Pre- and post-weaning nutritional and management strategies to increase piglet growth and reduce antimicrobial usage

A thesis presented in fulfilment of the requirements for the degree of

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By

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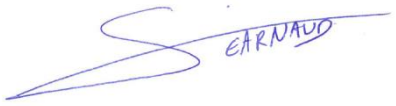
Declaration

No element of the work described in this thesis has been previously submitted for a degree at this or any other institution. The work in this thesis has been performed by the author, with supervision and guidance/support as outlined in the Author Contributions and Acknowledgments sections.

Signature:



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List of abbreviations

Abbreviation	Explanation
ADFI	Average daily feed intake
ADG	Average daily gain
AMR	Antimicrobial resistance
AMU	Antimicrobial usage
ANOVA	Analysis of variance
ASV	Amplicon sequence variant
ATP	Adenosine triphosphate
BF	Back fat
BP	Base pair
BW	Body weight
BWB	Body weight birth
C_{ADG}	Carcass average daily gain
CD	Crypt depth
cDNA	Complementary deoxyribonucleic acid
CFU	Colony forming units
$C_{G:F}$	Carcass gain to feed
CL	Carcass lean meat percentage
COX	Cyclo-oxygenase
CP	Crude protein
CW	Carcass weight
CXCL8	Interleukin 8
DADA2	Divisive Amplicon Denoising Algorithm 2
DE	Digestible energy
DM	Dry matter
DMd	Dry matter disappearance
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
DPS	Dry pelleted starter
EFSA	European Food Safety Authority
ESFs	Electronic sow feeders
ETEC	Enterotoxigenic *E. coli*
EU	European Union
FABP2	Fatty acid binding protein
FCR	Feed conversion ratio
FI	Feed intake
Fig	Figure
FYT	Phytase units
G:F	Gain to feed ratio
GE	Gross energy
GI	Gastrointestinal
GIT	Gastrointestinal tract
GLN	Glutamine
GLT	Glutamate
GLU	Glutamine
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IGF-1	Insulin-like growth factor 1

KNU	Kilo Novozymes unit
IL	Interleukin
IL6	Interleukin 6
IL17	Interleukin 17
IL18	Interleukin 18
IL22	Interleukin 22
IL23	Interleukin 23
IM	Intramuscularly
ISO	International Organisation for Standardisation
L_{ADG}	Lean average daily gain
LAB	Lactic acid bacteria
LBW	Low birth weight
LOD	Limit of detection
LMR	Liquid milk replacer
LMR+S	Mixture of liquid milk replacer and liquid starter
LPS	Lipopolysaccharide
LW	Live weight
MCFA	Medium chain fatty acid
Mel	Meloxicam
MMP1	Matrix metalloproteinase 1
MMP3	Matrix metalloproteinase 3
MRD	Maximum recovery diluent
MSG	Monosodium glutamate
MUC1	Mucin 1
MUC2	Mucin 2
NA	Not applicable
NB	Total number of piglets born
NBW	Normal birth weight
NFP	New feed protein
NRC	National research council
NS	Not studied
NSAID	Non-steroidal anti-inflammatory drug
OCLN	Occludin
PBS	Phosphate buffered saline
PCA	Principal component analysis
PCR	Polymerase chain reaction
PDS	Postpartum dysgalactia syndrome and mastitis
PLI	Lipase unit
PROC	Procedure
PRRS	Porcine reproductive and respiratory syndrome
PW	Post-weaning
PWD	Post-weaning diarrhoea
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RT	Rectal temperature
SA	Start age of supplementation
SAS	Statistical Analysis System
SCFA	Short chain fatty acid
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean
SID	Standard ileal digestibility

SLC1A4	Solute carrier family 1 member 4
SLC15A1	Peptide transporter 1
SLC2A1	Glucose transporter 1
SCL2A5	Glucose transporter 5
SLC7A1	Solute carrier family 7 member 1
Split	Split-suckling
SW	Sow weight
TJP1	Tight junction protein 1
TLR4	Toll-like receptor 4
TNF	Tumour necrosis factor
VFA	Volatile fatty acid
VH	Villus height
VH:CD	Villus height to crypt depth ratio
WA	Weaning age
WC	Weight category
WG	Weight gain
WHO	World Health Organisation
WW	Weaning weight
ZO	Zonula occludens
ZnO	Zinc Oxide

Pre- and post-weaning nutritional and management strategies to increase piglet growth and reduce antimicrobial usage.

Elisa A. Arnaud

Abstract

The objectives of this thesis were to (1) investigate the application of split-suckling with/without postpartum provision of a nonsteroidal anti-inflammatory drug to the sow on colostrum intake in suckling pigs and on lifetime growth, health, and medicinal usage in pigs; (2) determine the effect of providing a dry pelleted starter diet, a liquid milk replacer, and a liquid mixture of milk replacer and starter diet to suckling pigs, (3) assess the effect of 1% L-glutamine or enzyme supplementation of liquid creep feed and (4) determine the effect of post-weaning supplementary milk and/or dietary inclusion of 1% L-glutamine; on lifetime growth, health and medicinal usage in pigs. A single injection of meloxicam provided to sows post-partum reduced clinical cases of disease, tended to reduce medication usage in piglets and increased growth in pigs during the suckling and early post-weaning periods, and carcass weight at slaughter. This was most likely due to an increase in colostrum intake. No benefits were observed for split-suckling. Supplementing suckling piglets with liquid milk replacer or dry pelleted starter diet increased growth up to weaning but the benefit did not persist to slaughter. L-glutamine- or enzyme-supplemented liquid creep feed did not improve lifetime growth in pigs; in fact, L-glutamine tended to decrease weaning weight and to increase diarrhoea prevalence. As a post-weaning strategy, supplementing pigs with liquid milk replacer for 10 days post-weaning increased feed intake and improved small intestinal morphology, leading to increased slaughter weight. It also increased abundance of beneficial faecal bacteria and decreased expression of genes encoding pro-inflammatory cytokines. However, L-glutamine supplementation in liquid milk replacer post-weaning had no benefits. Furthermore, supplementing suckling or early-weaned pigs with a liquid diet with/without feed additives (L-glutamine or enzymes) did not influence medicinal usage.

1. Literature Review – Part 1: Selected nutrition and management strategies in suckling pigs to improve post-weaning outcomes

Adapted from[1]: Arnaud E.A., Gardiner G.E., Lawlor P.G. (2023). Selected Nutrition and Management Strategies in Suckling Pigs to Improve Post-Weaning Outcomes. *Animals*. https://doi.org/10.3390/ani13121998.

[1]Adapted to remove data generated from the experimental chapters of this thesis which were included in the published review paper but are not included here

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1.1. Introduction

Weaning is a critical period in pigs' lives, during which, they have to cope with many changes in their physical and social environment, as well as in their management and nutrition. They are separated from their mother, and piglets from different litters are mixed together, often resulting in fighting. As a result, increased levels of cortisol are often observed in pigs at weaning, indicating increased stress (Colson *et al.*, 2012). Pigs also come into contact with 'new' microorganisms which can impact their health. The diet is also modified; up to weaning, pigs suckle ~20 small meals of milk each day, which is high in fat and lactose, and at weaning, this is normally replaced by large meals of a less digestible, plant-based, solid diet (Klobasa *et al.*, 1987; Lawlor *et al.*, 2020). The physiological changes associated with weaning have been described in several reviews and will be summarized in part 2 of this literature review (Heo *et al.*, 2013; Tang *et al.*, 2022) and so will not be discussed here. All of these changes/stresses often lead to a reduction in post-weaning feed intake and weight gain (commonly referred to as a post-weaning 'growth check') (Lawlor *et al.*, 2020) and intestinal dysbiosis (Gresse *et al.*, 2017). The extent of this 'growth check' and dysbiosis depends on how rapidly the pig is able to adapt to its new circumstances. Intestinal dysbiosis is one of the leading factors contributing to post-weaning diarrhoea (PWD) (Gresse *et al.*, 2017). As reviewed by Canibe *et al.* (2022), PWD is a widespread disease that has major consequences for productivity and mortality on pig farms. Until recently, pharmacological doses of zinc oxide (ZnO; 2500 ppm of zinc) were widely included in the diet during the 2 first weeks post-weaning to prevent PWD and in-feed antibiotics were also used. However, antimicrobial resistance (AMR) has been linked with antibiotic and ZnO use in pigs (Bednorz *et al.*, 2013). The emergence of antibiotic-resistant bacteria in pigs is considered a major risk for public health, as resistant organisms can spread from pigs to humans, limiting the number of effective antibiotics available to treat human disease (Iramiot *et al.*, 2020). Therefore, in 2022, in response to this rise in AMR, the European Union prohibited all forms of routine antibiotic use in farming, including preventive group treatments and the use of medicated feed for prophylaxis (European Commission, 2019), and banned the use of pharmacological levels of ZnO in pigs (European Commission, 2017).

Intensive genetic selection has led to hyperprolific sows that give birth to more piglets than the number of functional teats available on the sow (Oliviero, 2022). This increase

in litter size has led to more heterogenous litters, with a lower average weight at birth and a higher proportion of 'light' piglets born alive. Furthermore, although the demand for colostrum and milk by the litter increases with increasing litter size, sows have a finite ability to produce both (King, 2000). Therefore, the mean volumes of colostrum and milk available for individual pigs decrease as litter size increases. This is important, as piglets need to receive at least 200 g of colostrum within the first 24 h of life to survive (Devillers *et al.*, 2011), and milk consumption is directly correlated with pre-weaning growth. Furthermore, achieving a high weaning weight is key to limiting the growth check at weaning (Lawlor *et al.*, 2020) and increasing lifetime growth (Collins *et al.*, 2017).

Several recent reviews discuss the different strategies that can be used pre-weaning to address the challenges outlined above (see Table 1-1). Rather than duplicating the content of these reviews, this review will focus on the following areas: (1) post-farrowing pain relief provision to sows, (2) split-suckling of piglets, (3) pre-weaning provision of supplementary milk and/or liquid feed, (4) strategies to stimulate earlier enzyme production in the intestine (e.g., extraneous enzyme supplementation), (5) strategies to stimulate gut structure and function (e.g., supplementing piglets with L-glutamine) and (6) strategies to modulate gut microbiota (e.g., pro-, pre- and post-biotics). These areas have not been reviewed recently. Correctly implementing these strategies can, not only increase post-weaning growth and reduce mortality, but also maximise lifetime growth in pigs.

Table 1-1. Pre-weaning nutritional and management strategies to improve growth and health of piglets raised in large litters.

Area	Strategies	Review
Sow management	• Provision of pain relief	(Schoos *et al.*, 2019),(Blavi *et al.*, 2021)
	• Provision of oxytocin to prolong the colostral phase	(Farmer and Edwards, 2022)
	• Optimisation of the farrowing environment (e.g., hygiene, temperature, noise and provision of substrates such as straw)	(Farmer and Edwards, 2022),(Baxter *et al.*, 2020)
	• Farrowing supervision and assistance	(Blavi *et al.*, 2021)
Sow nutrition	• Dietary interventions during lactation: ○ L-glutamine; ○ Spray-dried plasma (porcine or bovine); ○ Polyunsaturated fatty acids (Omega 3 and 6); ○ Vitamin E and selenium; ○ Probiotics (e.g., *Bacillus*); ○ Prebiotics (e.g., fructo oligosaccharides).	(Wensley *et al.*, 2021) (Blavi *et al.*, 2021) (Blavi *et al.*, 2021),(Wensley *et al.*, 2021) (Blavi *et al.*, 2021) (Blavi *et al.*, 2021) (Farmer and Edwards, 2022)
Piglet management	• Split-suckling	(Farmer and Edwards, 2022)
	• Cross-fostering	(Blavi *et al.*, 2021)
	• Nurse sows	(Farmer and Edwards, 2022)
	• Optimised farrowing environment: ○ Supplementary heat; ○ Provision of substrates such as straw; ○ Optimisation of feeder type.	(Farmer and Edwards, 2022) (Farmer and Edwards, 2022) (Middelkoop, 2020)
	• Increasing weaning age	(Lawlor *et al.*, 2020)
	• Split-weaning	(De Vos *et al.*, 2014)
	• Intermittent suckling	(Wensley *et al.*, 2021)

	• Artificial rearing	(Farmer and Edwards, 2022) (Baxter *et al.*, 2020)
	• Socialisation (mixing piglets before weaning)	(Wensley *et al.*, 2021)
Piglet nutrition	• Injection of glucose (energy booster)	(Farmer and Edwards, 2022)
	• Oral supplementation:	
	○ Amino acids (e.g., glutamine);	(Huting *et al.*, 2021)
	○ Colostrum (bovine, sow or replacer);	(Blavi *et al.*, 2021)
	○ Medium chain fatty acids;	(Farmer and Edwards, 2022)
	○ Prebiotics (e.g., inulin, β-glucans and oligosaccharides);	(Huting *et al.*, 2021)
	○ Faecal microbiota transplant;	(Canibe *et al.*, 2019)
	○ Nucleotides.	(Blavi *et al.*, 2021)
	• Creep feeding:	
	○ Supplementary solid feed;	(Wensley *et al.*, 2021)
	○ Supplementary liquid feed (mix of feed and water or milk);	(Blavi *et al.*, 2021)
	○ Supplementary milk.	(Blavi *et al.*, 2021)
	• Dietary intervention through creep feed:	
	○ Amino acids (e.g., glutamine);	(Huting *et al.*, 2021)
	○ Fat sources rich in medium chain fatty acids;	(Huting *et al.*, 2021)
	○ Fibre sources;	(Huting *et al.*, 2021)
	○ Flavours;	(Tokach *et al.*, 2020)
	○ Prebiotics (e.g., oligofructose);	(Huting *et al.*, 2021)
	○ Synbiotics;	(Huting *et al.*, 2021)
	○ Probiotics (e.g., yeast).	(Huting *et al.*, 2021)

1.2. Management strategies in sows and suckling pigs to increase colostrum intake

A large litter size has consequences for uterine capacity and the post-natal life experience of piglets (Rutherford *et al.*, 2013). This review will focus on the latter. Piglets need to receive at least 200 g of colostrum within the first 24 h of life to survive (Devillers *et al.*, 2011), and 250 g to ensure optimal growth (Hasan *et al.*, 2019). In large litters, some piglets often fail to ingest a sufficient quantity of colostrum during the first 24 h. This is critical as colostrum contains immunoglobulins, 80% of which are immunoglobulin G (IgG), which are of primary importance for the transfer of passive immunity from the sow to the piglets (Curtis and Bourne, 1971). Colostrum also provides energy to new-born piglets, as pigs have low energy reserves when born (Dividich *et al.*, 2005). It contains other biological components of importance for pig development and health, such as leukocytes (Dividich *et al.*, 2005) and various growth factors (Xu *et al.*, 2002). It also has laxative properties which are essential in helping to eliminate the first stool. Colostrum quality decreases rapidly during the first 24 h postpartum (Quesnel *et al.*, 2012), with the highest immunoglobulin concentrations found within the first 4 h postpartum (Klobasa *et al.*, 1987). If the optimal quantity of colostrum ingested per piglet during the first 24 h of life is set at 250 g (Quesnel *et al.*, 2012), and given an average number of piglets born alive per litter of 15, a nursing sow needs to produce at least 3.75 kg of colostrum within the first day postpartum. From data collected from an experimental herd, Quesnel *et al.* (2012) estimated that among 200 sows, 35% do not even produce the 3.25 kg of colostrum required to fulfil the needs of 13 piglets. Therefore, considering the importance of achieving an adequate intake of colostrum for the development and health of the pig, it is necessary to implement strategies to ensure that each pig within a litter receives an equal and adequate share of the colostrum available within the first 24 h of birth. Such strategies are discussed below.

1.2.1. Pain management in sows

Piglets commence suckling their sow within minutes after their birth. The sow must be comfortable in order to facilitate suckling by her litter so that piglets consume adequate quantities of colostrum and then milk. If a sow lies quietly, it is assumed that

piglets have ready access to the udder and therefore unlimited access to colostrum and milk (Fraser, 1984). It is generally accepted that parturition is a painful process and that post-farrowing pain and inflammation can impede the sow's ability to nurse. Farrowing leads to both visceral pain (Blavi *et al.*, 2021) (e.g., 'pain from the inner organs including pain manifested at the udder and dependent on the conduction of pain information through activation of visceral afferent fibres' (Herskin and Di Giminiani, 2018)) and somatic pain (Blavi *et al.*, 2021) [e.g., 'pain arising from damaged skin, joints bones or muscles and dependent on activation of somatic afferent fibres' (Herskin and Di Giminiani, 2018)]. In sows, inflammatory damage can still be observed one week after farrowing, as demonstrated by high levels of C-reactive protein and haptoglobin in the blood (Kovac *et al.*, 2008). Several factors can impact the degree of inflammation and pain caused by the farrowing process, such as prolonged farrowing duration and parturition difficulties, also referred to as dystocia (Blavi *et al.*, 2021).

Providing non-steroidal anti-inflammatory drugs (NSAIDs) and NSAID-like drugs to the sow around farrowing can alleviate the associated pain in the sow and therefore increase her receptiveness to suckling by her piglets. As reviewed by Schoos *et al.* (2019), NSAIDs have antipyretic, analgesic and anti-inflammatory effects, while NSAID-like drugs have only antipyretic and analgesic effects. To our knowledge, in 2023, there were five NSAIDs (meloxicam, flunixin, tolfenamic acid, ketoprofen and sodium salicylic acid) and two NSAID-like drugs (paracetamol and metamizole) authorised by the European Medicines Agency for use in pigs (European Medicines Agency - Science Medicines Health, 2023). Most NSAIDs act by inhibiting the enzymes cyclo-oxygenase 1 (COX-1) and 2 (COX-2). Some of them, such as meloxicam, are selective COX-2 inhibitors. Cyclo-oxygenases are involved in the conversion of arachidonic acid into thromboxanes, prostaglandins and prostacyclins, which have a role in platelet adhesion, vasodilation, antinociception and body temperature set-point determined in the hypothalamus (Ghlichloo and Gerriets, 2021). Cyclo-oxygenase 1 is always expressed in the body and plays a role in maintaining gastrointestinal mucosal integrity, whereas COX-2 is only expressed during an inflammatory response (Ghlichloo and Gerriets, 2021). Therefore, selective COX-2 inhibitors, such as meloxicam, when administered to provide postpartum pain relief,

provide the required anti-inflammatory benefits without compromising intestinal mucosa integrity (Ghlichloo and Gerriets, 2021; Chaiamnuay *et al.*, 2006).

Table 1-2 summarises the findings of studies that have used NSAIDs or NSAID-like drugs in sows around the periparturient period. NSAIDs or NSAID-like drugs can be administered orally via gavage (Mainau *et al.*, 2016; Navarro *et al.*, 2021; Schoos *et al.*, 2020), intramuscularly (Mainau *et al.*, 2012; Tenbergen *et al.*, 2014; Tummaruk and Sang-Gassanee, 2013; Claeyé *et al.*, 2015; Viitasaari *et al.*, 2013; Homedes *et al.*, 2014; Ison *et al.*, 2018) or orally with feed (Kuller *et al.*, 2021). In general, the timing of administration ranges from 1.5 h (Mainau *et al.*, 2012; Ison *et al.*, 2018) to 12 h postpartum (Tenbergen *et al.*, 2014; Claeyé *et al.*, 2015; Homedes *et al.*, 2014) for intramuscular injections in healthy sows. When given orally, the drug was provided at the beginning of the farrowing process in two studies (Mainau *et al.*, 2016; Navarro *et al.*, 2021). In two other studies, oral administration started 2 to 3 days before parturition and was repeated daily for up to 4 days postpartum (Schoos *et al.*, 2020; Kuller *et al.*, 2021). The results from these studies indicate that the use of NSAID drugs can benefit both the sow (Tummaruk and Sang-Gassanee, 2013; Kuller *et al.*, 2021; Viitasaari *et al.*, 2013) and the piglets (Mainau *et al.*, 2016; Navarro *et al.*, 2021; Homedes *et al.*, 2014). Meloxicam does not reduce fever in sows postpartum (Mainau *et al.*, 2012; Tenbergen *et al.*, 2014), while flunixin (Tummaruk and Sang-Gassanee, 2013) and ketoprofen (Claeyé *et al.*, 2015) do. Ketoprofen (Viitasaari *et al.*, 2013) and paracetamol (Kuller *et al.*, 2021) reduced the back fat loss experienced by sows during lactation. The reduction in back fat loss can be explained by the decrease in feed refusal in sows treated with these drugs during the treatment period (Viitasaari *et al.*, 2013). When administered orally at the beginning of farrowing, meloxicam positively influences piglet growth during lactation, but it does not seem to affect piglet mortality (Mainau *et al.*, 2016; Navarro *et al.*, 2021). On the contrary, when administered intramuscularly after parturition, ketoprofen reduced mortality (Homedes *et al.*, 2014; Claeyé *et al.*, 2015) but failed to demonstrate a positive growth effect (Viitasaari *et al.*, 2013; Claeyé *et al.*, 2015). Few studies compared NSAIDs or NSAID-like drugs within the same study (Tummaruk and Sang-Gassanee, 2013; Schoos *et al.*, 2020; Hirsch *et al.*, 2003). However, Schoos *et al.* (2020) treated sows suffering from postpartum dysgalactia syndrome (PDS) with either meloxicam or paracetamol. Rectal temperature, piglet mortality and growth were not affected by either treatment

compared to control untreated sows. However, the rectal temperature was lower in sows treated with paracetamol compared with meloxicam (Schoos *et al.*, 2020). Likewise, Hirsch *et al.* (2003) did not observe any differences between meloxicam and flunixin regarding their ability to reduce clinical signs of mastitis–metritis–agalactia syndrome. However, in this study, mortality was lower in piglets born to meloxicam-treated sows compared with those treated with flunixin (Hirsch *et al.*, 2003). Comparing flunixin to metamizole administration, rectal temperature was lower in sows treated with flunixin (Tummaruk and Sang-Gassanee, 2013). Postpartum NSAID administration to sows can also increase immunoglobulin transfer from sows to piglets. Higher levels of IgG (Mainau *et al.*, 2016) and IgA (Navarro *et al.*, 2021) were observed on day 1 after birth in the serum of piglets suckling sows supplemented with meloxicam. In the study by Navarro *et al.* (2021), the higher level of IgA persisted until day 9 after birth. The better immunity acquired by piglets from sows which received meloxicam could explain the better growth experienced by those pigs pre-weaning. However, to our knowledge, there are no published studies investigating the effects of NSAID provision to sows on pig growth post-weaning or on medication usage pre-and post-weaning.

While it is evident that many of the NSAID and NSAID-like drugs, when administered around farrowing, can confer benefits to both sows and piglets, there is no clear consensus on the drug of choice to use. However, as meloxicam can benefit serum immunoglobulin status in piglets and increase piglet survivability and growth in suckling and weaned pigs, it might be particularly beneficial. From these studies, it appears that NSAIDs and NSAID-like drugs should be provided within 2 h postpartum to the sow when administered intramuscularly and at the beginning of farrowing when given via oral gavage.

Table 1-2. Overview of the efficacy of non-steroidal anti-inflammatory drugs administered to sows in sows and piglets during the periparturient period. Treated sows were compared with untreated sows, unless otherwise stated [modified from Schoos *et al.* (2019)].

Medication	Dose	Route of Administration	Timing	Effects on Sows	Effects on Piglets	Reference
Meloxicam	0.4 mg/kg BW [1]	Intramuscular	~90 min postpartum	↓ time lying during day 3 postpartum = FI [2] = RT [3]	= mortality ↑ ADG [4] of low birth weight piglets (<1180 g) from multiparous sows	(Mainau *et al.*, 2012)
Meloxicam	0.4 mg/kg BW	Intramuscular	~12 h postpartum	= RT	= mortality ↑ litter size at weaning ↗ weight gain in litter of 11–13 pigs	(Tenbergen *et al.*, 2014)
Meloxicam	0.4 mg/kg BW	Oral gavage	Beginning of farrowing	NS [5]	= mortality ↑ ADG and weaning weight ↑ IgG [6] in serum on day 1 and 2	(Mainau *et al.*, 2016)
Meloxicam/ paracetamol	0.4/30 mg/kg BW	Oral gavage	Once a day for 7 days from day 113 of gestation (sows with PDS [7])	= RT paracetamol ↓ RT vs. meloxicam	= mortality = ADG	(Schoos *et al.*, 2020)
Meloxicam	0.4 mg/kg BW	Oral gavage	Beginning of farrowing	↗ colostrum IgA [8] and IgG	= mortality ↗ ADG from day 9 to weaning ↑ IgA in serum on day 1 and 9	(Navarro *et al.*, 2021)

					↗ IL-2 [9] and IL-4 [9] in serum on day 9	
Meloxicam/Flunixin (no untreated sows)	0.4/2 mg/kg BW	Intramuscular	1.5–24 h post-clinical PDS signs	FI: meloxicam = flunixin RT: meloxicam = flunixin	Mortality: meloxicam < flunixin ADG: meloxicam = flunixin	(Hirsch *et al.*, 2003)
Flunixin/ Metamizole (no untreated sows)	0.5/50 mg/kg BW	Intramuscular	End of parturition +24 h later if needed	Flunixin: ↓ RT (day 1 vs. day 3 postpartum) Metamizole: = RT (day 1 vs. day 3 postpartum)	NS	(Tummaruk and Sang-Gassanee, 2013)
Paracetamol	20 mL of paracetamol (400 mg/mL)	Over the feed divided over two meals	6 days from 3 days before farrowing to 2 days postpartum	= RT ↑ back fat at weaning	= mortality = ADG = IgG in serum on day 1	(Kuller *et al.*, 2021)
Ketoprofen	3 mg/kg BW	Intramuscular	During 3 days postpartum	↓ incidence of feed refusal ↑ back fat in week 2 ↓ constipation duration	= ADG	(Viitasaari *et al.*, 2013)
Ketoprofen	3 mg/kg BW	Intramuscular	Within 12 h postpartum	NS	↓ mortality ↑ litter size at weaning	(Homedes *et al.*, 2014)
Ketoprofen	1 mg/kg BW	Intramuscular	Within 12 h postpartum	= back fat at weaning ↓ RT	↘ mortality = weight gain per litter	(Claeyé *et al.*, 2015)
Ketoprofen	3 mg/kg BW	Intramuscular	1.5 h postpartum	= putative pain behaviours = salivary cortisol	NS	(Ison *et al.*, 2018)

	= C-reactive protein
	= cytokines

[1] BW, body weight; [2] FI, feed intake; [3] RT, rectal temperature; [4] ADG, average daily gain; [5] NS, not studied; [6] IgG, immunoglobulin G; [7] PDS, postpartum dysgalactia syndrome and mastitis; [8] IgA, immunoglobulin A; [9] IL-2(4), Interleukin-2(4); ↑ significant increase; ↗ tendency to increase; ↓ significant decrease; ↘ tendency to decrease; = no difference.

1.2.2. Split-suckling

Cross fostering is sometimes used on-farm to help increase colostrum intake in piglets. However, it seems that only maternally-derived cells can cross the gut barrier in the neonate and that cells from a foster mother's colostrum are not well absorbed by cross-fostered piglets (Bandrick *et al.*, 2011). Additionally, cross-fostering is usually conducted too late after farrowing (normally after 24 h), and while it will help to ensure milk intake for all pigs, it does little to increase colostrum intake. Contrary to this, split-suckling can help to ensure that all piglets within large litters get a chance to suckle and therefore consume sufficient colostrum during the critical early postpartum window. Split-suckling is defined as the removal of part of the litter from the sow for a set period of time to allow the remaining piglets to suckle the sow without competition (Donovan and Dritz, 1996). The strategy is particularly useful when the number of piglets born alive per sow is high, exceeding the number of functional teats available on the sow, and where fostering options are limited (Baxter *et al.*, 2013). Split-suckling should allow all piglets to access colostrum, and thereby to acquire passive immunity (Baxter *et al.*, 2013) and sufficient energy immediately after birth. However, there is no consensus on how to apply split-suckling in terms of its duration and timing, the number of piglets removed and the number of piglets left on the sow, and the category (weight, birth order, etc.) of piglets to remove (Vandaele *et al.*, 2020). The litter can be split by removing only the heaviest piglets for a period of time while leaving the lightest piglets to suckle (Kyriazakis and Edwards, 1986; Donovan and Dritz, 2000). Another way to apply split-suckling is to take into consideration birth order and to remove the first-born piglets, giving the piglets born later time to access colostrum (Morton *et al.*, 2019). It might also be interesting to assess the litter and remove piglets with a 'full' belly (which have already ingested colostrum) regardless of weight or birth order. The length of time piglets are removed from the udder varies across studies (Muns *et al.*, 2015; Morton *et al.*, 2019; Vandaele *et al.*, 2020). However, it is generally recommended to leave the first group of pigs for 1 h at the udder before placing back the piglets which were removed. The first day of life is the most critical period to apply split-suckling, as the quantity and quality of colostrum decreases rapidly during the first 24 h following parturition (Quesnel *et al.*, 2012). However, as most piglet mortality occurs during the first 3 days of life (Galiot *et al.*,

2018), some propose the application of split-suckling until day 3 postpartum, alternating groups every 3 h for 12 h per day (Vandaele *et al.*, 2020). In addition, a study from Donovan and Dritz (2000), in which ADG within litters was more homogeneous in litters with more than nine pigs born alive, suggests that split-suckling is only beneficial in large litters.

As outlined above, there is no consensus on the best split-suckling strategy to apply, and results vary considerably between studies. Muns *et al.* (2015) failed to demonstrate beneficial outcomes in terms of piglet growth and survival when they removed the heaviest piglets (>1.30 kg) for 2 h within the first 24 h after birth (Muns *et al.*, 2015). However, other authors reported increased piglet growth (Morton *et al.*, 2019) and increased survival of small piglets (Huser *et al.*, 2015) when split-suckling was applied during the 1st day after birth. In one of these studies, Morton *et al.* (2019) reported a ~17% increase in piglet growth up to day 7 postpartum for all pigs when the six heaviest piglets were removed from the udder for 1.5 h, leaving the remainder of the litter to suckle without competition during this time window. Huser *et al.* (2015) reported a 13% increase in the pre-weaning survival rate for small piglets in litters where heavy piglets (>2.08 kg) were removed once for 2 h in the morning following farrowing.

The timing and duration of the split-suckling bout and how many times split-suckling bouts are conducted will all influence the success observed with split-suckling. For example, Vandaele *et al.* (2020) conducted split-suckling during the first 3 days of life by alternating two groups of the heavier piglets at the udder every 3 h for 12 h per day, while the smallest piglets always remained with the sow, the aim being to provide them with a nursing advantage (Vandaele *et al.*, 2020). However, this split-suckling strategy reduced the growth of all pigs and did not increase the colostrum intake or survival of the smallest piglets (Vandaele *et al.*, 2020). To our knowledge, there is no published study investigating the effects of split-suckling on pig growth post-weaning. Based on the above, it would seem important for the success of split-suckling that it is conducted within the first 24 h of the piglet's life and that piglets are not removed from their sow for more than 2 h during split-suckling bouts. In the case of successful split-suckling strategies, increases in piglet growth up to weaning could lead to

improved post-weaning growth, as weaning weight is highly correlated to subsequent lifetime growth in pigs (Collins *et al.*, 2017).

1.3. Nutritional strategies in suckling pigs to improve growth and intestinal maturity at weaning

1.3.1. Strategies to help maximise dry matter intake in piglets prior to weaning

Pre-weaning strategies that effectively increase nutrient intake and growth in piglets up to weaning are important since weaning weight is positively correlated with subsequent health and growth in pigs (Collins *et al.*, 2017). Increasing weaning weight and ensuring good intestinal health at weaning can help pigs to overcome the normal stresses associated with weaning. Creep feeding suckling piglets with dry feed, liquid milk replacer and/or liquid feed are strategies which can help increase pre-weaning dry matter intake (DMI) and consequently growth, resulting in heavier pigs at weaning.

