25	0.28	0.48
21-day litter weight (kg)	42.2	40.7	59.7	64.0	3.59	<0.001	0.54	0.25
21-day piglet weight (kg)	4.85	4.60	6.37	6.26	0.239	<0.001	0.30	0.68
21-day weight intra-litter CV (%)	18.6	20.8	23.7	22.7	1.72	0.006	0.68	0.19
Litter daily gain (kg/day)	1.33	1.27	2.04	2.17	0.113	<0.001	0.48	0.18

6.5.3 Post-Weaning Performance

Gilt progeny were lighter than SP at day 7 (7.18 vs. 9.02 kg, $p < 0.001$) and day 14 after weaning (9.61 vs. 11.8 kg, $p < 0.001$). There was no effect of dietary POL on liveweight at day 7 ($p = 0.61$) or day 14 ($p = 0.97$) after weaning. Live weight gain was lower in GP compared with SP from day 7 to 14 (347 vs. 394 g/day, $p = 0.042$), but was unaffected by dietary POL ($p = 0.40$). Feed intake from weaning to day 7 was not affected by parity ($p = 0.60$) or diet ($p = 0.77$). Feed intake between days 7 and 14 after weaning was lower in GP than in SP (375 vs. 428 g/day, $p = 0.018$), regardless of diet ($p = 0.67$). There was no effect of either parity ($p = 0.37$) or dietary POL ($p = 0.29$) on feed conversion ratio between days 7 and 14 after weaning and no significant interactions were observed ($p = 0.18$) (Table 6.2). No mortalities were observed in the post weaning period.

Table 6.2 The effect of parity (gilt vs. sow) and diet (control, CON vs. 0.5% dietary Polygain, POL) on the post-weaning performance of dam progeny.

Parity (P) Diet (D)	GILT		SOW		SED	<i>p</i> -value		
	CON	POL	CON	POL		P	D	PxD
(n)	47	32	49	42				
Day 7 liveweight (kg)	7.19	7.17	9.17	8.86	0.359	<0.001	0.61	0.58
Day 14 liveweight (kg)	9.66	9.55	11.8	11.8	0.49	<0.001	0.97	0.88
Day 7-14 live weight gain (g/day)	353	341	372	415	30.9	0.042	0.40	0.20
Day 0-7 feed intake (g/day)	184	181	185	193	16.1	0.60	0.77	0.59
Day 7-14 feed intake (g/day)	385	366	414	443	29.8	0.018	0.67	0.25
Day 7-14 feed conversion ratio	1.12	1.16	1.32	1.11	0.129	0.37	0.29	0.18

6.5.4 Plasma IL-1 β Concentrations

Plasma IL-1 β concentrations were lower in GP than in SP (2264 vs. 2568 pg/mL, $p = 0.021$) and decreased after weaning (2556, 2449, and 2244 pg/mL at pre-weaning, 3 days post-weaning, and 14 days post-weaning, respectively, $p = 0.002$), while no main effect of dietary POL (2354 vs. 2478 pg/mL for CON and POL, respectively, $p = 0.15$) was observed. However, there were significant interactions between diet and parity ($p = 0.041$), such that CON GP had lower plasma IL-1 β concentrations than GP from dams supplemented with POL (Table 3), while no significant differences were observed between CON SP and POL SP. Moreover, the effect of parity was most pronounced at 14 days after weaning, where plasma IL-1 β concentrations were 35% higher in SP than in GP (2577 vs. 1910 pg/mL), although there were no differences in pre-weaning and 3 days post-weaning (Table 6.3).

Table 6.3 The effect of parity (gilt progeny (GP) vs. sow progeny (SP)), diet (control, CON vs. 0.5% dietary Polygain, POL) and time (pre-weaning, PreW vs. 3 days post weaning, 3 days PoW vs. 14 days post-weaning, 14 days PoW) on interleukin-1 β (IL-1 β) concentrations in plasma of progeny.

Diet (D)	Parity (P)	Timepoint (T)			SED	Significance ¹
		PreW	3 days PoW	14 days PoW		
CON	GP	2190	1982	1838	320	P ⁺ , T ^{**} , D $\times$ P [*] , D $\times$ T [*] , P $\times$ T [*]
	SP	2830	2555	2728		
POL	GP	2584	3010	1982		
	SP	2618	2250	2427		

¹⁺ $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Other main and interactive effects $p > 0.10$.

6.6 Discussion

This study confirmed that GP perform more poorly than SP during the pre-weaning and immediate post-weaning period. Moreover, feeding POL to primiparous sows in gestation and lactation did not improve pre-weaning and post-weaning performance of their progeny. Furthermore, the POL diet was unable to reduce plasma IL-1 β at weaning and GP had lower plasma IL-1 β at 14 days post-weaning compared with SP. An additional finding was that GP had reduced concentrations of the inflammatory marker IL-1 β overall, possibly indicating delayed development of the immune system.

During the pre-weaning period, litters born to gilts were lighter than those born to sows, and this extended to 21 days and beyond. Furthermore, gilt litters grew slower compared with sow litters, which exacerbated the weight difference later on, which is consistent with the literature (Wijesiriwardana, Pluske et al. 2019). Low birth weights can limit pre-weaning and post-weaning growth (Quiniou, Dagorn et al. 2002, Dunshea, Kerton et al. 2003), which was seen in this study. Furthermore, while gilts and sows have similar milk composition (Craig, Dunshea et al. 2019a), their progeny grow at different rates and, therefore, this poor pre-weaning growth must be partially attributed to lower milk yield of the gilt (Beyer, Jentsch et al. 2007). Individual piglet and litter weight are strong drivers of dam milk production (King, Mullan et al. 1997), hence the reduction in suckling pressure from the lighter GP may further contribute to the lower milk production of gilts.

Gilt progeny were lighter at day 7 after weaning and grew slower from day 7 to day 14 after weaning in comparison with SP. Slower growth after weaning has been previously

reported especially in low birth- and weaning-weight piglets (Dunshea, Kerton et al. 2000, Dunshea, Kerton et al. 2002), and is often attributed to the abrupt shift from highly digestible milk constituents to more complex solid feed. This post-weaning growth check seems to be exacerbated in GP, reflected particularly by the reduced feed intake and live weight gain in this study, which has been previously reported (Wijesiriwardana, Pluske et al. 2019). Interestingly, there was no difference in feed intake between GP and SP in the immediate 7-day post-weaning period. However, there was a clear difference in the second week following weaning, where SP consumed 14% more feed than GP. This further validates the impacts of the post-weaning growth check that newly weaned pigs experience, but also demonstrates the ability of SP to overcome the challenge of weaning more quickly than GP. In this context Pluske, Kerton et al. (2003) found that light pigs at weaning have a less developed gastrointestinal tract than heavier pigs and that there was a progressive increase in small intestinal brush border maltase, glucoamylase and sucrose, and pancreatic trypsin activity over the first 2 weeks after weaning, whereas brush border lactase activity declined in these light piglets. These changes in enzyme activities reflect the transition from a liquid milk-based diet to a complex solid cereal-based diet and indicate that heavier pigs are better able to make this adjustment, which may have been the case of SP in comparison with GP.

A key finding of this study was that GP had increased concentrations of IL-1 β at 3 days post-weaning, but reduced concentrations overall compared with SP, regardless of POL supplementation. This was especially evident at day 14 following weaning and was largely owing to the maintenance of plasma IL-1 β concentrations across the entire period in SP, whereas there was a decrease with time in GP. While SP had increased IL-1 β concentrations at this timepoint, they also ate more and grew faster than GP. On the basis of the concentrations of circulating IL-1 β found in this study along with their consistent underperformance, it seems that GP display compromised immune development. While this must be viewed as a preliminary observation, it seems that slow growing piglets displayed impaired transcription of genes associated with acquired and innate immunity (Lessard, Blais et al. 2018). Furthermore, studies of foetuses that suffer from intrauterine growth restriction (IUGR) have lower circulating cytokines in comparison with non-IUGR foetuses (Briana, Liosi et al. 2012), with IUGR piglets having reduced circulating IL-1 β concentrations in comparison with non IUGR piglets at 21 days of age (Hu, Peng et al. 2017). Gilt progeny have been reported to exhibit a degree of IUGR owing to their asymmetric organ and tissue growth patterns (Craig, Dunshea et al. 2019c), and this may contribute to the attenuated immune responses seen in GP.

Of course, there are other pro-inflammatory markers that could be been investigated such as Tumour Necrosis Factor Alpha (TNF- α), which has been shown to increase markedly in response to lipopolysaccharide (LPS) challenge in weaned pigs (Xu, Chen et al. 2018), and these should be investigated in future studies.

Gilts and sows supplemented with the POL diet ate less than their control counterparts, and this may be in part owing to its bitter flavour profile a result of the high concentration of phenolic compounds (Soares, Kohl et al. 2013). However, the POL diet did not reduce feed intake after weaning as it did for gilts and sows. The lack of dietary effect on feed intake in weaned piglets compared with their dams may have been owing to the exposure of the POL diet before weaning (King 1979, Berkeveld, Langendijk et al. 2007, Sulabo, Tokach et al. 2010). It is possible that the amount of supplemental POL was insufficient to increase dietary polyphenols adequately to elicit any production against oxidative stress. Alternatively, the background polyphenol concentrations in the control diets may have been too high to mask any effects of POL. The dam and weaner diets contained canola meal and/or soybean meal, both of which are rich sources of polyphenols (Nandasiri, Eskin et al. 2019, Freitas, Alves da Silva et al. 2019). In this context, dietary POL increased growth performance and improved meat quality in broiler chickens, with responses being most pronounced in the finisher period and when chickens were in a state of oxidative stress induced by high temperatures (Shakeri 2019). The responses were dose-dependent in a linear manner up to a dose that was twice as high as that used in the present study. Encouragingly, a holly extract rich in polyphenols and tannins has been shown to alleviate gastrointestinal inflammation in LPS challenged weaned piglets with reduced IL-6 and TNF- α concentrations being reported (Xu, Hua et al. 2020). Furthermore, holly supplemented diets altered intestinal microbiota in LPS challenged weaned piglets and improved their intestinal barrier function with upregulation of tight junction proteins such as claudin-1 and occludin observed (Xu, Hua et al. 2020). These results were seen in response to an inclusion of a commercial holly polyphenol product containing 65.5% total polyphenols. Therefore, it is proposed that further studies should investigate higher inclusion rates and should focus on weaner pigs.

6.7 Conclusions

Dietary supplementation of POL did not improve GP growth performance with GP performing poorly in the pre-weaning and post-weaning period compared with SP. The POL

diet was unable to reduce IL-1 β in GP and SP overall. By the end of the weaner component of this study, GP weighed less, ate less, and had reduced plasma IL-1 β . Collectively, this indicates that GP may exhibit reduced immunocompetence compared with SP, and further work is required in characterizing the immune response of GP.

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Chapter 7 Feeding lucerne, or diets with similar energy and nutritional profiles to lucerne, improves pre-weaning performance in gilt progeny

7.1 Abstract

Gilt progeny (GP) are characterised by their low birth weights and poor growth which present a significant cost to the pork industry. Dietary inclusion of fibre and branched-chain amino acids (BCAA) in sow gestation and lactation diets, respectively, have been shown to improve litter weights at weaning. Therefore, lucerne, which is high in fibre and BCAA, may be able to improve GP performance in the pre-weaning period. Experiment 1 investigated the effects of feeding 229 gilts either (i) a control diet; (ii) a control diet with 2 kg of lucerne chaff (LUC); or (iii) a diet formulated to have a similar fibre, amino acid and energy content as LUC (SIMLUC 1) on growth during the pre-weaning period. Diets were fed from 108 days of gestation (108 ± 0.1 days) until 3 days after farrowing. The LUC and SIMLUC 1 diets improved litter weight ($p = 0.055$) and average piglet weight ($p = 0.029$) on day 21 of life, and piglet average daily gain during the pre-weaning period ($p = 0.02$). Experiment 2 used 111 primiparous sows (gilts) and 112 multiparous sows (parities 2-5) to determine whether a diet similar in composition to SIMLUC 1 (Experiment 1) could be fed beyond parturition to improve performance of progeny (SIMLUC 2). Dams were fed either the control diet or SIMLUC 2 from day 110 of gestation to farrowing (PreF) or to day 10 post-farrowing (PreF+PostF). The heaviest litters at D21 were born to dams fed SIMLUC 2 PreF while those fed SIMLUC 2 PreF+PostF were the lightest ($p = 0.019$). Overall, gilts had lighter litters at birth ($p < 0.001$) and D21 ($p < 0.001$) compared to sows regardless of the diet they were allocated to, but SIMLUC 2 tended to be more beneficial to gilts compared to sows when fed PreF as indicated by an interaction between diet and parity ($p = 0.078$). GP on average were 170 grams heavier from the SIMLUC 2 PreF diet compared to their CON counterparts while SP were 410 grams lighter from the SIMLUC 2 PreF diet compared to their CON counterparts. Furthermore, gilt litters grew faster when gilts were fed SIMLUC 2 PreF while this was not observed in sow litters ($p = 0.048$). The improvement of D21 litter weights in gilts in response to the LUC and SIMLUC diets fed in late gestation is an important finding which demonstrates the importance of targeted feeding strategies towards gilts to maximize GP performance.

7.2 Introduction

The pig has evolved to have a high milk energy content to aid the survival of piglets born with very scant energy reserves or insulation (Mellor and Cockburn 1986). However, modern housing systems are such that pigs don't need such a high energy: protein milk to prosper, except perhaps for the first few days of life. The protein to energy content of sow's milk varies but has been reported to be less than 8.0 g/MJ (King, Toner et al. 1993) whereas the young pig requirement is approximately 11.0 g/MJ (Campbell and Dunkin, 1983, Auldist, Stevenson et al. 1997, Dunshea, Kerton et al. 2000) and independent of birth weight. Gilt progeny (GP) represent a considerable cost to the pork industry as they are characterised by low birth weights and slow growth and could benefit from strategies that could increase the protein content of sow's milk (Craig et al. 2019). However, gilts are still growing and depositing protein during lactation which may limit total milk and milk protein secretion (Pluske, Williams et al. 1998). Indeed, interventions that have stimulated maternal protein deposition in gilts during lactation such as dietary ractopamine can decrease milk protein concentrations (van Wettere, Pain et al. 2016a). Conversely, if there are strategies that can increase milk protein secretion in gilts without necessarily changing milk yield, growth performance of GP may be increased. One strategy may be the provision of additional dietary branch chain amino acids (BCAA) (Dunshea, Bauman et al. 2005).

Lucerne hay can contain high concentrations of branched-chain amino acids (BCAA), particularly leucine, relative to other amino acids (Homolka, Koukolová et al. 2008). The use of BCAA in lactation has been shown to change milk composition of the lactating sow. Dunshea, Bauman et al. (2005) found that increasing the dietary protein and BCAA contents can increase milk protein secretion but not milk yield in multiparous lactating sows. In the same study, a combined infusion of insulin and dextrose increased milk and milk protein yields but had no effect on milk protein content. Interestingly, these effects were additive and increased piglet growth rate in lactation by 24%. While infusion or provision of insulin on farm is problematic, manipulation of dietary protein and BCAA contents could provide a simple means of increasing milk protein and growth of GP. In support of this approach, Zhao, Li et al. (2019) found that increasing the dietary concentration of valine during late gestation and lactation resulted in improved colostrum lactose and IgG concentrations and increased piglet growth rate in during lactation. Therefore, it is possible that increasing the BCAA content of

late gestation and lactation diets may increase milk protein secretion particularly in gilts who are depositing protein in maternal as well as foetal tissues and milk.

In addition to having a high protein and BCAA content, lucerne is also relatively high in fibre. The inclusion of dietary fibres in late gestation diets have been reported to increase feed intake of sows during lactation and is mostly attributed to the effects on the gastrointestinal capacity of the dam (Meunier-Salaün and Bolhuis. 2014). Rooney, O'Driscoll et al. (2019) reported that feeding sugar-beet pulp at 40% to primiparous sows during late gestation resulted in piglets having increased live-weight, carcass weight and muscle depth at slaughter. Quesnel, Meunier-Salaün et al. (2008) also reported an increased feed intake and piglet weight at weaning in primiparous sows when dietary fibre was fed at 12.4 %. Moreover, dietary fibre has been shown to have beneficial impacts on behaviour and welfare of gestating sows. Edwards, Plush et al. (2019) reported an improvement in sow welfare during farrowing and throughout lactation when 1 kg of lucerne hay was given daily.

Taken together, feeding lucerne in late gestation may improve growth rate and survival of GP. However, feeding supplemental lucerne may present difficulties in effluent management, cost, quality and biosecurity issues and so alternative dietary strategies that provide a similar nutrient profile to lucerne but without the bulk may be useful. Therefore, the aims of the following studies were to determine whether supplementation of late gestation and lactation diets with lucerne or diets with similar energy and nutritional profiles can maximize GP growth performance.

7.3 Materials and Methods

The two experiments were conducted under commercial conditions at a commercial piggery in South Australia (SunPork Pty Ltd., Wasleys, SA, Australia) in April 2018 and September 2019.

7.3.1 Ethics statement

All experimental procedures were approved by PIRSA Animal Ethics Committee (licence number 247 and approval number 09/19) under the Southern Australian Animal

Welfare Act in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

7.3.2 Experiment 1

The study involved 229 gilts fed one of three dietary treatments in late gestation and during lactation. The three diets were fed either at 2.5 kg/day of a commercial gilt lactation diet (CON, $n = 72$), 2.5 kg/day of a commercial gilt lactation diet plus 2 kg/day of lucerne chaff which was delivered at the same time to a separate feeder (LUC, $n = 78$), or 3.5 kg/day of a diet formulated to have the same fibre, amino acid and energy content as the combined CON plus lucerne diet (SIMLUC 1, $n = 79$) (Table 1). All diets were fed from day 108 of gestation (108 ± 0.1 ; mean $\pm$ SE). The diets continued to be fed at *ad libitum* for the first 3 days of lactation after which all gilts received a commercial lactation diet *ad libitum*. Gilts were housed in farrowing crates fitted with nipple drinks for both dams and piglets, with piglets having access to a heat lamp in the creep area. All litters were weighed at day 1 and cross-fostering was carried out as per commercial procedures with piglets fostered within parity and diet where possible. The number of piglets born alive, born dead and mummified foetuses were recorded for each litter. Litters were weighed again at 21 days. The lucerne hay was cut after budding and was estimated to contain 18 % crude protein and 27 % crude fibre. The SIMLUC diets were formulated to have similar but not identical nutritional profiles to a combination of the 2.5 kg of the CON diet plus 2.0 kg of Lucerne and were formulated from readily available ingredients. The major changes were a decrease in wheat and an increase in barley and millrun.