1.3.1.1. Provision of solid creep feed pre-weaning

The provision of dry creep feed to suckling piglets is a common practice which has previously been well reviewed (Huting *et al.*, 2021; Tokach *et al.*, 2020). Creep feed provision has the primary objective of supporting sow milk production, as this becomes a limiting factor for piglet growth during mid-lactation, especially in large litters (Tokach *et al.*, 2020). In addition, providing dry creep feed to suckling piglets can help to habituate them to solid feed prior to weaning, increase feed intake and growth and improve intestinal structure and function post-weaning. The effects of dry creep feeding on pre- and post-weaning growth are not always consistent; some studies find the practice beneficial, while others do not [reviewed by Tokach *et al.* (2020)]. Inconsistencies in the response to the dry creep feeding of suckling piglets can be explained by the different approaches to creep feed provision taken by the authors. The duration of creep feeding and piglet age at weaning were reviewed by Tokach *et*

al. (2020) as two important factors affecting the response to creep feeding. Studies in which litters were weaned at 35 days of age or greater demonstrated a consistent gain in weaning weight with creep feeding (Tokach *et al.*, 2020), which was most likely due to increased creep feed consumption with increasing weaning age. Creep feeding can start as early as two days of age to as late as a couple of days before weaning (Tokach *et al.*, 2020). The percentage of piglets within each litter eating creep feed (i.e., piglets considered as "eaters") can also explain differences in outcomes between studies, with litters with a higher proportion of 'eaters' benefitting most from creep feeding. Several factors can affect the creep feed intake of individual pigs, such as the availability of the sow's milk (e.g., if the pig has access to a teat producing a low quantity of milk), the piglet's birth weight, the size of the pellets provided, the creep feeding duration, the composition of the creep feed itself and its accessibility and organoleptic properties [as reviewed by Huting *et al.* (2021) and Tokach *et al.* (2020)]. The use of flavours in dry creep feed has also been well reviewed to date, and it would appear that there is a lack of effect on creep feed intake and pig growth, most likely due to variable palatability preferences and perceptions between piglets (Tokach *et al.*, 2020). Providing dry creep feed can help to develop the intestinal tract so that it can cope better with the post-weaning diet, and this is a principal benefit of the practice. It may stimulate earlier enzyme secretory capacity in the gastrointestinal tract (GIT) of piglets, thereby enabling the digestion of non-milk ingredients normally found in diets after weaning. The effects of providing dry creep feed on gut structure are not always consistent either [as reviewed by Huting *et al.* (2021)]. In order to obtain the greatest benefit, it is generally accepted that creep feed should be offered in small amounts to avoid feed wastage and to keep the feed as fresh as possible. Creep feed supplementation should be started on day 7 to 10 of age to maximise intake. In practice, creep feed intakes can be very variable. Therefore, providing creep feed in liquid form might be a solution to promote intake.

1.3.1.2. Provision of supplemental milk pre-weaning

Providing piglets with a liquid diet (supplementary milk or a diet mixed with milk/water) pre-weaning could be a promising strategy to increase creep feed intake

prior to weaning. This strategy could reduce the feed neophobia experienced by suckling piglets toward solid feed, increasing DMI and the number of eaters, thereby positively influencing weaning weight (De Greeff *et al.*, 2016) and post-weaning growth (Wolter *et al.*, 2002). The provision of a supplementary milk replacer to suckling piglets enables the rearing of large litters while they continue suckling their mother (Pustal *et al.*, 2015). However, there is no consensus regarding when milk replacer provision should commence, how often milk replacer should be offered during the day and for how long during lactation the practice should be implemented. Supplemental milk can be provided during the entire lactation period starting from 24 h after farrowing (Azain *et al.*, 1996; Douglas *et al.*, 2014) or for a shorter amount of time starting 5 to 10 days before weaning (Dunshea *et al.*, 1999; Van Oostrum *et al.*, 2016). Milk can be supplemented ad libitum (Wolter *et al.*, 2002; Pustal *et al.*, 2015), or access to milk may be restricted to a set period of time each day (Park *et al.*, 2014; De Greeff *et al.*, 2016). Milk can be prepared and fed to piglets manually or through an automated delivery system. Automated delivery systems for supplemental milk replacer are now quite common on European farms (see schematic, Figure 1). These systems mix milk replacer powder with warm water at a pre-determined concentration. The feeding frequency can be set to approximate ad libitum feeding. Usually, fresh milk is prepared at least twice daily. Where milk cups are used, the cups contain a push lever which piglets use to release milk (Kobek-Kjeldager *et al.*, 2021b) and milk is available on demand (e.g., Neopigg™ RescueCare system by Cargill, United States; CulinaCup by Big Dutchman, Germany). Alternatively, some systems contain sensors within troughs, and when the milk in the trough is below the level of the sensor, fresh milk is delivered to that trough at the next pre-determined feeding time (e.g., Babyfeed system by Schauer Agrotronic GmbH, Austria; CulinaFlex by Big Dutchman, Germany). Regardless of the milk feeding system type, it is essential for the system to be hygienic; systems are normally flushed with an acid daily and an alkaline detergent flush is performed once per week, but sometimes as infrequently as once a month. This is carried out to minimise biofilm formation and to help to ensure good microbial quality in the milk. By cleaning with peracetic acid daily and using an alkaline detergent once a month, Pustal *et al.* (2015) did not observe any increases in bacterial counts in milk sampled from the tank at the end of the day every 5 days from the 3rd

to the 23rd day of supplementation. However, bacterial growth was not monitored in the tank during the day.

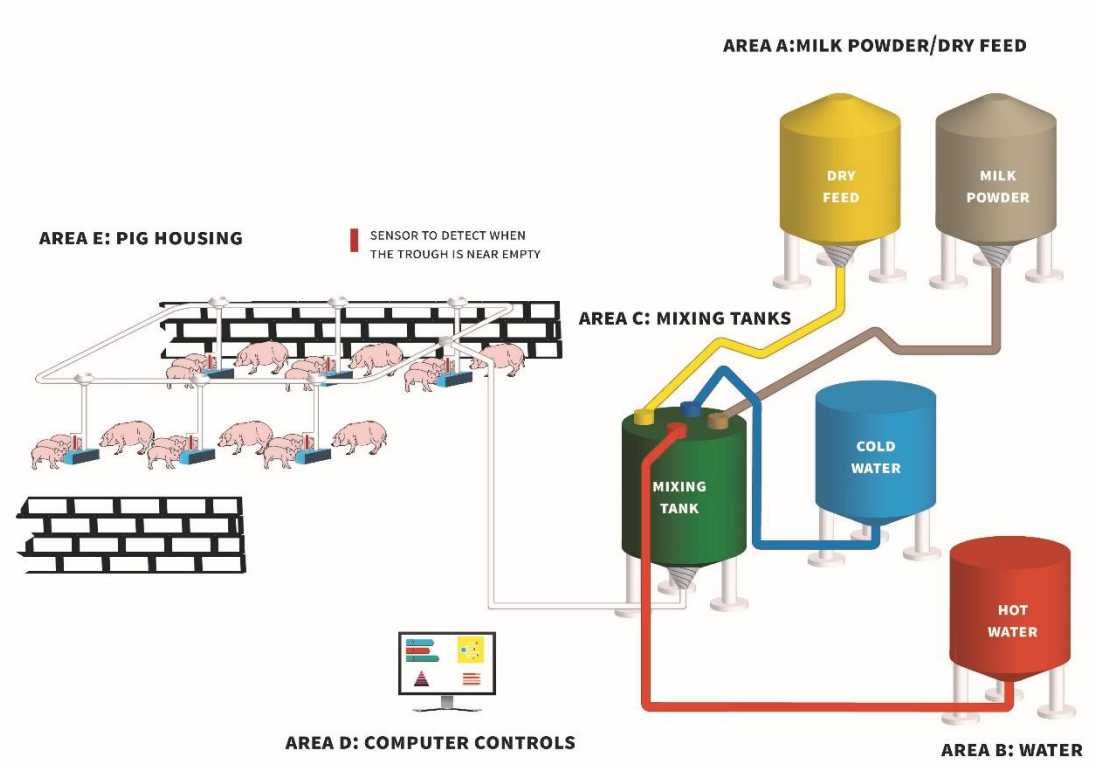

Figure 1-1. Diagram of a typical automated liquid milk/feed delivery system (based on the Babyfeed system; Schauer Agrotronic GmbH, Austria) demonstrating how milk powder/dry feed from feed bins (Area A) and water (Area B) are delivered to a central mixing tank (Area C) and agitated, followed by the delivery of liquid milk/feed to farrowing pens via a series of pipes for consumption by piglets (Area E).

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Table 1-3 summarises the effect of milk replacer supplementation to suckling litters on pre-weaning and post-weaning piglet growth and health. Milk intakes are very variable within and between litters. Intakes can be influenced by several factors, such as room temperature or the quantity of milk produced by the sow [as reviewed by Huting *et al.* (2021)]. Several studies showed an increase in pre-weaning ADG and weaning weight when suckling piglets were supplemented with liquid milk (Dunshea *et al.*, 1998; Dunshea *et al.*, 1999; Wolter *et al.*, 2002; Van Oostrum *et al.*, 2016; De Greeff *et al.*, 2016). Wolter *et al.* (2002) observed a 16% increase in weaning weight when piglets were supplemented with liquid milk from day 3 post-farrowing to day 21 (weaning). This observation was confirmed in a study conducted by De Greeff *et al.* (2016) in which piglets supplemented with milk from day 2 to 21 (weaning) were 8% heavier at weaning. Contrary to this, Pustal *et al.* (2015) failed to find an increase in pre-weaning piglet ADG and weaning weight when supplementary milk was provided to piglets from day 2 post-farrowing to day 28 (weaning). However, in the latter study, milk-supplemented litters weaned 1.1 piglets more than unsupplemented litters, and the litter weaning weight was increased as a consequence. Wolter *et al.* (2002) also found that pre-weaning milk supplementation increased the number of pigs weaned by 0.5 piglets per litter. The effect of milk supplementation on pre-weaning mortality is variable, with some studies finding a reduction in pre-weaning mortality when piglets were supplemented with milk (Wolter *et al.*, 2002; Park *et al.*, 2014) and others showing no effect (Azain *et al.*, 1996; Miller *et al.*, 2012; Douglas *et al.*, 2014). Relatively few studies have followed the growth of milk-supplemented piglets into the post-weaning period and beyond. Wolter *et al.* (2002) did not observe any effects of pre-weaning milk supplementation on pig growth immediately post-weaning (from weaning to 25 kg). However, they found that pigs supplemented with milk replacer pre-weaning had a 4.5% increase in average daily feed intake (ADFI) and tended to have an increase in ADG (of 3.3%) during the middle of the grower period (from 25 to 65 kg body weight (BW)). As a consequence, supplemented pigs reached the target slaughter weight (110 kg) 3 days before their non-supplemented counterparts (Wolter *et al.*, 2002). Park *et al.* (2014) also monitored the post-weaning growth of pigs that had been provided with supplementary milk from day 4 after birth up to weaning on day 21. In an experiment conducted in the autumn, weaning weight and pre-weaning

mortality were not influenced by milk provision to suckling pigs. In another conducted in July, weaning weight was increased and pre-weaning mortality reduced in pigs supplemented with milk replacer. Therefore, the prevailing temperatures during the period in which pre-weaning milk supplementation to suckling litters is performed may influence intake of supplemental milk and therefore the response observed. This is possibly because of reduced milk production in sows due to the reduced lactation feed intake normally observed during periods of high temperature. However, in the latter study, there was no effect on the final slaughter weight in either experiment. Overall, providing supplementary milk can be an effective strategy to increase creep feed intake prior to weaning. We believe that the provision of supplementary milk to suckling piglets is particularly beneficial in large litters (≥16 piglets born alive). However, there is a possibility that it could reduce the consumption of sow milk by piglets.

Few studies have investigated the effect of supplementing milk replacer pre-weaning on gut maturity at weaning. De Greeff *et al.* (2016) observed a 26% increase in small intestinal weight in suckling piglets supplemented with milk replacer for 21 days, as well as a higher relative weight:length ratio compared with control non-supplemented piglets, indicating that the milk supplement stimulated intestinal growth. These authors also observed an increase in crypt depth and a lower villus height:crypt depth ratio in the ileum of milk-supplemented piglets on day 21 (weaning). This indication of higher cell-proliferation rates could imply an impairment of intestinal integrity in this study. However, Hu *et al.* (2020) did not observe any differences in villus height or crypt depth in the jejunum on day 28 (weaning) and day 35 (8 days post-weaning) in pigs supplemented with milk during the suckling period compared to non-supplemented pigs. Regarding enzyme production, they found lower lactase and higher sucrase activity on day 28 and higher maltase activity on day 35 in the jejunum of pigs supplemented with milk, suggesting that pre-weaning supplementary milk provision to suckling pigs may induce earlier maturation of the jejunum. The effect of pre-weaning liquid milk supplementation on intestinal microbiota composition is not consistent, with some studies demonstrating a benefit from pre-weaning milk supplementation (Hu *et al.*, 2020) and others not (Jin *et al.*, 2020). Hu *et al.* (2020) observed a greater abundance of *Clostridium XI*, *Turicibacter* and *Moraxella* at 28

days of age in the jejunum of piglets supplemented with milk from day 4 to 28 after birth in comparison to control unsupplemented pigs. In addition, they demonstrated an increased abundance of *Lactobacillus* and a decreased abundance of *Streptococcus* and *Blautia* in the jejunum on day 35 (7 days post-weaning), indicating that the milk supplement may have increased the abundance of beneficial bacteria in the small intestine, therefore helping to maintain intestinal homeostasis. In a study in which pigs were supplemented with milk from day 7 postpartum until day 21 (weaning), Jin *et al.* (2020) observed that the supplemented group had higher bacterial species richness estimates (ACE and Chao1) in the jejunum compared to control unsupplemented pigs, indicating a higher number of bacterial species. However, the supplemented group had similar Simpson and Shannon diversity indices compared to the control, indicating that there were no differences in the abundance of each species. The supplemented group had lower abundances of *Romboutsia*, *Actinobacillus*, *Bacteroides* and *Lactobacillus* than the control group, indicating that the abundance of some beneficial bacteria (such as *Lactobacillus)* was reduced in pigs supplemented with milk. The authors surmise that the decrease in *Lactobacillus* abundance could be the result of reduced ingestion of sow milk containing oligosaccharides. However, this would also have been the case in the study by Hu *et al.* (2020), and they observed the opposite. This lack of agreement across studies is likely due to differences in the composition of the supplementary milk fed. De Greeff *et al.* (2016) observed an increase in concentrations of the volatile fatty acids (VFAs), acetate, propionate, butyrate and valerate in the colon of milk-supplemented versus non-supplemented piglets at 21 days of age (weaning). Volatile fatty acids are fermentation end-products of the colonic microbiota, and the higher concentrations in the milk-supplemented pigs reflect a change in the composition of the colonic microbiota, which is likely explained by the high total dietary fibre content of the milk used in this study compared to sow milk. However, no microbiome analysis was conducted in this study.

It would appear that supplementing suckling piglets with milk from 1 to 4 days after birth until weaning can increase the weaning weight (Azain *et al.*, 1996; Dunshea *et al.*, 1998; Dunshea *et al.*, 1999; De Greeff *et al.*, 2016; Miller *et al.*, 2012; Wolter *et al.*, 2002; Van Oostrum *et al.*, 2016) and the number of piglets weaned (Pustal *et al.*, 2015; Wolter *et al.*, 2002) and reduce mortality pre-weaning (Wolter *et al.*, 2002; Park

et al., 2014). The benefit of pre-weaning milk supplementation on intestinal maturation and microbiota after weaning is not consistent and likely linked to milk composition (De Greeff *et al.*, 2016; Hu *et al.*, 2020). Although not extensively studied, some studies report increased post-weaning growth in response to providing a liquid milk replacer to suckling pigs (Wolter *et al.*, 2002; Van Oostrum *et al.*, 2016; Dunshea *et al.*, 1999).

Table 1-3. Effect of pre-weaning milk replacer supplementation on pre-weaning and post-weaning piglet growth and health. Litters provided with milk replacer are compared to litters not provided with milk replacer, unless otherwise stated [modified from Middelkoop (2020)].

SA [1] (days)	WA [2] (days)	Pattern of Provision	Pre-Weaning Effects (d0 = Birth)				Post-Weaning Effects (d0 = Weaning)				Reference
Litter size			Supplemental Milk Intake	ADG [3]	Weaning Weight	Other	ADFI [4]	ADG	FCR [5]	Other	
1	21	Ad libitum	2.5 L of milk/pig (375 g DM [6] cool season) 9.9 L of milk/pig (1.49 kg DM warm season)	NA [7]	↑	↑ total litter weight = mortality ↑ glucose, IGF-I [8] and thyroxine in serum at weaning	NA	NA	NA	NA	(Azain *et al.*, 1996)
10.4											
4	28	Ad libitum	4.76 L of cow's milk/pig; 10.96 L artificial milk/pig (200 g total solids/L)	= from d0 to d14 ↑ from d14 to 28 ↑ from d0 to 28	↑	NA	NA	NA	NA	NA	(Dunshea *et al.*, 1998)
12											
10	20	Ad libitum	3.9 L of milk/pig (200 g of skim milk powder/L)	↑	↑	NA	↑ from d0 to d21	↑ from d0 to d21	NA	↑ weight on d21	(Dunshea *et al.*, 1999)
12											
3	21	Ad libitum		=	↑	↘ % mortality			=		

12			1000 g of milk powder/pig			↗ number weaned	↑ from 25 to 65 kg (grower period)	↑ from 25 to 65 kg (grower period)		reached slaughter weight 3 days earlier	(Wolter *et al.*, 2002)
3	26	Ad libitum	13.8 mL to 10.35 L of milk/pig (winter); 43.7 mL to 17.25 L of milk/pig (summer) (150 g powder/L of water)	=	↑	= % mortality = % medicated piglets	= from d0 to 42	= from d0 to d42	= from d0 to 42	= % mortality = % medicated pigs	(Miller *et al.*, 2012)
10 to 11											
4	21	From 8:00 to 16:00 h daily	NA in Trial 1(late fall) 22 g of milk powder/pig in Trial 2 (summer)	= (Trial 1) ↑(Trial 2)	= (Trial 1) ↑(Trial 2)	↘ % mortality (Trial 2)	NA	↑ d21 to d54 (trial 1) = (trial 2)	NA	= carcass weight, back fat thickness, dressing percentage	(Park *et al.*, 2014)
10											
1	28	Twice a day or as needed	3.86 L/pig or 138 mL/pig/day (150 g of powder/L of water)	=	=	= % mortality ↑ antibiotic treatments	NA	= from d0 to d21, d21 to d72, d72 to 115	NA	= % mortality	(Douglas *et al.*, 2014)
11 to 12											
2	28	Ad libitum	520 g of powder/pig (20 g/pig/day)	=	=	↑ number weaned ↑ total litter weight = mortality, diarrhoea ↓ treatment of facial lesions	NA	NA	NA	NA	(Pustal *et al.*, 2015)
16.8											
2	21		From d0–d7:	NA	↑		NA	NA	NA	NA	

13 to 14		Twice a day from 7:00 to 8:00 h and from 15:00 to 16:00 h	75 g DM [6] (litter/day) From d7–d14: 225 g DM (litter/day) From d14–21: 773 g DM (litter/day)			↑ IGF-1 [8] gene expression on d21 in jejunum mucosa ↑ small intestine weight on d21 ↑ crypt depth and ↓ villus height:crypt depth ratio in the ileum on d21 ↑ VFA [11] in the colon on d21					(De Greeff *et al.*, 2016)
22	27	200 mL/pig per day	172.5 g of creep/pig	↑	↑	NA	↑ from d0 to d14 ↑ from d14 to d28	↑ from d0 to d14 ↗ from d14 to d28	= from d0 to d14 ↑ from d14 to d28	NA	(Van Oostrum *et al.*, 2016)
NA											
4	28	Ad libitum	NA	=	NA	At d28 in colon: = bacterial diversity [9] = bacterial species richness [10] ↑ VFA ↓ *Lactobacillus, Clostridium XI, Blautia, Clostridium sensu stricto, Escherichia* ↑ *Paraprevotella* ↗ *Ruminococcus, Clostridium XIVa and IV, Succiniclasticum* ↑ TLR4 [12] gene expression, ↓ IL-6 [13] gene expression in mucosa	= from d0 to d7	= from d0 to d7	NA	↘ diarrhoea frequency	(Shi *et al.*, 2018)
8											

4 8	28	Ad libitum access, provision of fresh milk at 9:00 and 19:00 h	NA	=	NA	= villus height, crypt depth in jejunum on d28 ↓ lactase activity and ↑ sucrase activity in jejunum	=	=	NA	In jejunum on d7: = villus height, crypt depth ↑maltase activity ↑ *Lactobacillus* ↓ *Streptococcus*	(Hu *et al.*, 2020)
7 NA	21	Ad libitum access	NA	↑	↑	↓ diarrhoea At d21, in jejunum: ↑ bacterial species richness [14] = bacterial diversity ↓ *Romboutsia*, *Actinobacillus*, *Bacteroides* and *Lactobacillus*	NA	NA	NA	NA	(Jin *et al.*, 2020)
1 14 or 17	28	From 15:00 h on day 1 until weaning	For all piglets alive: From d1 to d12, 1.67 L/pig or 125 mL/pig/day) From d12 to d28, 3.2 L/pig or 200 mL/pig/day (150 g of powder/L of water)	NA	↑ in litters of 17 piglets on d1 = in litters of 14 piglets on d1	↓ risk of piglets dying	NA	NA	NA	NA	(Kobek-Kjeldager *et al.*, 2020)
1	28	From 15:00 h on day 1 until weaning	NA	=	=	= body fat and body protein content	NA	NA	NA	NA	(Kobek-Kjeldager *et al.*, 2021a)

↑ Significant increase; ↗ tendency to increase; ↓ Significant decrease; ↘ tendency to decrease; = No difference; [1] SA: start age of supplementation; [2] WA: weaning age; [3] ADG: average daily gain; [4] ADFI: average daily feed intake; [5] FCR: feed conversion ratio; [6] DM: dry matter; [7] NA: not applicable; [8] IGF-1: insulin-like growth factor 1; [9] Shannon and Simpson; [10] observed species and Chao1; [11] VFA: volatile fatty acids, [12] TLR4: Toll-like receptor 4 ;[13] IL-6: interleukin 6; [14] ACE and Chao1.

1.3.1.3. Provision of supplemental liquid feed pre-weaning

The provision of supplemental milk pre-weaning can increase pre-weaning DMI and growth and reduce pre-weaning mortality, as outlined in Section 1.3.1.2. However, it does little to expose piglets to the plant-based ingredients that they will encounter in the dry diets fed post-weaning. A solution to this is to provide suckling piglets with liquid feed pre-weaning (i.e., dry feed in a gruel form or an enriched milk containing plant-based compounds). Few studies to date have compared the effect of providing supplementary liquid feed to suckling piglets, with dry creep feeding and/or with no creep feeding (dry or liquid). One such study by Lawlor *et al.* (2002) supplemented a liquid mixture of milk and feed to suckling piglets from 12 days of age to weaning, with creep-fed litters only standardised at eight piglets. In this study, creep feeding the liquid mixture and standardising litters at eight piglets increased the weaning weight by 7%. However, the authors concluded that the increase in weaning weight was most likely achieved due to the reduced number of suckling pigs per sow. Kobek-Kjeldager *et al.* (2021b) supplemented milk to suckling piglets from day 2 to 12 of lactation, followed by liquid feed from day 12 to weaning. This trial also compared two different weaning ages (day 24 or 35). Providing the liquid diet before weaning was found to shorten the latency period to first feed consumption post-weaning but had no impact on the latency to first water consumption following weaning. Interestingly, a change in feeding behaviour was observed at the transition from supplementary milk to liquid feed on day 12, with a reduction in the number of feeding bouts observed the day following the diet change.

Some studies demonstrated a benefit to supplementing liquid creep feed in comparison to dry creep feed in terms of increased pre-weaning ADFI (Byrgesen *et al.*, 2021; Martins *et al.*, 2020). Martins *et al.* (2020) observed that pigs supplemented with a gruel feed (pre-gelatinised rice, micronised soybean and whey mixed with water at a 1:1 ratio) from day 3 of age to day 21 (weaning) had a ~566% higher ADFI during the first days of supplementation (day 3 to 7) than pigs supplemented with dry creep feed. Similarly, Byrgesen *et al.* (2021) showed that pigs supplemented with liquid creep from day 10 of age to day 28 (weaning) had ~270% higher dry matter disappearance during the first week of supplementation (day 10 to 18) than pigs supplemented with dry creep. However, these studies found no increase in pre- and post-weaning ADG in

response to the pre-weaning supplementation of liquid creep feed. Despite higher intakes, Byrgesen *et al.* (2021) and Martins *et al.* (2020) found that the weaning weight in piglets offered liquid creep feed did not differ from piglets offered dry creep feed. This could have been due to the higher number of piglet eaters observed in litters offered dry creep feed compared to litters offered liquid creep feed, even though piglets supplemented with liquid creep had a higher average intake. In the study by Byrgesen *et al.* (2021), suckling pigs supplemented with dry creep feed were 9.6% heavier on day 61 post-weaning compared to pigs fed liquid creep feed during the suckling period. Furthermore, Martins *et al.* (2020) showed that suckling pigs supplemented with dry creep feed had less variation in BW on day 133 post-weaning and a similar slaughter weight compared to pigs fed gruel creep feed during the suckling period.

In a recent study, Amdi *et al.* (2021) compared the growth and intestinal morphology and function of piglets fed a milk replacer to that of piglets fed the same milk replacer with added wheat from day 3 to 25 post-farrowing. No treatment differences were found for weaning weight, jejunal morphology (villus height, crypt depth and villus height to crypt depth ratio) and intestinal gene expression. However, an increase in the activity of sucrase and maltase in the small intestine was found just prior to weaning (~25 days of age) in response to the addition of wheat to the liquid milk. These enzymes are important for the digestion of vegetable-based ingredients, and an increase in their activity at weaning suggests that these pigs should be better equipped to digest ingredients in the normally dry diet fed post-weaning. In another study, enzyme activities just before weaning (~25 days of age) were compared between piglets offered liquid creep feed and piglets offered dry creep feed (Byrgesen *et al.*, 2021). Here, the activities of sucrase and maltase in the proximal part of the small intestine were highest in piglets supplemented with dry creep even though DMI was highest when liquid creep feed was provided. Therefore, it is possible that the form of the creep feed (solid vs. liquid) may influence enzyme activity more than DMI (Byrgesen *et al.*, 2021). This could be due to the occurrence of spontaneous fermentation in liquid creep feed, which changes its physicochemical properties and its effect on the GIT.

From these studies, it appears that supplementing liquid creep feed instead of dry creep feed can increase the pre-weaning ADFI (Byrgesen *et al.*, 2021; Martins *et al.*, 2020). In addition, supplementing liquid creep feed instead of milk can improve intestinal enzyme maturation (Amdi *et al.*, 2021; Byrgesen *et al.*, 2021). However, increases in liquid creep feed intake and changes in intestinal function do not always result in increased growth pre- and post-weaning due to the low number of piglets consuming creep feed within litters.

1.3.2. Other pre-weaning strategies to stimulate earlier enzyme production

Van den Borne *et al.* (2007) found a positive correlation between pancreatic enzyme secretion and pig growth during the suckling period. During the suckling period, the intestinal tract is well adapted for the digestion and absorption of maternal milk. At weaning, the transition from maternal milk to solid feed leads to a remodelling of the GIT. This includes a switch in enzyme production; for example, within the intestinal brush-border disaccharidases, production switches from lactase to sucrase and maltase. Similarly, enzymes with proteolytic activity are found in relatively low concentrations during the suckling period (Pierzynowski *et al.*, 1990; Pierzynowski *et al.*, 1993). Studies suggest that intestinal tract remodelling can be accelerated when exogenous enzymes are administered to suckling mammals (Słupecka *et al.*, 2012; Prykhodko *et al.*, 2015; Prykhodko *et al.*, 2016). Prykhodko *et al.* (2015) observed an increase in gastric secretion and a switch in intestinal disaccharidases, with a decrease in lactase and an increase in maltase and sucrase in the proximal part of the small intestine, in rats supplemented with pancreatic enzymes (amylase, protease and lipase extracted from porcine pancreas) or pancreatic-like enzymes (microbially-derived alpha-amylase, proteinase and lipase). Enzyme supplementation also increased amylase and trypsin production in the pancreas (Prykhodko *et al.*, 2015). Słupecka *et al.* (2012) observed that supplementing suckling piglets twice a day for a week with porcine pancreatic enzymes increased villus height, reduced crypt depth and increased adult-type enterocyte appearance in the distal jejunal epithelium on day 16 after birth (1 day after the end of the treatment). Adult-type enterocytes are enterocytes that have

differentiated into those with an absorptive function. In the same study, supplementing pigs with a complex of microbially-derived amylase, protease, and lipase also increased adult-type enterocyte appearance but decreased villus height and crypt depth on day 16 after birth, which could indicate a reduced tolerance to supplementation with exogenous enzymes. The increase in adult-type enterocyte appearance could indicate an early maturation of the intestinal epithelium. In another study by Prykhodko *et al.* (2016), supplementing suckling pigs with a complex of microbially-derived amylase, protease and lipase once or twice between 7 and 14 days postpartum increased BW and improved the feed conversion ratio (FCR) during the grow–finishing period. In the same study, pigs supplemented with enzymes also reached the target slaughter weight earlier than non-supplemented pigs (Prykhodko *et al.*, 2016). From these findings, it appears that pancreatic and microbially-derived enzyme supplementation of piglets during the suckling period may benefit lifetime growth, due to earlier maturation of the GIT. In addition, Prykhodko *et al.* (2016) observed a decrease in nitrogen excretion per kilogram BW gain in pigs supplemented with enzymes prior to weaning compared with non-supplemented pigs, suggesting that enzyme supplementation of suckling piglets could also help in reducing the environmental impact of pig production.

To our knowledge, only the two studies outlined above investigated the effect of supplementing suckling piglets with a cocktail of enzymes on pig growth up to slaughter and on intestinal structure and function pre-weaning. Therefore, there is a need for additional studies investigating the effects of supplementing suckling piglets with an enzyme cocktail on intestinal structure and function post-weaning and pig growth up to slaughter to confirm the benefit of using this strategy on commercial farms and to understand the underlying mechanisms of action.

1.3.3. Other pre-weaning strategies to stimulate gut structure and function

Beneficial effects on pig growth in response to supplementing weaned pigs with glutamine or glutamate have been demonstrated (Teixeira *et al.*, 2014), as well as benefits in terms of feed efficiency (Wu *et al.*, 1996), intestinal function (Domeneghini

et al., 2006; Teixeira *et al.*, 2014) and structure (Molino *et al.*, 2012) and reduced incidence of diarrhoea (Rezaei *et al.*, 2013; Teixeira *et al.*, 2014). Glutamine and glutamate are the most abundant protein-bound amino acids in sow's milk (Wu and Knabe, 1994). Sow's milk is also rich in free glutamine, which increases in concentration from 0.1 mM on day 1 of lactation to 3.4 mM on day 29 of lactation (Wu and Knabe, 1994). Studies suggest that glutamine and glutamate are major fuels for enterocytes in the piglet small intestine (Watford, 2015). Until recently, glutamine and glutamate were not considered essential amino acids (i.e., ones that need to be supplemented in the diet as they cannot be synthesised by the pig from a metabolic intermediate). However, additional functions have recently been assigned to amino acids, and some, including glutamine and glutamate, are now considered essential at key times [as reviewed by Watford (2015)]. Glutamine is synthesised from glutamate and ammonia via the action of glutamine synthetase (Watford, 2015). Glutamate can be synthesised via the action of glutaminase, which degrades glutamine that enters into the mitochondria into glutamate and ammonia (Watford, 2015). Glutamine can be metabolised into purines and pyrimidines for the synthesis of nucleotides to support cell proliferation, while glutamate cannot (Watford, 2015). For this reason, this review focuses on glutamine supplementation of suckling piglets.

Few studies to date have investigated the effects of glutamine supplementation of suckling piglets on their growth and intestinal health pre-and post-weaning. Haynes *et al.* (2009) observed a 12% increase in growth in piglets orally supplemented with glutamine (dissolved in 20 mL of water at a concentration of 3.42 mmol/kg BW or 0.5 g/kg BW) twice daily from day 7 to 14 of age compared with piglets receiving oral treatment with alanine or water. It is important to note that the dose is critical with regard to piglet growth. Indeed, a preliminary study conducted by the same authors showed that oral supplementation with glutamine twice daily from day 7 to 21 of age at a concentration of 6.84 mmol/kg BW (i.e., 1 g of glutamine/kg BW) reduced piglet daily weight gain by 19%. Although not explained by the authors, excess glutamine can generate ammonia, which is not fully excreted by the kidneys and can therefore cause adverse effects. As the supplementation was performed twice daily, the piglets received in total 2 g of glutamine/kg BW daily, and it is likely that this rate of supplementation was above the detoxifying capacity of the animal. In the study by

Haynes *et al.* (2009), growth was also enhanced in piglets supplemented with glutamine following a lipopolysaccharide (LPS) challenge. Glutamine administration reduced the rectal temperature by 0.5 °C, improved intestinal structure (increased villus height in the jejunum) and ameliorated intestinal injury in piglets following LPS challenge (Haynes *et al.*, 2009). In addition, they demonstrated that the addition of L-glutamine to the medium of cultured neonatal enterocytes prevented LPS- or hydrogen peroxide-induced cell death (Haynes *et al.*, 2009). Noticeably, this effect was specific to the L-isomer. These findings support the role of L-glutamine in preventing intestinal damage in endotoxin-infected piglets, suggesting that it may have a role to play in protection against infection with Gram-negative pathogens such as enterotoxigenic *Escherichia coli*, one of the main causes of PWD. To our knowledge, L-glutamine supplementation of creep feed has only been investigated in one study to date (Cabrera *et al.*, 2013). Cabrera *et al.* (2013) demonstrated that supplementing creep feed and nursery feed (fed after weaning) with 1% glutamine improved intestinal structure 7 days post-weaning and post-weaning FCR. However, as the supplementation was continued after weaning, it is difficult to identify the contribution of creep feed supplementation with glutamine on the positive outcomes observed post-weaning. In addition, the authors did not observe an effect of pre-weaning glutamine supplementation on weaning weight.

There is growing interest in L-glutamine supplementation for low-birth-weight (LBW) piglets as a means of improving intestinal health. Low-birth-weight piglets have higher mortality rates (Quiniou *et al.*, 2002) and impaired small intestinal morphology and function (Ayuso *et al.*, 2021) compared with normal-birth-weight (NBW) piglets. Few studies have investigated the effects of oral supplementation with glutamine at 1 g/kg of BW/day in suckling pigs. Li *et al.* (2022) observed a 14.7% increase in milk intake and a 7.5% increase in BW in LBW piglets on days 11–12 postpartum when supplemented with glutamine at 1 g/kg of BW/day from day 1 via oral gavage. In both piglet categories (LBW and NBW), Schulze Holthausen *et al.* (2022) noted a higher number of CD3+ intraepithelial lymphocytes in colon tissue and a tendency for an increase in CD3+ intraepithelial lymphocytes in the lamina propria in piglets supplemented with glutamine. A higher number of CD3+ intraepithelial lymphocytes

may indicate a more mature intestinal immune system, as these cells have a signalling role in the defence of the intestinal epithelium.

During the neonatal period, gains in protein mass in skeletal muscle contribute most to growth (Rudar *et al.*, 2019). Glutamine plays an important role in skeletal muscle development in piglets, and therefore subsequent growth (Wu *et al.*, 2011). Growth can be impaired in LBW piglets, which makes glutamine supplementation an interesting strategy to support their development. A recent study by Zhao *et al.* (2021) demonstrated that glutamine can increase cell proliferation in the muscle of LBW piglets. In support of this, Zhao *et al.* (2020) found larger muscle fibres in glutamine-supplemented piglets. However, the authors noted that the effects on skeletal muscle were minor in both studies (Zhao *et al.*, 2020; Zhao *et al.*, 2021).

In summary, the provision of glutamine at 1 g/kg of BW/day via oral gavage to piglets for 7 to 10 days before weaning can benefit piglet intestinal immunity (Schulze Holthausen *et al.*, 2022; Haynes *et al.*, 2009) and growth (Haynes *et al.*, 2009; Li *et al.*, 2022). However, additional work should be conducted to assess how to implement this strategy at commercial farm scale (e.g., adding glutamine to creep feed or liquid milk). Future work should also aim to study the effect of pre-weaning glutamine supplementation on post-weaning pig growth.

1.3.4. Other pre-weaning strategies to modulate gut microbiota

During recent decades, a number of strategies have been developed to modulate the composition of microbial (mainly bacterial) populations within the porcine gut, with the aim of improving pig growth and health. In particular, shaping the pig intestinal microbiota early in life has the potential to influence lifetime growth and health. Among the strategies investigated to date, the use of probiotics and prebiotics has been most researched, with a range of different microorganisms and compounds used across studies. Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill *et al.*, 2014). Probiotic administration to sows during gestation and/or lactation has been shown to improve colostrum/milk quality and quantity and to modulate the piglet gut microbiome, which are likely some of the mechanisms by which maternal probiotic supplementation

benefits piglet growth and health (Liao and Nyachoti, 2017; Barba-Vidal *et al.*, 2019). A study from our group, among others, has demonstrated probiotic transfer from sows to suckling piglets, proving that maternal probiotic administration can be an effective means of early-life inoculation of piglets (Crespo-Piazuelo *et al.*, 2022). To our knowledge, our study is also the only one to date to show lifetime benefits in the offspring of probiotic-supplemented sows, namely improved growth during the finisher period and increased carcass weight at slaughter (Crespo-Piazuelo *et al.*, 2022). Studies also demonstrate that the administration of probiotics directly to suckling piglets can accelerate the response to enterotoxigenic *E. coli* challenge post-weaning (Hansen *et al.*, 2022) and can increase pre-weaning and early post-weaning pig growth, possibly via gut microbiota/immune modulation (Kiros *et al.*, 2019; Xin *et al.*, 2020).

A prebiotic is "a substrate that is selectively utilised by host microorganisms conferring a health benefit" (Gibson *et al.*, 2017). Some substances are well-accepted prebiotics (galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), inulin and lactulose), while others are considered candidates (Scott *et al.*, 2020; Lawlor *et al.*, 2020). Similar to probiotics, prebiotic administration to sows during gestation and/or lactation has been shown to improve colostrum quality and quantity (Hasan *et al.*, 2018; Davis *et al.*, 2021) and to modulate piglet microbiome (Hasan *et al.*, 2018), and these effects are sometimes accompanied by improved piglet growth pre-weaning (Davis *et al.*, 2021). Prebiotic administration to suckling piglets has also been shown in a study by Alizadeh *et al.* (2016) to modulate intestinal microbiome and to improve intestinal structure, although pig growth was not improved. However, this study used pigs as a model for humans, and the measurement of production parameters was not one of the main objectives. Recently, the concept of postbiotics has emerged. A postbiotic is defined as a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (Salminen *et al.*, 2021). Zhong *et al.* (2022) reviewed the use of postbiotics in livestock, and from this, it appears that only one study to date has administered postbiotics to suckling piglets. In that study, Busanello *et al.* (2015) demonstrated that the administration of inactivated *Lactobacillus* pre-weaning increased feed intake and growth post-weaning but did not impact faecal counts of lactic acid bacteria or coliform; however, a full microbiome

analysis was not conducted. The stability and safety of postbiotics make them interesting alternatives to probiotics (Zhong *et al.*, 2022).

1.4. Conclusions

Several management and nutritional strategies can be implemented to increase piglet growth to weaning and consequently improve subsequent post-weaning outcomes. Such strategies are particularly important considering recent bans on the use of pharmacological levels of ZnO and in-feed antibiotics, and continued increases in litter size. Most pre-weaning management and nutrition studies only record post-weaning growth in pigs for a limited period of time, if at all. Consequently, there is limited information on how these strategies influence lifetime growth and health in pigs, and therefore, on their economic impact. Some of the pre-weaning interventions examined in this review are inexpensive and easily implemented (e.g., split-suckling or postpartum provision of analgesia to sows). However, in some cases, additional work is needed to determine their effect on post-weaning pig growth and health. For solid creep feeding in the farrowing house, the available data suggest inconsistent effects on post-weaning pig growth due to often low and variable creep feed intake. Providing milk or liquid feed as a creep provides an opportunity to increase DMI and the proportion of eaters per litter. In addition, milk/liquid feed could be an effective route for the early-life administration of feed additives (e.g., enzymes; L-glutamine; and pro-, pre- and postbiotics). However, liquid feeding of suckling piglets in farrowing rooms needs to be conducted hygienically and requires substantial financial investment. In summary, there are many nutritional and management approaches that can be recommended to improve the pre- and post-weaning growth and health of piglets raised in large litters. However, solutions should be selected and combined on a case-by-case basis to suit the particular production system.

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1. Literature review – Part 2: Selected nutritional strategies in weaned pigs to improve post-weaning outcomes and limit antimicrobial usage.

1.6 Introduction

As outlined in Chapter 1 Part 1, weaning is associated with reduced feed intake and a growth check (Lawlor *et al.*, 2020). The extent of this depends on how rapidly the pig is able to adapt to its new circumstances. At weaning the pig's diet changes abruptly from highly digestible liquid milk to dry pelleted feed. When milk intake ceases, feed intake reduces and the structure of the small intestine changes rapidly, with a reduction in villus height and an increase in crypt depth after weaning (Miller *et al.*, 1986; Cera *et al.*, 1988; Nabuurs *et al.*, 1993; Vente-Spreeuwenberg *et al.*, 2003). Shorter villi reduces the surface area of the small intestine and deeper crypts are associated with an increase in cell production in the crypts and cell turn-over in the small intestine (van Beers-Schreurs *et al.*, 1992; Al-Mukhtar *et al.*, 1982). Together, these result in reduced digestive and absorptive capacity of the small intestine (Al-Mukhtar *et al.*, 1982). All of these changes can lead to intestinal dysbiosis, which is one of the leading factors contributing to post-weaning diarrhoea (Gresse *et al.*, 2017). Several post-weaning strategies have been investigated to alleviate the growth check experienced by pigs at weaning. This second part of the literature review will begin by outlining the changes in intestinal structure and function experienced by pigs at weaning and the associated challenges and will then focus on the following post-weaning nutritional strategies: (1) supplementing pigs with milk or liquid feed during the early post-weaning period to improve feed intake at weaning and to alleviate weight loss, (2) use of feed additives (i.e. L-glutamine) to improve intestinal health of the pig early post-weaning.

1.7 Changes in intestinal structure and function at weaning

1.7.1 Intestinal structure

At weaning, the structure of the intestine changes rapidly. Several studies report a reduction in small intestinal villus height and an increase in crypt depth after weaning (Miller *et al.*, 1986; Cera *et al.*, 1988; Nabuurs *et al.*, 1993; Vente-Spreeuwenberg *et al.*, 2003) with minimal values for villus height:crypt depth ratio occurring around 5 days after weaning and a recovery by 11 days post-weaning (van Beers-Schreurs *et*

al., 1992). Shorter villi and deeper crypts are considered adverse as it reduces the surface area of the small intestine and the number of mature enterocytes(van Beers-Schreurs *et al.*, 1992). An increase in crypt depth is correlated with an increase in crypt-cell production rate and a stimulation of cell turn-over in the small intestine that has generally been associated with reduced digestive and absorptive capacity (Al-Mukhtar *et al.*, 1982). Several factors contribute to these adverse changes in intestinal morphology post-weaning, including reduced feed intake and dietary antigenicity, with the lack of feed intake being the central cause (Dong and Pluske, 2007).

1.7.2 Enzyme production and digestibility

The digestive physiology of suckling pigs centres around the digestion of milk components. Sow's milk is composed of water (~80%) and solids (~20%), with the latter consisting of proteins, lipids, carbohydrates (mainly lactose), minerals, vitamins and cells (Hurley, 2015). The pool of digestive enzymes is therefore adapted to these components. For example, the activity of lactase, an enzyme that breaks down lactose, is high during the first days of life and reaches a peak at ~3 weeks of age. Conversely, the activity of sucrase and maltase is low during the first days of life but rises considerably during the first 2 weeks after birth (Cunha, 1977). The activities of enzymes in the stomach, pancreas and intestine change at weaning, as the intestine has to adapt suddenly to a new diet, mainly based on cereals. The activity of some enzymes decrease abruptly; for example, it takes at least 5 days post-weaning for gastric pepsin to return to weaning levels (Hedemann and Jensen, 2004). Weaning stress is also characterized by a decrease in activity of most of the pancreatic enzymes (Jensen *et al.*, 1997; Hedemann and Jensen, 2004), including lactase (Hampson and Kidder, 1986) and sucrase (albeit activities of the latter do recover) (Hampson and Kidder, 1986).

1.7.3 Intestinal microbiota

The piglet intestine is sterile at birth. It is subsequently colonized by micro-organisms from the mother and the environment (Lallès *et al.*, 2007). These bacteria compete and

interact to form a complex stable intestinal microbiota population which provides "colonisation resistance" against more transient bacteria or potentially pathogenic species (Hopwood and Hampson, 2003). There are age-related changes that occur within the intestinal microbiome of the pig. The dietary and environmental changes that pigs overcome at weaning disrupt the mass, function and composition of intestinal microbiota populations, allowing pathogenic bacteria to proliferate. This disruption is termed 'dysbiosis'(Cullen *et al.*, 2022). One of the main factors affecting the intestinal microbiota at weaning is the consumption of solid plant-based feed, which results in altered availability of specific microbial substrates. As reviewed by Cullen *et al.* (2022), the introduction of solid feed results in a reduction in *Lactobacillaceae* in the faeces of pigs. Weaning has also been associated with a shift from a *Bacteroidaceae* and *Enterobacteriaceae*-dominated faecal microbiota pre-weaning to a high abundance of *Prevotellaceae*- and *Ruminococcaceae* post-weaning (Motta *et al.*, 2019). In the case of intestinal dysbiosis, several authors have suggested that onset of diarrhoea could be associated with the alteration in the relative abundance of *Escherichia* and *Prevotella,* with *Escherichia-Shigella* being a core microorganism in diarrhoeic piglets and *Prevotellaceae UCG-003* the dominant genus in non-diarrhoeic piglets [reviewed by Cullen *et al.* (2022)]. The pig develops a more stable microbiota as it matures (Zhao *et al.*, 2015). Figure 1-2 shows the microbiota profile of pig faeces at 1, 2, 3, and 6 months of age at the phylum level (Zhao *et al.*, 2015). It shows that the ratio of *Firmicutes* to *Bacteroidetes* in the faeces of 2, 3 and 6 month-old pigs was 10-fold higher than that of 1-month old piglets.

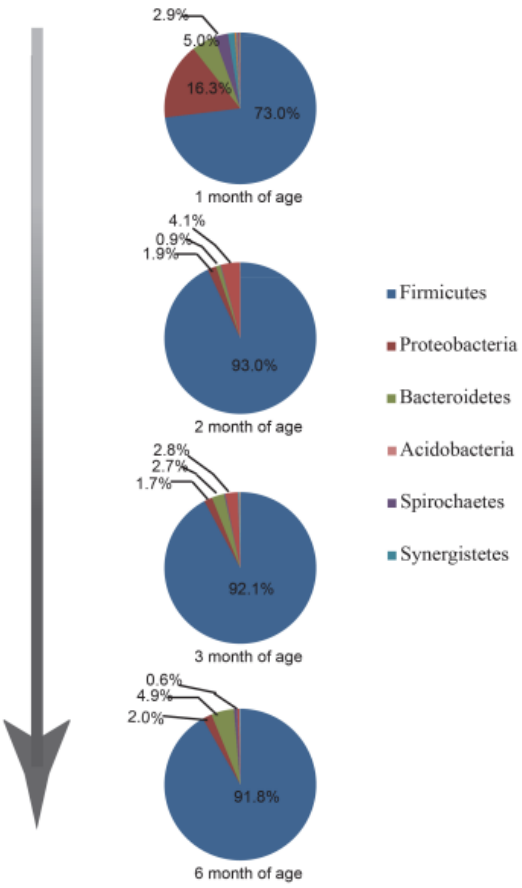

Figure 1-2. Profile of bacteria at the phylum level in the faeces of pigs at 1, 2, 3, and 6 months of age Adapted from Zhao *et al.* (2015).

1.7.4 Intestinal immunity

The function of the intestinal epithelium is not only to absorb nutrients, but also to provide a barrier against a diverse range of ingested antigens (King *et al.*, 2013). The intestinal barrier is composed of the basement membrane, the epithelial cells, tight junctions that join adjacent cells and the cell glycocalyx (King *et al.*, 2013). The intestinal epithelium provides information regarding the content of the intestinal lumen to the mucosal immune system through the production of cytokines. Epithelial cells, such as goblet cells, secrete mucin which reinforces the barrier and protects the mucosa from luminal antigens and pathogens (Deplancke and Gaskins, 2001).

Similarly, paneth cells secrete antimicrobial peptides into the gut lumen, creating a barrier against colonization (Zhang *et al.*, 2000). In addition to these mechanical barriers, the epithelial monolayer contains a population of intraepithelial T lymphocytes (Vega-López *et al.*, 2001). T lymphocytes are also present in the lamina propria, as well as other immune cells, such as B lymphocytes, dendritic cells, macrophages, neutrophils, eosinophils and biologically active fibroblasts (King *et al.*, 2013). The disruption of the intestinal barrier can cause antigens to enter and be processed in the lamina propia. Therefore, antigen recognition by T helper cells activates them. Once activated, they secrete diverse cytokines which recruit other lymphocytes and components of the innate immune system leading to the production of pro-inflammatory cytokines and other inflammatory mediators (King *et al.*, 2013). Several studies demonstrate an activation of the intestinal immune system at weaning with an increase in jejunal CD4+ (helper) and CD8+ (cytotoxic) T lymphocytes within 2 to 7 days post-weaning (Pluske *et al.*, 1999; McCracken *et al.*, 1999) but also, an increase in plasma pro-inflammatory cytokines, such as Interleukin-I (McCracken *et al.*, 1995).

1.8 Post-weaning diarrhoea

1.8.1 Common pathogens causing PWD and factors predisposing pigs to PWD

Post-weaning diarrhoea is a multifactorial disease which occurs frequently within 2 weeks after weaning. It is characterized by severe diarrhoea, dehydration, a significant loss of body weight and increased mortality (Fairbrother *et al.*, 2005). Post-weaning diarrhoea is usually attributed to the proliferation of enterotoxigenic *E. coli* (ETEC) in young pigs (Nagy and Fekete, 2005). These bacteria attach to the brush border of villus enterocytes in the pig small intestine via pilus adhesins (fimbriae) and produce enterotoxins (Heo *et al.*, 2013). These enterotoxins cause an increase in water and electrolyte secretion into the intestinal lumen and this increased secretion reduces nutrient absorption (Nagy and Fekete, 1999). Particular serotypes of *E. coli* with

specific fimbriae and toxin genes are associated with PWD. These are summarized in Table 1.4.

Post-weaning diarrhoea is a complex disease but most studies attempting to identify the causative agent to date have focused on monitoring only one pathogen. However, as reported in a review by Rhouma *et al.* (2017), rotavirus is considered to be an important enteric pathogen in weaned pigs, as well as coccidia, sapovirus and *Cryptosporidium parvum*. In addition, other diseases such as Porcine Reproductive and Respiratory Syndrome (PRRS) weaken the pig's immune response and permit ETEC to cause septicaemia, leading to death.

Table 1-4. Enterotoxigenic *E. coli* O serotypes most frequently implicated in post-weaning diarrhoea in pigs (Heo et al., 2013).

O serotypes	**Associated fimbrial antigens**
8	F4ab (K88ab) F4ac (K88ac)
138	F18, F4ac
139	F18
141	F18, F4ab, F4ac
147	F4ac, F18
149	F4ac, F18
157	F4ac

The factors that predispose pigs to PWD can be classified into pre- and post-weaning factors (Heo *et al.*, 2013; Rhouma *et al.*, 2017). This review does not aim to detail all of these factors; however, some are outlined. For example, at weaning, the immaturity of the intestinal immune system and the removal of milk containing Immunoglobulin A and other biological compounds limits the ability of the weaned pig to mount the appropriate immunological response, thereby increasing susceptibility to infectious diseases, including PWD (Heo *et al.*, 2013). In addition, the abrupt change in the diet, and over-eating after a period of feed intake reduction, can lead to an increase in gastric pH after weaning. As acidification of the stomach is a primary physiological defence

mechanism, this could increase PWD as high intestinal pH values provide an optimal milieu for ETEC survival/growth (Heo *et al.*, 2013). Another predisposing factor is the intestinal dysbiosis associated with weaning (discussed in Section 1.7.3), which increases susceptibility to pathogenic bacteria, such as ETEC (Pluske *et al.*, 2002).

1.8.2 Antimicrobial usage post-weaning to limit enteric disorders

In many countries, pig production is a high consumer of antimicrobials for veterinary use, with studies showing that they are primarily administered to pigs post-weaning for prophylactic or metaphylactic purposes (UK-VARSS, 2022; O'Neill *et al.*, 2020; FDA, 2021). In a study conducted in Ireland in 2017 across 67 pig farms, O'Neill *et al.* (2020) estimated that pig production represents 40% of veterinary antimicrobial usage (AMU). They observed that tetracyclines, potentiated sulphonamides, penicillins, and macrolides are the antimicrobials most commonly used in Ireland (O'Neill *et al.*, 2020). They found that 50% of the farms administered prophylactic antimicrobial treatments during the first week of life and 88% of farms provided antimicrobials in medicated feed at, or just after, weaning. In the past, there has also been widespread use of pharmacological levels of zinc oxide (ZnO) in pig feed (i.e. 2500 mg/kg of zinc), specifically around the time of weaning, to limit the incidence of PWD and improve gut health.

Because of the large quantities of antibiotics and ZnO used in the pig industry, antimicrobial resistance (AMR) is a major concern. Antimicrobial resistance is defined as ‘a change of bacteria, viruses, fungi and parasites over time making them no longer responsive to medicines’ (WHO, 2021). Infections are therefore harder to treat and there are increased risks of disease spread, severe illness and death, both in humans and animals (WHO, 2021). In fact, it has been estimated that AMR could cause the death of 10 million people a year by 2050 (O'Neill, 2016). A study investigating the evolution of antibiotic resistance in Denmark showed that 70% of *E. coli* strains isolated from Danish pigs were resistant to tetracycline (Holmer *et al.*, 2019). They also demonstrated that a number of strains, which are problematic for the swine industry, are also resistant to other antimicrobials (Holmer *et al.*, 2019). The

emergence of antimicrobial resistant microbes in pigs is a public health concern, as antimicrobial resistant organisms and/or genes can spread from pigs to humans, thereby limiting the number of antimicrobials effective to treat human diseases (Iramiot *et al.*, 2020).

In 2006, because of concerns around AMR, the European Union (EU) banned the use of antibiotics as growth promoters in livestock. Since January 2022, additional EU legislation has prohibited all forms of routine antibiotic use in farming, including preventive group treatments and the use of medicated feed for prophylaxis [European Commission (2019b); European Commission (2019a)]. In addition, the use of pharmacological levels of ZnO has been banned by the EU since June 2022 (European Commission, 2017), as zinc is excreted in pig manure, thereby impacting the environment (EMA, 2017). Since the ZnO ban and antibiotic restrictions were announced, a diverse range of feed additives has been researched as replacements. However, only a limited number are effective in stimulating early gut development and health in pigs. No single alternative has yet been found that improves growth or reduces the occurrence of diarrhoea to the same extent as AMU. There is therefore an urgent need to identify management and nutritional strategies with growth-promoting, microbiome-optimising and immune-stimulating properties as suitable alternatives to the use of in-feed antimicrobials. Some of the alternative post-weaning nutritional strategies being investigated in pigs to improve growth and limit antimicrobial usage will now be discussed.

1.9 Increasing feed intake in pigs post-weaning – provision of milk/milk replacer or liquid feed post-weaning

1.9.1 Feeding milk post-weaning

Studies have demonstrated the benefits of feeding milk instead of a solid starter diet during the first days post-weaning on growth, feed intake and intestinal morphology (Pluske *et al.*, 1996a; Pluske *et al.*, 1996b; Dunshea *et al.*, 1999). Pluske *et al.* (1996b) demonstrated that pigs fed ewe's milk only for 5 days after weaning at day 28 of age had a live weight daily gain that was 35% higher (435 ± 47.6 *vs* 307 ± 47.6 g/day;

P<0.01) and an empty body weight (BW) daily gain that was 39% higher (375 ± 50.6 *vs* 252 ± 50.6 g/day; *P*<0.01) than pigs fed a starter diet. These findings were confirmed in another study by Pluske *et al.* (1996a) which demonstrated that pigs fed cow's milk ad libitum for 5 days after weaning at 29 days of age had a live weight daily gain that was 56% higher (514 ± 80.1 *vs* 288 ± 80.1 g/day; *P*<0.001) and an empty BW daily gain that was 67% higher (463 ± 74.2 *vs* 231 ± 74.2 g/day; *P*<0.001) than pigs fed a starter diet alone. Similar to the study conducted by Pluske *et al.* (1996b) in which a 5% increase in energy intake (7.4 ± 0.55 *vs* 5.7 ± 0.55 MJ gross energy (GE)/ day; *P*<0.001) was observed in pigs fed ewe's milk compared to pigs fed a starter diet, Pluske *et al.* (1996a) observed a 33% increase in voluntary feed intake (400 ± 74.2 *vs* 286 ± 74.2 g/day; *P*<0.001) and an 8% increase in energy intake (8.9 ± 0.76 *vs* 5.1 ± 0.76 MJ GE/day; *P*<0.001) in pigs fed cow's milk instead of a starter diet. However, feed conversion ratio was not affected (Pluske *et al.*, 1996a). In both studies, the improvements in growth performance were accompanied by improvements in intestinal morphology. Proximal jejunal and mid-jejunal villus heights were greater by 53% (569 ± 53.7 *vs* 330 ± 53.7 µm; *P*<0.001) and 30% (428 ± 45.9 *vs* 316 ± 45.9 µm; *P*<0.01), respectively in pigs fed ewe's milk compared to those fed starter alone (Pluske *et al.*, 1996b). Likewise, proximal jejunal, mid-jejunal and distal ileal villus heights were greater by 21% (508 ± 47.7 *vs* 413 ± 47.7 µm; *P*<0.001), 35% (547 ± 50.6 *vs* 384 ± 50.6 µm; *P*<0.01) and 38% (442 ± 37.9 *vs* 300 ± 37.9 µm; *P*<0.001) respectively, in pigs fed cow's milk compared to those fed starter diet alone (Pluske *et al.*, 1996a). In these studies, the authors also measured lactase and maltase activity as an indicator of intestinal maturity and physiological changes (Pluske *et al.*, 1996a; Pluske *et al.*, 1996b). However, Pluske *et al.* (1996b) failed to demonstrate differences in lactase and sucrase activities in pigs fed ewe's milk compared to pigs fed starter alone, despite improvements in intestinal structure and pig growth. This was explained by the large variations in digestive enzyme activity measured. Pluske *et al.* (1996a) observed lower lactase (52 ± 17.4 *vs* 105 ± 17.4 nmol/min per g protein; *P*<0.05) and sucrase (45 ± 12.6 *vs* 86 ± 12.6 nmol/min per g protein; *P*<0.05) activities in pigs fed cow's milk post-weaning compared to pigs fed a starter diet.

1.9.2 Supplementation with milk/milk replacer or a liquid diet post-weaning

Providing milk instead of a starter diet at weaning delays the nutritional stress caused by the change from a liquid to a solid diet, but it does not avoid this abrupt transition and in fact results in a "second weaning" (Zijlstra *et al.*, 2009). To alleviate this nutritional stress, one solution is to provide milk/milk replacer as a supplement to a starter diet during the first days post-weaning. Studies show a positive effect of supplementing milk/milk replacer in addition to a starter diet on growth and feed intake of pigs post-weaning (Zijlstra *et al.*, 1996; Dunshea *et al.*, 1999; Rault *et al.*, 2015; Vasa *et al.*, 2023). Zijlstra *et al.* (1996) demonstrated a 30% increase in average daily gain (ADG; 319 ± 16 *vs* 245 ± 16 g/day; $P<0.01$) in pigs weaned at day 21 of age onto a milk replacer plus starter diet compared to pigs weaned conventionally onto starter diet alone. Similarly, Dunshea *et al.* (1999) observed a higher ADG from weaning to day 41 of age and a higher BW at day 41 in pigs weaned at day 20 that received milk replacer plus a pelleted diet in comparison to pigs weaned onto a pelleted diet only. They also observed that the dry matter intake (DMI) of pigs supplemented with milk was 8 times higher (257 ± 30.4 *vs* 30 ± 30.4 g; $P<0.001$) over the first 2 days post-weaning compared with pigs that received a pelleted diet only and remained higher in these pigs until day 41. Consequently, the post-weaning growth check was alleviated. These findings are in agreement with those of Rault *et al.* (2015) who weaned pigs at day 21 of age onto either a pelleted diet or the same pelleted diet supplemented with reconstituted skim milk. The DM content of the diet was gradually increased over the first 7 days post-weaning by reducing the amount of skim milk and increasing the quantity of dry pelleted feed until pigs received only the dry pelleted diet at day 7. Milk supplementation alleviated the growth check at weaning, by reducing pig weight loss by 2.5 times over the initial 2 days post-weaning compared with non-supplemented pigs (-135 ± 25.4 *vs* -373 ± 25.4 g/day; $P<0.01$). Pigs supplemented with milk had a 48% higher ADG (124 ± 9.6 *vs* 76 ± 9.6 g/day; $P<0.01$) over the first week post-weaning compared with pigs fed pelleted feed alone. This higher ADG resulted in heavier pigs at day 28 (7.6 ± 0.17 *vs* 7.2 ± 0.17 kg; $P=0.02$).

The authors also observed that the DMI of pigs supplemented with skim milk was 10 times higher (692 ± 16.7 *vs* 51 ± 16.7 g/day; $P<0.01$) over the first 2 days post-weaning than for pigs weaned onto a pelleted diet alone. This higher feed intake was maintained until 7 days post-weaning, during which time the feed gradually changed to a solid diet. Unfortunately, across all of the studies outlined above, only growth performance was monitored.

A more recent study conducted by Vasa *et al.* (In press) compared the effects of supplementary milk replacer or liquid starter diet provision for either 4 or 11 days post-weaning, on newly weaned pig growth and intestinal development. Supplementing pigs with liquid milk replacer for 11 days increased their DMI and ADG compared to unsupplemented pigs (Vasa *et al.*, In press). However, this growth advantage was not maintained after day 28 post-weaning. In addition, the authors observed a 37% increase in jejunal villus height when pigs received the liquid milk replacer for 11 days compared to pigs fed a dry pelleted starter diet only. Pigs supplemented with the liquid milk replacer for 11 days had a higher ileal sucrase activity than pigs supplemented with liquid milk replacer for 4 days only and those supplemented with the liquid starter diet (Vasa *et al.*, In press). To our knowledge, this is the only study which investigated the effect of liquid supplementation post-weaning on intestinal structure and enzyme activity along with the pig's lifetime growth performance. In order to fully understand the mechanisms behind this growth improvement, future studies should investigate the effect of milk replacer supplementation post-weaning on the pig intestinal microbiome and some markers of inflammation.

1.10 Improving intestinal structure and function – dietary supplementation with L-glutamine or glutamate post-weaning

In a recent review, Ji *et al.* (2019) summarised the effect of dietary supplementation pre- or post-weaning with glutamine and/or glutamate on the growth performance, gastrointestinal structure and function of weaned pigs (see Table 1-5). These studies demonstrated positive effects of supplementing weaned pig diets with 0.2 to 2% glutamine and/or glutamate. As discussed in Chapter 1 Part 1, glutamine and glutamate are major fuels for the enterocytes (Watford, 2015) and the mechanisms of action are outlined there. Chapter 1 Part 1 also outlines the benefits of glutamine and/or glutamate when fed to suckling pigs. However, several benefits of feeding to weaned pigs have also been demonstrated in terms of feed efficiency (Wu *et al.*, 1996), intestinal function (Domeneghini *et al.*, 2006; Teixeira *et al.*, 2014) and structure (Molino *et al.*, 2012; Ewtushik *et al.*, 2000; Hsu *et al.*, 2010; Johnson and Lay, 2017) and reduced incidence of diarrhoea (Rezaei *et al.*, 2013; Teixeira *et al.*, 2014). These will be discussed here and are summarised in Table 1-5.