Table 7.1 Composition of experimental diets fed in late gestation and 3 days after farrowing (% as fed) in Experiment 1.

Ingredients	CON	SIMLUC 1	Lucerne
Barley	0	39	
Wheat	46.8	10	
Millrun	12.7	21.9	
Field Peas	5.2	0	
Canola Meal	10	12	
Full Fat Soyabean	1.1	0	
Blood Meal	2	0	
Meat Meal	5.3	3	
Salmate Oil	0.4	0.4	
Vege Oil Blend	3.5	1	
Limestone	0.42	0.97	
Monocalcium Phosphate	0	0.4	
Betaine Liq 40%	0.6	0.3	
Lactation Concentrate Mash	12	6	
Pre-farrow Concentrate mash	0	5	
Estimated composition			
Energy (MJ DE/kg) (%)	14.3	12.8	7.5
Crude Protein (%)	21.2	17.4	18.0
Crude Fibre (%)	4.50	6.30	27.0
Ash (%)	5.3	5.9	9.5
Available SID lysine (%)	1.05	0.72	0.44
Tryptophan:Lysine (%)	0.20	0.25	0.36
Isoleucine (%)	0.77	0.73	0.71
Leucine (%)	1.57	1.19	1.28
Valine (%)	1.07	1.11	0.8
Total available BCAA: SID lysine (%/%)	3.24	4.21	6.41

7.3.3 Experiment 2

7.3.3.1 Animals, Diets and Experimental Design

A total of 223 dams, including 111 primiparous sows (gilts) and 112 multiparous sows (parities 2-5 sows) using a 2 x 3 factorial design were employed in this experiment. Dams were allocated to one of three dietary treatments (Table 2): (i) a standard commercial lactation diet fed until farrowing (control; CON) at 2.5 kg/day and then *ad libitum* to weaning; (ii) SIMLUC 2-supplemented diet fed until farrowing (pre-farrowing; PreF) at 5 kg/day; and then the CON diet *ad libitum* to weaning (iii) SIMLUC 2-supplemented diet fed at 5kg/day to farrowing, *ad libitum* until day 10 after farrowing and then the CON diet *ad libitum* to weaning (post-farrowing; PreF+PostF) (Figure 1). Following on from Experiment 1, the SIMLUC 1 diet was reformulated based on commercially available ingredients to make SIMLUC 2 since there were seasonal differences in the availability of ingredients and the nutrient content of available ingredients. Feeding of all diets commenced upon entry of sows to the farrowing house at day 110 of gestation.

Table 7.2 Composition of experimental diets fed in late gestation and lactation (% as fed) in Experiment 2.

Ingredients	CON	SIMLUC 2	Lucerne
Barley	15.6	35.4	
Wheat	41.9	19.7	
Millrun	6.5	25	
Field Peas	7.5	0	
Lentils	2.55	1.5	
Canola Meal	7.15	2.15	
Soyabean Meal	0	3.5	
Blood meal	0.8	0.7	
Meat meal	6.2	1.9	
Salmate Oil	0.4	0.4	
Vege Oil Blend	3.25	0.8	
Limestone	0.62	1.26	
Monocalcium phosphate	0	0.37	
Betaine Liquid 40%	0.48	0.40	
Breeder concentrate mash	2	4	
Lactation concentrate mash	5	0	
Pre-farrow concentrate mash	0	3	
Estimated composition			
Energy (MJ DE/kg) (%)	14.1	12.6	7.50
Crude Protein (%)	18.3	16.1	18.0
Crude Fibre (%)	4.51	6.18	27.0
Ash (%)	5.03	5.55	9.50
Available SID lysine (%)	0.85	0.70	0.44
Tryptophan:Lysine (%)	0.19	0.26	0.36
Isoleucine (%)	0.66	0.69	0.71
Leucine (%)	1.27	1.09	1.28
Valine (%)	0.89	1.06	0.80
Total available BCAA: SID lysine (%/%)	3.33	4.07	6.41

Table 7.3

Piglets were weighed at day 1 (D1) of age and individually ear tagged (n = 2,677). Farrowing performance was assessed by the total piglets born (TB), born alive (BA), still borns (SB) and mummified (MU) piglets recorded for each litter. A sub-sample of piglets (n = 1578) were weighed on day 21 (D21) to ascertain pre-weaning growth rates. The number of piglets weaned pre-weaning removals and pre-weaning mortalities were recorded

7.3.3.2 Blood sample collection

Blood samples were taken from the dams at D1 and D10 after farrowing (Figure 1). Piglet blood samples were also taken at D1 and D10. Blood samples were collected from the external jugular vein into lithium heparin Vacutainers® tubes (BD Australia, North Ryde NSW,

Australia) using a 21-gauge needle. Tubes were then inverted to ensure mixing with anti-coagulant and placed on ice. Plasma was collected following centrifugation at 1,500 x g for 10 min then stored at -20°C until analysis.

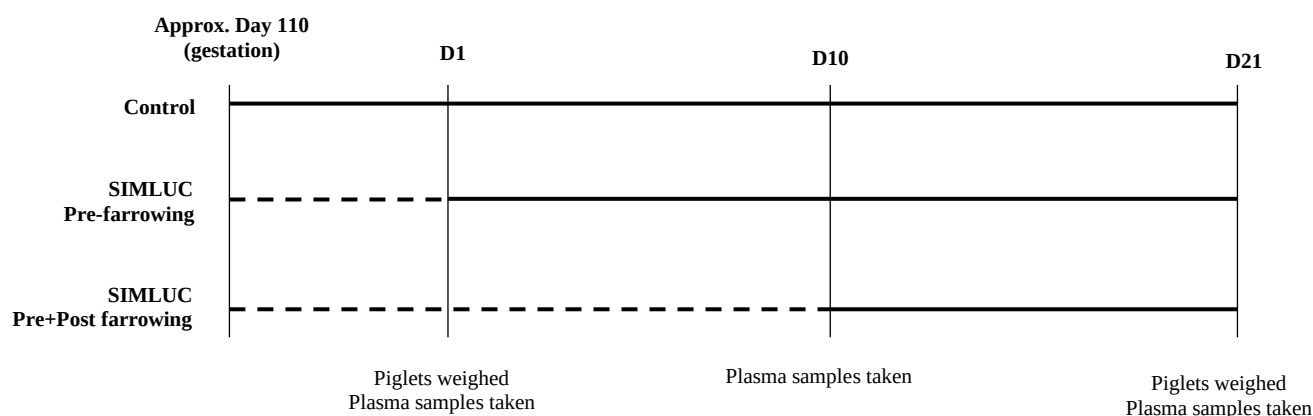


Figure 7.1 Timeline of diets and measurements taken at day 1 (D1), day 10 (D10) and day 21 (D21) after farrowing. Dashed line indicates feeding of SIMLUC and solid line CON diets.

7.3.3.3 Metabolite analysis

Plasma samples were assayed for glucose, urea, protein (catalogue no's TR15221, TR12421 and 23200 respectively, Thermo Fisher Scientific), non-esterified fatty acids (NEFA, catalogue no. 27975401, Wako Chemical Industries) and BCAA (catalogue no. ab83374) concentrations. All assays were carried out as per the manufacturer's instructions, with the exception of NEFA which was performed following a 5-fold dilution of reagents A and B with 0.025M phosphate buffer (pH 7.8) (Cottrell et al., 2003). The intra- and inter-assay variations (%CV) were 1-7% and 7-8% for glucose, 1-4% and 3% for NEFA, 1-3% and 2% for urea, 2-5% and 5% for protein and 0.1-2.3% and 4% for BCAA.

7.3.4 Statistical analyses

All data were analysed by linear mixed models using Genstat (16th edition). For analysis of Experiment 1, diet (control vs. LUC vs. SIMLUC 1) was allocated as the main factor and room allocated as the random term. The litter size on D1 and the number of days on diet prior to farrowing were fitted as a covariate when a significant contribution to the model was made. For Experiment 2, analysis of litter data was performed by designating parity (gilt vs sow) and

diet (CON vs PreF vs PreF+PostF) as the main and interactive factors while room was designated as the random term. For D1 and D10 sow and piglet metabolite data in Experiment 2, parity (gilt vs sow) and diet (CON vs SIMLUC 2) were allocated as the main and interactive factors. For sow metabolite analysis, room was designated as the random term while for piglet metabolite analysis, individual sow was designated as the random term. The number of days on diet prior to farrowing was fitted as a covariate for all analysis when a significant contribution to the model was made. Pre-weaning mortality data were analysed using a chi-square (χ^2) test. Data are presented as a $\pm$ standard error of the differences (SED). A value of $p < 0.05$ was used to indicate statistical significance and a value of $p < 0.10$ was considered a statistical trend.