1.10.1 Effects of dietary supplementation of weaned pigs with L-glutamine

Wu *et al.* (1996) supplemented weaned pig diets with 0, 0.2, 0.6 and 1% L-glutamine. Pigs supplemented with 1% L-glutamine had a higher gain to feed ratio than unsupplemented pigs. They also had longer villi in the jejunum at day 7 post-weaning (358 ± 32 vs 270 ± 32 μm; $P<0.05$) and a smaller lamina propria depth at day 14 post-weaning (279 ± 16 vs 301 ± 16 μm; $P<0.05$) than unsupplemented pigs, demonstrating the ability of glutamine to improve small intestinal morphology. Glutamine supplementation (1%) increased the concentration of glutamine in the duodenal digesta 8-fold. However, it did not affect the concentration of glutamate. These results are in line with those reported by Wang *et al.* (2015) who found that pigs fed a diet supplemented with 1% L-glutamine had a greater ADG the first week post-weaning and increased jejunal villus height (334 ± 9.4 vs 254 ± 6.8 μm, $P<0.05$) compared to unsupplemented pigs ($P<0.05$). Glutamine also increased jejunal expression of

occludin, *claudin-1*, *zonula occludens (ZO) 2*, and *ZO-3* genes, all of which encode tight junction proteins, one week after weaning (Wang *et al.*, 2015). This likely means that glutamine improved intestinal permeability during this period. When added at a rate of 1% to a low crude protein diet (17% crude protein) fed for 28 days from day 7 post-weaning, L-glutamine increased the BW of pigs at 35 days post-weaning (P<0.001) compared to diets containing 0, 2 and 3% L-glutamine (Li *et al.*, 2024). Dietary inclusion of 1% L-glutamine also increased the ADG (P<0.001) and decreased the feed conversion ratio (FCR; P<0.001) during the glutamine supplementation period. Contrary to this, dietary inclusion of 2 or 3% L-glutamine decreased the average daily feed intake (ADFI; P<0.001) and ADG (P<0.001) and increased the FCR (P<0.001) of pigs from day 7 to 35 post-weaning compared with dietary inclusion of 0 and 1% L-glutamine (Li *et al.*, 2024). This highlights the potential negative effects of using doses >2% of L-glutamine in post-weaning diets. It is likely that the additional L-glutamine contained in the diets supplemented at 2 and 3% could not be metabolized, as shown by the increase in blood urea nitrogen, causing the detrimental effects on growth (Li *et al.*, 2024). However, it is interesting to note that supplementation with 2% L-glutamine can alleviate the negative effects on pig growth and intestinal integrity post-weaning created by a challenge with *E. coli* K88 (Yi *et al.*, 2005).

1.10.2 Effects of dietary supplementation of weaned pigs with glutamate

As regards glutamate supplementation, Rezaei *et al.* (2013) supplemented weaned pig diets with 0, 0.5, 1, 2, and 4% monosodium glutamate (MSG). Piglets were weaned at 21 days of age and supplementation was to a basal corn and soybean meal-based diet. Feed intake was not affected by supplementation with 0, 0.5, 1, or 2% MSG. However, the authors observed a 15% reduction in feed intake in pigs supplemented with 4% MSG compared with 0%. Body weight was dose dependently increased with 1, 2 and 4% MSG in comparison to 0% MSG at day 7, 14 and 21 post-weaning (BW at day 21: 12.2 ± 0.26, vs 13.4 ± 0.31 kg, for 0 and 4% MSG respectively; P<0.05). ADG was dose dependently increased with 1, 2 and 4% MSG in comparison to 0% MSG during the day 14 to 21 post-weaning period (460 ± 8 vs 497 ± 9, for 0 and 4% MSG

respectively; $P<0.05$). Jejunal villus height was increased with dietary supplementation with 0.5, 1, 2 and 4% MSG ($P<0.05$). However, crypts were deeper in the jejunum of pigs supplemented with 4% MSG compared to 0% MSG ($P<0.05$). In addition, pigs supplemented with 0.5, 1% and 2% MSG had a lower incidence of diarrhoea during the first week post-weaning ($P<0.05$). Lin *et al.* (2014) found comparable results, where pigs supplemented with 2% glutamate had longer villi in the duodenum and jejunum, and deeper crypts in the jejunum compared to unsupplemented pigs ($P<0.05$). Lin *et al.* (2014) also observed that supplementation with 2% glutamate increased expression of *occludin* and *ZO-1* genes in the jejunal mucosa ($P<0.05$), which could indicate an improvement of the gastrointestinal structure, as these genes encode tight junction proteins. In a recent study, Liu *et al.* (2023) observed that dietary inclusion of 1% glutamate in pig diets from weaning to day 21 post-weaning increased ADFI ($P<0.05$) and ADG ($P<0.05$) and improved FCR ($P<0.05$) from weaning to day 21 (Liu *et al.*, 2023). They also reported that glutamate increased villus height ($P<0.05$) and villus height to crypt depth ratio ($P<0.05$), and decreased crypt depth ($P<0.05$) in the ileum at day 21. Glutamate also increased the expression of *interleukin 10 gene*, and decreased expression of *interleukin-1β, 6, 8, 17* and *21 genes* in the ileum, indicating that glutamate may play a role in regulating inflammation in the pig small intestine.

1.10.3 Effects of dietary supplementation of weaned pigs with mixtures of L-glutamine and glutamate

Finally, some studies report the effects of dietary supplementation combining L-glutamine and L-glutamate (Molino *et al.*, 2012; Teixeira *et al.*, 2014; Luise *et al.*, 2022). Molino *et al.* (2012), supplemented weaned pig diets with 0.8% of a mixture of L-glutamine and L-glutamate from day 21 of age (weaning) to day 35 of age. Pigs supplemented with the glutamine and glutamate were 4.6% heavier at day 35 than pigs not supplemented. They had 11.1% higher ADG and 7.9% higher feed intake with a 3.2% improvement in feed conversion efficiency. Body weight and ADG also increased at day 49 by 7.1 and 10.2%, respectively, in pigs supplemented with the glutamine-glutamate mixture compared to control pigs. Supplementation resulted in a

7.7, 10.4 and 6.6% increase in villus height in the duodenum, jejunum and ileum, respectively. Villus height:crypt depth ratio was higher in the duodenum and jejunum for pigs supplemented with the combination of glutamine and glutamate. Luise *et al.* (2022), supplemented weaned piglets with mixed doses of glutamate and glutamine: 0.6+0%, 0.45+0.15%, 0.3+0.3%, 0.15+0.45%, 0+0.6% (glutamate+glutamine%) for 21 days post-weaning. They observed a tendency for a linear increase in pig BW at day 21 post-weaning (P=0.07) and the number of goblet cells in the ileum of pigs at day 8 post-weaning (P=0.09) with glutamine. Therefore, L-glutamine inclusion in the mixture benefitted the growth of the pigs at day 21 post-weaning and certainly stimulated the differentiation of stem cells into goblet cells which secrete mucin creating a protective mucin layer in the small intestine. Glutamine also increased the number of duodenal glands in the pig small intestine at day 8 post-weaning compared with mixed doses of glutamine and glutamate (P=0.05). Similarly, duodenal glands are known to secrete an alkaline fluid composed of mucin, thereby protecting the duodenum (Krause, 1988). Regarding blood parameters, at day 8 post-weaning, the glutamate:glutamine ratio had a quadratic effect on neutrophils (P=0.01) and lymphocyte (P=0.01) percentages, in which the 0.3+0.3% dose of glutamate + glutamine had the highest and lowest values, respectively. It had a linear effect on the percentage of monocytes in the blood (P=0.03).These results confirm that L-glutamine can be used as a fuel for the proliferation of immune cells, such as lymphocytes. They also highlight the potential interactions between glutamate and glutamine on some blood parameters linked to immune response. The glutamate:glutamine ratio had a quadratic effect on the diarrhoea score and number of days with diarrhoea, in which the 0.15+0.45% dose of glutamate + glutamine had the lowest score. In addition, supplementing pigs with 1% of a combination of glutamine and glutamate was shown to reduce the diarrhoea score during the first week post-weaning (Teixeira *et al.*, 2014).

1.10.4 Effects of dietary supplementation of weaned pigs with L-glutamine and glutamate on the intestinal microbiota composition and activity

As reported in Table 1-5, several studies to date have investigated the effect of supplementing post-weaning diets with glutamine and/or glutamate on the intestinal microbiota of weaned pigs (Luise *et al.*, 2022; Liu *et al.*, 2023; Duttlinger *et al.*, 2021; Wang *et al.*, 2024; Li *et al.*, 2024). In-vitro studies have demonstrated that up to 20 to 40% of added L-glutamate can be utilized by bacteria in the small intestinal tract (Dai *et al.*, 2010). In a recent study, Van den Abbeele *et al.* (2022) incubated glutamine and glutamate separately for 48 h in-vitro with an inoculum of porcine-derived colonic bacteria. Interestingly, glutamine and glutamate stimulated the production of acetate and butyrate in the medium. This was linked to the stimulation of bacterial families which contain short chain fatty acid (SCFA)-producing species (*Ruminococcaceae*, *Oscillospiraceae*, and *Christensenellaceae*). Liu *et al.* (2023) observed an increase in bacterial taxa considered beneficial (*Lactobacillus*, *Prevotellaceae* NK3B31 group, and *Oscillospiraceae* UCG-005) in the colonic content of pigs supplemented with 1% glutamate in their diet. The increased abundance of UCG-005, belonging to the *Oscillospiraceae* family, confirmed the observations made by Van den Abbeele *et al.* (2022). Increase in concentrations of the SCFAs, acetate, valerate, isovalerate, propionate, isobutyrate and butyrate were also reported following dietary inclusion of 1% glutamate (Liu *et al.*, 2023), confirming the results obtained in-vitro by Van den Abbeele *et al.* (2022). Similarly, Li *et al.* (2024) observed an increase in the abundance of bacterial genera considered beneficial (*Lactobacillus*, *Prevotella*, *Gemmiger*) when weaned pig diets were supplemented with 1% L-glutamine. In addition, they observed a decrease in the abundance of *Streptococcus*, a genus with potentially pathogenic, but also beneficial, species. Luise *et al.* (2022) did not observe an effect of supplementing mixed doses of glutamine and glutamate to pig diets from day 24 (weaning) to day 45 of age on alpha or beta diversity indices of the pig caecal microbiota. This indicates that adding mixed doses of glutamine and/or glutamate to post-weaning diets did not profoundly influence the microbial composition of the large intestine. Some minor changes in relative abundance of some bacteria genera were reported, demonstrating that 3 weeks of glutamine supplementation could favour the growth of amino acid-fermenting bacteria, such as *Selenomonas, Pediococcus and Enterococcus* (Luise et

al., 2022). They also observed a decrease in *Lactobacillus* abundance when glutamate only was added to the diet compared to mixed addition and to the addition of glutamine alone (Luise *et al.*, 2022). *Lactobacillus* displays glutaminase activity, an enzyme which converts glutamine to glutamate (Vermeulen *et al.*, 2007). This could explain the increased abundance of *Lactobacillus* when glutamine was available.

In conclusion, glutamine and/or glutamate can benefit growth and intestinal structure and function of pigs when used at inclusion rates of between 0.2 and 2% in post-weaning diets. Dietary supplementation with glutamine and/or glutamate can increase the abundance of beneficial bacteria, producing SCFAs in the intestine. In addition, studies demonstrated potential interactions between glutamine and glutamate in terms of epithelial and immune cell proliferation.

Table 1-5. The effects of dietary supplementation with glutamine and/or glutamate supplementation post-weaning on the growth performance, gastrointestinal structure and function of weaned pigs [adapted from Ji *et al.* (2019)].

Weaning age	**Experimental treatment**	**Response to supplementation**	**Reference**
28 days of age	Basal diet supplemented with 0.5% monosodium GLT (; creep feed) for suckling pigs on day after farrowing until weaning, and to weaned pigs during first 13 days post-weaning.	↑ suckling pigs' feed intake by 36%. = weaning weight. ↑ weaning pigs ADFI by 10%. ↑ daily BW gain by 7% during first 13 days PW	Gatel and Guion (1990)
21 days of age	Corn and soybean meal basal diet supplemented with 0, 0.2%, 0.6% or 1.0% L-GLN, for 14 days PW.	1% GLN ↑ jejunal VH by 33% during the first week. ↑ gain:feed ratio by 25%. ↑ plasma GLN by 24%, GLT by 74% and alanine by 35% during second week.	Wu *et al.* (1996)
12.5 days of age	Six treatments including corn, whey, oat basal diet (control), basal diet supplemented with either arginine, GLT, citrulline, ornithine or polyamines, at 0.93%, 6.51%, 0.94%, 0.90% and 0.39%, respectively, for 12 days PW.	6.51% GLT ↑ duodenum VH compared with control or polyamine	Ewtushik *et al.* (2000)
21 days of age	Corn soybean meal, barley meal basal diet supplemented with either 0 or 0.5% GLN for 28 days PW.	↑ ileal VH by 23.5% and CD by 51.4%. ↓ VH:CD ratio by 18.3%. ↑ number of mitotic mucosal cells by 4.4%. ↓apoptosis of enterocytes by 5.6% and lymphatic follicles by 25.4%.	Domeneghini *et al.* (2006)
28 days of age	Corn soybean meal basal diet supplemented with either 0, 1% or 2% GLN for 21 days PW.	GLN groups ↗ VH in duodenum and jejunum compared to control. 2% GLN ↑ plasma net xylose absorptive concentration by 10% at 7 days PW and 21% at 14 days PW	Hsu *et al.* (2010)

21 days of age	• Phase 1: 21 (weaning) to 35 days of age, 0.8% mixture of GLN and GLT in diets with no lactose or with 4.0% or 8.0% lactose. • Phase 2**:** 36 to 49 days, all animals received the same diet but with no lactose inclusion in corn and soybean meal basal diet.	0.8% mix of GLN and GLT ↑ BW by 7% and weight gain by 10% from 21 to 49 days of age. ↑ VH in duodenum by 7.7%, jejunum by 10.4% and ileum by 6.6% at 49 days of age	Molino *et al.* (2012)
21 days of age	Corn soybean meal basal diet supplemented with 0, 0.5%, 1%, 2% and 4% monosodium GLT for 21 days PW.	1,2 and 4% monosodium GLT dose-dependently ↑ BW at day 7, 14 and 21 PW compared with 0%. 4% monosodium GLT ↓ ADFI by 15% compared with 0%. 0.5, 1 and 2% monosodium GLT ↓ diarrhoea incidence during 1st week PW compared with 0%.. 0.5, 1,2 and 4% monosodium GLT ↑ jejunal VH. 4% monosodium GLT ↑ jejunal CD compared with 0%. ↑ jejunal concentrations of ATP by 54.8%, DNA by 35.0%, RNA by 30.7%, during the first week after weaning.	Rezaei *et al.* (2013)
21 days of age	Mixtures of GLN and GLT (ratio unknown); five treatments: basal diet supplementation with 0, 0.5%, 1.0%, 1.5% mixtures and positive control (basal diet + 4% porcine plasma) for 21 days in corn- and soybean meal-based diet.	1.0% mixtures ↑ weight gain by 68.3%, feed intake by 29.8%, ↓ FCR by 29.7%. 1.0% mixtures ↓ diarrhoea score by 12.1% compared with porcine plasma control group during the first week after weaning. 1.0% mixtures ↓ duodenal CD by 17.3% and ileum digesta pH by 2.2%. ↑ VH:CD ratio by 36.8% at age 28 days compared to porcine plasma diet.	Teixeira *et al.* (2014)

21 days of age	Corn soybean meal basal diet supplemented with 0 or 1% GLN for 7 days PW compared with piglets reared by sows.	Pigs in control group grew 89% slower than sow-reared piglets over 7 days. Those offered diet with 1% GLN grew 68% slower than sow-reared piglets	Wang *et al.* (2015)
18.8 days of age	Corn soybean meal basal diet supplemented with dietary antibiotics [chlortetracycline (0.44 g/kg) + tiamulin (0.04 g/kg)], or GLN (0.20% GLN as-fed), or not supplemented (no dietary antibiotics/no GLN) for 14 days PW.	Pigs supplemented with GLN had 60.3% ↑ feed intake than those supplemented with antibiotics or not supplemented throughout 14 days of treatment. BW ↑ 8.7% in group supplemented with GLN compared with non-supplemented group. ↑ VH in jejunum and ileum of pigs supplemented with GLN compared with pigs not supplemented. ↑ VH:CD ratio in duodenum (12.1%), jejunum (12.8%) and ileum (15.6%) of pigs supplemented with GLN compared with non-supplemented.	Johnson and Lay (2017)
18.4 days of age	Corn soybean meal basal diet supplemented with antibiotics [A; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (NA), or 0.20% GLN fed for 14 days PW.	↑ BW at day 14 and ADG from day 0 to by 14 3.8% and 11.4%, respectively in GLN pigs compared with NA. = ADFI from day 0 to 14 in GLN pigs compared with NA. = growth between treatments detected after day 14. Day 13 plasma TNF-α ↓ 43% in GLN compared with NA.	Duttlinger *et al.* (2019)
19.1 days of age	Corn soybean meal basal diet supplemented with dietary antibiotics (positive control [PC]; chlortetracycline [441 mg/kg] + tiamulin [38.6 mg/kg]), no antibiotics or added GLN (negative control [NC]), 0.20% GLN, 0.40% GLN,	Increasing GLN in the diet ↗ ADG. Overall, BW at day 35 ↑ in 0.8% GLN and 0.4% GLN compared with NC, 0.2% GLN, and 0.6% GLN pigs. BW at day 35 ↑ for 0.40% GLN and 1.00% GLN pigs vs. 0.20% GLN pigs.	Duttlinger *et al.* (2020)

	0.60% GLN, 0.80% GLN, or 1.00% GLN fed for 14 days PW.	= BW between PC, 0.4%, 0.8% and 1.05 GLN at day 35. 0.4% GLN most effective level while experimental diets were fed and 0.8% most effective for duration of trial considering costs.	
18.4 days of age	Corn soybean meal basal diet supplemented with antibiotics [A; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (NA), or 0.20% GLN fed for 14 days PW.	GLN ↑ VH:CD ratio by 7.0% compared with NA at day 33 PW **Ileal microbiome:** Difference in beta diversity of ileal microbiota of GLN compared with NA at day 33 PW. No difference in alpha diversity. ↑ *Elusimicrobium* and *Neisseria* in GLN compared with NA at day 33 PW. *Lactobacillus* ↑ 2-fold in AB compared with GLN and NA at day 11 PW.	Duttlinger *et al.* (2021)
24 days of age	Six experimental treatments: (1) Basal diet (CO); (2) CO plus 0.6+0%, 0.45+0.15%, 0.3+0.3%, 0.15+0.45%, 0+0.6% (GLT+GLN%) fed for 21 days PW..	= BW; at day 21 PW linear increase GLT ↘ BW. Amino acid supplementation ↑ ADG from day 7 to 14 compared to CO. Amino acid ↓ ADG from day 14 to 21 PW compared to CO. 0GLT+0.6GLN ↑ number of duodenal glands at day 8 PW. Quadratic effect of amino acid supplementation on lymphocyte count in % in the blood at day 8 PW. **Caecal microbiome:** no effect on alpha or beta diversity	Luise *et al.* (2022)

		Mixtures of GLT and GLN ↑ *Lactobacillus* compared to GLT. GLN ↑ abundance of amino acid-fermenting bacteria e.g. *Selenomonas*.	
Not mentioned	Corn soybean meal basal diet supplemented with 0 or 1% GLT for 21 days PW.	GLT ↑ ADFI, ADG and ↓ FCR from weaning to day 21 PW. GLT ↑ VH and VH:CD ratio, and ↓ CD in ileum at day 21 PW. GLT ↑ expression of interleukin 10, and ↓ expression of interleukin-1β, 6, 8, 17 and 21 in ileum. **Colonic microbiome:** GLT↓ alpha and beta diversity . GLT ↑ abundance of *Lactobacillus*, *Prevotellaceae* NK3B31 group, and *Oscillospiraceae* UCG-005.	Liu *et al.* (2023)
24 days of age	Corn soybean meal low protein basal diet (17% crude protein) supplemented with 0, 1, 2 or 3% GLN from day 7 to 35 PW (7 days adaptation phase PW).	↑ BW at day 35 PW, ↑ ADG and ↓ FCR from day 21 to 35 PW for 1% GLN vs 0, 2 and 3%. ↓ BW at day 35 PW, ↓ ADFI, ↓ ADG and ↑ FCR from day 21 to 35 PW for 2 and 3% GLN vs 0 and 1%. ↑ serum glutathione for 1% GLN compared with 0% GLN at day 21 PW. ↓ Hydrogen peroxide for GLN 1, 2 and 3% compared with GLN 0% at day 21 PW. **Faecal microbiome:** no effect of diets on alpha diversity at day 35 PW. Difference in beta diversity of microbiota of pigs fed 0 and 1% vs. 2 and 3% GLN. 1% GLN ↑ abundance of *Erysipelotrichaceae* p_75_a5, *Clostridium, Lactobacillus, Prevotellaceae_Prevotella,*	Li *et al.* (2024)

		and *Gemmiger* and ↓ abundance of *Streptococcus* compared with 0% GLN at day 21 PW.	
21 days of age	Corn soybean meal basal diet supplemented with 0 or 0.81% of GLN from day 3 to day 23 PW (3 days adaptation phase PW).	GLN ↑ VH, VH:CD ratio in duodenum, jejunum and ileum, ↓ plasma diamine oxidase and HSP70 in jejunum at day 23 PW. **Colonic microbiome:** GLN ↑ abundance of *Lactobacillus*, and ↓ abundance of *Clostridium*, *Coccoides*, *Enterococcus* and *Enterobacterium*	Wang *et al.* (2024)

↑Significant increase, ↗ tendency to increase
↓ Significant decrease, ↘ tendency to decrease
= No difference
BW, body weight; ADFI, average daily feed intake; FCR: feed conversion ration; VH, villus height; CD, crypt depth; NA, No antibiotics; GLT, glutamate; GLN, L-glutamine; PW, post-weaning; ATP, adenosine trisphosphate.

1.11 Conclusions

Supplementing pigs with milk/liquid milk replacer or liquid feed post-weaning can increase growth early post-weaning and improve intestinal structure and function. However, there is limited information regarding the effects of these strategies on lifetime growth up to commercial slaughter. Although medication usage is a relatively simple parameter to monitor, there is also no information on the impact of these strategies on antibiotic and anti-inflammatory treatments in pigs. Moreover, there is no information on the effect of supplementing milk/liquid milk replacer or liquid feed on the pig intestinal microbiome, which can give an indication of pig intestinal health early post-weaning. Dietary inclusion of L-glutamine and/or glutamate in dry diets also provides an opportunity to improve the intestinal structure and function of pigs post-weaning, resulting in increased growth. Recent studies also highlight the benefit of adding L-glutamine and glutamate to the diet on the pig intestinal microbiome, but did not monitor medication usage. In addition, liquid milk replacer/liquid feed could be an effective route for the administration of L-glutamine and/or glutamate or other feed additive immediately post-weaning and this should be investigated in future studies.

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1.13 Rationale and objectives of the research performed in this thesis

As outlined in both parts of this literature review, several management and nutritional strategies can be implemented to increase piglet growth to weaning and early post-weaning intake and growth, and consequently have the potential to improve lifetime growth and health of the pig. Such strategies are particularly important considering the recent ban on the use of pharmacological levels of ZnO in pig diets, restrictions on the use of in-feed antibiotics, and continued increases in litter size. However, most pre-weaning management and nutrition studies only monitor post-weaning growth and health in pigs for a limited period of time, if at all. Consequently, there is limited information on how these strategies influence lifetime growth and health in pigs, and therefore, on their economic impact. Some of the pre-weaning interventions examined in this review are inexpensive and easily implemented (e.g., split-suckling or post-partum provision of analgesia to sows). However, in some cases, additional work is needed to determine their effect on post-weaning pig growth and health so that cost-benefit analyses can be conducted. Regarding solid creep feeding in farrowing houses, the available data suggest inconsistent effects on post-weaning pig growth due to often low and variable creep feed intake. Providing milk/milk replacer or liquid feed as creep feed provides an opportunity to increase DMI and the proportion of eaters per litter. In addition, milk/milk replacer/liquid feed could be an effective route for early-life administration of feed additives (e.g., enzymes; L-glutamine; and pro-, pre- and post-biotics). However, liquid feeding of suckling piglets in farrowing rooms needs to be conducted hygienically and requires substantial financial investment. Some of the post-weaning interventions examined in this review demonstrated a strong benefit in terms of increasing DMI and growth in pigs early post-weaning (e.g. liquid milk replacer supplementation early post-weaning and dietary inclusion of feed additives such as L-glutamine). However, information regarding the effects of supplementing liquid milk replacer along with a pelleted diet post-weaning on pig lifetime growth and intestinal integrity and health is very limited. Several studies demonstrated the benefit of adding L-glutamine and/or glutamate in dry pelleted diets fed to pigs post-weaning on pig growth and intestinal integrity and function post-weaning. However, information is missing regarding the effect of adding L-glutamine to liquid feed post-weaning on pigs' lifetime growth and health.

The gaps in the literature highlighted above need to be addressed so that effective nutritional and/or management strategies can be recommended for the improvement of pre- and post-weaning growth and health of piglets raised in large litters. Priorities include obtaining additional information on early management strategies pre-weaning (e.g. split-suckling and anti-inflammatory provision to sows post-partum); a direct comparison of dry and liquid creep feeding of suckling piglets; the use of liquid creep feed for early dietary supplementation with feed additives; and supplementation with liquid milk replacer with or without the inclusion of feed additives in weaned pigs. In all cases, monitoring pig growth and health until commercial slaughter is critical so that cost-benefit analyses can be conducted in order to help inform investment decisions for farmers in the future.

The overall objectives of this thesis therefore are:

- To investigate the application of split suckling with/without postpartum provision of a nonsteroidal anti-inflammatory drug to the sow on colostrum intake in suckling pigs and on lifetime growth, health, and medicinal usage in pigs.
- To determine the effect of providing supplementary dry pelleted starter diet, liquid milk replacer, and a liquid mixture of milk replacer and starter diet as creep feed to suckling pigs on sow body weight and back fat thickness and lifetime growth, health and medicinal usage in pigs.
- To assess the effect of L-glutamine or enzyme supplementation of liquid creep feed on sow body weight and back fat thickness and lifetime growth, health and medicinal usage in pigs.
- To determine the effect of post-weaning supplementary liquid milk replacer and/or dietary inclusion of 1% L-glutamine on lifetime growth, health and medicinal usage in pigs.

2. Postpartum meloxicam administration to sows but not split-suckling increases piglet growth and reduces clinical incidence of disease in suckling piglets

E.A. Arnaud, G.E. Gardiner, K.M. Halpin, C. Ribas, J.V. O' Doherty, T. Sweeney, P.G. Lawlor (2023). Postpartum meloxicam administration to sows but not split-suckling increases piglet growth and reduces clinical incidence of disease in suckling piglets. *Journal of Animal Science*. https://doi.org/10.1093/jas/skad275

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2.1 Abstract

Each suckling pig should receive ≥200 g of colostrum within the first 24 h of life, but with increased litter size this is now difficult to achieve. The aim of this study was to assess the effect of split-suckling and postpartum meloxicam provision to sows as a means of ensuring adequate colostrum intake, on growth and health in pigs pre- and post-weaning. One hundred and four sows (Large White × Landrace) and their litters, averaging 16.3 piglets born alive, were assigned to one of four treatments in a two-by-two factorial arrangement. Factors were provision of meloxicam (yes/no; Mel/N-Mel) and split-suckling (yes/no; Split/N-Split). Meloxicam was administered intramuscularly at 0.4 mg/kg body weight to sows on release of the placenta (~2 h postpartum). Split-suckling commenced 4 h after birth of the first piglet, with the six heaviest piglets removed from the sow for 1 h to allow the lightest piglets to suckle. This was repeated after 1.5 h. Pigs were weighed at birth and at days 1, 6, 14, and 27 after birth and at days 6, 14, 21, 28, 47, and 129 post-weaning. Carcass data were collected at slaughter. Medication usage was recorded from birth to slaughter. There was a split-suckling by meloxicam interaction effect at days 1 to 6 (P<0.001) and days 6 to 14 (P<0.001) after birth. Meloxicam administration had no effect on average daily gain (ADG) when split-suckling was applied; however, when split-suckling was not applied, postpartum meloxicam administration increased ADG. There was a meloxicam × split-suckling interaction for ADG from weaning to day 6 post-weaning (P=0.03). Meloxicam increased ADG when split-suckling was applied but not in its absence. Carcass weight was increased by meloxicam (P=0.01) but was not affected by split-suckling (P>0.05). Meloxicam use in sows reduced the number of clinical cases of disease (P=0.04) in suckling pigs which tended to reduce the volume of antibiotics (P=0.08) and anti-inflammatories (P=0.08) administered. Split-suckling had no effect on medication usage in sows and piglets during lactation but increased their use from weaning to slaughter. In conclusion, postpartum administration of meloxicam to sows is an easily implemented strategy. It reduced clinical cases of disease, increased ADG in pigs during the first two weeks of life and early post-weaning and increased carcass weight at slaughter. However, no split-suckling benefit was observed.

2.2 Introduction

It is essential that all pigs receive at least 200 g of colostrum in the first 24 h of life (Devillers *et al.,* 2011). This is because colostrum is not only an energy source for piglets, but it is also a source of passive immunity (Rutherford *et al.,* 2013). This is because colostrum is not only an energy source for piglets, but it is also a source of passive immunity (Teagasc, 2022). Being born into a large litter reduces the chances of all piglets receiving adequate amounts of colostrum, as heavier and more vigorous pigs can monopolize the sows' teats in the first 48 h postpartum. Split-suckling is a strategy sometimes used to address this. Split-suckling involves removing the more advantaged pigs from the sow for a period of time to allow the smaller and weaker littermates time to suckle without competition. This ensures that all pigs have sufficient opportunity to consume an adequate quantity of colostrum. There are a number of ways to conduct split-suckling. One way is that the heaviest piglets are temporarily denied access to the sow, allowing the lighter piglets greater access to the sow's udder (Kyriazakis and Edwards, 1986; Donovan and Dritz, 2000; Alonso *et al.*, 2012). Another way is to separate piglets based on birth order, with the first half of the litter born being temporarily removed to allow those born later greater access to the sow's udder (Morton *et al.*, 2019). Recent studies suggest that conducting split-suckling of piglets during the first day of life is enough to improve neonatal growth (Morton *et al.,* 2019) and reduce piglet mortality (Huser *et al.*, 2015). Most piglet mortality occurs during the first days of life and is higher among the smaller/lighter piglets within the litter (Quiniou *et al.*, 2002). Conducting split-suckling for three consecutive days has been shown to reduce piglet growth (Vandaele *et al.*, 2020). These data suggest that the first day of life should be targeted when split-suckling is applied.

Pain management in sows following the farrowing process can increase suckling and therefore increase colostrum intake, consequently reducing neonatal mortality, as reviewed by Baxter *et al.* (2013). It has been suggested that postpartum anti-inflammatory treatment of sows may facilitate increased colostrum intake in neonatal pigs by minimizing discomfort and pain in the sows, thereby making them more receptive to suckling by their litter (Mainau *et al.,* 2012). This increased colostrum intake leads to increased pre-weaning piglet growth and immunological status at

weaning (Mainau *et al.*, 2016). Furthermore, anti-inflammatory drugs such as meloxicam can be transferred to piglets through colostrum/milk, resulting in anti-inflammatory effects in piglets (Bates *et al.*, 2014). Therefore, it is reasonable to assume that the resulting stronger, heavier and more immunologically equipped piglets might be more resistant to disease pressure, requiring less medicinal treatment, which could help in reducing antimicrobial usage (AMU) and subsequent development of antimicrobial resistance.

However, there is a lack of information on the effect of combining split-suckling and anti-inflammatory administration to sows postpartum on piglet growth rate, health, and AMU. The objective of this study was to determine the effect of split-suckling with/without postpartum provision of a nonsteroidal anti-inflammatory drug (meloxicam) to the sow, on colostrum intake, growth, health, and medicinal usage in suckling piglets. The residual effects of split-suckling and postpartum meloxicam administration to sows on post-weaning growth, health, and medicinal usage to target slaughter weight (120 kg) were also determined. The hypothesis was that split-suckling and/or meloxicam would increase colostrum intake and pre-weaning growth and health and consequently reduce the need for injectable therapeutic (anti-inflammatory and antibiotic) use in both sows and piglets. Furthermore, it was hypothesized that these benefits would increase lifetime growth in pigs.

2.3 Materials and methods

2.3.1 Ethical approval

This study was performed between March and December 2021, at the Teagasc Pig Development Department, Moorepark, Fermoy, Co. Cork, Ireland. Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. TAEC2020-272) and Waterford Institute of Technology Ethics Committee (approval no. WIT2021REC011). The project was authorized by the Irish Health Products Regulatory Authority (project authorization no. AE19132/P129). The experiment was conducted in accordance with the legislation for commercial pig production set out in

the European communities (welfare of farmed animals) regulations 2010 and in Irish legislation (SI no. 311/2010).

2.3.2 Experimental design and animal management

One hundred and four sows (Large White × Landrace; PIC, Hermitage Genetics, Sion Road, Co. Kilkenny, Ireland) were used in this study, which was conducted in four batches. Sows were artificially inseminated at onset of standing estrus and again 24 h later using pooled semen (Topigs Norsvin Tempo; Premier Pig Genetics Limited, Ireland). Gestating sows were managed in a dynamic group of ~120 animals. Sows were introduced to the dynamic group 3 to 6 d after service. The pen had fully-slatted floors, insulated concrete lying bays, and two electronic sow feeders (ESFs; Schauer Feeding System [Competent 6], Prambachkirchen, Austria). Water was available ad libitum from single-bite drinkers in the ESFs and from five drinker bowls located around the group pen. On day 107 of gestation, sows were blocked within farrowing batch into 26 blocks of four sows on the basis of parity group (mean ± SD; 2.3 ± 0.99), number of born alive piglets at the previous farrowing (12 ± 7.7) and body weight (BW) (269.8 ± 31.58 kg). Sow parity group distribution was as follows: group one, parity 0 (24%); group two, parity 1 to 2 (31%); group three, parity 3 to 5 (33%); and group four, parity > 5 (12%). The experiment was a two-by-two factorial arrangement with the factors being split-suckling (yes/no; Split/N-Split) and provision of meloxicam postpartum (yes/no; Mel/N-Mel). Within block, sows were randomly assigned to the following treatments: 1) no split-suckling application and no postpartum provision of meloxicam to the sow (N-Split/N-Mel), 2) no split-suckling application and postpartum provision of meloxicam to the sow (N-Split/Mel), 3) split-suckling application and no postpartum provision of meloxicam to the sow (Split/N-Mel), and 4) split-suckling application and postpartum provision of meloxicam to the sow (Split/Mel).

Approximately 5 d before sows were due to farrow they were moved into standard farrowing crates in pens (dimension: 2.5 m × 1.8 m) with cast-iron slats under the sow and plastic slats for the piglets. Where meloxicam (Loxicom Injection, Norbrook, Newry, Northern Ireland) was provided to sows, it was administered intramuscularly

(IM) at 0.4 mg/kg of BW on release of the placenta (~2 h postpartum) using a 10 mL syringe (Becton Dickinson, Franklin Lakes, New Jersey, USA) and a 1.65 × 38 mm needle (Agriject disposable needles, AgriHealth, Monaghan, Ireland). Where split-suckling was conducted it commenced 4 h after the birth of the first piglet with the six heaviest piglets removed from the sow for 1 h to allow the lightest piglets to suckle. During the separation time, the six heaviest piglets were marked and restrained in a bottomless wooden box placed on a water-heated pad (Big Dutchman heating plates, Vechta, Germany) beside the farrowing crate. This procedure was repeated after a period of 1.5 h.

Farrowing room temperature was maintained at ~24 °C. The temperature of the heat pads was 38 to 40 °C for the first 2 d after farrowing and was reduced by 1 °C each day to 30 °C at 10 d after farrowing and it was maintained at this until weaning. Artificial lighting was provided daily from 0800 to 1630 hours. Where possible, litter size was standardized between 24 and 48 h after parturition, with cross fostering only being conducted within treatment group, so that there was an average litter size of 14.3 ± 1.80 piglets per sow at 48 h postpartum. The final number of piglets remaining on each sow at 48 h postpartum was affected by the rearing capacity of the sow (i.e., the number of functional teats), the availability of foster sows to take surplus piglets, and the time of farrowing. Piglets' teeth were clipped within 24 h of birth. On day 5 postpartum tails were docked and all piglets were injected with 1 mL of iron (Gleptosil, Ceva Santé Animale, Libourne, France). Male pigs remained fully intact and piglets were weaned at day 28 ± 0.8 of lactation. To study the residual effects of split-suckling and postpartum analgesia provision to sows in progeny, a subsample of 506 pigs (8.0 ± 1.36 kg) were selected at weaning (four to five male and female pigs per sow). Within each treatment group, pigs were formed into single sex groups of 10 to 12 pigs of even weight while ensuring that pigs from individual litters were not over-represented within the pen group. Pens were blocked on sow treatment, sex (entire male or female) and birth weight category (light birth weight [<1.25 kg] or heavy birth weight [>1.30 kg]). Pen groups for treatment N-Split/NMel (n = 14), treatment Split/N-Mel (n = 11), treatment Split/ Mel (n = 13) and treatment N-Split/Mel (n = 12) were moved to weaner accommodation at weaning. Pigs were monitored to slaughter, with feed disappearance and pig weight recorded. Temperature in the weaner rooms was maintained at 28 °C during the first week after weaning and

reduced by 2 °C each week to 22 °C at the end of 4 wk. Weaner pens were 2.5 m × 2 m with fully-slatted plastic floors. Ventilation was from a punched ceiling with air exhausted via a variable speed fan linked to a thermostat which was controlled by computer (Big Dutchman 135). At day 47 post-weaning, pen groups were moved to finisher accommodation, with pig weight recorded prior to slaughter and feed intakes monitored weekly to target slaughter weight. Temperature in the finisher rooms was maintained at 20 to 22 °C with the same type of ventilation system used as in the weaner house. Finisher pens were 4.2 m × 2.4 m with a slatted concrete floor. All rooms were equipped with windows for natural light. Pigs in each experimental treatment group were slaughtered over 2 wk when they reached the target slaughter weight of ~120 kg live weight (average age at slaughter 157 d). The heaviest pigs in each pen group were slaughtered during the first week and the remaining pigs in the pen were slaughtered 7 d later.

2.3.3 Diet preparation and feeding

Diets were formulated to meet or exceed National Research Council (NRC) recommendations (NRC, 2012). The ingredient composition and nutrient content of the diets are shown in Table 2-1. During gestation, sows were fed a gestation diet (diet 1) in meal form at a feed allowance of 2.2 kg/d between days 0 to 90 of gestation. From day 90 of gestation to parturition, gestation feed allowance was increased to 2.7 kg/d. In the farrowing room, diets were fed using a computerized feed delivery system (DryExact Pro, Big Dutchman). Sows were fed a lactation diet (diet 2; Table 2-1) in meal form twice daily from farrowing to day 6 of lactation and three times daily from day 7 to weaning at 28 d. Sows were fed according to a lactation feeding curve which started at 60 MJ DE/d at day 0 of lactation and gradually increased to 107, 125, 133, and 137 MJ DE/d at days 7, 14, 21, and 26 of lactation, respectively. During lactation, feeding curves for individual sows were adjusted up or down, as required, to ensure that sow feed intake was as close to ad libitum feeding as possible and to prevent feed wastage. Water was provided on an ad libitum basis to sows from a single-bite drinker in the feed trough and to suckling piglets from a bowl in the farrowing pen. Starter diet (diet 3; Table 2-1) was fed in pelleted form (3 mm diameter pellets) as creep feed to suckling pigs from day 14 after birth until weaning using a creep feeder (Easy pan;

Rotecna, Lleida, Spain) placed at the bottom of the heat pad. Creep feed intake per litter was determined weekly. Between weaning and service, sows were provided with ad libitum access to a lactation diet and following insemination were restrictively fed a gestation diet (diet 1; Table 2-1), both in meal form.

Following weaning, pigs were fed a sequence of diets in accordance with their growth stage. Starter diet (diet 3; Table 2-1) was provided from weaning to day 6 post-weaning, link diet (diet 4; Table 2-1) from days 6 to 17 post-weaning, weaner diet (diet 5; Table 2-1) from days 17 to 47 post-weaning, and a finisher diet (diet 6; Table 2-1) from day 47 post-weaning to slaughter (~ day 157 of age). All diets fed post-weaning were in pelleted form (3 mm diameter) and all were provided on an ad libitum basis. Each weaner pen had a single-space (33 cm) wet-dry feeder (Verba, Sint-Oedenrode, Netherlands) with inset nipple drinker. Each finisher pen had one shelf-type single-space (33 cm) wet-dry feeder (Verba) with inset nipple drinker. Water was available on an ad libitum basis from a single bowl drinker (Rotecna) per pen in weaner and finisher rooms. Pigs were inspected daily and any pig showing signs of ill health was treated appropriately. Assessment of clinical signs of disease and treatment protocols were followed in accordance with farm policies. All veterinary treatments were recorded including antibiotic and anti-inflammatory treatments.

2.3.4 Sow body weight and back fat thickness

Sow BW and back fat (BF) were recorded on day 110 of gestation, at weaning, and at their subsequent service (~day 6 post-weaning). Sow BW was recorded using an electronic sow scales (EziWeigh 7i, O'Donovan Engineering, Coachford, Co. Cork, Ireland). Empty farrowing weight was calculated using the following equation from the NRC, 1998[1]:

$$^{1}SW_{farr} = [SW_{d110} – (NB \times 2.25)].$$

Where, SW_{farr} = Empty sow farrowing weight, SW_{d110} = Sow weight at day 110 of gestation, NB = total number of piglets born. The 2.25 kg is an estimate of the increased weight in the gravid uterus and in mammary tissue attributed to each pig in a litter (NRC, 1998).

Back fat was measured using a digital BF indicator (Renco LEANMEATER, Renco Corporation, Golden Valley, MN, USA) by placing the probe of the digital indicator on the back of the sow at the level of the last rib, 6 cm from the side of the backbone. A reading was taken from the right and left side of the sow and the average reading was recorded.

2.3.5 Farrowing performance and pre-weaning piglet growth performance

The number of piglets born (total, live, stillborn, and mummified) was recorded for each litter at birth. Farrowing duration and time of birth of the first piglet were recorded for each sow. The weight and sex of each piglet was recorded at birth when each piglet was tagged for identification purposes. Piglets were individually weighed at birth and 24 h after birth and on days 7, 14, and 27 postpartum using an electronic piglet scale (Defender 3000 XtremeW, O'Donovan Engineering) and these data were used to determine the litter weight at each weighing, and piglet pre-weaning average daily gain (ADG). Piglet mortality between birth and weaning was also recorded.

2.3.6 Post-weaning pig growth performance

Pen groups were weighed on days 6, 14, 21, 28, and 47 post-weaning and individual pig weights were recorded just prior to slaughter (at ~day 129 post-weaning) using an electronic scale (EziWeigh 7i, O'Donovan Engineering). Pigs were fasted for 15 to 18 h prior to slaughter before weighing. Feed disappearance was recorded on a pen basis from weaning to slaughter at the same time points that pig weights were recorded. These data were used to determine the ADG, average daily feed intake (ADFI), and gain to feed (G:F) per pen.

2.3.7 Carcass data

Pigs were transported 95 km to the abattoir (Dawn Pork & Bacon, Grannagh, Co. Waterford, Ireland) where they were killed by exsanguination after CO2 stunning. At the abattoir, carcass cold weight of individual pigs was calculated by multiplying the

hot carcass weight, recorded within 45 min of the pig being exsanguinated, by 0.98. Back fat and muscle depth, measured at 6 cm from the edge of the split back at the level of the third and fourth last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean meat content was estimated according to the following formula(Department of Agriculture and Food and Rural Development, 2001):

$$CL=60.3 - 0.847x + 0.147y$$

The following equations were used to determine parameters of interest relating to carcass growth (Lawlor and Lynch, 2005):

$$C_{ADG} = [(CW – WW \times 0.65) \times 1{,}000] / D1$$

$$C_{G:F} = C_{ADG}/ FI$$

$$L_{ADG} = (CW \times CL \times 10) / D2$$

Where C_{ADG} = carcass ADG (from weaning to slaughter), CW = carcass weight (kg), WW = weaning weight (kg), D1 = number of days from weaning to slaughter, $C_{G:F}$ = Carcass G:F, FI = daily feed intake (g), L_{ADG} = Lean ADG (from birth to slaughter), CL = carcass lean meat percentage, and D2 = number of days from birth to slaughter.

2.3.8 Colostrum intake

Colostrum intake was estimated 24 h after the birth of the last piglet using the equation of Theil et al. (2014) as follows:

$$\text{Colostrum intake (g)} = -106 + (2.26 \times WG) + (200 \times BWB) + (0.111 \times D) – [1{,}414 \times (WG/D)] + [0.0182 \times (WG/BWB)].$$

Where WG is piglet weight gain (g) from birth to 24 hours of life, BWB is piglet BW at birth (kg) and D is the duration in minute of suckling between birth of last piglet and weighing at ~ 24 h (1440 min).

2.3.9 Milk sampling and compositional analysis

On day 14 of lactation, milk samples were collected from sows (n = 13 sows/treatment) by milking the first four teats immediately distal to the sow's head on one side of the udder following administration of a 1 mL (10 IU) IM injection of oxytocin (Oxytocin, AgriHealth) to induce milk let-down. Samples for compositional analysis were stored at −20 °C until analysis. Milk samples were defrosted at room temperature. When fully thawed, samples were mixed by inverting several times to disrupt settled solids, and mixed well. Each sample was analyzed for total solids, lactose, fat and protein content by near-infrared absorption using a Bentley Dairyspec FT (Bentley Instruments Inc., Chaska, MN, USA).

2.3.10 Health monitoring

Fecal consistency scores were determined for piglets at days 1, 7, 14, 21, and 28 before weaning and at days 6, 14, 21, 28, 47, and 129 post-weaning (the latter was just prior to slaughter). A four-point scoring system (Casey *et al.*, 2007) was used and the average score from five pigs was determined as the average score for each crate/pen. In brief: 0 = normal (dry pelleted feces), 1 = soft (soft with shape), 2 = mild diarrhoea (very soft or viscous liquid), and 3 = severe diarrhoea (watery or with blood).

Antibiotic and anti-inflammatory usage was recorded in sows during lactation and in pigs during each production stage from birth until they reached target slaughter weight. Medication was administered when joint-ill, lameness, malaise or diarrhoea were observed in piglets and when malaise or vaginal discharge was observed in sows. Only one antibiotic (Unicillin, Univet Limited, Cootehill, Co. Cavan, Ireland) and one anti-inflammatory (Loxicom, Norbrook), were used during this experiment. Animal ID, pen number, product name, product code, dose administered (mL), frequency of administration, date of administration, and reason for use were recorded when an animal was treated. From this, the total number of piglet injections per litter, the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a litter basis and per sow, and the total number of piglet clinical cases (i.e., when an animal was treated one or more times) per litter were calculated pre-weaning. The

average volume of medication (antibiotic and anti-inflammatory) administered per pig on a pen basis was calculated post-weaning, for both the weaner and finisher periods.

2.3.11 Statistical analysis

All data were tested for normality using the Univariate procedure. Residuals were inspected in all models to confirm normality. Model fit was determined by choosing models with the minimum finite-sample-corrected Akaike Information Criteria.

Growth parameters pre-weaning (ADG, BW, and litter weight), growth parameters post-weaning (BW, ADFI, ADG, and G:F), carcass quality, deaths or removals, colostrum intake, sow BW, sow BF, weaning to service interval, milk composition, total number of piglet injections per litter, average volume of medication (antibiotic and anti-inflammatory) administered per pig on a litter basis and per sow, total number of piglet clinical cases per litter pre-weaning and average volume of medication (antibiotic and anti-inflammatory) administered per pig on a pen basis post-weaning were analysed in SAS using the linear mixed models procedure (PROC MIXED) in the Statistical Analysis Systems (SAS) software package version 9.4 (SAS Institute Inc., Cary, NC, US) for a two-by-two factorial arrangement. The incidence of diarrhoea in the farrowing houses from days 2 to 28 was analyzed using the PROC Genmod procedure of SAS for a two-by-two factorial arrangement. Data from batches one, two, three, and four were analyzed together as all measurements were recorded at the same time points.

For piglet pre-weaning growth parameters, sow BW, sow BF and weaning to service interval, split-suckling, meloxicam provision and their associated interactions were included in the model as fixed effects. For analysis of pre-weaning piglet growth parameters; piglet birth weight and litter size at 48 h were included as covariates, when significant in the model. For analysis of sow BW; sow BW at day 110 of gestation was included as a covariate in the model. For analysis of sow BF; BF at day 110 of gestation was included as a covariate in the model. For analysis of weaning to service interval; parity rank was included in the model as a covariate. For analysis of pre-weaning growth, sow BW and sow BF; day was included in the model as the repeated variable. Block was included as a random effect. Pig nested within sow/litter was the experimental unit for pre-weaning piglet growth parameters and sow/litter was the experimental unit for analysis of sow BW, sow BF, weaning to service interval and litter weight.

For the analysis of the total number of piglet injections per litter, the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a litter basis and per sow, the total number of piglet clinical cases of disease per litter pre-weaning, colostrum intake per pig, number of deaths and removals per litter (birth to weaning and 48 h to weaning); split-suckling, meloxicam provision and their associated interactions were included in the model as fixed effects. As cross fostering was completed at 48 h; litter size at 48 h was included in the model as a covariate for the analysis of the number of deaths between 48 h and weaning. For analysis of the number of clinical cases per litter; birth weight was included in the model as a covariate. Block was included as a random effect and sow/litter was the experimental unit.

For the analysis of diarrhoea incidence; split-suckling, meloxicam provision, day and the associated two-way and three-way interactions were included in the model. A fecal score of two or greater was considered representative of diarrhoea. For the analysis of milk composition; split-suckling, meloxicam provision and their associated interactions were included in the model as fixed effects. Parity rank was included in the model as a covariate when significant in the model. The sow was the experimental unit. To determine the effect of sow treatment (split-suckling and meloxicam provision) on piglet colostrum intake, ADG from birth to weaning and weaning weight for pigs from each birth weight category, low (<1.25 kg) and high (>1.25 kg); the model included birth weight category (low and high), sow treatment (split-suckling or meloxicam provision) and their associated interactions as fixed effects. Block was included as a random effect and pig nested within sow/litter was the experimental unit. For post-weaning growth parameters; split-suckling, meloxicam provision, and their associated interactions were included in the model as fixed effects. Weaning weight was included in the model as a covariate when significant in the model. Day was included as a repeated variable in the model and block was included as a random effect. The pen group was considered the experimental unit. For carcass quality data and the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a pen basis post-weaning (for both the weaner and finisher periods) the model was the same but day was not included as a repeated variable. In all cases, differences between least square means were investigated using the t-test after Tukey adjustment for multiple comparisons. Results are presented in the text and tables as

the least square means together with their pooled standard error. Differences between treatments were considered significant for $P \leq 0{\cdot}05$, whereas $0.05 < P \leq 0{\cdot}10$ was considered as a tendency.

2.4 Results

2.4.1 Pig removals and deaths

In total, 15 sows were removed from the study. Four sows were removed before applying the experimental treatment because the number of piglets born alive was <9 (one in the N-Split/Mel treatment and three in the Split/N-Mel treatment). Three sows were removed because they aborted in the farrowing house more than 3 d before their expected farrowing date (one from the N-Split/N-Mel treatment, one from the Split/N-Mel treatment, and one from the Split/Mel treatment). Eight sows were removed because they were medicated around farrowing due to sickness or because of farrowing complications (two from the N-Split/N-Mel treatment, two from the N-Split/Mel treatment, two from the Split/N-Mel treatment and two from the Split/Split treatment). All removals were unrelated to experimental treatment.

Eighteen percent of all piglets on trial died pre-weaning. Among the dead piglets, 36% died in the first 24 h, 46% between days 1 and 6, 8% between days 6 and 14 and 10% between days 14 and 27. Pre-weaning mortality was 16%, 14%, 18%, and 21% for N-Split/N-Mel, N-Split/Mel, Split/N-Mel, and Split/Mel treatments, respectively. Deaths during the first 24 h and from days 1 to 6 were mainly due to starvation or crushing. After day 6, causes of mortality were more variable and included crushing, starvation, injury, and sudden death.

Three percent of all pigs on trial died post-weaning. Among the dead pigs, 38% of the pigs died between days 28 and 47 post-weaning and 62% died after day 47 post-weaning. Post-weaning mortality was 0%, 6%, 3%, and 2% for N-Split/ N-Mel, N-Split/Mel, Split/N-Mel, and Split/Mel treatments, respectively. Deaths and removals were due to starvation, lameness, or injury (only one animal).

2.4.2 Sow growth and reproductive performance

2.4.2.1 Sow body weight and back fat thickness

Meloxicam × split-suckling interactions for sow weight, BF depth, and reproductive performance from farrowing to service are shown in Supplementary Table S 2-1. No meloxicam × split-suckling interaction (P>0.05) was found for any variable of interest. Sows from the meloxicam group tended to be lighter at weaning than those which did not receive meloxicam (235.4 vs. 241.6, SEM = 2.39 kg; P=0.06). Treatment did not influence sow BF depth or weaning to service interval (P>0.05; Supplementary Table S 2-1).

2.4.2.2 Litter size, fostered and pre-weaning deaths

Meloxicam × split-suckling interactions for sow litter size, deaths per litter and the number of piglets fostered are shown in Supplementary Table S 2-2 and treatment main effects are in Supplementary Tables S 2-3 and S 2-4. There was no meloxicam × split-suckling interaction for litter size at birth, 48 h after birth or at weaning and for the number of piglets fostered or deaths per litter (P>0.05). Meloxicam administration (P>0.05) or split suckling (P>0.05) had no effect on any parameter of interest.

2.4.3 Pre-weaning piglet growth performance

Meloxicam × split-suckling interactions for piglet weight and growth during the suckling period are shown in Table 2-2 and treatment main effects in Supplementary Tables S 2-5 and S 2-6. There was no meloxicam × split-suckling interaction for litter weight during the whole lactation period (P>0.05). Litters from the meloxicam group were heavier at day 6 (33.0 vs. 30.0, SEM = 0.93 kg; P=0.02) and day 14 (60.5 vs. 56.0, SEM = 1.54 kg; P=0.04) after birth than litters of sows which did not receive meloxicam. Split-suckling did not affect litter weight at any time (P>0.05). There was

no meloxicam × split-suckling interaction for average pig weight at birth (day 0) and day 1 after birth (P>0.05). There was a meloxicam × split-suckling interaction

for pig weight at day 6 (P=0.04), 14 (P<0.001) and 27 (P<0.001) after birth and overall (P<0.001). At days 6 and 14 postpartum, meloxicam had no effect on piglet weight when split-suckling was applied; however, when split-suckling was not applied, meloxicam increased piglet weight (P<0.05). At day 27, whether split-suckling was applied or not, meloxicam increased piglet weight (P<0.001); however, the increase in weight was significantly higher when split-suckling was not practiced. Meloxicam tended to increase piglet weight at day 6 (2.29 vs. 2.21, SEM = 0.046 kg; P=0.06), and increased piglet weight at day 14 (4.38 vs. 4.16, SEM = 0.047 kg; P<0.001) and day 27 (8.18 vs. 7.83, SEM = 0.047 kg; P<0.001) after birth. Overall, meloxicam administration increased piglet weight (P<0.001). Split-suckling reduced piglet weight at day 14 (4.20 vs. 4.34, SEM = 0.046 kg; P<0.01) and day 27 (7.92 vs. 8.09, SEM = 0.047 kg; P<0.001). Overall, split-suckling reduced piglet weight (P=0.01).

There was a meloxicam × split-suckling interaction for piglet ADG from days 1 to 6 (P<0.001), from days 6 to 14 (P<0.001) and overall (P<0.001). From days 1 to 6 and 6 to 14, meloxicam had no effect on ADG when split-suckling was applied; however, when split-suckling was not applied, it increased ADG. Meloxicam increased ADG from days 1 to 6 (171 vs. 160, SEM = 4.4 g/d; P<0.001), days 6 to 14 (252 vs. 242, SEM = 4.8 g/d; P=0.03), and overall (P<0.01). Split-suckling reduced ADG from days 1 to 6 (161 vs. 171, SEM = 4.4 g/d; P<0.01) and days 6 to 14 (242 vs. 252, SEM = 4.7 g/d; P=0.04). Overall, split-suckling did not affect ADG (P=0.12).

The effect of birth weight category and treatment on ADG from birth to weaning and weight at weaning are presented in Supplementary Tables S 2-7 and S 2-8. There was no meloxicam × weight category interaction for pig ADG from birth to weaning (P>0.05) or for pig weight at weaning (P>0.05). There was a split-suckling × weight category interaction for ADG from birth to weaning (P<0.01). Heavy pigs at birth had a lower ADG when split-suckling was applied, while split-suckling had no effect on the ADG of light birth weight pigs. There was a split-suckling × weight category interaction for pig weight at weaning (P=0.02). Heavy pigs at birth had a lower weaning weight when split-suckling was applied, while split-suckling had no effect on the weaning weight of light birth weight pigs.

2.4.4 Post-weaning pig growth and carcass quality

Meloxicam × split-suckling interactions for feed intake and pig growth from weaning to slaughter are shown in Table 2-3 and treatment main effects in Supplementary Tables S 2-9 and S 2-10.

2.4.4.1 Pig weight

There was no meloxicam × split-suckling interaction for pig weight at any time point, except slaughter (P<0.001). Split-suckling reduced pig weight at slaughter when meloxicam was not applied; however, meloxicam increased pig weight at slaughter whether split-suckling was applied or not. Meloxicam had no effect on pig weight at any time point, except at slaughter when it increased weight (121.7 vs. 118.1; SEM = 0.57 kg; P<0.001). Overall, pig weight was not affected by meloxicam. Split-suckling had no effect on pig weight at any time point, except at slaughter when it reduced weight (119.0 vs. 120.8; SEM = 0.58 kg; P=0.02). Overall, pig weight post-weaning was not affected by split-suckling (P>0.05).

2.4.4.2 Pig feed intake

There was no meloxicam × split-suckling interaction for pig ADFI from weaning to day 6, days 6 to 14, days 14 to 21, days 21 to 28, days 28 to 47, day 47 to slaughter, and overall (P>0.05). Meloxicam had no effect on pig ADFI from weaning to day 6, days 6 to 14, days 14 to 21, days 21 to 28, days 28 to 47, day 47 to slaughter, and overall (P>0.05). Split-suckling tended to reduce pig ADFI from days 6 to 14 (P=0.07) but had no effect during any of the other time periods or overall (P>0.05).

2.4.4.3 Pig growth

There was a meloxicam × split-suckling interaction for pig ADG from weaning to day 6 post-weaning (P=0.03). Meloxicam increased ADG when split-suckling was conducted but not when split-suckling was not conducted. Meloxicam increased ADG from weaning to day 6 post-weaning (228 vs. 200; SEM = 11.0 g/d; P=0.04) and

tended to increase ADG from day 47 to slaughter (1,079 vs. 1,046; SEM = 14.2 g/d; *P*=0.07) but had no effect during any of the other time periods or overall (*P*>0.05). Split-suckling had no effect on pig ADG during any time period or overall.

2.4.4.4 Pig feed efficiency

There was a meloxicam × split-suckling interaction for G:F from weaning to day 6 post-weaning (*P*=0.04). Combining meloxicam and split-suckling increased G:F whereas the provision of either alone, did not affect G:F. There was a tendency for a meloxicam × split-suckling interaction for G:F from day 47 to slaughter (*P*=0.09). Meloxicam administration without split-suckling tended to increase G:F compared to meloxicam with split-suckling. There was no meloxicam × split-suckling interaction for pig G:F for any of the other time periods or overall (*P*>0.05). From weaning to day 6, meloxicam increased G:F (1.10 vs. 0.99; SEM = 0.035 g/g; *P*=0.04). From day 46 to slaughter, meloxicam tended to increase G:F (0.46 vs. 0.44; SEM 0.006 g/g; *P*=0.10). Meloxicam did not affect G:F during any of the other time periods or overall (*P*>0.05). From weaning to day 6, split-suckling tended to increase G:F ratio (1.09 vs. 0.99; SEM = 0.020 g/g; *P*=0.06). However, split-suckling did not affect G:F during any of the other time periods. Overall, split-suckling tended to increase the G:F ratio (*P*=0.07).

2.4.4.5 Carcass data

Meloxicam × split-suckling interactions for carcass parameters are shown in Table 2-4 and treatment main effects in Supplementary Tables S 2-11 and S 2-12. There was no meloxicam × split-suckling interaction for carcass weight, fat depth, muscle depth, lean meat percentage, kill out percentage, carcass G:F from weaning to slaughter, or lean ADG (*P*>0.05). There was a meloxicam × split-suckling interaction for carcass ADG from weaning to slaughter (*P*<0.01). Meloxicam decreased carcass ADG when split-suckling was applied. There was no meloxicam effect on fat depth, muscle depth, lean meat percentage, kill out percentage, carcass G:F from weaning to slaughter or lean ADG. Meloxicam increased carcass weight (93.0 vs. 90.2 kg; SEM 0.75 kg;

P=0.01) and carcass ADG from weaning to slaughter (872 vs. 838; SEM 2.4 g/g; *P*<0.001). Split-suckling had no effect on carcass weight, muscle depth, kill out percentage, carcass G:F from weaning to slaughter or lean ADG. It reduced fat depth (13.1 vs. 14.4; SEM 0.30 mm; *P*<0.01), increased lean meat percentage (58.8 vs. 58.0; SEM 0.24%; *P*=0.02), and reduced carcass ADG from weaning to slaughter (844 vs. 865; SEM 3.3 g/g; *P*<0.01).

2.4.5 Sow and pig health

2.4.5.1 Colostrum intake and milk composition

Meloxicam × split-suckling interactions for piglet colostrum intake are shown in Table 2-5 and treatment main effects in Supplementary Tables S 2-13 and S 2-14. There was no meloxicam × split-suckling interaction for piglet colostrum intake (*P*>0.05). Split-suckling had no effect on piglet colostrum intake (*P*>0.05). However, meloxicam tended to increase colostrum intake (345 vs. 327; SEM = 7.2 g/pig; *P*=0.06). The effect of birth weight category and treatment on colostrum intake are presented in Supplementary Tables S 2-15 and S 2-16. There was no meloxicam × weight category interaction effect on colostrum intake (*P*>0.05). There was a tendency for a split-suckling × weight category interaction effect on colostrum intake (*P*=0.08). Heavy pigs at birth tended to have a lower colostrum intake when split-suckling was applied. Split-suckling had no effect on the colostrum intake of light birth weight pigs (*P*>0.05).

Meloxicam × split-suckling interactions for the composition of sow milk at day 14 postpartum are shown in Supplementary Table S 2-17. There was no meloxicam × split-suckling interaction for the total solids, lactose, fat, or protein content of sow milk (*P*>0.05). Neither postpartum meloxicam administration nor split-suckling had an effect on any of the compositional parameters (*P*>0.05).

2.4.5.2 Pre-weaning diarrhoea incidence, antibiotic and anti-inflammatory treatment and clinical cases of disease

Meloxicam × split-suckling interactions for diarrhoea incidence from days 2 to 28 after birth, the total number of clinical cases of disease per litter, the total number of injections per litter pre-weaning, and the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a litter basis and per sow are shown in Table 2-5 and treatment main effects in Supplementary Tables S 2-13 and S 2-14. There was no meloxicam × split-suckling interaction for diarrhoea incidence from days 2 to 28 (P>0.05). Meloxicam had no effect on diarrhoea incidence from days 2 to 28 (P>0.05). Split-suckling decreased diarrhoea incidence from days 2 to 28 (5.1 vs. 14.8; SEM = 2.19%; P<0.01).

There was no meloxicam × split-suckling interaction for the average volume of antibiotic used per sow, the average volume of antibiotic used per pig, the average volume of anti-inflammatory used per sow, the average volume of anti-inflammatory used per pig, total number of injections per litter and total number of clinical cases of disease per litter (P>0.05). Split-suckling had no effect on the average volume of antibiotic or anti-inflammatory used per sow or per pig, the total number of injections per litter, and the total number of clinical cases per litter (P>0.05). Meloxicam had no effect on the average volume of antibiotic used per sow (P>0.05) but it tended to reduce the average volume of antibiotic used per pig (0.1 vs. 0.2; SEM = 0.05 mL/pig; P=0.08). Meloxicam tended to reduce the average volume of anti-inflammatory used per sow (0.61 vs. 2.78; SEM = 0.785 mL/ sow; P=0.06) and the number of injections per litter (2.8 vs. 5.6; SEM = 1.06; P=0.06). It reduced the number of clinical cases per litter (1.0 vs. 2.1; SEM = 0.40; P=0.04) and tended to reduce the average volume of anti-inflammatory used per pig (0.02 vs. 0.04; SEM = 0.007 mL/pig; P=0.08).

2.4.5.3 Post-weaning fecal consistency scores and antibiotic and anti-inflammatory treatment

Statistical analysis of the effect of treatment on post-weaning diarrhoea could not be conducted, as the occurrence of fecal consistency scores > zero was rare (a score of

two or greater is required to be considered diarrhoea). Out of the 250 fecal consistency scores assigned up to day 28 post-weaning, a score of one was given fourteen times to the N-Split/N-Mel treatment, five times to the N-Split/Mel, eleven times to the Split/N-Mel and eight times to the Split/Mel. A score of two (considered diarrhoea) was given three times to the N-Split/N-Mel, once to the N-Split/Mel, four times to the Split/N-Mel and five times to the Split/Mel. A score of three (considered diarrhoea) was given three times to the N-Split/N-Mel, zero times to the N-Split/Mel, zero times to the Split/N-Mel and once to the Split/Mel. No scores higher than zero were given after 28 d post-weaning.