7.4 Results

7.4.1 Experiment 1

Litters born to dams on the CON diet tended to have lower D21 litter weights compared to both LUC and SIMLUC 1 (48.1 vs 53.4 and 53.7 kg respectively, $p = 0.055$) (Table 7.3). Average piglet weights were lower when born to dams fed the CON diet compared to those fed the LUC and SIMLUC 1 diets (4.69 vs. 4.89 and 5.12 respectively, $p = 0.029$) at D21 (Table 7.3). Furthermore, litters of gilts fed the CON diet grew slower than litters born to gilts fed the LUC and SIMLUC 1 diets (1.80 vs 2.10 and 2.06 kg, $p = 0.031$) during the pre-weaning period. There were no other significant main or interactive effects observed (Table 7.3).

Table 7.4 Litter data of gilts fed a control (CON), lucerne (LUC) or a diet formulated to have the similar fibre, energy and amino acid content as lucerne (SIMLUC 1).

Parameter	CON	LUC	SIMLUC 1	SED	<i>p</i> - value
<i>n</i>	43	33	42		
Total born	14.1	13.9	13.9	0.342	0.77
Born alive	13.7	13.4	13.5	0.341	0.77
Still born	0.42	0.43	0.41	0.170	0.96
D1 litter size	12.1	12.9	12.2	0.217	0.37
D1 total litter weight (kg)	14.6	14.8	14.9	0.465	0.86
D1 average piglet weight (kg)	1.20	1.25	1.21	0.032	0.38
D21 litter size	9.98	10.7	10.4	0.386	0.19
D21 total litter weight (kg)	48.1 ^a	53.4 ^{ab}	53.8 ^b	2.687	0.055
D21 average piglet weight (kg)	4.69 ^a	4.89 ^{ab}	5.12 ^b	0.170	0.029
Litter ADG (kg/day)	1.80 ^a	2.10 ^b	2.06 ^b	0.125	0.031
Litter size weaned	9.97	10.3	10.5	0.391	0.40

7.4.2 Experiment 2

7.4.2.1 Pre-weaning performance

Gilt litters were lighter than sow litters on D1 (12.8 vs 15.1 kg, $p < 0.001$) and individual GP were lighter on average compared to SP (1.23 vs 1.41 kg, $p < 0.001$) (Table 7.4). Gilt litters had a lower intra-litter variation compared to sow litters (16.6 vs 19.2%, $p = 0.003$) (Table 7.4). At D21, gilts had smaller litter sizes than sows (9.31 vs 9.99, $p = 0.003$) (Table 7.4). Dams fed the CON and SIMLUC 2 PreF diets had larger litters at D21 compared to those fed SIMLUC 2 PreF+PostF (9.72 vs 10.1 vs 9.15, $p = 0.024$) (Table 7.4). Gilt litters were lighter than sow litters at D21 (45.6 vs 58.3, $p < 0.001$) (Table 7.4). Dams fed the CON and SIMLUC 2 PreF diets had heavier litters than those fed SIMLUC 2 PreF+PostF (53.3 vs 53.9 vs 48.6 kg respectively, $p = 0.019$) (Table 7.4). GP were lighter on average compared to SP at D21 (4.88 vs 5.85 kg, $p < 0.001$) (Table 7.4). Furthermore, an interaction between parity and time was observed such that GP from gilts fed SIMLUC 2 PreF+PostF tended to be the lightest out of all groups with SP of the CON diet tended to be the heaviest of all groups ($p = 0.078$) (Table 7.4). Gilt litters grew slower than sow litters during the pre-weaning period (1.678 vs 2.104, $p < 0.001$) (Table 7.4). Furthermore, dams fed the CON and SIMLUC 2 PreF diets had faster growing litters compared to those on the SIMLUC 2 diet PreF+PostF (1.99 vs 1.92 vs 1.76 kg/day, $p = 0.05$) (Table 7.4). An interaction between parity and time was observed on ADG where gilts fed SIMLUC 2 PreF+PostF diet tended to have the slowest growing litters while sows fed the CON diet tended to have the fastest growing litters ($p = 0.098$) (Table 7.4).

Table 7.5 Litter performance of gilts and sows fed either a commercial control diet (CON) or the SIMLUC 2 diet fed either pre-farrowing (PreF) or pre- and 10 days post-farrowing (PreF+PostF).

Parity (P)	Gilt			Sow			SED	<i>p</i> - value		
Diet (D)	CON	PreF	PreF+PostF	CON	PreF	PreF+PostF		P	D	P*D
Total born	12.4	12.2	11.9	12.0	13.1	12.5	0.720	0.48	0.57	0.38
Born alive	11.6	11.7	11.3	11.1	12.4	11.7	0.691	0.78	0.36	0.42
Still born	0.76	0.53	0.64	0.85	0.74	0.79	0.260	0.30	0.66	0.94
Mummified	0.23	0.08	0.35	0.29	0.26	0.25	0.134	0.54	0.31	0.32
D1 litter size	10.5	10.8	10.1	10.6	11.4	10.6	0.670	0.31	0.24	0.84
D1 total litter weight (kg)	12.9	13.3	12.1	14.7	15.9	14.8	0.808	<0.001	0.11	0.66
D1 average piglet weight (kg)	1.23	1.24	1.22	1.41	1.41	1.40	0.043	<0.001	0.84	0.98
D1 litter CV (%)	16.1	16.5	17.1	18.7	20.0	19.0	0.015	0.003	0.69	0.75
D21 litter size	9.37	9.92	8.65	10.1	10.3	9.64	0.488	0.013	0.024	0.68
D21 total litter weight (kg)	45.8	50.0	41.1	60.9	57.9	56.2	3.041	<0.001	0.019	0.19
D21 average piglet weight (kg)	4.86	5.03	4.74	6.07	5.66	5.84	0.184	<0.001	0.30	0.078
D21 litter CV (%)	18.2	19.4	18.7	18.1	19.2	19.3	0.019	0.94	0.65	0.94
D1 to D21 ADG (kg/day)	1.69	1.85	1.50	2.29	2.00	2.03	0.147	<0.001	0.05	0.098

7.4.3 Pre-weaning mortalities

7.4.3.1 Experiment 1

Overall, liveborn mortalities were highest in GP born to gilts on the CON diet compared to those on the Lucerne or SIMLUC 1 diet ($p = 0.017$) (Figure 7.2).

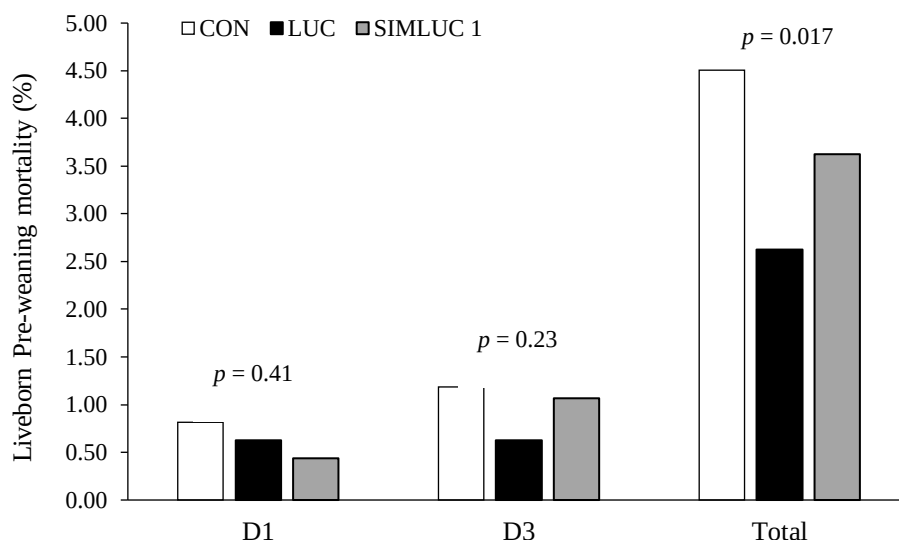


Figure 7.2 The effect of diet (CON vs LUC vs SIMLUC 1) on pre-weaning liveborn piglet mortality on day 1 (D1), day 3 (D3) and over the total pre-weaning period (Total)

7.4.3.2 Experiment 2

A higher proportion of GP died on D1 compared to SP in the first day of life ($p < 0.001$) (Figure 7.3a). No differences in mortalities between GP and SP were observed on D2 ($p = 0.93$) or D3 ($p = 0.33$) (Figure 7.3a). Overall liveborn mortalities were higher in GP compared to SP ($p < 0.001$) (Figure 7.3a). No differences between the CON and SIMLUC 2 diets were observed on liveborn mortalities on D1 ($p = 0.13$), D2 ($p = 0.51$) and D3 ($p = 0.58$) (Figure 7.3b). However, piglets born to dams fed the SIMLUC 2 PreF+PostF had higher liveborn mortalities overall ($p = 0.010$) (Figure 7.3b).