Meloxicam × split-suckling interactions for post-weaning antibiotic and anti-inflammatory treatments in pigs are shown in Table 2-6 and treatment main effects in Supplementary Tables S 2-18 and S 2-19. There was no meloxicam × split-suckling interaction effect on antibiotic or anti-inflammatory usage per pig during either the weaner or finisher periods or during the entire period from weaning to slaughter ($P > 0.05$). Neither meloxicam nor split-suckling affected antibiotic or anti-inflammatory usage per pig during the weaner period ($P>0.05$). Meloxicam had no effect on antibiotic or anti-inflammatory usage per pig during the finisher period ($P>0.05$). Split-suckling tended to increase antibiotic (0.59 vs. 0.23; SEM 0.152 mL/pig; $P=0.06$) and anti-inflammatory usage per pig (0.20 vs. 0.08; SEM 0.043 mL/pig; $P=0.06$) during the finisher period. It also increased antibiotic (0.77 vs. 0.34; SEM 0.130 mL/pig; $P=0.02$) and anti-inflammatory usage per pig (0.29 vs. 0.13; SEM 0.064 mL/pig; $P=0.02$) during the period from weaning to slaughter. Meloxicam tended to increase antibiotic (0.72 vs. 0.40; SEM 0.130 mL/pig; $P=0.09$) and anti-inflammatory usage per pig (0.27 vs. 0.15; SEM 0.045 mL/pig; $P=0.07$) during the weaning to slaughter period.

2.5 Discussion

To our knowledge, this study is the first to determine the effect of split suckling, postpartum provision of meloxicam to sows, and their interaction on pig growth and health to slaughter weight in pigs. Both strategies aim to increase piglet colostrum intake and as a consequence increase pig growth and health. Successful strategies may

help to increase lifetime growth and reduce antibiotic usage in pigs born into large litters. This is important now that there are restrictions on the use of antibiotics and pharmacological levels of zinc oxide in pig production.

2.5.1 Pig growth

In the current study, meloxicam was provided to sows after release of the placenta (at the end of farrowing), as earlier meloxicam administration could inhibit the release of prostaglandins, thereby increasing farrowing duration (Rao and Knaus, 2008). Data in the literature suggests that meloxicam can reduce inflammatory pain (Engelhardt *et al.*, 1995) and this was expected in the current study since administration was conducted at a time when the inflammatory process was established. The increase in colostrum and milk intake in piglets following meloxicam administration to sows, assumed from weaning weight, supports the hypothesis that pain relief was provided to the sows, thereby facilitating nursing activity.

Pre-weaning piglet growth was improved by meloxicam administration. Sows from the meloxicam group had a lower BW at weaning, most likely indicating increased mobilization of body resources during lactation. It is likely that this resulted in increased milk production in these sows (Strathe *et al.*, 2017). Increased pre-weaning growth in piglets from these sows, particularly when split-suckling was not practiced, supports this. The benefit of providing meloxicam postpartum to sows on piglet ADG observed in the current study is similar to results from previous studies where meloxicam was administered orally to sows at the beginning of farrowing (Mainau *et al.*, 2016; Navarro *et al.*, 2021). However, in other studies, when meloxicam was administered by IM injection at the same dose and time as in the current study, no effect on piglet ADG from birth to weaning was observed (Mainau *et al.*, 2012). However, our study design differed from that of Mainau *et al.* (2012) where weaning age was 21 d postpartum rather than 28 d. Furthermore, the number of piglets born alive was 12.2 compared with 16.3 in the current study, and this most likely explains why results differed across studies. Interestingly, Mainau *et al.* (2012) found that the pre-weaning ADG of low BW piglets (e.g., BW < 1,180 g) from multiparous sows, with a number of piglets born alive of ~14.2, was increased in response to meloxicam

administration. This study indicated that lighter piglets from large litters benefit most from meloxicam administration to sows; however, this was not found to be the case in the current study.

Based on the study results and those from the literature, it is evident that pre-weaning piglet growth can be increased when meloxicam is provided IM to sows as soon as possible after birth of the last piglet (Mainau *et al*., 2012; our study) or orally at the beginning of farrowing (Mainau *et al.*, 2016; Navarro *et al.*, 2021). A single dose (Mainau *et al.*, 2012; Mainau *et al.*, 2016; Navarro *et al.*, 2021; our study) at these times appears to be more effective than repeated administration (Schoos *et al.*, 2020) before or after farrowing. Furthermore, administration too late after farrowing should be avoided (Tenbergen *et al.*, 2014).

In the current study, split-suckling reduced pre-weaning ADG and BW at weaning when the six heaviest piglets were twice excluded from suckling the sow for a period of 1.5 h. On closer examination of the data, split-suckling decreased colostrum intake, ADG from birth to weaning and BW at weaning in high birth weight piglets (>1.25 kg). The reduction of colostrum intake in heavy pigs likely contributed to reduced piglet growth. Likewise, Vandaele *et al.* (2020) found that piglet growth was reduced when they implemented an intensive regime (removing the heaviest piglets for three consecutive days every 3 h for a period of 12 h/d). It could be argued that similar to Vandaele et al. (2020), the protocol implemented in the current study was overly intensive. With a similar protocol to the one implemented in our study, but with only one separation period Morton *et al.* (2019), found that weight gain and BW at day 7 postpartum was increased when the six heaviest piglets were excluded from the sow for a period of 1.5 h. Taken together, these results suggest that separating heavy piglets more than once, as we did, is too intensive and therefore, detrimental. On the other hand, others have found no growth response even when split-suckling was less intensive (Donovan and Dritz, 2000; Muns *et al*., 2015).

In the current study, the application of split-suckling also negated the effect of meloxicam on piglet growth and BW. As outlined above, split-suckling reduced colostrum intake and weaning weight in heavy birth weight pigs and it appears than meloxicam administration could not compensate for the negative impact of split-suckling these pigs. There is no consensus in the literature regarding the optimum

protocol for split-suckling in terms of separation time, frequency of separation, and type of piglets to be separated. The findings of the current study indicate that the duration and frequency of split-suckling implemented were not effective. It may be that the removal of piglets was performed too frequently and/or that split-suckling commenced too early in the piglets' lives. However, split-suckling was purposely commenced 4 h after the birth of the first piglet, as the quality of colostrum in terms of immunoglobulin content drops rapidly > 4 h postpartum (Klobasa *et al.*, 1987).

To our knowledge, most of the work performed to date on provision of meloxicam to sows has only evaluated the impact on piglet growth up to weaning. However, the current study demonstrated that BW at slaughter, as well as carcass weight and carcass ADG from weaning to slaughter, were increased as a result of postpartum meloxicam provision to sows. Meloxicam administration in this study increased piglet weaning weight. Heavier piglets at weaning have previously been found to have increased post-weaning and lifetime growth (Collins *et al.*, 2017), possibly explaining the increased slaughter weight observed in the current study. Additionally, G:F and ADG were increased during the first week post-weaning. No histology measurements were taken; however, G:F is a good proxy for digestive and absorptive capacity of the intestinal tract following weaning. The better feed efficiency observed early post-weaning could also have contributed to the heavier weight obtained at slaughter. In line with this, the decreased pig BW at slaughter and carcass ADG found in the current study as a result of split-suckling is most likely a consequence of the reduced pre-weaning growth and weaning BW found in these pigs.

2.5.2 Pig health

The current study demonstrates that providing postpartum meloxicam to sows decreased the number of clinical cases and injections required in suckling pigs, and tended to decrease pre-weaning antibiotic and anti-inflammatory treatments per piglet. Colostrum intake tended to increase in pigs raised by sows which received meloxicam and the reduced need for medication in these piglets is most likely due to the increase in passive immunity. Colostrum intake is critical for the development of piglet immunity, as it contains immunoglobulins, 80% of which are immunoglobulin (Ig) G,

which is of primary importance for the transfer of passive immunity from the sow to the piglets (Curtis and Bourne, 1971). In our study, estimated colostrum intake per pig in the first 24 h was 73% higher than the 200 g which is regarded as the minimum intake after birth to ensure piglet survival (Devillers *et al.*, 2011). Although not evaluated, it is likely that the immunological status of piglets reared by sows administered meloxicam was improved due to increased colostrum intake. This is in line with the study from Mainau *et al.* (2016) which showed that administration of meloxicam to sows at the beginning of the farrowing process resulted in increased plasma IgG concentration in piglets 24 h after birth. Additionally, transmammary transfer of meloxicam from sows to piglets has previously been demonstrated (Bates *et al.*, 2014) which could also help to explain the reduced number of clinical cases of disease and injections required per litter. The combination of increased passive immunity and the anti-inflammatory pain relief provided through the colostrum/milk to piglets from sows supplemented with meloxicam are both likely drivers for the resulting increased pre-weaning piglet growth. It was expected that practicing split-suckling would increase colostrum intake in light birth weight piglets; however, this was not the case and it actually reduced colostrum intake in heavy birth weight piglets. Therefore, it is not surprising that split-suckling reduced pre-weaning growth and that clinical cases of disease and the requirement for medication usage were not reduced. To our knowledge, our study is the first to monitor medication usage in pigs in response to split-suckling.

Pre-weaning mortality observed in the present study was higher than the Irish industry average of 11.1%, which is likely explained by the higher number of piglets born alive in the present study, which was 16.3 on average compared to the industry average of 14.8 (Teagasc, 2022). In agreement with other studies (Mainau *et al.*, 2012; Mainau *et al.*, 2016; Navarro *et al.*, 2021), there was no effect of meloxicam provision to the sow on piglet pre-weaning mortality. The current study was conducted in a high health status herd where sanitary conditions were good and this most likely explains the lack of effect of meloxicam on mortality. However, when mastitis-metritis- agalactia syndrome was an issue in a previous study, meloxicam reduced piglet mortality from 32 to 14% when it replaced flunixin as the anti-inflammatory used (Hirsch *et al.*, 2003). Likewise, split-suckling, as conducted in the current study, did not reduce pre-weaning piglet mortality and this is in agreement with the results of Muns *et al.* (2015).

Only one treatment in the current study had a pre-weaning mortality rate higher than 20%, the rate above which causes of mortality should be paid particular attention (Koketsu *et al.*, 2021) and this was where litters were split-suckled. Split-suckling might have been expected to reduce mortality, especially since the practice reduced diarrhoea incidence by one third; however, it involves a disturbance of both piglets and the sow and this likely negated any positive effect on pre-weaning piglet mortality (Muns *et al.*, 2015). In our study, split-suckling piglets involved personnel lifting pigs on four separate occasions during the first hours after birth and temporary removal of the heaviest littermates reduced colostrum intake in these pigs at this critical time.

Unlike the situation pre-weaning, postpartum analgesia had little effect on medication usage during the post-weaning period. This is possibly not surprising since the effect of meloxicam would have long since waned by this time. However, split-suckling increased medication usage during the weaning to slaughter period, thereby demonstrating a negative residual effect from this practice. Colostrum intake was reduced in heavy birth weight pigs in response to split-suckling and this most likely explains this increased post-weaning medication usage in pigs that had been split-suckled.

2.6 Conclusions

In conclusion, a single IM injection of meloxicam provided to sows as soon as possible after delivery of the placenta can increase colostrum intake. This likely led to the observed reduction in clinical cases of disease, increased ADG in pigs during the first two weeks of life and early post-weaning and increased carcass weight at slaughter. Furthermore, the strategy tended to reduce pre-weaning antibiotic and anti-inflammatory usage in pigs. Contrary to this, commencing split-suckling 4 h after birth of the first piglet by twice removing the six heaviest piglets from the sow, reduced pig growth prior to weaning and up to slaughter, having no effect on pre-weaning medication usage. In conclusion, postpartum meloxicam administration to sows is recommended as a strategy to increase lifetime pig growth and health whereas split-suckling, as conducted in the current study, is not advised.

2.7 Tables

Table 2-1. Composition of the experimental diets (on an air-dry basis; kg/tonne).

Diet Number **Diet type**	**1** **Dry sow**	**2** **Lactation**	**3** **Starter**	**4** **Link**	**5** **Weaner**	**6** **Finisher**
Ingredients						
Barley	759.7	259.7	50.0	68.4	495.9	410.5
Wheat	0	455.2	0	100.0	216.8	390.0
Maize	0	0	231.0	300.0	0	0
Soybean meal	76.2	179.8	143.4	186.9	163.2	165.0
Full fat soybean meal	0	0	130.8	70.0	50.0	0
Lactoflo[1]	0	0	200.0	150.0	0	0
Skim milk powder	0	0	125.0	50.0	0	0
Soya hulls	125.3	0	0	0	0	0
Soya oil	14.0	66.0	85.0	38.2	40.0	11.0
Premix[2]	1.5	1.5	3.0	3.0	3.0	1.0
L-Lysine HCl	2.3	5.0	6.2	6.7	5.9	4.3
DL-Methionine	0.4	1.5	3.6	3.2	2.2	1.0
L-Threonine	1.0	2.7	3.7	3.4	2.7	1.9
L-Tryptophan	0	0.8	1.4	1.3	0.6	0.2
L-Valine	0	2.7	1.3	1.3	0.6	0
Limestone flour	8.5	11.5	7.0	7.5	10.5	11
Mono dicalcium phosphate	7.0	8.5	5.5	7.0	5.5	1.0
Salt	4.0	5.0	3.0	3.0	3.0	3.0
Phytase[3]	0.1	0.1	0.1	0.1	0.1	0.1
Chemical composition						
Dry matter[4]	883.0	893.0	907.0	897.0	888.0	887.0
Crude protein[4]	125.0	163.0	188.0	166.0	178.0	177.0
Ash[4]	42.0	48.0	57.0	53.0	47.0	46.0
Ether extract[4]	33.7	85.4	119.1	58.4	65.2	31.0
Crude fibre[4]	87.0	26.0	16.0	33.0	32.0	38.0
Lysine[5]	7.8	11.5	16.2	15.0	13.0	10.9
Methionine[5]	2.4	3.9	7.0	6.1	4.7	3.4
Cystine[5]	2.5	3.0	2.7	2.9	3.1	3.1
Threonine[5]	5.6	8.3	10.9	10.1	8.8	7.6
Tryptophan[5]	3.66	3.36	2.66	2.22	1.54	2.79
Digestible energy (MJ/Kg)[5]	12.51	14.86	16.20	15.00	14.27	13.73
Net energy (MJ/Kg)[5]	8.86	10.90	12.06	10.94	10.30	9.80
SID lysine[5,6]	6.6	10.7	15.3	14.1	12.0	10.0
Total calcium[5]	7.2	8.3	8.2	7.5	7.4	6.5
Digestible phosphorus[5]	3.5	3.8	4.6	4.2	3.3	2.5

[1]Lactoflo, non-hygroscopic whey permeate powder (Volac, Royston, United Kingdom)

[2] Premix provided per kg of complete diet (Diets 1 and 2): Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 70 mg; Mn from manganese oxide, 62 mg; Zn from zinc oxide, 80 mg, I from potassium iodate, 0.6 mg; Se from sodium selenite, 0.2 mg; vitamin A as retinyl acetate, 3.44 mg; vitamin D3 as cholecalciferol, 25 mg; vitamin E as DL-alpha-tocopheryl acetate, 100 mg; vitamin

K, 2 mg; vitamin B12, 15 µg; riboflavin, 5 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 500 mg; Biotin, 200µg; folic acid, 5mg; vitamin B1,2 mg; and vitamin B6, 3 mg.
Premix provided per kilogram of complete diet Diets 3, 4 and 5): Cu from copper sulphate, 100 mg; Fe from ferrous sulphate monohydrate, 90 mg; Mn from manganese oxide, 47 mg; Zn from zinc oxide, 120 mg; I from potassium iodate, 0.6 mg; Se from sodium selenite, 0.3 mg; vitamin A as retinyl acetate, 2.1 mg; vitamin D3 as cholecalciferol, 25 µg; vitamin E as DL-alpha-tocopheryl acetate, 100 mg; vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B1, 2 mg; and vitamin B6, 3 mg.
Premix provided per kilogram of complete diet (Diet 6): Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; vitamin A as retinyl acetate, 0.7 mg; vitamin D3 as cholecalciferol, 12.5 µg; vitamin E as DL-alpha-tocopheryl acetate, 40 mg; vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B1, 2 mg; vitamin B6, 3 mg.
[3]The diet contained 1000 phytase units (FYT) per kg feed (RONOZYME HiPhos GT; DSM, Belfast, UK).
[4]Analysed nutrient composition
[5]Calculated nutrient composition
[6]SID lysine = Standardized ileal digestible lysine.

Table 2-2. Effect of split-suckling and/or post-partum meloxicam provision to sows on piglet weight and growth during the suckling period.

Split-suckling (Split)	**N-Split**	**N-Split**	**Split**	**Split**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**Split**	**Mel x Split**
Number of sows	23	23	20	23				
Litter weight, kg								
Day 0 (birth)	22.3	22.2	21.2	22.7	0.82	0.32	0.71	0.51
Day 1	21.5	22.0	20.7	22.1	0.68	0.10	0.54	0.32
Day 6	30.7^{B}	33.9^{A}	29.4^{B}	$32.0^{A,B}$	1.26	0.02	0.19	0.06
Day 14	56.6^{B}	62.9^{A}	55.4^{B}	$58.1^{A,B}$	2.14	0.04	0.17	0.07
Day 27	105.6	111.4	102.1	107.2	3.99	0.17	0.34	0.34
Overall					1.61	0.07	0.26	0.88
Mean piglet BW[1], kg								
Day 0	1.42	1.35	1.48	1.41	0.054	0.12	0.16	0.23
Day 1	1.45	1.41	1.53	1.44	0.054	0.12	0.22	0.27
Day 6	2.20^{b}	2.36^{a}	2.22^{b}	2.22^{b}	0.057	0.06	0.21	0.04
Day 14	4.12^{b}	4.56^{a}	4.20^{b}	4.19^{b}	0.057	<0.001	<0.01	<0.001
Day 27	7.80^{c}	8.37^{a}	7.85^{c}	7.98^{b}	0.057	<0.001	<0.001	<0.001
Overall					0.039	<0.001	0.01	<0.001
ADG[2], g/pig/day								
Day 0 to 1	47^{c}	70^{a}	$65^{a,b}$	$52^{b,c}$	6.9	0.39	0.99	0.02

Split-suckling (Split)	N-Split	N-Split	Split	Split		*P*-value		
Meloxicam (Mel)	N-Mel	Mel	N-Mel	Mel	SEM	Mel	Split	Mel x Split
Day 1 to 6	158[b]	184[a]	162[b]	160[b]	5.0	<0.001	<0.01	<0.001
Day 6 to 14	237[b]	266[a]	247[b]	238[b]	5.7	0.03	0.04	<0.001
Day 14 to 27	260	256	262	263	5.8	0.73	0.36	0.72
Overall	176	194	184	178	4.4	<0.01	0.12	<0.001

[1]BW, body weight.
[2]ADG, average daily gain.
[a, b, c] Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).
[A, B] Values within a row that do not share a common superscript tended to differ ($0.05 < P \leq 0.10$).

Table 2-3. Effect of split-suckling and/or post-partum meloxicam provision to sows on pig growth and feed intake from weaning to slaughter.

Split-suckling (Split)	**N-Split**	**N-Split**	**Split**	**Split**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**Split**	**Mel x Split**
Number of pens	14	12	11	13				
BW[1], kg								
Day 0 (weaning)	8.2	7.8	8.3	8.4	0.80	0.83	0.66	0.94
Day 6 post-weaning	9.3	9.0	9.1	9.8	0.80	0.73	0.72	0.88
Day 14 post-weaning	12.0	11.7	11.6	12.4	0.80	0.72	0.80	0.89
Day 21 post-weaning	15.2	15.0	14.8	15.6	0.80	0.73	0.93	0.92
Day 28 post-weaning	18.9	19.1	18.9	19.5	0.80	0.60	0.83	0.94
Day 47 post-weaning	33.0	33.2	32.5	33.8	0.80	0.34	0.93	0.70
Day of slaughter (day 157 of age)	119.2[b]	122.4[a]	116.9[c]	121.0[a]	0.80	<0.001	0.02	<0.001
Overall					0.52	0.12	0.84	0.39
ADFI[2], g/pig/day								
Day 0 to 6	221	205	187	213	11.3	0.60	0.20	0.12
Day 6 to 14	477	483	430	460	19.6	0.36	0.07	0.23
Day 14 to 21	610	655	601	618	25.8	0.22	0.36	0.46
Day 21 to 28	833	881	821	831	35.9	0.42	0.38	0.65
Day 28 to 47	1083	1075	1045	1083	33.6	0.65	0.65	0.84
Day 47 to slaughter	2409	2383	2321	2390	56.0	0.70	0.48	0.72

Split-suckling (Split)	N-Split	N-Split	Split	Split		*P*-value		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**Split**	**Mel x Split**
Overall					22.7	0.37	0.24	0.60
ADG[3], g/pig/day								
Day 0 to 6	210[b]	208[b]	191[b]	248[a]	14.4	0.04	0.45	0.03
Day 6 to 14	348	331	336	349	20.0	0.90	0.89	0.88
Day 14 to 21	471	469	466	456	24.6	0.81	0.71	0.97
Day 21 to 28	530	576	588	568	40.8	0.74	0.54	0.75
Day 28 to 47	672	661	656	688	18.2	0.53	0.76	0.57
Day 47 to slaughter	1062	1090	1030	1070	19.2	0.07	0.16	0.18
Overall	549	556	544	563	12.7	0.25	0.91	0.60
G:F[4], g/g								
Day 0 to 6	0.95[b]	1.04[a,b]	1.03[a,b]	1.15[a]	0.050	0.04	0.06	0.04
Day 6 to 14	0.73	0.72	0.77	0.74	0.025	0.48	0.25	0.61
Day 14 to 21	0.76	0.74	0.76	0.73	0.028	0.28	0.89	0.74
Day 21 to 28	0.63	0.66	0.70	0.67	0.034	0.99	0.28	0.61
Day 28 to 47	0.62	0.63	0.63	0.63	0.010	0.61	0.77	0.92
Day 47 to slaughter	0.44[B]	0.47[A]	0.44[B]	0.44[B]	0.009	0.10	0.14	0.09
Overall					0.014	0.35	0.07	0.67

[1]BW, body weight.
[2]ADFI, average daily feed intake.
[3]ADG, average daily gain.

[4]G:F, gain to feed ratio.
[a, b, c] Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).
[A, B] Values within a row that do not share a common superscript tended to differ ($0.05 < P \leq 0.10$).

Table 2-4. Effect of split-suckling application and/or meloxicam provision to sows post-partum on pig carcass parameters.

Split-suckling (Split)	**N-Split**	**N-Split**	**Split**	**Split**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**Split**	**Mel x Split**
Number of pens	14	12	11	13				
Cold carcass weight, kg	90.9	93.0	89.4	93.0	1.07	0.01	0.53	0.50
Fat depth, mm	14.0	14.7	12.9	13.4	0.43	0.15	<0.01	0.80
Muscle depth, mm	54.3	55.9	54.7	54.7	0.77	0.30	0.62	0.31
Lean meat, %	58.1	57.8	59.0	58.7	0.33	0.34	0.02	0.99
Kill out, %	76.1	76.5	76.5	76.5	0.40	0.65	0.68	0.68
Carcass ADG weaning to slaughter, g/d[1,2]	667	671	649	682	9.4	0.06	0.72	0.15
Carcass G:F weaning to slaughter, g/g[3,4]	0.37	0.38	0.37	0.37	0.006	0.12	0.69	0.31
Lean ADG, g/d[5]	412	411	407	424	5.77	0.17	0.45	0.13

[1]ADG, average daily gain.
[2]Carcass ADG (from weaning to slaughter) = [(carcass weight in kg – weaning weight in kg × 0.65) × 1,000]/number of days from weaning to slaughter (Lawlor and Lynch, 2005).
[3]G:F, gain to feed.
[4]Carcass G:F (from weaning to slaughter) was calculated as follows: carcass G:F = carcass ADG (g)/ daily feed intake (g) (Lawlor and Lynch, 2005).
[5]Lean ADG (from birth to slaughter) = (carcass weight × carcass lean meat percentage × 10)/number of days to slaughter (Lawlor and Lynch, 2005).

Table 2-5. Effect of split-suckling and/or post-partum meloxicam provision to sows on piglet diarrhoea incidence pre-weaning, colostrum intake, pre-weaning antibiotic and anti-inflammatory treatment of sows and piglets and number of clinical cases.

Split-suckling (Split)	**N-Split**	**N-Split**	**Split**	**Split**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**Split**	**Mel x Split**
Number of sows	23	23	20	23				
Diarrhoea incidence, % (day 2-28)[1]	12.8	17.1	5.5	4.8	3.02	0.81	<0.01	0.53
Colostrum intake per pig, g[2]	334	352	320	338	9.8	0.06	0.14	0.99
Number of clinical cases per litter[3]	1.7	1.0	2.6	1.1	0.55	0.04	0.36	0.49
Number of injections per litter	4.7	2.5	6.5	3.2	1.48	0.06	0.38	0.70
Antibiotic usage per sow, ml[4]	21.0	12.7	24.8	12.0	6.98	0.13	0.82	0.75
Antibiotic usage per pig, ml[5]	0.2	0.1	0.3	0.1	0.06	0.08	0.27	0.70
Anti-inflammatory usage per sow, ml[6]	1.6	0.9	4.0	0.3	1.11	0.06	0.39	0.19
Anti-inflammatory usage per pig, ml[7]	0.03	0.01	0.04	0.02	0.010	0.08	0.35	0.89

[1]A faecal score of 2 or greater was considered representative of diarrhoea.
[2]Estimated value: Colostrum intake (g) = -106 + (2.26 × WG) + (200 × BWB) + (0.111 × D) – (1,414 × (WG/D)) + (0.0182 × (WG/BWB)). Where WG is piglet weight gain (g), BWB is piglet body weight at birth (kg) and D is the duration of colostrum suckling (min) (Theil *et al.*, 2014).
[3]Number of piglets per litter treated one or more times.
[4]Volume of antibiotic administered per sow.
[5]Volume of antibiotic administered per piglet.

[6]Volume of anti-inflammatory administered per sow.
[7]Volume of anti-inflammatory administered per piglet.

Table 2-6. Effect of split-suckling and/or post-partum meloxicam provision to sows on post-weaning antibiotic and anti-inflammatory treatments in pigs.

Split-suckling (Split)	**N-Split**	**N-Split**	**Split**	**Split**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**Split**	**Mel x Split**
Number of pens	14	12	11	13				
Weaner period								
Antibiotic usage per pig, ml[1]	0.11	0.10	0.10	0.27	0.057	0.16	0.18	0.11
Anti-inflammatory usage per pig, ml[2]	0.06	0.05	0.05	0.13	0.028	0.16	0.18	0.11
Finisher period								
Antibiotic usage per pig, ml[1]	0.06	0.41	0.52	0.66	0.183	0.20	0.06	0.58
Anti-inflammatory usage per pig, ml[2]	0.02	0.14	0.17	0.22	0.061	0.20	0.06	0.58
Weaning to slaughter period								
Antibiotic usage per pig, ml[1]	0.17	0.50	0.61	0.93	0.185	0.09	0.02	0.97
Anti-inflammatory usage per pig, ml[2]	0.08	0.18	0.22	0.35	0.640	0.07	0.02	0.84

[1]Volume of antibiotic administered to each pig.
[2]Volume of anti-inflammatory administered to each pig.

2.8 Supplementary material

Table S 2-1. Effect of split-suckling application and/or meloxicam provision to sows post-partum on sows' reproductive performance, body weight and back fat.

Split-suckling (Split)	**N-Split**	**N-Split**	**Split**	**Split**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**Split**	**Mel x Split**
Number of sows	23	23	20	23				
Lactation length, days	27.7	27.7	27.4	28	0.24			
Weaning to service interval, days	4.6	4.3	4.3	4.4	0.14	0.31	0.45	0.27
BW, kg[1]								
Day 110 of gestation	275.3	275.0	275.2	275.7	3.26	0.98	0.93	0.99
Farrowing[2]	241.5	242.0	240.5	236.2	3.29	0.55	0.29	0.54
Weaning[3]	240.4	232.5	242.8	238.4	3.28	0.06	0.19	0.37
Service[3]	228.8	225.4	232.1	232.7	3.48	0.69	0.12	0.13
Overall					2.04	0.21	0.40	0.82
BF, mm[4]								
Day 110 of gestation	16.2	16.1	16.1	16.2	0.33	0.95	0.86	0.99
Weaning	13.0	12.9	13.4	12.8	0.33	0.23	0.65	0.48
Service	12.7	12.5	12.7	12.6	0.34	0.56	0.76	0.92

Split-suckling (Split)	N-Split	N-Split	Split	Split		*P*-value		
Meloxicam (Mel)	N-Mel	Mel	N-Mel	Mel	SEM	Mel	Split	Mel x Split
Overall					0.24	0.36	0.75	0.79
Sow BW change, kg								
Day 110 to weaning[5]	-34.8	-41.6	-31.4	-37.6	4.34	0.10	0.35	0.32
Farrowing to weaning[5]	-1.0	-8.9	3.3	2.0	4.36	0.25	0.06	0.14
Weaning to service[6]	-8.0	-4.8	-6.3	-9.0	4.60	0.95	0.77	0.90
Overall					2.97	0.13	0.16	0.92
Sow BF change, mm								
Day 110 to weaning[7]	-3.2	-3.2	-2.7	-3.4	0.31	0.20	0.58	0.38
Weaning to service[8]	-0.1	-0.4	-0.6	-0.3	0.32	0.99	0.49	0.61
Overall					0.23	0.33	0.89	0.92

[1]BW, body weight.
[2]Estimated value: empty farrowing weight = (sow weight at day 110 – (total born × 2.25)). The value of 2.25 kg is an estimate of the increased weight in the gravid uterus and in mammary tissue attributed to each pig in a litter (NRC, 1998).
[3]Weaning = day 28 ± 0.2 of lactation; service = day 4 ± 0.1 post-weaning.
[4]BF, back fat.
[5]Sow BW change = (sow BW at weaning – sow BW at day 110 of gestation or at farrowing).
[6]Sow BW change = (sow BW at service – sow BW at weaning).
[7]Sow BF change = (sow BF at weaning – sow BF at day 110 of gestation).
[8]Sow BF change = (sow BF at service – sow BF at weaning).

Table S 2-2. Effect of split-suckling and/or post-partum meloxicam provision to sows on sow litter-size, the number of piglets fostered and the number of deaths per litter.

Split-suckling (Split)	**N-Split**	**N-Split**	**Split**	**Split**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**Split**	**Mel x Split**
Number of sows	23	23	20	23				
Litter								
Total born[1]	17.2	16.1	17.9	17.5	0.72	0.28	0.14	0.59
Live born	16.6	15.4	16.5	16.7	0.69	0.44	0.40	0.30
Litter size at 48 h	14.5	14.0	14.4	14.4	0.39	0.46	0.71	0.51
Litter size at weaning	13.4	13.6	13.2	13.4	0.23	0.35	0.32	0.92
Deaths and cross fostered								
Cross fostered[2]	-0.5	0.5	-0.5	-0.3	0.42	0.11	0.32	0.33
Deaths total	2.5	2.5	2.7	3.0	0.44	0.73	0.46	0.73
Deaths after 48 h	0.9	0.7	1.1	0.9	0.23	0.36	0.32	0.92

[1]Total number born = number of piglets born alive, stillborn, and mummified.
[2]Cross fostered = piglets cross foster on – piglets cross fostered off

Table S 2-3. Effect of post-partum meloxicam provision to sows on litter-size, the number of piglets fostered and the number of deaths per litter.

Meloxicam (Mel)	**N-Mel**	**Mel**	**SEM**	***P*-value[1]**
Litter				
Total born	17.6	16.8	0.53	0.28
Live born	16.6	16.0	0.49	0.44
Litter size at 48 h	14.5	14.2	0.28	0.46
Litter size at weaning	13.3	13.5	0.16	0.36
Deaths and cross fostered				
Cross fostered	-0.5	0.1	0.32	0.11
Deaths total	2.6	2.7	0.31	0.73
Deaths after 48 h	1.0	0.8	0.16	0.36

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.

Table S 2-4. Effect of split-suckling on litter-size, the number of piglets fostered and the number of deaths per litter.

Split-suckling (Split)	**N-Split**	**Split**	**SEM**	***P*-value[1]**
Litter				
Total born	16.7	17.7	0.53	0.14
Live born	16.0	16.6	0.49	0.40
Litter size at 48 h	14.3	14.4	0.28	0.71
Litter size at weaning	13.5	13.3	0.16	0.32
Deaths and cross fostered				
Cross fostered	-0.0	-0.4	0.32	0.32
Deaths total	2.5	2.8	0.31	0.46
Deaths after 48 h	0.8	1.0	0.16	0.32

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.