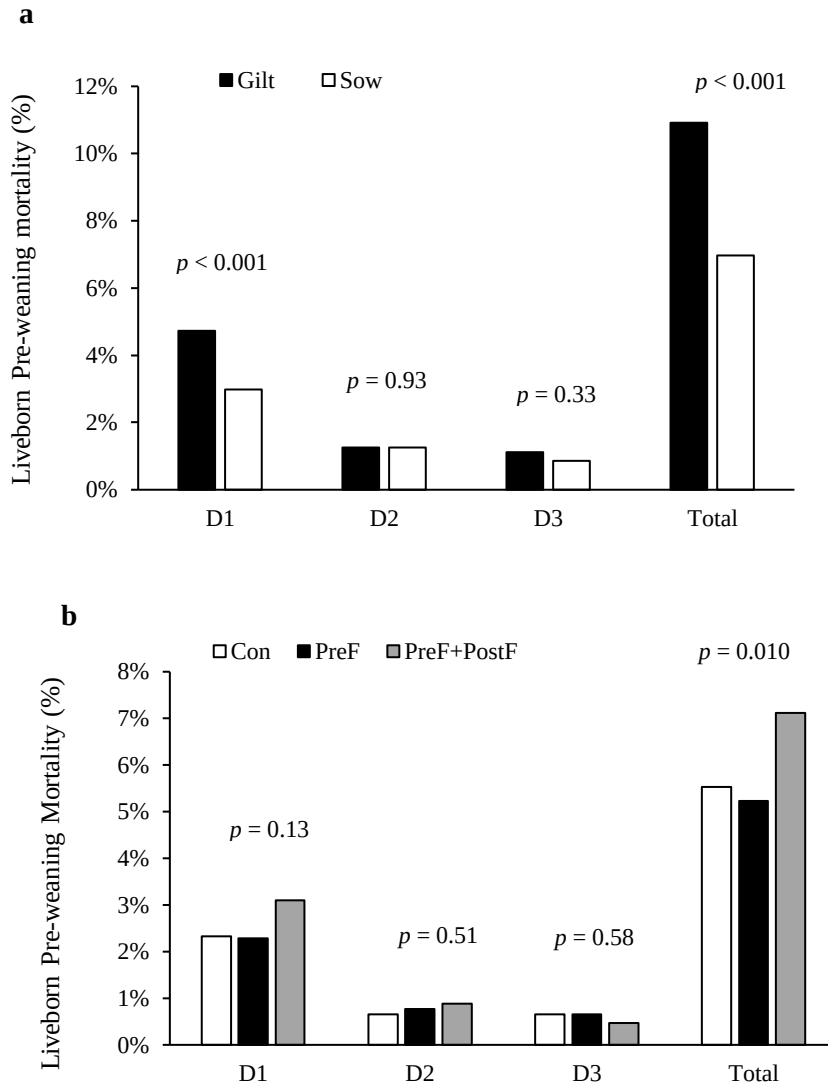


Figure 7.3 The effect of **(a)** dam parity (gilt vs. sow) and **(b)** diet (CON vs PreF vs PreF+PostF) on pre-weaning liveborn piglet mortality on day 1 (D1), day 2 (D2), day 3 (D3) and over the total pre-weaning period (Total).

7.4.4 Experiment 2 dam plasma metabolites

7.4.4.1 Day 1

Dams fed the CON diet tended to have lower plasma BCAA concentrations compared to those fed SIMLUC 2 (0.52 vs 0.64 nmol respectively, $p = 0.088$) (Table 7.5). Plasma NEFA concentrations were lower in gilts compared to sows (151 vs 327 μM respectively, $p < 0.001$) (Table 7.5). Dams fed the CON diet had higher plasma glucose concentrations overall (6.56 vs 5.67 mM respectively, $p = 0.03$) (Table 7.5). A trend for an interaction between parity and diet was observed such that sows fed SIMLUC 2 had lower glucose plasma concentrations

compared to all other groups ($p = 0.088$) (Table 7.5). There were no other significant main or interactive effects observed (Table 7.5).

Table 7.6 Plasma metabolites of gilts and sows fed either the control diet (CON) or SIMLUC 2 at D1 post-farrowing

Parity (P)	Gilt		Sow		SED	<i>p</i> - value		
Diet (D)	CON	SIMLUC 2	CON	SIMLUC 2		P	D	P*D
BCAA ⁺ nmol	-0.61 (0.54)	-0.45 (0.64)	-0.69 (0.50)	-0.43 (0.65)	0.162	0.65	0.088	0.66
NEFA ⁺ μ M	5.07 (159)	4.96 (143)	5.83 (340)	5.75 (314)	0.308	<0.001	0.64	0.96
Glucose mM	6.28 ^a	6.29 ^a	6.84 ^a	5.05 ^b	0.462	0.14	0.03	0.088
Urea mM	17.8	17.5	17.5	17.5	0.23	0.64	0.50	0.33
Total Protein pg/mL	630	631.5	610	641	33.1	0.87	0.57	0.53

^aDue to skewed data the values were Log_e transformed before statistical analysis. Back transformed means are presented in parentheses

7.4.4.2 Day 10

Dams fed the CON and SIMLUC 2 PreF had lower concentrations of plasma BCAA compared to those fed SIMLUC 2 PreF+PostF (0.45 vs 0.38 vs 0.52 nmol respectively, $p = 0.03$) (Table 7.6). Furthermore, dams fed CON or SIMLUC 2 PreF had lower plasma NEFA concentrations compared to dams fed SIMLUC 2 PreF+PostF (119 vs 138 vs 250 μ M respectively, $p = 0.017$) (Table 7.6). Dams fed the CON diet or SIMLUC 2 PreF had higher plasma glucose concentrations compared to those fed SIMLUC 2 PreF+PostF (5.69 vs 5.62 vs 4.51 mM respectively, $p < 0.001$). There were no other significant main or interactive effects observed (Table 7.6).

Table 7.7 Plasma metabolites of gilts and sows on D10 fed either a control (CON) diet or SIMLUC 2 fed pre-farrowing (PreF) or administered both pre-farrowing and 10 days post-farrowing (PreF+PostF).

Parity (P)	Gilt			Sow			SED	<i>p</i> - value		
Diet (D)	CON	PreF	PreF+PostF	CON	PreF	PreF+PostF		P	D	P*D
BCAA nmol	0.44	0.43	0.52	0.46	0.33	0.52	0.063	0.67	0.030	0.44
NEFA ⁺ μ M	4.85 (127)	4.94 (139)	5.47 (238)	4.72 (111)	4.91 (136)	5.57 (262)	0.363	0.82	0.017	0.90
Glucose mM	5.58	5.92	4.95	5.79	5.31	4.07	0.421	0.16	<0.001	0.16
Urea mM	18.0	17.9	17.9	18.1	18.2	17.5	0.262	0.76	0.21	0.11
Total Protein pg/mL	674	641	627	668	639	690	42.5	0.53	0.57	0.44

⁺Due to skewed data the values were Log_e transformed before statistical analysis. Back transformed means are presented in parentheses

7.4.5 Piglet plasma metabolites

7.4.5.1 Day 1

Piglets born to dams fed the CON diet had higher circulating glucose compared to those born to dams fed SIMLUC 2 (8.62 vs 7.61 mM respectively, $p = 0.017$) on day 1 of life. There were no other significant main or interactive effects observed (Table 7.7).

7.4.5.2 Day 10

GP had higher plasma NEFA concentrations overall in comparison to SP (570 vs 489 μM respectively, $p = 0.028$). There were no other significant main or interactive effects observed (Table 7.8).

Table 7.8 Plasma metabolites of piglets on D1 born to dams fed either a control diet (CON) or the SIMLUC 2 diet.

Parity (P)	Gilt		Sow		SED	<i>p</i> - value		
Diet (D)	CON	SIMLUC 2	CON	SIMLUC 2		P	D	P*D
BCAA nmol	0.41	0.40	0.38	0.40	0.071	0.81	0.93	0.70
NEFA ⁺ μM	5.78	5.68	5.60	5.58	0.132	0.20	0.50	0.69
Glucose mM	8.83	7.20	8.42	8.02	0.566	0.22	0.017	0.14
Urea ⁺ mM	2.86	2.87	2.84	2.90	0.024	0.35	0.63	0.68
Total Protein pg/mL	395	380	425	396	29.7	0.24	0.31	0.75

⁺ Due to skewed data the values were Log_e transformed before statistical analysis. Back transformed means are presented in parentheses

Table 7.9 Plasma metabolites of piglets on D10 born to dams fed either a control diet (CON) or SIMLUC 2 diet administered pre-farrowing (PreF) or administered both pre-farrowing and 10 days post-farrowing (PreF+PostF).