Table S 2-5. Effect of meloxicam provision to sows post-partum on litter weights, individual piglet body weight and piglet average daily gain to weaning.

Meloxicam (Mel)	N-Mel	Mel	SEM	*P*-value[1]
Litter weight, kg				
Day 0	21.7	22.5	0.63	0.32
Day 1	21.1	22.0	0.54	0.10
Day 6	30.0	33.0	0.93	0.02
Day 14	56.0	60.5	1.54	0.04
Day 27	103.8	109.3	2.83	0.17
Overall			1.16	0.07
Piglet BW, kg[2]				
Day 0	1.45	1.38	0.045	0.12
Day 1	1.49	1.43	0.045	0.12
Day 6	2.21	2.29	0.046	0.06
Day 14	4.16	4.38	0.047	<0.001
Day 27	7.83	8.18	0.047	<0.001
Overall			0.036	<0.001
ADG, g/pig/day[3]				
Day 0 to 1	55.8	60.7	5.49	0.39
Day 1 to 6	159.7	171.8	4.41	<0.001
Day 6 to 14	242.4	251.9	4.78	0.03
Day 14 to 27	261.1	259.5	4.82	0.73
Overall			4.02	<0.01

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]BW, body weight.
[3]ADG, average daily gain.

Table S 2-6. Effect of split-suckling application on litter weights, individual piglet body weight and piglet average daily gain to weaning.

Split-suckling (Split)	N-Split	Split	SEM	*P*-value[1]
Litter weight, kg				
Day 0	22.2	22.0	0.63	0.71
Day 1	21.7	21.4	0.54	0.54
Day 6	32.3	30.7	0.93	0.19
Day 14	59.7	56.8	1.54	0.17
Day 27	108.5	104.6	2.83	0.34
Overall			1.16	0.26
Piglet BW, kg[2]				
Day 0	1.39	1.45	0.045	0.16
Day 1	1.43	1.49	0.045	0.22
Day 6	2.28	2.22	0.046	0.21
Day 14	4.34	4.20	0.046	<0.01
Day 27	8.09	7.92	0.047	<0.001
Overall			0.036	0.01
ADG, g/pig/day[3]				
Day 0 to 1	58.2	58.3	5.49	0.99
Day 1 to 6	170.7	160.8	4.40	<0.01
Day 6 to 14	251.7	242.7	4.77	0.04
Day 14 to 27	258.2	262.3	4.82	0.36
Overall			4.02	0.12

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]BW, body weight.
[3]ADG, average daily gain.

Table S 2-7. Effect of meloxicam provision to sows post-partum and/or weight category (heavy or light birth weight piglet) on piglet growth and weight at weaning.

Weight category (WC)	**Light**	**Light**	**Heavy**	**Heavy**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**WC**	**Mel*WC**
Number of sows	23	23	20	23				
Body weight at weaning, kg	6.54	6.73	8.48	8.78	0.14	0.02	<0.001	0.60
ADG[1] from birth to day 27, g/pig/day	203	203	250	259	3.58	0.23	<0.001	0.18

[1]ADG, average daily gain

Table S 2-8. Effect of split-suckling and/ weight category (heavy or light birth weight) on piglet weight at weaning.

Weight category (WC)	**Light**	**Light**	**Heavy**	**Heavy**		***P*-value**		
Split-suckling (Split)	**N-Split**	**Split**	**N-Split**	**Split**	**SEM**	**Split**	**WC**	**Split *WC**
Number of sows	23	23	20	23				
Body weight at weaning, kg	6.54^{c}	6.70^{c}	8.80^{a}	8.48^{b}	0.14	0.42	<0.001	0.02
ADG[1] from birth to day 27, g/pig/day	199^{c}	207^{c}	260^{a}	250^{b}	3.55	0.79	<0.001	0.01

[1]ADG, average daily gain

[a, b, c] Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$)

Table S 2-9. Effect of post-partum meloxicam provision to sows on growth of pigs from weaning to slaughter.

Meloxicam (Mel)	**N-Mel**	**Mel**	**SEM**	***P*-value[1]**
BW, kg[2]				
Day 0 (weaning)	8.2	8.1	0.57	0.83
Day 6 post-weaning	9.2	9.4	0.57	0.73
Day 14 post-weaning	11.8	12.1	0.57	0.72
Day 21 post-weaning	15.0	15.3	0.57	0.73
Day 28 post-weaning	18.9	19.3	0.57	0.60
Day 47 post-weaning	32.7	33.5	0.57	0.34
Day of slaughter (157 days)	118.1	121.7	0.57	<0.001
Overall			0.39	0.12
ADFI, g/pig/day[3]				
Day 0 to 6	204	209	8.9	0.60
Day 6 to 14	454	471	14.4	0.36
Day 14 to 21	605	637	18.6	0.22
Day 21 to 28	827	856	25.7	0.42
Day 28 to 47	1064	1079	24.1	0.65

Meloxicam (Mel)	N-Mel	Mel	SEM	*P*-value[1]
Day 47 to slaughter	2365	2386	39.8	0.70
Overall			16.5	0.37
ADG, g/pig/day[4]				
Day 0 to 6	200	228	11.0	0.04
Day 6 to 14	342	340	14.7	0.90
Day 14 to 21	469	463	17.9	0.81
Day 21 to 28	559	572	29.2	0.74
Day 28 to 47	664	675	13.5	0.53
Day 47 to slaughter	1046	1079	14.2	0.07
Overall			9.9	0.25
G:F, g/g[5]				
Day 0 to 6	0.99	1.10	0.035	0.04
Day 6 to 14	0.75	0.73	0.018	0.48
Day 14 to 21	0.76	0.73	0.020	0.28
Day 21 to 28	0.67	0.67	0.024	0.99
Day 28 to 47	0.62	0.63	0.007	0.61

Meloxicam (Mel)	N-Mel	Mel	SEM	*P*-value[1]
Day 47 to slaughter	0.44	0.46	0.006	0.10
Overall			0.010	0.35

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]BW, body weight.
[3]ADFI, average daily feed intake.
[4]ADG, average daily gain.
[5]G:F, gain to feed ratio.

Table S 2-10. Effect of split-suckling application on growth of pigs from weaning to slaughter.

Split-suckling (Split)	N-Split	Split	SEM	*P*-value[1]
BW, kg[2]				
Day 0 (weaning)	8.0	8.3	0.58	0.66
Day 6 post-weaning	9.2	9.5	0.58	0.72
Day 14 post-weaning	11.8	12.0	0.58	0.80
Day 21 post-weaning	15.1	15.2	0.58	0.93
Day 28 post-weaning	19.0	19.2	0.58	0.83
Day 47 post-weaning	33.1	33.1	0.58	0.93
Day of slaughter (d.157) days)	120.8	119.0	0.58	0.02
Overall			0.39	0.84
ADFI, g/pig/day[3]				
Day 0 to 6	213	200	8.9	0.20
Day 6 to 14	480	445	14.4	0.07
Day 14 to 21	633	610	18.7	0.36
Day 21 to 28	857	826	25.7	0.38
Day 28 to 47	1079	1064	24.1	0.65

Split-suckling (Split)	N-Split	Split	SEM	*P*-value[1]
Day 47 to slaughter	2396	2356	39.8	0.48
Overall			16.5	0.24
ADG, g/pig/day[4]				
Day 0 to 6	209	219	11.0	0.45
Day 6 to 14	340	342	14.8	0.89
Day 14 to 21	470	461	17.9	0.71
Day 21 to 28	553	578	29.2	0.54
Day 28 to 47	667	672	13.6	0.76
Day 47 to slaughter	1076	1050	14.3	0.16
Overall			9.9	0.91
G:F, g/g[5]				
Day 0 to 6	0.99	1.09	0.035	0.06
Day 6 to 14	0.72	0.75	0.018	0.25
Day 14 to 21	0.75	0.75	0.020	0.89
Day 21 to 28	0.65	0.69	0.024	0.28
Day 28 to 47	0.62	0.63	0.007	0.77

Split-suckling (Split)	N-Split	Split	SEM	*P*-value[1]
Day 47 to slaughter	0.45	0.44	0.006	0.14
Overall			0.010	0.07

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]BW, body weight.
[3]ADFI, average daily feed intake.
[4]ADG, average daily gain.
[5]G:F, gain to feed ratio.

Table S 2-11. Effect of meloxicam provision to sows post-partum on pig carcass parameters.

Meloxicam (Mel)	N-Mel	Mel	SEM	*P*-value[1]
Cold weight, kg	90.2	93.0	0.75	0.01
Fat depth, mm	13.4	14.1	0.30	0.15
Muscle depth, mm	54.5	55.3	0.55	0.30
Lean meat, %	58.6	58.3	0.24	0.34
Kill out, %	76.3	76.5	0.29	0.65
Carcass ADG weaning to slaughter, g/d[2,3]	658	676	6.6	0.06
Carcass G:F weaning to slaughter, g/g[4,5]	0.37	0.38	0.004	0.12
Lean ADG, g/d[6]	410	418	4.06	0.17

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]ADG, average daily gain.
[3]Carcass ADG (from weaning to slaughter) = [(carcass weight in kg – weaning weight in kg × 0.65) × 1,000]/number of days from weaning to slaughter (Lawlor and Lynch, 2005).
[4]G:F, gain to feed.
[5]Carcass G:F (from weaning to slaughter) was calculated as follows: carcass G:F = carcass ADG (g)/daily feed intake (g).
[6]Lean ADG (from birth to slaughter) = (carcass weight × carcass lean meat percentage × 10)/number of days to slaughter (Lawlor and Lynch, 2005).

Table S 2-12. Effect of split-suckling application on pig carcass parameters.

Split-suckling (Split)	N-Split	Split	SEM	*P*-value[1]
Cold weight, kg	91.9	91.2	0.76	0.53
Fat depth, mm	14.4	13.1	0.30	<0.01
Muscle depth, mm	55.1	54.7	0.54	0.62
Lean meat, %	58.0	58.8	0.24	0.02
Kill out, %	76.3	76.5	0.29	0.68
Carcass ADG weaning to slaughter, g/d[2,3]	669	666	6.7	0.72
Carcass G:F weaning to slaughter, g/g[4,5]	0.37	0.37	0.004	0.69
Lean ADG, g/d[6]	411	416	4.08	0.13

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]ADG, average daily gain.
[3]Carcass ADG (from weaning to slaughter) = [(carcass weight in kg – weaning weight in kg × 0.65) × 1,000]/number of days from weaning to slaughter (Lawlor and Lynch, 2005).
[4]G:F, gain to feed.
[5]Carcass G:F (from weaning to slaughter) was calculated as follows: carcass G:F = carcass ADG (g)/daily feed intake (g).
[6]Lean ADG (from birth to slaughter) = (carcass weight × carcass lean meat percentage × 10)/number of days to slaughter (Lawlor and Lynch, 2005).

Table S 2-13. Effect of meloxicam provision to sows post-partum on piglet colostrum intake and antibiotic and anti-inflammatory treatments pre-weaning.

Meloxicam (Mel)	**N-Mel**	**Mel**	**SEM**	***P*-value[1]**
Diarrhoea incidence, % (day 2-28)[2]	8.5	9.2	2.29	0.81
Colostrum intake per pig, g[3]	327	345	7.2	0.06
Number of clinical cases/litter[4]	2.1	1.0	0.40	0.04
Number of injections per litter	5.6	2.8	1.06	0.06
Antibiotic usage per sow, mL[5]	22.92	12.35	4.9	0.14
Antibiotic usage per pig, mL[6]	0.2	0.1	0.05	0.08
Anti-inflammatory usage per sow, mL[7]	2.78	0.61	0.785	0.06
Anti-inflammatory usage per pig, mL[8]	0.04	0.02	0.007	0.08

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]A faecal score of 2 or greater was considered representative of diarrhoea.
[3]Estimated value: Colostrum intake (g) = -106 + (2.26 × WG) + (200 × BWB) + (0.111 × D) – (1,414 × (WG/D)) + (0.0182 × (WG/BWB)). Where WG is piglet weight gain (g), BWB is piglet body weight at birth (kg) and D is the duration of colostrum suckling (mins) (Theil *et al.*, 2014).
[4]Number of piglets per litter treated one or more times.
[5]Volume of antibiotic administered per sow.
[6]Volume of antibiotic administered per piglet.
[7]Volume of anti-inflammatory administered per sow
[8]Volume of anti-inflammatory administered per piglet.

Table S 2-14. Effect of split-suckling application on piglet colostrum intake and antibiotic and ant-inflammatory treatments pre-weaning.

Split-suckling (Split)	N-Split	Split	SEM	*P*-value[1]
Diarrhoea incidence, % (day 2-28)[2]	14.8	5.1	2.19	<0.01
Colostrum intake per pig, g[3]	343	329	7.2	0.14
Number of clinical cases/litter[4]	1.3	1.8	0.40	0.36
Number of injections per litter	3.6	4.9	1.06	0.38
Antibiotic usage per sow, mL[5]	16.87	18.40	4.940	0.83
Antibiotic usage per pig, mL[6]	0.1	0.2	0.05	0.27
Anti-inflammatory usage per sow, mL[7]	1.22	2.17	0.785	0.39
Anti-inflammatory usage per pig, mL[8]	0.02	0.03	0.007	0.35

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]A faecal score of 2 or greater was considered representative of diarrhoea.
[3]Estimated value: Colostrum intake (g) = -106 + (2.26 × WG) + (200 × BWB) + (0.111 × D) – (1,414 × (WG/D)) + (0.0182 × (WG/BWB)). Where WG is piglet weight gain (g), BWB is piglet body weight at birth (kg) and D is the duration of colostrum suckling (mins) (Theil *et al.*, 2014).
[4]Number of piglets per litter treated one or more times.
[5]Volume of antibiotic administered per sow.
[6]Volume of antibiotic administered per piglet.
[7]Volume of anti-inflammatory administered per sow
[8]Volume of anti-inflammatory administered per piglet.

Table S 2-15. Effect of meloxicam provision to sows post-partum and/or weight category (heavy or light birth weight piglet) on piglet colostrum intake.

Weight category (WC)	**Light**	**Light**	**Heavy**	**Heavy**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**WC**	**Mel*WC**
Number of sows	23	23	20	23				
Colostrum intake per pig, g[1]	262	261	370	376	3.3	0.25	<0.001	0.20

[1]Estimated value: Colostrum intake (g) = -106 + (2.26 × WG) + (200 × BWB) + (0.111 × D) – (1,414 × (WG/D)) + (0.0182 × (WG/BWB)). Where WG is piglet weight gain (g), BWB is piglet body weight at birth (kg) and D is the duration of colostrum suckling (min) (Theil *et al.*, 2014).

Table S 2-16. Effect of split-suckling and/or weight category (heavy or light birth weight piglet) on piglet colostrum intake.

Weight category (WC)	**Light**	**Light**	**Heavy**	**Heavy**		***P*-value**		
Split-suckling (Split)	**N-Split**	**Split**	**N-Split**	**Split**	**SEM**	**Split**	**WC**	**Split *WC**
Number of sows	23	23	20	23				
Colostrum intake per pig, g[1]	262^{C}	261^{C}	378^{A}	369^{B}	3.4	0.04	<0.001	0.08

[1]Estimated value: Colostrum intake (g) = -106 + (2.26 × WG) + (200 × BWB) + (0.111 × D) – (1,414 × (WG/D)) + (0.0182 × (WG/BWB)). Where WG is piglet weight gain (g), BWB is piglet body weight at birth (kg) and D is the duration of colostrum suckling (min) (Theil *et al.*, 2014).
[A, B, C,]Values within a row that do not share a common superscript tended to differ ($0.05<P\leq0.10$)

Table S 2-17. Effect of split-suckling application and/or meloxicam provision to sows post-partum on sow milk composition at day 14 post-partum.

Split-suckling (Split)	**N-Split**	**N-Split**	**Split**	**Split**		***P*-value**		
Meloxicam (Mel)	**N-Mel**	**Mel**	**N-Mel**	**Mel**	**SEM**	**Mel**	**Split**	**Mel x Split**
Milk								
Total solids, %	17.14	17.29	17.55	16.92	0.447	0.60	0.96	0.39
Lactose, %	5.81	5.76	5.98	5.90	0.100	0.51	0.88	0.12
Fat, %	7.17	7.07	7.46	6.77	0.451	0.40	0.99	0.52
Protein, %	4.32	4.35	4.19	4.42	0.134	0.34	0.84	0.46

Table S 2-18. Effect of meloxicam provision to sows post-partum on antibiotic and anti-inflammatory treatments in pigs post-weaning.

Meloxicam (Mel)	N-Mel	Mel	SEM	*P*-value[1]
Weaner period				
Antibiotic usage per pig, mL[2]	0.10	0.18	0.040	0.16
Anti-inflammatory usage per pig, mL[3]	0.05	0.09	0.020	0.16
Finisher period				
Antibiotic usage per pig, mL[2]	0.29	0.53	0.129	0.20
Anti-inflammatory usage per pig, mL[3]	0.10	0.18	0.043	0.20
Weaning to slaughter				
Antibiotic usage per pig, mL[2]	0.40	0.72	0.130	0.09
Anti-inflammatory usage per pig, mL[3]	0.15	0.27	0.045	0.07

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]Volume of antibiotic administered to each pig.
[3]Volume of anti-inflammatory administered to each pig.

Table S 2-19. Effect of split-suckling application on antibiotic and anti-inflammatory treatments in pigs post-weaning.

Split-suckling (Split)	N-Split	Split	SEM	*P*-value[1]
Weaner period				
Antibiotic usage per pig, mL[2]	0.10	0.18	0.040	0.18
Anti-inflammatory usage per pig, mL[3]	0.05	0.09	0.020	0.18
Finisher period				
Antibiotic usage per pig, mL[2]	0.23	0.59	0.152	0.06
Anti-inflammatory usage per pig, mL[3]	0.08	0.20	0.043	0.06
Weaning to slaughter				
Antibiotic usage per pig, mL[2]	034	0.77	0.130	0.02
Anti-inflammatory usage per pig, mL[3]	0.13	0.29	0.064	0.02

[1]Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
[2]Volume of antibiotic administered to each pig.
[3]Volume of anti-inflammatory administered to each pig.

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3. Effect of creep feeding pelleted starter diet, liquid milk replacer and a liquid mixture of starter diet and milk replacer to suckling pigs on their growth and medication usage

E.A. Arnaud, G.E. Gardiner, M. Chombart, J.V. O' Doherty, T. Sweeney, P.G. Lawlor (2023). Effect of creep feeding pelleted starter diet, liquid milk replacer and a liquid mixture of starter diet and milk replacer to suckling pigs on their growth and medication usage. *Translational Animal Science*. https://doi.org/10.1093/tas/txae041

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3.1 Abstract

The aim of this study was to assess the effect of creep feeding solid starter diet, liquid milk replacer and a liquid mixture of starter diet and milk replacer to suckling pigs on their growth and medication usage up to target slaughter weight (~120 kg). Ninety-one sows and their litters were randomly assigned to one of four post-farrowing treatments at day 107 of gestation; 1) no creep feed provided to weaning at day 28 of age (CONTROL; n=20), 2) dry pelleted starter diet provided as creep feed from day 10 of age to weaning (DPS; n=25), 3) liquid milk replacer provided as creep feed from day 3 of age to weaning (LMR; n=23), and 4) liquid milk replacer provided from day 3 to 6 of age followed by a mixture of liquid milk replacer with an increasing proportion of liquid starter diet to weaning provided as creep feed (LMR+S; n=23). Pig weight and dry matter disappearance (DMd) were recorded during lactation and post-weaning until pigs reached target slaughter weight (~120 kg). At target slaughter weight, carcass weight and quality were recorded. Medication (antibiotic and anti-inflammatory) usage per pig on a litter basis, and number of injections and clinical cases of disease per litter were recorded from birth to slaughter. At day 5 post-weaning, a subset of pigs (n=40) were sacrificed and intestinal samples were collected for histological analysis. Piglets supplemented with DPS had higher DMd of creep feed than those supplemented with LMR or LMR+S (P<0.001). Providing LMR+S to suckling piglets reduced the coefficient of variation (CV) for within-litter piglet weaning weight (P<0.01) compared to DPS and LMR, but CV of LMR+S was similar to that of CONTROL. Providing DPS or LMR to suckling piglets increased piglet weaning weight compared to CONTROL (P<0.001) but pig weight was not significantly different from CONTROL at time-points thereafter. Gain to feed ratio from weaning to day 6 post-weaning was less for LMR pigs compared to all other treatments (P<0.001). Providing DPS or LMR+S to suckling piglets tended to increase post-weaning ileal villus height (P=0.07). Diarrhoea incidence, as well as the number of clinical cases of disease and injections per litter and volume of antibiotic and anti-inflammatory administered per pig pre- and post-weaning were not affected by treatment (P>0.05). In conclusion, supplementing suckling pigs with liquid milk replacer or dry pelleted starter improved growth at weaning, but the benefit did not persist to slaughter.

3.2 Introduction

Weaning is an extremely challenging event for piglets. Pigs are often mixed and moved to an unfamiliar environment. Diet abruptly changes from sows' milk, a highly digestible diet in liquid form, to a dry solid diet mainly of vegetable origin. This results in delayed and reduced feed intake and growth, and sometimes diarrhoea in piglets during the early post-weaning period (Hampson and Smith, 1986; Wolter and Ellis, 2001; Collins *et al.*, 2017). Litter size in sows has increased dramatically, particularly in the past decade [+2.5 piglets born alive between 2011 and 2022; Teagasc (2022)]. Although demand for milk by the litter increases with increasing litter-size, sows have a finite ability to produce milk and therefore, the mean volume of milk available for individual pigs is decreased (King, 2000). This negatively affects piglet pre-weaning weight gain and weaning weight, as lighter pigs at weaning have reduced lifetime growth and higher mortality rates (Collins *et al.*, 2017). Providing supplementary feed (i.e. creep feed) to suckling piglets is a good strategy to increase pre-weaning growth and weaning weight. Furthermore, it can familiarise suckling pigs with feed prior to weaning and thereby reduce latency to first feed after weaning and increase post-weaning feed intake and growth (Muns and Magowan, 2018; Pluske *et al.*, 2007). However, creep feed consumption varies greatly within and between litters. This is one reason that the response to creep feeding has been found to vary greatly, being particularly influenced by the proportion of 'eaters' and 'non-eaters' of creep-feed within the litter (Bruininx *et al.*, 2002; R. C. Sulabo *et al.*, 2010). Providing supplementary milk to suckling piglets can increase weaning weight and has the potential to increase the proportion of 'eaters' within litters (Van Oostrum *et al.*, 2016; Wolter *et al.*, 2002). However, supplemental milk does little to expose piglets to the plant-based ingredients that they will encounter in their post-weaning diet. A solution to this is to supplement suckling piglets with a post-weaning diet in liquid or gruel form. This has been found to increase piglet feed consumption compared to feeding the same diet in dry form (Byrgesen *et al.*, 2021; Martins *et al.*, 2020).

Increasing creep feed consumption prior to weaning can increase weaning weight (Lee and Kim, 2018; Wolter *et al.*, 2002) and improve intestinal maturity in pigs at weaning

(Cabrera *et al.*, 2013; Amdi *et al.*, 2021). Therefore, the practice might be expected to result in more robust pigs at weaning, and as a consequence reduce their need for antimicrobial usage (AMU). However, information is lacking on the effect of creep-feeding strategies on pig intestinal integrity, clinical cases of disease and medication usage (e.g. AMU). The objective of this study was to determine the effect of providing supplementary dry pelleted starter diet, liquid milk replacer, and a liquid mixture of milk replacer and starter diet as creep feed to suckling pigs on piglet growth and AMU. Additionally, sow body weight and back fat thickness were monitored in order to determine if this strategy could reduce loss of sow body reserves during lactation. The residual effects of these pre-weaning dietary treatments on post-weaning growth, health and medicinal usage to target slaughter weight were also determined.

3.3 Materials and methods

3.3.1 Ethical approval

This study was performed between August 2021 and September 2022, at the Teagasc Pig Development Department, Moorepark, Fermoy, Co. Cork, Ireland. Ethical approval was granted by the Teagasc Animal Ethics Committee (approval no. TAEC2020-273) and South East Technological University Ethics Committee (approval no. WIT2021REC011). The project was authorised by the Irish Health Products Regulatory Authority (project authorisation no. AE19132/P129). The experiment was conducted in accordance with the legislation for commercial pig production set out in the European communities (welfare of farmed animals) regulations 2010 and in Irish legislation (SI no. 311/2010).

3.3.2 Experimental design and animal management

Ninety-one sows (Large White × Landrace, PIC®, Hermitage Genetics, Sion Road, Co. Kilkenny, Ireland) were used in this study, which was conducted over four batches (with a batch being a group of sows inseminated on the same week). The first batch

included nineteen sows, the second batch included twenty six sows, the third batch included twenty two sows and the fourth batch included twenty four sows. Sows were artificially inseminated at onset of standing oestrus and again 24 h later using pooled semen (Topigs Norsvin Tempo, Premier Pig Genetic Limited, Ireland). Gestating sows were housed in dynamic groups of ~120 animals. Sows were introduced to the dynamic group between 3 and 6 days after service and fed from electronic sow feeders [ESFs; Schauer Feeding System (Competent 6), Prambachkirchen, Austria]. On day 107 of gestation, sows were blocked within farrowing batch into 25 blocks on the basis of parity group (mean ± SD; 2.4 ± 0.99), number of pigs weaned/sow in the previous cycle (13.4 ± 1.86 for multiparous sows) and body weight (BW) (270.5 ± 32.61 kg). Sow parity group distribution was as follows: group one, parity 0 (19%); group two, parity 1 to 2 (38%); group three, parity 3 to 5 (25%); and group four, parity 6 to 8 (18%).

Within block, suckling litters were randomly assigned to the following pre-weaning dietary treatments: 1) no creep feed provided to weaning (CONTROL; n=20), 2) dry pelleted starter diet provided as creep feed from day 10 of age to weaning (DPS; n=25), 3) liquid milk replacer provided as creep feed from day 3 of age to weaning (LMR; n=23), and 4) creep feed provided as liquid milk replacer from day 3 to day 6 of age followed by a mixture of liquid milk replacer and liquid starter diet to weaning (LMR+S; n=23). Day 10 was selected as the creep feeding start date for DPS to conform to farm creep feeding practices where creep feeding generally commences after day 10 post-farrowing. However, automated liquid feed delivery systems allow earlier commencement of liquid feeding and we selected to start liquid creep feeding at day 3 after farrowing when piglets had consumed sufficient quantities of colostrum.

Prior to the housing of the sows in farrowing accommodation, each farrowing room was cleaned, disinfected, and allowed sufficient time to dry according to standard practices in the facility. At ~ day 110 of gestation sows were moved into standard farrowing crates in pens (2.5 m x 1.8 m) with cast-iron slats under the sow and plastic slats with a water-heated floor pad for the piglets (BigDutchman; Vechta, Germany). Farrowing room temperature was maintained at 24 ± 3.0 °C at the time of farrowing and gradually reduced to 21 °C by day 7 of lactation. The temperature of the heat pads was 38-40 °C for the first 2 days after farrowing and was reduced by ~1 °C each day

to 30 °C at 10 days after farrowing and it was maintained at this temperature until weaning. Artificial lighting was provided daily from 0800 h to 1630 h. The average number of piglets born alive was 15.9 ± 4.44 piglets. Where possible, litter size was standardized between 24 h and 48 h after parturition, with cross-fostering being conducted so that there was an average litter size of 14.6 ± 0.95 piglets per sow at 48 h postpartum. The final number of piglets remaining on each sow at 48 h postpartum was affected by the rearing capacity of each sow (i.e. the number of available functional teats) and the availability of foster sows to take surplus piglets. Piglets' teeth were clipped within 24 h of birth. On day 5, postpartum tails were docked and all piglets were injected with 1 mL of iron (Gleptosil, Ceva santé animale, Libourne, France). Male pigs remained fully intact and piglets were weaned at day 28 ± 1.0 of lactation.

To study the residual effect of the pre-weaning dietary treatment in progeny, a subsample of 566 pigs (8.1 ± 1.31 kg) were selected at weaning. Within sow treatment groups, pens of 10 to 12 pigs of the same sex (entire male or female) of even weight were formed and blocked by sow treatment, sex and BW. Pen groups for CONTROL (n=12), DPS (n=12), LMR (n=12), and LMR+S (n=12) were moved to weaner accommodation at weaning. Pig BW, feed disappearance and health were monitored up to target slaughter weight. Weaner pens were equipped with fully slatted plastic floors (2.5 × 2 m) with automatic environmental control. Each pen had a shelf-type single-space (33 cm) wet-dry feeder (BA19100, Verba, Verbakel, Netherlands) with inset nipple drinker and a supplementary bowl drinker (SS Drinker, Rotecna, Lleida, Spain). A spiked rubber ball (Easyfix Luna 142, Easyfix, Galway, Ireland) was provided for each pen as environmental enrichment. Temperature in the weaner rooms was maintained at 28 °C during the first week after weaning and reduced by 2 °C each week to 22 °C at the end of 4 weeks. Ventilation was from a punched ceiling with air exhausted via a variable speed fan linked to a thermostat which was controlled by computer (Big Dutchman 135). At day 47 post-weaning, pen groups were moved to finisher accommodation. Finisher pens had fully slatted concrete floors (2.4 × 4.2 m) with automatic environmental control. Each pen had one shelf-type single-space (33 cm) wet-dry feeder (MA19100, Verba) with inset nipple drinker and a supplementary bowl drinker (SS Drinker, Rotecna). A wooden (larch) post was provided for each pen

as environmental enrichment. All rooms were equipped with windows for natural light. Temperature in the finisher rooms was maintained at 22-20 °C with the same type of ventilation system used as in the weaner house. Pigs in each treatment group were slaughtered over 2 weeks when they reached the target slaughter weight of ~120 kg live weight (LW; average age at slaughter 157 days). The heaviest pigs in each pen group were slaughtered during the first week and the remaining pigs in the pen were slaughtered 7 days later.

3.3.3 Diet preparation and feeding

Diets were formulated to meet or exceed National Research Council (NRC, 2012) recommendations. Diet samples were analysed for dry matter (DM) (oven drying), ash (furnace drying and gravimetry), crude protein (Dumas method), total fat (Weibul acid hydrolysis) and crude fibre (Ankom 200 fibre analyser, Macedon, New York, United States) by Sciantec Analytical Services Ltd, Selby, United Kingdom according to European Union Commission Regulation No 152/2009 of 27 January 2009 . The ingredient composition and nutrient content of the diets are shown in Table 3-1. During gestation, sows were fed a gestation diet (Diet 1) in meal form at a feed allowance of 2.2 kg/day between days 0 to 90 of gestation. From day 90 of gestation to parturition, gestation feed allowance was increased to 2.7 kg/day. In the farrowing room, sows were fed a lactation diet (Diet 2; Table 3-1) in meal form using a computerized feed delivery system (DryExact Pro, Big Dutchman). Sows were fed twice daily from farrowing to day 6 of lactation and three times daily from day 7 to weaning at 28 days. Sows were fed according to a lactation feeding curve which started at 60 MJ DE/d at day 0 of lactation and gradually increased to 107, 125, 133, and 137 MJ DE/d at days 7, 14, 21, and 26 of lactation, respectively. During lactation, feed allocation for individual sows was adjusted up and down from the curve, as necessary, to ensure that sow feed intake was as close as possible to *ad libitum* feed allowance and to prevent feed wastage. Between weaning and service, sows were provided with ad libitum access to a lactation diet for 4 days followed by a gestation diet (Diet 1; Table 3-1) in meal form. Water was provided on an *ad libitum* basis to sows from a

single-bite drinker in the feed trough and to suckling piglets from a bowl in the farrowing pen.