Parity (P)	Gilt			Sow			SED	<i>p</i> - value		
Diet (D)	CON	PreF	PostF	CON	PreF	PostF		P	D	P*D
BCAA nmol	0.29	0.31	0.27	0.35	0.28	0.32	0.052	0.68	0.77	0.29
NEFA μM	587	545	578	525	505	437	60.2	0.028	0.63	0.45
Glucose mM	9.36	9.55	8.86	9.36	9.16	9.50	0.451	0.91	0.58	0.24
Urea mM	17.7	18.1	17.8	17.6	18.2	18.5	0.410	0.19	0.23	0.34
Total Protein pg/mL	472	457	460	518	476	467	28.7	0.18	0.36	0.65

7.5 Discussion

The litters of primiparous sows are born and weaned lighter and exhibit poorer growth in the pre-weaning period compared to those of multiparous sows (Craig, Collins et al. 2017, Wijesiriwardana, Pluske et al. 2019). Results from the current study demonstrated that the litter weights of primiparous sows can be improved by supplementing late gestation diets with lucerne or feeding a diet nutritionally similar to lucerne. However, it seems that to increase litter weights, these diets should be administered during late gestation and ceased no later than 3 days after farrowing before the gilts are switched to a standard lactation diet. Furthermore, the beneficial effects of these diets do not seem to extend to litters of multiparous sows to the same extent as primiparous sows.

Increasing the growth of litters of primiparous sows is challenging in part due to their poorer milk yield. Milk availability is the limiting factor for piglet growth with milk yield being lower in gilts due to underdeveloped mammary tissue (Balzani, Cordell et al. 2016), lower voluntary feed intake and prolonged catabolic states during lactation (Moeller, Goodwin et al. 2004, Mallmann, Betiolo et al. 2018). Therefore, investigating nutritional interventions to target these challenges is required to improve the performance of GP. Depending on the source of dietary fibre fed during gestation, beneficial effects on sow welfare and feed intake during lactation have been observed with greater effects being reported in younger sows (Matte, Robert et al. 1994, Renteria-Flores, Johnston et al. 2008, Meunier-Salaün and Bolhuis 2014). This increased feed intake will presumably support lactation and growth of the litter. Both the LUC and SIMLUC diets contained higher crude fibre concentrations than the control diet, which may assist in explaining the improved gilt litter weights at D21. According to Jørgensen, Theil et al. (2010), dietary fibres sources such as potato pulp and sugar beet pulp can improve satiation due to increased swelling and water binding capacity resulting in delayed gastric emptying. This can result in a greater gut capacity which may stimulate feed intake during lactation. Increased gut capacity would likely be particularly beneficial for gilts due to their smaller size compared to multiparous sows, and this may also be one of the reasons for their lower feed intake and milk yield (Theil, Nielsen et al. 2012). Low feed intake during lactation has been reported in gilts (Speer and Cox 1984, Beyer, Jentsch et al. 2007, Devillers, Farmer et al. 2007, Strathe, Bruun et al. 2016, Pedersen, van Vliet et al. 2019, Craig, Dunshea et al. 2019a, Wijesiriwardana, Pluske et al. 2020) and feed intake is generally insufficient to meet their nutrient requirements during lactation. It was unfortunate that due to commercial

constraints feed intake was not measured in this study. However, the higher D21 litter weights found in response to the LUC and SIMLUC suggests improved feed intake in these gilts.

Dams consuming the SIMLUC diets consumed more BCAA with a greater ratio of BCAA to lysine compared to those consuming the control diets and when fed prior to farrowing, this also seemed to improve litter weights of both GP and SP. Inclusion of dietary BCAA in gestation can support mammary gland growth and development (Zhang, Zeng et al. 2017). Mammary gland development of the gilt increases immensely during the last third of gestation (Sørensen, Sejrsen et al. 2002, Ji, Hurley et al. 2006) with mammary uptake and utilization of BCAA increased when circulating BCAA are available (Li, Knabe et al. 2009). Isoleucine and leucine are involved in protein synthesis in the mammary gland (Lei, Feng et al. 2012) while valine supplementation has been reported to enhance milk fat synthesis (Che, Xu et al. 2019) and increase lactose in colostrum (Zhao, Li et al. 2019) when supplemented during late gestation. Increased development of the mammary gland during gestation for the gilt is imperative with mammary gland size being an indicator of milk yield (Nielsen, Pedersen et al. 2001). Taken together, it seems that the dietary inclusion of more BCAA is a promising nutritional intervention to support mammary development of the gilt and therefore a possible way to improve milk composition and yields. It must also be noted that along with increased BCAA prior to farrowing, gilts consumed more energy when switched to the CON diet after farrowing. This may have also supported higher milk yields and contributed to the increased growth of GP.

Although the SIMLUC diets were able to improve D21 litter weights in both Experiments 1 and 2, it seems that the timing and duration of feeding SIMLUC is critical. Feeding SIMLUC 2 from D110 of gestation to farrowing and feeding LUC and SIMLUC 1 to 3 days after farrowing were able to improve litter weights at D21 overall. However, extending the duration of feeding SIMLUC 2 to 10 days after farrowing had a deleterious effect on D21 litter weights of both gilts and sows. Furthermore, higher pre-weaning mortality was also seen in these piglets. This could be attributed to the lower digestible energy (DE) and lysine available in the SIMLUC diets in comparison to the CON. The fact that the CON diet had greater energy and lysine values in comparison to the SIMLUC 2 diet also cannot be disregarded and indeed the greater ADG and D21 weights observed in piglets from dams fed the CON diet in Experiment 2 during lactation maybe attributed to this and also suggests that

the low DE and lysine consumed may have been insufficient to support milk production in mid-lactation. When energy intake is limited, sows will increase lipid mobilisation to support lactation. The higher plasma NEFA concentrations in both gilts and sows fed the SIMLUC 2 diet PreF+PostF indicate a mobilization of adipose tissue lipid as plasma NEFA concentrations and NEFA entry rate closely related in pigs (Dunshea, Harris et al 1992). Furthermore, this may have been particularly detrimental for gilt litters, with the lowest litter weights at D21 found from gilts fed SIMLUC 2 PreF+PostF. With less lipid reserves to mobilize than multiparous sows and reduced energy intake, gilts of the SIMLUC 2 PreF+PostF diet were unable to support the metabolic demands of lactation. Unfortunately, P2 backfat measurements were not taken in this study and therefore, more work is required to support this theory. While the LUC and SIMLUC diets were higher in BCAA which have shown to improve protein content in milk and increase piglet weight during lactation (Dunshea, Bauman et al. 2005), the suspected effect of fibre on feed intake may have negated the beneficial effects of BCAA on piglet growth.

While feeding SIMLUC during late gestation (experiment 2) and up to 3 days after farrowing (experiment 1) was able to improve litter weights in gilts at D21, this beneficial effect did not extend to sow litters. This was also evidenced by the faster growth of GP of dams fed SIMLUC 2 PreF compared to gilts of the other groups. This finding was not observed in SP, rather the SP from the CON diet grew faster than those exposed to the SIMLUC 2 diet. This could be due to a variety of factors. Gilts experience intense growth of mammary tissue during the last third of gestation in comparison to sows who are already developed (Farmer and Hurley 2014). The amount of BCAA consumed from the SIMLUC diets may have provided more nutritional support to gilts to aid in the growth and development of mammary tissue and therefore, satisfying gilt maternal protein deposition. The beneficial effect of dietary fibre on the reduction of stereotypies in younger sows should also be considered (Meunier Salaün, Edwards et al. 2001). Edwards, Plush et al. (2019) reported that administering LUC as an enrichment was shown to reduce stereotypical behaviour in the 18-hour period prior to farrowing and more interactions with their piglets during early lactation. Alternatively, the improvement seen in GP may be attributed to the increased gastrointestinal capacity and increased intestinal residence time of digesta in the mature heavier sow compared to the growing gilt (Goff, Milgen et al. 2002). The retention time of the digesta in the gastrointestinal tract may have been longer in sows. This in turn may have suppressed their appetite for an

increased period of time resulting in reduced feed intake even after the SIMLUC 2 diet was stopped at D10. Unfortunately, feed intake was not measured in this study and therefore this is speculative and further work is required to support this.

Overall, SP outperformed GP during the pre-weaning period reflected in their heavier weights and faster growth which is in agreement with numerous studies (Town, Putman et al. 2004, da Silva, Dalto et al. 2013, Terry, Kind et al. 2015, van Wettere, Willson et al. 2016b, Craig, Collins et al. 2017, Wijesiriwardana, Pluske et al. 2019,). The poor growth of GP has been attributed to a variety of factors such as reduced milk yield (Beyer, Jentsch et al. 2007, Craig, Dunshea et al. 2019b), reduced passive immunity in the perinatal period and poor growth and development in the pre-weaning period (Craig, Dunshea et al. 2019c). Furthermore, GP have higher mortalities compared to SP in the first day of life and in the pre-weaning period overall. The neonatal period is marked by a high risk of mortality (Tuchscherer, Puppe et al. 2000) due to crushing, starvation and hypothermia (Noblet, Dourmad et al. 1997, Theil, Lauridsen et al. 2014). Furthermore, the low birth weight of GP puts them at a higher risk in this period due to low energy reserves (Vanden Hole, Ayuso et al. 2019). Nutritional interventions targeted towards late gestation may mitigate the stressors associated with the neonatal period.

7.6 Conclusion

Taken together, these experiments demonstrate that feeding lucerne or diets with similar nutritional profiles such as SIMLUC in late gestation diets can improve GP growth performance. These diets contain higher levels of BCAA and fibre that can improve mammary gland growth and support GP growth during the pre-weaning period. Furthermore, the SIMLUC 2 diet was more beneficial to GP in comparison to SP which suggests that gilts would benefit from targeted feeding strategies. However, feeding these diets to mid lactation resulted in poorer growth performance of GP and SP. Therefore, feeding LUC or SIMLUC may be more successful in improving GP performance if fed only from late gestation to very early lactation and then switched to a standard lactation diet.

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Chapter 8 General Discussion

8.1 Introduction

The main objectives of this thesis were to contribute to strategies that can improve GP performance by:

- (1) Identifying underlying biological causes that can contribute to GP underperformance;
- (2) Clarify the timeframes of when this disparity occurs;
- (3) Develop I-FABP as a biomarker to accurately quantify barrier function;
- (4) Investigate nutritional strategies that can be implemented during these timeframes to reduce the performance variation between GP and SP.

The general hypotheses examined in this thesis were that the underperformance of GP could in part be explained by their poorer gastrointestinal (GI) tract function (Chapter 3) compared to SP, and that GI tract integrity could be quantified by a biomarker, namely intestinal fatty acid binding protein (I-FABP) (Chapter 4). Results from Chapter 3 suggested that differences in GI tract function between GP and SP are exacerbated during the pre-weaning period with GP entering weaning with poorer GI function. This suggests that development of GI function is highly dependent on colostrum and milk intake, which is most likely lower in GP which contributes to their poorer GI function at weaning. However, GI barrier function could not be accurately quantified using I-FABP as a biomarker for intestinal damage and therefore, other methods of barrier quantification were used to quantify this. In order to improve GP growth in the pre-weaning period, Chapters 5, 6 and 7 investigated nutritional strategies targeted towards late gestation and lactation. Chapter 3 focused on improving the growth and survival of GP by supplementing late gestation diets with 1,3-Butanediol (1,3-BD) and hypothesised that this would increase energy available for the neonatal piglet thereby increasing birth weights of GP and improving their growth and survival in the pre-weaning period. Chapter 4 hypothesised that GP growth would be increased by supplementing late gestation and lactation diets with a sugarcane-derived polyphenol (Polygain) while also improving growth after weaning when weaner diets were supplemented with Polygain. Finally, Chapter 5 hypothesised that feeding gilts a diet high in lucerne hay (a source of branched-chain amino acids; BCAA) or a diet with a similar nutritional profile as lucerne hay (SIMLUC) could improve GP growth and improve their metabolic status.

The main findings from this thesis are listed below:

- (1) Gilt progeny are consistently born and weaned lighter and grow slower than SP;
- (2) Gilt progeny display characteristics consistent with less tract development largely in the pre-weaning period, with GP being weaned with reduced GI tract integrity;
- (3) Gilt progeny show signs of lower immunocompetence compared to SP which impedes their ability to cope with the stressors of weaning;
- (4) Nutritional interventions that support lactation performance and GP growth and metabolic parameters offer promising nutritional strategies to improve GP performance such as the supplementation of gestation diets with lucerne or diets with similar nutritional profiles to lucerne;
- (5) While nutritional interventions that support survival and growth of GP within the neonatal period are important, these interventions must also support their growth during the pre-weaning period to be successful.

8.2 Differences between GP and SP are established during the pre-weaning period

Gilt progeny in these studies were consistently born lighter (Table 8.1) and weaned lighter (Table 8.2) than SP and therefore, grew at a slower rate in the pre-weaning period. This finding was consistent across the two commercial piggeries used and is in agreement with other reports (Miller., Collins. et al. 2012, Carney-Hinkle, Tran et al. 2013, Miller, Collins et al. 2013, Craig, Collins et al. 2017, Craig, Muller et al. 2019a). The lower birth weights of GP observed in Chapters 5, 6 and 7 suggest that GP experience restricted growth *in utero* compared to their SP counterparts. This was confirmed in previous studies where GP exhibited asymmetrical growth patterns *in utero*, which are typically found in piglets suffering from intra-uterine growth restriction (IUGR) (Craig, Dunshea et al. 2019b). This restricted growth in GP would be expected based on the size of the gilt at mating and during gestation (Town, Patterson et al. 2005) and, therefore, it would be expected that GP would be born lighter than SP.

Table 8.1 Individual birth weight and litter birth weights of gilt progeny (GP) in comparison to those of sow progeny (SP) and percentage difference between progenies from different parities (%) difference

Chapter	Individual Birth weight (kg)			Litter birth weight (kg)		
	GP	SP	% Difference	GP	SP	% Difference
5	1.34	1.51	12.0	15.5	18.5	17.6
6	1.29	1.55	18.3	13.8	18.4	28.6
7	1.23	1.41	13.6	12.9	14.7	13.0

Table 8.2 Individual 21-day (21D) weight and 21D litter weights of gilt progeny (GP) in comparison to those of sow progeny (SP).

Chapter	Individual 21D weight (kg)			21D litter weight (kg)		
	GP	SP	<i>p</i> value	GP	SP	<i>p</i> value
5	5.02	6.18	20.7	47.1	66.4	34.0
6	4.85	6.37	27.1	42.2	59.7	34.3
7	4.86	6.07	22.1	45.8	60.9	28.3

The asymmetrical growth patterns in GP could also explain their poorer GI tract growth and development discussed in Chapter 1. Craig, Dunshea et al. (2019b) reported that GP have greater brain: liver and brain: body weight ratios in comparison to SP that is most likely a result of the nervous system being developed earlier in gestation to ensure the development of the brain (Hammond 1944). As the foetus grows in size throughout gestation, other organ, such as the GI tract, development at later stages of gestation (McPherson, Ji et al. 2004) may be restricted in the gilt and could result in poorer long-term function and compromised growth (Craig, Collins et al. 2017). The optimal growth and development of GI tract barrier integrity is imperative for piglet performance and health, and to this author's knowledge, this is the first study that has characterised GI tract development of GP in compared to SP. A novel finding of this thesis was that GP and SP initially displayed similar levels of intestinal barrier integrity in the first 24 hours of life, which was surprising given their different birth weights. However, by weaning, GP exhibited higher macromolecular permeability and lower barrier function (as assessed by transepithelial electrical resistance, permeability of 4kDa and 150 kDa sized molecules and tight junction abundance) compared to SP, which would likely increase their susceptibility to the post-weaning malaise and may contribute to their poorer growth after weaning (Craig, Collins et al. 2017, Craig, Muller et al. 2019a). Collectively, results from Chapter 3 indicated that the differences between GP and SP is mostly established during the

pre-weaning period culminating in poorer GI tract growth and barrier integrity at weaning. Furthermore, this disparity seems to be developed or exacerbated during the pre-weaning period, suggesting that colostrum and (or) milk consumption may be a limiting factor of GP performance.

Based on lower birth weights alone, GP are at a higher risk of pre-weaning mortality compared to SP (Quiniou, Dagorn et al. 2002). Indeed, this was observed in Chapters 5 and 7. However, no differences in pre-weaning mortality were observed in Chapter 6. This may be due to the unexpected reduction in feed intake by dams of the treatment diet and its possible confounding effect on pre-weaning mortality. Regardless, the higher pre-weaning mortality of GP compared to SP seen in Chapters 5 and 7 concurs with other studies (Holyoake 2006, Edwards, Campbell et al. 2013, Craig, Muller et al. 2019a). Interestingly, in Chapter 7, the GP had significantly higher mortality rates at day 1 after birth than SP, but this response was not present on days 2 and 3. This was surprising as sow litters have been reported to have higher piglet mortality in the first 3 days of life as higher intra-litter variation can result in a higher proportion of smaller runt piglets being overlain by the sow (Mahan 1994). The difference between Chapter 7 and the other studies may be due to different farming locations and management practices.

The disparity between GP and SP is largely established in the pre-weaning period resulting in GP displaying inferior intestinal function and lighter weights at weaning (Chapter 3, 5 and 7). Because of this, it seems that GP are unable to overcome the stressors of weaning to the same extent as SP (Chapter 6). Evidence of this was found in Chapter 6, where although feed intake was similar between GP and SP in the first week after weaning, SP ate more than GP in the second week of weaning. On a physiological level, GP and SP both suffer from significant loss of barrier integrity in the GI tract in response to weaning (Chapter 3). Despite this, SP consistently weighed more and grew faster than GP regardless of feed intake after weaning. This difference can most likely be explained by the compromised barrier integrity reflected in the higher permeability and lower TER of GP during the weaning period, specifically in distal portions of the GI tract (ileum and colon). Additionally, the increased expression of claudin-2 found in GP also support this. However, this finding is novel and therefore, further investigations must be performed in this area as previous studies of claudin-2 expression in response to stress are mainly in cultured monolayer epithelial cells. It is important to note that the full negative ramifications of weaning are at their peak at 3-5 days

after-weaning (Cera, Mahan et al. 1988, Pluske, Hampson et al. 1997), and perhaps if measurements of intestinal integrity were taken at timepoints past the 1st day after weaning, more variation in GI tract barrier integrity between GP and SP might have been observed.

To further quantify differences in barrier integrity between GP and SP after weaning, the use of I-FABP as a potential biomarker for GI tract damage was examined in Chapter 3. The development of circulating biomarkers which potentially can assess barrier integrity allow for streamlined investigations into GI tract structure and function without invasive techniques. However, the use of I-FABP in plasma to quantify differences in barrier integrity between GP and SP at weaning yielded inconclusive results and this was primarily due to the extremely low and variable concentrations obtained in young pigs. Therefore, further investigations into its use as a viable biomarker to accurately quantify plasma I-FABP were undertaken in Chapter 4. Findings from this chapter showed that commercially available kits marketed towards analysis in human plasma samples can accurately quantify I-FABP in pig plasma samples. Indeed, the use of human kits for analysing I-FABP in pig plasma has been reported in previous studies (Niewold, Meinen et al. 2004, Berkeveld, Langendijk et al. 2007). However, the plasma samples used in Chapter 4 had values below the lower limit of quantitation than the commercially available ELISA kits. Therefore, conclusions could not be drawn from these experiments based on the number of samples with undetectable concentrations of I-FABP (approximately 45%). Furthermore, the plasma samples that yielded detectable concentrations of I-FABP were highly variable within groups (weaned vs. unweaned), a finding that has also been previously been reported, specifically when information about GI function in piglets was measured during the weaning period (Berkeveld, Langendijk et al. 2007, Celi, Verlhac et al. 2019). Therefore, the use of I-FABP as a biomarker for intestinal integrity requires further investigation with more sensitive methodologies and could not be used as a reliable biomarker for intestinal integrity using the weaning or parity model in this thesis.

In addition to the reduced intestinal integrity observed in GP, findings from Chapters 3 and 6 suggested that immune development in GP do not exhibit differs from SP, and this may culminate in lower immunocompetence when dealing with the stressors of weaning. Findings from Chapters 3 and 6 demonstrated that after weaning, GP had lower jejunal concentrations of TNF- α and lower circulating IL-1 β concentrations compared to SP. While this could possibly be perceived as SP having higher levels of inflammation compared to GP, the fact that

SP are heavier, eat more and grow faster after weaning while also having higher concentrations of these pro-inflammatory markers seems to indicate that they display higher levels of immunocompetence compared to GP. This finding may help to explain the poorer performance seen in GP after weaning in Chapter 6 and in other studies (Craig, Collins et al. 2017). However, it must be stated that this is a preliminary finding and therefore more research in this area is required. Nevertheless, studies have shown that slow growing piglets do not elicit an optimal immune response compared to faster growing piglets (Lessard, Blais et al. 2018), while (Hu, Peng et al. 2017) showed that IUGR piglets have abnormal cellular and innate immune responses. Impaired or maladaptive immune responses can be a result of early excessive exposure to pro-inflammatory cytokines to the foetus (Pohl, Medland et al. 2015). In Chapter 3, high IL-8 concentrations were observed at birth in GP. While IL-8 is responsible for stimulating foetal intestinal maturation without the influx of neutrophils (Maheshwari, Voitenok et al. 2009), the fact that GP exhibited poorer intestinal development and maturation over the pre-weaning period but displayed higher amounts of IL-8 at birth indicates that, at least in this context, IL-8 abundance appear to provide some evidence for the limited immune response seen in both Chapters 3 and 6.

8.4 Supporting the growth of GP during the pre-weaning period

The success of the piglet following birth is influenced by birth weight, piglet body composition, colostrum and milk intake and subsequent growth rates, all of which can be affected by sow nutrition. Results from this thesis consistently support the theory that the pre-weaning period is where the majority of the variation in between GP and SP occurs and, therefore, represents a crucial time for GP. Targeting interventions to specifically support growth and survival during the early life for the piglet may improve GP growth and longevity as found in Chapter 5, when primiparous sows were fed 1,3-Butanediol. Furthermore, interventions targeted specifically towards supporting the nutritional requirements of gilts seem to be an effective strategy. Previous research has consistently shown that gilts are in negative energy balance during late gestation (Thomas, Goodband et al. 2018) and have lower feed intake during lactation compared to multiparous sows (Gatford, Smits et al. 2010). Results from Chapter 7 demonstrated that dietary requirements are different between primiparous and multiparous sows as reflected in the significant effects of SIMLUC on GP but not SP. It would be interesting to investigate the outcome of feeding parity-specific diets based on requirements

of the sow for maintenance and maternal protein and lipid deposition, which have been shown to differ between parities (Thomas, Goodband et al. 2018).

Previous reports of higher levels of oxidative stress in primiparous sows during gestation (Roy, Lavoie et al. 2016) and possibly lactation (Berchieri-Ronchi, Kim et al. 2011) may contribute to the poor growth of GP during the pre-weaning period. In order to improve GP performance, Polygain, a polyphenol mixture derived from sugarcane, was fed to primiparous sows due to its ability to reduce oxidative stress, which has been shown in chickens under heat stressed conditions (Shakeri, Cottrell et al. 2020). However, this targeted nutritional strategy was unsuccessful in improving growth performance of both GP and SP and therefore, it was decided not to assess indicators of oxidative stress in gilts and sows fed this diet. The experiment by (Shakeri, Cottrell et al. 2020) quantified the dose response of Polygain and observed that the more effective inclusion rate was 1% and doses of 0.5% were not effective. Therefore it appears that the dose used in this experiment may be too low. It is possible that higher doses may be beneficial, but a reduction in feed intake was observed. In other experiments molasses extracts have also reduced feed intake (Brooks and Iwanaga 1967) and it may be that pigs find higher inclusion rates of sugar cane extracts aversive. Therefore further research is required to properly ascertain the dose response relationship between Polygain inclusion rates and sow productivity.

The SIMLUC diet investigated in Chapter 7 also provided evidence of the importance of diets targeted specifically towards primiparous sows. Another likely reason for the success of this diet on improving GP performance was the inclusion of high levels of BCAA in the SIMLUC diet. The inclusion of BCAA may have provided more support to primiparous sows and aided in the growth and development of the mammary tissue in comparison to the multiparous sow who has already matured. Thomas, Goodband et al. (2018) has shown that ADG was greatest in parity 1 sows and could in part be attributed to the growth of the mammary gland. Since mammary gland size has been correlated to milk yield (Nielsen, Pedersen et al. 2001), supporting mammary gland growth during gestation of the primiparous gilt may be an effective strategy to improve GP performance.

8.5 Alternative methods to improve GP performance

While nutritional strategies may serve as short-term solutions improve GP performance, other management practices have the potential to achieve the same outcome. Gilt progeny perform poorly compared to SP due to a variety of complex reasons and management practices that directly target these reasons may improve GP performance. According to MaŁ, Tuz et al. (2018), selection criteria of gilts that include structural soundness and condition, reproductive organs, body weight and litter size at birth of the gilt selected and growth rates (of the gilt) can all be used in conjunction with nutritional strategies from this thesis.

The lower birth weights that were observed in GP in this thesis suggest that uterine capacity is restricted in gilts. Selection for increased uterine capacity in gilts has been reported to increase litter size by 1.6 pigs and increase retention in the herd between parity 1 and 2 (Freking, Lents et al. 2016), and may therefore, provide a long-term option for improving gilt performance. Litter size is a primary driver for increased milk production, and this may result in increased milk yield to support the growth of GP in the pre-weaning period.

Improving sow longevity is also an alternative strategy to reduce the impacts of poorer GP performance. Reducing the number of gilts on farm and thereby reducing the significant proportion of GP may be a possible option (MaŁ, Tuz et al. 2018). Longevity is primarily influenced by management, gilt development, nutrition, identification of genes and markers (Stalder, Knauer et al. 2004). Further studies into investigating these factors on sow longevity and their effect on GP performance is required.

8.6 Conclusions

The findings from these studies indicate that the effects of parity on piglet production is strong and the biological basis for this can be explained somewhat by poorer GI tract development during the pre-weaning period in GP, as quantified by measuring indices of intestinal barrier integrity. Circulating I-FABP concentrations appear to be low in piglets up to weaning and further work is required to ascertain if this is due to low expression of I-FABP at this time, or low appearance in the plasma. And to properly assess plasma methodologies such as mass spectrometry should be investigated, but if a relationship is established then the development of more sensitive ELISA's will be required. The pre-weaning period represents a critical time for growth and development of GP and therefore, the use of late gestation and

lactation diets which can support colostrum and (or) milk production are required. This thesis has collectively shown that signs of the disparity between GP and SP are present just after birth and are increased throughout the pre-weaning period compromising GP's ability to cope with weaning.

Directions for future research:

- (1) Nutritional interventions that improve milk yield of gilts by improving feed intake to meet the metabolic demands of lactation.
- (2) Investigations into the immunocompetence of GP during the pre-weaning period and the effect of weaning on immunity in GP.
- (3) Increasing pre-weaning growth of GP to improve their ability to cope with the stressors of weaning.
- (4) Selection for reproductive traits such as uterine capacity to improve birthweights and ultimately GP performance.
- (5) Increase longevity of sows to reduce the proportion of GP entering the herd.

8.7 References

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