Starter diet (Diet 3; Table 3-1) when in dry form was fed as pellets (3 mm diameter pellets) as creep feed to suckling pigs from day 10 after birth until weaning using a circular creep feeder (Easy pan, Rotecna) placed at the bottom edge of the heat pad. Creep feed was provided frequently in small quantities to ensure freshness and to minimise wastage. Dry matter disappearance (DMd) was recorded at day 21 and 28 of lactation for DPS by weighing the total amount of dry starter provided to each pen during the period. The milk replacer powder [Opticare, Swinco, Helmond, The Netherland] used in treatments LMR and LMR+S was probiotic-free. The milk replacer powder contained the following, in descending order of inclusion: sweet whey powder, vegetable oils, porcine dried plasma powder, whey powder, digestible starch, dextrose, hyper-immunised egg powder, soya protein concentrate, hydrolysed wheat gluten, premix of amino acids, vitamins and trace minerals. The milk replacer powder contained 11.9 MJ/kg net energy, 21.5% crude protein, 9% fat, 0.1% crude fibre, 6.5% crude ash, 1.8% lysine, 0.46% methionine, 0.7% calcium, 0.55% phosphorus, and 0.7% sodium. Liquid milk replacer was prepared at a mixing ratio of 150 g of milk replacer powder per 1 L of water and fed through an automatic feeder (Babyfeed, Schauer Agrotronic GmbH, Prambachkirchen, Austria). The liquid feeding trough was positioned to one side of the sow's head at the front of the farrowing pen. For treatment LMR+S, liquid milk replacer was fed from day 3 to 6 of age followed by liquid milk replacer with an increasing proportion of starter diet (Diet 3; Table 3-1) added to the mixture as weaning age approached (Table S 3-1). The liquid starter for the LMR+S treatment was prepared by mixing 200 g of dry pelleted starter diet with 1 litre of warm water (55 ˚C) to achieve a feed to water ratio of 1:5. From day 3 to 6 of age, 100% liquid milk replacer was provided to suckling pigs. The percentage of liquid milk replacer in the mixture decreased to 80% from day 7 to 10, 60% from day 11 to 14, 40% from day 15 to 17, 20% from day 18 to 21 and 0% from day 21 to 28, while the percentage of liquid starter simultaneously increased.

Following weaning, pigs were fed a sequence of diets in accordance with their growth stage. Starter diet (Diet 3; Table 3-1) was provided from weaning to day 6 post-weaning, link diet (Diet 4; Table 3-1) from day 6 to 17 post-weaning, weaner diet (Diet

5; Table 3-1) from day 17 to 47 post-weaning, and a finisher diet (Diet 6; Table 3-1) from day 47 post-weaning to slaughter (~ day 129 post-weaning). All diets for post-weaning pigs were provided dry as 3 mm diameter pellets and on an *ad libitum* basis. Pigs were inspected daily and any pigs demonstrating visual signs of illness were treated appropriately. Assessment of clinical signs of disease and treatment protocols were followed in accordance with farm protocol. All veterinary treatments were recorded including antibiotic and anti-inflammatory treatments.

3.3.4 Pre-weaning liquid feed system

The supplementary liquid milk replacer and the liquid mixture of milk replacer and starter diet was provided through an automatic feeder (Babyfeed). Fresh feed was prepared twice daily at 0835 h and at 1645 h. Ingredients (milk replacer powder and starter diet) were mixed with water at 55 ˚C for ~10 minutes. Ten feeding cycles (each lasting ~ 2 h) were programmed between 0930 h and 0400 h. During each cycle the in-situ trough sensors checked the amount of liquid feed present in the trough 3 times. Whenever the feed level was below the level of the sensor, the trough was detected as empty, and milk replacer or the liquid mixture of milk replacer and starter diet, depending on treatment, was delivered to the trough and the amount delivered to the trough was recorded in the system computer at each re-fill. Therefore, each pen could potentially have been supplied with milk replacer or the liquid mixture of milk replacer and starter diet, depending on treatment, up to 30 times in a 24 h period. Each day after the last feeding, the system was cleaned in closed circuit, which included mixing tanks and all of the pipelines, with a 1% acid solution (Deosan Acidbrite AG313, Diversey Europe Operations BV, Utrecht, The Netherlands). In addition, the system was cleaned once a week with a 0.5% solution of an alkaline detergent (AvalKsan Gold Standard CF, Carbon Group, Ringaskiddy, Ireland), to help remove lime scale from the circuit. The troughs were cleaned each morning with air pressure and rinsed with acidified water and a 0.5% solution of the alkaline detergent was applied to troughs, as for the acid rinse, once weekly. Every morning, before the cleaning of the troughs, any remaining uneaten feed was quantified in each trough. The pH of samples from the LMR tank and from the LMR+S tank was measured at 0900 h and 1530 h on days

4, 8, 12, 15, 18 and 25 of lactation for quality control purposes. The pH reduced between morning and afternoon measurements (6.08 vs 4.35 ± 0.166 pH units and 5.40 vs 4.11 ± 0.179 pH units, for the LMR and LMR+S, respectively; see Table S 3-2). The lactic acid bacteria count increased between morning and afternoon samples, and especially for LMR+S (Fig S 3-1).

3.3.5 Sow body weight and back fat thickness

Sow BW and back fat (BF) were recorded on day 110 of gestation, at weaning, and at their subsequent service (~day 4 post-weaning). Sow BW was recorded using an electronic sow scales (EziWeigh 7i, O'Donovan Engineering, Co. Cork, Ireland). Empty farrowing weight was calculated using the following equation from the NRC (1998)[1]:

$$^{1}SW_{farr} = [SW_{d110} – (NB \times 2.25)].$$

Where, SW_{farr} = Empty sow farrowing weight, SW_{d110} = Sow weight at day 110 of gestation, NB = total number of piglets born. The 2.25 kg is an estimate of the increased weight in the gravid uterus and in mammary tissue attributed to each pig in a litter (NRC, 1998).

Body fat was measured using a digital BF indicator (Renco LEANMEATER, Renco Corporation, Golden Valley, MN) by placing the probe of the digital indicator on the back of the sow at the level of the last rib, 6 cm to the side of the backbone. A reading was taken from the right and left side of the sow and the average reading was recorded.

3.3.6 Farrowing performance and pre-weaning piglet growth performance

The number of piglets born (total, live, stillborn, and mummified) was recorded for each litter at birth. The individual weight and sex of each piglet was recorded at birth, when each piglet was tagged for identification purposes, at 48 h after birth, on day 7, 11, 19 and 27 postpartum using an electronic piglet scale (Defender 3000 XtremeW, O'Donovan Engineering). These data were used to determine the litter weight at each

weighing, and piglet pre-weaning average daily gain (ADG). The coefficient of variation for within-litter BW was calculated for each weighing day. Piglet mortality between birth and weaning and between 48 h and weaning was also recorded. The average creep feed disappearance per litter was monitored at day 21 and 28 for DPS by weighing the total amount of dry starter provided to each pen. For LMR and LMR+S, the amount of liquid creep feed provided to each trough/pen was recorded daily by the system computer. The average creep feed disappearance per piglets was obtained by dividing the quantity provided per litter by the number of piglets alive in that litter at the recording time-point.

3.3.7 Live observation of trough-directed behaviour per litter

The feeding behaviour of individual piglets within pen groups was observed in batches 1 and 2 of the experiment using instantaneous scan sampling at day 12, 18, 22 and 26 after birth. To enable the easy identification of piglets during scan sampling, piglets in each litter were marked with a number from 1 to 17 (linked to their tag number) using black hair dye (Pro color plus, healthpoint, Blackpool, United Kingdom) on the day before scan sampling was conducted. Six 1-hour sessions were conducted between 0900 h and 1600 h on each scan sampling day. During each 1-hour session of live observations, each pen was scanned (i.e. the behaviour of the group was recorded) every 3 minutes, leading to 21 scans/pen/session. A simple ethogram was used for scoring feeding behaviour. At every scan, trough-directed activity (solid or liquid) was recorded. Trough-directed activity was defined as when a piglet snout was inserted into the solid feed trough or immersed in the liquid feed for at least 2 seconds. Piglets were categorized as eaters if they were engaged in two or more trough-directed activity at any time during an observation day. The percentage of piglet eaters per pen was calculated on a pen basis for each observation day and for all observation days combined. This was carried out by dividing the number of piglets considered as eaters by the total number of piglet present in the pen, then multiplying the results by 100 to express as a percentage.

3.3.8 Post-weaning pig growth performance

Pen groups were weighed on day 0 (weaning), 6, 14, 21, 28, 47 post-weaning and individual pig weights were recorded just prior to slaughter (at ~day 129 post-weaning) using an electronic scale (EziWeigh 7i, O'Donovan Engineering). Pigs were fasted prior to recording BW before slaughter. Feed disappearance was recorded on a pen basis between weaning and slaughter for the periods between which BWs were recorded. These data were used to determine the average daily feed intake (ADFI), ADG, and Gain to Feed (G:F).

3.3.9 Carcass data

In total, 552 pigs (~138/treatment) were transported 95 km to the abattoir (Dawn Pork & Bacon, Grannagh, Co. Waterford, Ireland) where they were killed by exsanguination after CO2 stunning. At the abattoir, carcass cold weight of individual pigs was calculated and muscle depth and BF measured as outlined in Chapter 2. Lean meat content, carcass ADG, carcass G:F and lean ADG were calculated as outlined in Chapter 2.

3.3.10 Intestinal sampling and small intestinal histology

A subset of 40 pigs (n=10 per pre-weaning dietary treatment) were euthanized at day 5 post-weaning by captive bolt followed by immediate exsanguination. After euthanasia, the entire intestinal tract was removed. Samples (~2 cm) of tissue were excised from the duodenum (15 cm distal to the pyloric junction), jejunum (1.5 m distal to the pyloric junction) and ileum (15 cm proximal to the ileo-caecal junction). Tissue samples were rinsed in phosphate-buffered saline (Scientific Laboratory Supplies, Nottingham, United Kingdom) immediately post-harvest and placed in an alcohol/aldehyde fixative (No-Tox; Scientific Device Laboratory, Des Plaines, Illinois, United States) and that evening placed on a shaker for 48 h prior to storage at room temperature for histological analysis. Duodenal, jejunal and ileal tissue samples were sent to an external company (NationWide Laboratories, Devon, United Kingdom). There, the tissue samples were removed from the No-Tox fixative and

dehydrated through a graded alcohol series, cleared with xylene and embedded in paraffin wax. Tissue samples were sliced into 5 micrometre sections using a microtome (Leica RM2135, Wetzlar, Germany), mounted on microscope slides and stained with haematoxylin and eosin for determination of gross morphological parameters of intestinal structure (villus height, villus width and crypt depth and crypt width). For each pig, 10 villi and 10 crypts, where villi were attached to the lumen, were measured on five fields of view, and the means were used for statistical analysis.

3.3.11 Health monitoring

Faecal consistency scores on a pen basis were determined weekly at day 2, 7, 11, 19, 27 before weaning and day 6, 14, 21 and 28 post-weaning. A 4-point scoring system (Casey *et al.*, 2007) was used and the average score from five pigs was determined as the average score for each pen. In brief: 0 = normal (dry pelleted faeces), 1 = soft (soft with shape), 2 = mild diarrhoea (very soft or viscous liquid) and 3 = severe watery diarrhoea (watery or with blood). The diarrhoea incidence was determined at each time point by considering a faecal score of 2 or greater as indicative of diarrhoea for each litter/pen.

Antibiotic and anti-inflammatory usage was recorded in sows during lactation and in pigs from birth until they reached their target slaughter weight (separately for the weaner and finisher periods, respectively). Medication was administered when joint-ill, lameness, malaise or diarrhoea were observed in piglets and when malaise or vaginal discharge was observed in sows by trained farm technicians. One antibiotic (Unicillin; Univet Ltd, Cootehill, Co. Cavan, Ireland) and one anti-inflammatory (Loxicom, Norbrook, Newry, United Kingdom) only, were used during this experiment. Animal ID, pen number, product name, product code, dose administered (ml), frequency of administration, date of administration, and reason for use were recorded when an animal was treated. From this, the total number of piglet injections per litter/pen, the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a litter basis and per sow, and the total number of clinical cases of disease (i.e. when an animal was treated one or more times) per litter were calculated pre-weaning. The average volume of medication (antibiotic and anti-

inflammatory) administered per pig per pen was also calculated between weaning and target slaughter weight.

3.3.12 Statistical analysis

All data were tested for normality using the Univariate procedure and residuals were inspected in all models to confirm normality. Model fit was determined by choosing models with the minimum finite-sample-corrected Akaike Information Criteria.

All data were analysed in Statistical Analysis Systems (SAS) using the linear mixed models procedure (PROC Mixed) using the software package version 9.4 (SAS Institute Inc., Cary, North Carolina, United States), except the data for incidence of diarrhoea. The incidence of diarrhoea in the farrowing accommodation from day 2 to 28 and in the weaner accommodation from weaning to day 28 post-weaning was analysed using the PROC Glimmix procedure of SAS with a binomial distribution. Data from batches 1, 2, 3 and 4 were analysed together as all measurements were recorded at the same time points.

For the analysis of pre-weaning litter weight, piglet BW, sow BW and sow BF, the percentage of piglets classified as "eaters", the total number of piglet injections per litter, the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a litter basis and per sow, the total number of clinical cases of disease per litter, number of deaths and removals per litter, post-weaning growth parameters, carcass quality data and the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a pen basis post-weaning and diarrhoea incidence; treatment was included in the model as a fixed effect. For analysis of pre-weaning piglet growth parameters, piglet weight and litter size at 48 h were included as co-variates, when significant in the model. For analysis of sow BW and BF, initial value at day 110 of gestation was included as a covariate in the model. For the analysis of post-weaning growth and carcass quality parameters, weaning weight was included as a co-variate. For BW at slaughter and cold carcass weight, the number of days from weaning to slaughter was included as a co-variate. Day was included in the above models as a repeated variable when relevant and block was included as a random

effect. The litter/sow was the experimental unit for the analysis of all pre-weaning parameters, except piglet weight and growth, where pig nested within-sow/litter was the experimental unit. The experimental unit post-weaning was the pen group.

For analysis of intestinal morphology parameters, treatment was included in the model as a fixed effect. Sow was included as a random effect and the pig was the experimental unit.

In all cases, differences in least square means were investigated using the t test after Tukey adjustment for multiple comparisons. Results are presented in the text and tables as the least square means together with their pooled standard error. Differences between treatments were considered significant when $P \leq 0{\cdot}05$, whereas $0{\cdot}05 < P \leq 0{\cdot}10$ was considered as a tendency.

3.4 Results

3.4.1 Sow body weight and back fat thickness

The effect of treatment on sow BW, BF depth and reproductive performance from farrowing to service (~4 days after weaning) is presented in Table S 3-3. There was no effect of treatment (P>0.05) on any parameter of interest at any time point.

3.4.2 Litter size, number fostered, pre- and post-weaning deaths and removals

The effect of treatment on sow litter-size at birth, 48 h and weaning, the number of piglets fostered between 24 and 48 h and the number of piglet deaths per litter from birth to weaning and 48 h to weaning is presented in Table S 3-4. The total number of piglets born tended to be higher for treatment LMR compared to all other treatments (P=0.06). The number of piglets born alive was higher for treatments LMR and LMR+S compared to CONTROL (P=0.01). The number of piglets cross-fostered onto

sows was higher in CONTROL compared to all other treatments (P=0.05). There was no treatment effect (P>0.05) on litter size at 48 h, litter size at weaning, number of deaths between birth and weaning and number of deaths between 48 h and weaning. Seven per-cent of all piglets on trial died between 48 h after birth and weaning. Among these, 52% died between day 2 and 7, 24% died between day 7 and 11, 14% died between day 11 and 19, and 10% died between day 19 and 28. Mortality rate from 48 h to weaning was 9%, 7%, 6% and 6% for CONTROL, DPS, LMR and LMR+S treatments, respectively. Deaths between day 2 and 6 were mainly due to starvation and crushing. After day 6, causes of mortality were more variable and included crushing, starvation, injury and sudden death.

Two per cent of all pigs on trial died or were removed post-weaning. Among the dead or removed pigs, 50% died or were removed between weaning and day 47 post-weaning and 50% died or were removed after day 47 post-weaning. Post-weaning mortality and removal rate was 6%, <1%, <1% and 3% for CONTROL, DPS, LMR and LMR+S treatments, respectively. Deaths and removals were due to lameness or injury.

3.4.3 Pre-weaning piglet growth performance

The effect of treatment on piglet creep feed DMd, weight and growth during the suckling period is presented in Table 3-2. Pre-weaning total DMd of creep feed per litter and per piglet was higher for DPS compared to LMR+S (P=0.03). The DMd per litter and per piglet of the LMR treatment was similar to DPS and LMR+S (P>0.05). There was no effect of treatment on litter weight at day 2, 7, 11, 19 and overall (P>0.05). At day 27 of lactation, LMR piglets tended to have a higher litter weight than CONTROL piglets (P=0.08). There was no effect of treatment on mean piglet BW at day 2, 7, 11 and overall (P>0.05). At day 19 of lactation, LMR piglets tended to be heavier than DPS and LMR+S piglets (P=0.06), whereas CONTROL piglets had a similar BW to all other treatments (P>0.05). At day 27 of lactation, LMR piglets were heavier than piglets on all other treatments (P<0.001), whereas DPS piglets were heavier than CONTROL piglets only. Piglets from the LMR+S treatment had a similar BW at weaning as CONTROL pigs and DPS pigs.

From day 2 to 7 of lactation, DPS and LMR+S piglets had a higher ADG than CONTROL piglets (P<0.01), whereas LMR piglets had similar ADG to all other treatments (P>0.05). From day 7 to 11, LMR and LMR+S piglets had a higher ADG than DPS, but a similar ADG to CONTROL (P<0.001). From day 11 to 19, LMR piglets had a higher ADG than for all other treatments (P<0.001). From day 19 to 27, DPS and LMR piglets had a higher ADG than CONTROL piglets or LMR+S piglets (P<0.001) and LMR+S piglets had a higher ADG than CONTROL piglets.

There was no treatment effect on the coefficient of variation (CV) of within litter BW at day 2 (P>0.05). At day 7, CONTROL and LMR+S piglets had a lower CV than LMR piglets (P=0.03). The LMR+S piglets had a lower CV than DPS and LMR piglets at day 11 (P =0.01), 19 (P=0.02) and 27 (P<0.05), however, all treatments had a similar CV to that of CONTROL piglets.

3.4.4 Live observation of trough-directed feeding behaviour per litter

The proportion of piglets having two or more feeder-directed activities within-litter is presented in Table S 3-5. There was no treatment effect on the percentage of eaters at day 12 of lactation. At day 18, 22, 26 of lactation and overall, DPS had a higher percentage of eaters than all other treatments (P<0.001). At day 22 and overall, LMR+S had a higher eater percentage than LMR only (P<0.001).

3.4.5 Pre-weaning diarrhoea scores and antibiotic and anti-inflammatory treatment

The effect of treatment on diarrhoea incidence is presented in Table 3-3. There was no effect of treatment on diarrhoea incidence between day 2 and 28 after birth (P>0.05).

The effect of treatment on the total number of clinical cases of disease per litter, the total number of injections per litter, and the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a litter basis, and per sow, during the pre-weaning period is presented in Table 3-3. There was no effect of treatment on any of these parameters (P>0.05).

3.4.6 Post-weaning pig growth and carcass quality

The effect of treatment on pig growth, feed intake and feed efficiency from weaning to slaughter is presented in Table 3-4. There was no effect of treatment on pig BW and ADFI at any time point post-weaning (P>0.05). There was no effect of treatment on ADG from day 6 to 14, and on G:F ratio or ADG from day 14 to 21, day 21 to 28, day 28 to 47, day 47 to slaughter and overall. Compared to all other treatments, LMR pigs had a lower G:F ratio and ADG from weaning to day 6 post-weaning (P<0.001) and LMR+S piglets had a lower G:F ratio from day 6 to day 14 post-weaning (P<0.001). The effect of treatment on carcass parameters is presented in Table 3-5. There was no effect of treatment on any parameter of interest (P>0.05).

3.4.7 Post-weaning diarrhoea scores, antibiotic and anti-inflammatory treatment

The effect of treatment on diarrhoea incidence from weaning to day 28 post-weaning is presented in Table 3-6. There was no effect of treatment on diarrhoea incidence between weaning and day 28 post-weaning (P>0.05). No scores higher than 0 were given after 28 days post-weaning.

The effect of treatment on the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a pen basis post-weaning is presented in Table 3-6. There was no treatment effect on the volume of antibiotic or anti-inflammatory administered per pig on a pen basis during the weaner or finisher periods.

3.4.8 Intestinal morphology

The effect of treatment on intestinal morphology at day 5 post-weaning is presented in Table 3-7. There was no effect of treatment on villus height, crypt depth, villus height to crypt depth ratio (VH:CD) or villus width in the duodenum and jejunum of

pigs. The DPS and LMR+S pigs tended to have greater villus height in the ileum than CONTROL piglets or those supplemented with LMR (P=0.07).

3.5 Discussion

This study is novel, in that it compares the effect of creep feeding dry pelleted starter diet, liquid milk replacer, and a liquid mixture of milk replacer and starter diet to suckling pigs on pre- and post-weaning growth to target slaughter weight (~120 kg) and medication usage. The results should provide valuable practical information for the design of optimal pre-weaning nutritional and management strategies to maximise pre- and post-weaning pig growth.

3.5.1 Pre-weaning

It was hypothesised that feeding creep feed in liquid form would increase the creep feed DMd of suckling piglets. However, this was not the case, as piglets supplemented with DPS had a higher total pre-weaning DMd (565 g/pig) than those supplemented with LMR+S (353 g/pig) while being similar to those supplemented with LMR (471 g/pig). In general, intake of dry pelleted creep feed in the current study was higher than that reported in previous studies (Bruininx *et al.*, 2002; Byrgesen *et al.*, 2021) and intakes of liquid creep were lower than that found in other studies (Wolter *et al.*, 2002; Pustal *et al.*, 2015; Lawlor *et al.*, 2002). Based on recent work from our group (Vasa *et al.*, 2023), it is likely that the frequency of sensor checks in the liquid feed troughs in the current study was insufficient, particularly during the final week prior to weaning, and this may have negatively impacted intake of liquid feed. Further to this, the feeder space of the liquid feeders was less than half that of the dry creep feeders (226 cm^2 with a circumference of 34.5 cm vs. 573 cm^2 with a circumference of 84.8 cm) and this may also have contributed to the lower liquid feed intake (Appleby *et al.*, 1991). It is likely that increasing the proportion of milk replacer powder in the LMR+S mixture or feeding it for additional days during early supplementation would increase DMd and pig growth. However, milk replacer powder

is ~3 times more expensive than starter diet and increasing its proportion in the mixture is likely to be uneconomic.

In the current study, pre-weaning provision of DPS and LMR increased piglet BW at weaning compared to the control, whereas this was not the case when LMR+S was provided. This is in agreement with previous findings where weaning weight was increased when DPS (Lee and Kim, 2018) or LMR (Wolter *et al.*, 2002; Van Oostrum *et al.*, 2016) were provided as creep feed. Despite LMR and LMR+S having similar pre-weaning DMd and LMR resulting in similar DMd to DPS, it was surprising that weaning weights were higher for LMR than for all other treatments and that litter weights tended to be higher for LMR than the control. The milk replacer powder in our study contained ~40% lactose and it could be that the higher intake of lactose contributed to the better pre-weaning growths observed when LMR was fed. Suckling piglets have high lactase activity in their small intestine and only a poorly developed capacity to digest starch and other substrates from plant-based ingredients (Pluske *et al.*, 1997). Regarding the lack of response of LMR+S on weaning weight, others have also found that liquid creep feeding did not increase pig weight at weaning (Byrgesen *et al.*, 2021; Martins *et al.*, 2020; Vodolazska *et al.*, 2023) even when DMd was higher than for dry creep feed [45 g/piglet vs. 25 g/piglet; Byrgesen *et al.* (2021)]. In the current study, it might be that the period of supplementation with liquid milk replacer was too short (only 3 days) for the LMR+S treatment. Vodolazska *et al.* (2023) supplemented piglets for 10 days and did not observe an increase in weight at weaning. It is likely that liquid milk replacer should be supplemented for a longer period in order to increase weaning weight. In the current study, liquid creep feed treatments also had a higher number of live born than the control, suggesting that piglets were generally smaller in those litters. Although there was no significant growth improvement for piglets supplemented with the liquid mixture compared to the control, this suggests that results could have been poorer in these litters without the provision of the liquid mixture. In the current study, although not significant, medication usage in sows, clinical cases of disease and number of injections per litter were numerically reduced in all of the creep feed treatments compared to the control which was not provided with creep feed. Additionally, deaths from 48 h to weaning for LMR and LMR+S were numerically reduced compared to the control (-0.4 pig deaths). These results, although

not significant, suggest that creep feeding in dry or liquid form improves the health of suckling pigs, thereby reducing the need for medication and resulting in increased survival of pigs to weaning.

Others have shown that the growth response to creep feeding is largely influenced by the proportion of piglets within the litter that consume creep feed before weaning (i.e. eaters) as reviewed by Middelkoop (2020). It is interesting to note that the DPS diet not only resulted in higher pre-weaning DMd in the current study but the highest proportion of piglets within each litter categorized as "eaters" was also found for this treatment compared with the liquid feed treatments. Nonetheless, piglets supplemented with dry creep did not have the highest weaning weight which suggests that feed wastage may have been an issue with DPS. Every effort was made to minimise feed wastage in the current study. However, feeder design and accessibility can influence feed wastage (Tokach *et al.,* 2020) and although the dry creep feeders used in the current study had plastic separators to stop the animals from lying in the feeder, they were open and had no hopper. This could have promoted creep wastage by allowing piglets to root in the feed, as suggested by Rommel Sulabo *et al.* (2010). It was not expected that a lower percentage of piglets from the liquid creep feed treatments would be classified as "eaters" compared to those from the dry creep treatment. One could speculate that, as dry pelleted creep feed was always present in the feeders, that piglets had frequent and smaller meals compared to those supplemented with liquid milk/mixture. This would have increased their chances of being observed in feeder-directed activity while live observations were being conducted. Further to this, as sensors on the liquid feed troughs were checked only three times every two hours, it is possible that troughs could have been void of feed for long periods of time during which live observations were being conducted. Both of these factors might explain the lower proportion of pigs classified as "eaters" where liquid as opposed to dry creep feed was provided in the current study.

3.5.2 Post-weaning

Supplementing suckling piglets with liquid milk replacer resulted in reduced growth and poorer feed efficiency during the first week post-weaning despite these pigs

having been heavier at weaning. A possible explanation for this might be that pre-weaning milk replacer provision to suckling piglets did little to expose them to the largely vegetable-based substrates that dominate post-weaning diets. For this reason, the development of the enzyme secretory capacity necessary to utilise the vegetable-based components of post-weaning diets, although not measured in the current study, may have been delayed in comparison to where a starter diet was provided as creep feed, as demonstrated by others (Hampson and Kidder, 1986; de Passillé *et al.*, 1989). As milk replacer is rich in lactose, a higher lactase activity was likely present at weaning when it was provided as creep feed. In contrast, the development of the necessary enzyme secretory capacity for starch degradation, for example, was likely delayed in comparison to that for all other treatments, explaining the reduced growth observed with this treatment in the immediate post-weaning period. It is likely that pigs familiarised with a liquid milk-based diet prior to weaning were less equipped to digest plant-rich dry pelleted starter diet offered post-weaning. This is supported by the reduced VH in the ileum of LMR pigs compared to those provided with DPS or LMR+S as a creep feed.

With a very high proportion of 'eaters' (78% of piglets observed having at least two feeder-directed activities at day 26 of age), very high total DMd per pig and increased weaning weight in pigs supplemented with DPS pre-weaning, a positive effect on intakes early post-weaning and possibly post-weaning growth may have been expected based on the work of Rommel Sulabo *et al.* (2010). However, no increase in post-weaning feed intake or growth was observed for pigs on this treatment compared to the control. On the other hand, the lack of post-weaning response is not surprising considering that the proportion of 'eaters' was most likely over-estimated and feed wastage was increased on this treatment, as outlined above.

In the present study there was a tendency for increased villus height in the ileum of piglets supplemented with DPS and LMR+S, at 5 days post-weaning, compared to control and liquid milk replacer-fed pigs. The latter may indicate a positive effect of this supplementation on intestinal structure post-weaning. The effects of creep feeding on post-weaning intestinal morphology are not consistent in the literature, as they are dependent on feed intake and diet composition (Huting *et al.*, 2021). As intake and growth of pigs supplemented with DPS and LMR+S were not increased early post-

weaning, the tendency for an increase in villus height in the ileum in the current study must be interpreted with caution. This tendency to increase villus height could be explained by the exposure to different sources of carbohydrates and plant-based proteins pre-weaning with these treatments. Soya was the main protein source of the starter diet, while whey and casein are the main protein sources of sow's milk (Theil and Hurley, 2016). Despite the lack of a significant effect of pre-weaning creep feeding on medication usage and clinical cases post-weaning, it is interesting to note that pigs supplemented with LMR+S had a numerically lower incidence of diarrhoea (-13%) post-weaning compared to the control. This is in agreement with Vodolazska *et al.* (2023) who observed an increase in faecal dry matter early post-weaning when liquid feed had been provided to suckling pigs. Supplementing a liquid mixture of milk replacer and starter or dry starter alone could therefore have familiarized the pig to these indigenous ingredients pre-weaning, thereby limiting their damaging effect on the intestinal tract early post-weaning. However, as outlined above, this did not result in a concomitant improvement of feed intake or growth.

The feed cost per pig during lactation based on the current study was €0.59, €0.71 and €1.41 per pig for DPS, LMR+S and LMR, respectively. These costs do not include the capital and running costs of the liquid feeding system. Therefore, milk replacer powder is expensive and its use must be economically justified.

3.6 Conclusions

The provision of liquid milk replacer to suckling piglets from day 3 to 28 after birth and dry pelleted starter diet from day 10 to 28 after birth increased piglet weaning weight. However, this weaning weight advantage did not persist post-weaning and the slaughter weight of pigs on both treatments was similar to that of the control where no creep feed was provided. Providing a liquid mixture of milk replacer and starter diet did not result in increased growth pre-or post-weaning compared to the control. Providing creep feed as a dry pelleted starter diet or as a liquid mixture of milk replacer and starter diet to suckling piglets tended to increase post-weaning ileal villus height. The management of the automated milk delivery system should be further optimised in order to ensure, in future studies, more frequent feed deliveries to troughs. This

would increase liquid creep feed availability, and most likely increase the DMd of liquid creep-fed piglets. This, in turn, should result in greater benefits from liquid creep feeding of piglets.

3.7 Tables

Table 3-1. Composition of the experimental diet (on an air-dry basis; kg/tonne).

Diet Number	**1**	**2**	**[1]3**	**4**	**5**	**6**
Diet type	**Dry sow**	**Lactation**	**Starter**	**Link**	**Weaner**	**Finisher**
Ingredients						
Barley	759.7	259.7	50.0	68.4	495.9	410.5
Wheat	0	455.2	0	100.0	216.8	390
Maize	0	0	231	300	0	0
Soybean meal	76.2	179.8	143.4	186.9	163.2	165
Full fat soybean meal	0	0	130.8	70.0	50.0	0
Whey permeate	0	0	200	150	0	0
Skim milk powder	0	0	125	50	0	0
Soya hulls	125.3	0	0	0	0	0
Soya oil	14	66	85	38.2	40	11
Premix	1.5[2]	1.5[2]	3.0[3]	3.0[3]	3.0[3]	1.0[4]
L-Lysine HCl	2.3	5.0	6.2	6.7	5.9	4.3
DL-Methionine	0.4	1.5	3.6	3.2	2.2	1.0

L-Threonine	1.0	2.7	3.7	3.4	2.7	1.9
L-Tryptophan	0	0.8	1.4	1.3	0.6	0.2
L-Valine	0	2.7	1.3	1.3	0.6	0
Limestone flour	8.5	11.5	7.0	7.5	10.5	11.0
Mono dicalcium phosphate	7.0	8.5	5.5	7.0	5.5	1.0
Salt	4	5	3	3	3	3
Phytase[5]	0.1	0.1	0.1	0.1	0.1	0.1
Chemical composition						
Dry matter[6]	883.0	864.0	912.0	904.0	890.5	880.0
Crude protein[6]	117.0	157.0	182.0	174.0	162.5	163.0
Ash[6]	47.0	50.0	58.0	51.0	44.0	46.5
Ether extract[6]	34.8	86.4	120.9	83.4	74.8	36.6
Crude fibre[6]	83.0	30.0	18.0	19.5	31.0	33.5
Lysine[7]	7.8	11.5	16.2	15.0	13.0	10.9
Methionine[7]	2.4	3.9	7.0	6.1	4.7	3.4
Cystine[7]	2.5	3.0	2.7	2.9	3.1	3.1
Threonine[7]	5.6	8.3	10.9	10.1	8.8	7.6
Tryptophan[7]	3.66	3.36	2.66	2.22	1.54	2.79

Digestible energy (MJ/Kg)[7]	13.20	15.20	16.20	15.00	14.27	13.73
Net energy (MJ/Kg)[7]	8.59	10.93	12.06	10.94	10.30	9.80
SID lysine[7,8]	6.6	10.7	15.3	14.1	12.0	10.0
Total calcium[7]	7.2	8.3	8.2	7.5	7.4	6.5
Digestible phosphorus[7]	3.5	3.8	4.6	4.2	3.3	2.5

[1]The starter diet was used as solid creep feed. It was also used to prepare the liquid mixture supplemented to piglets pre-weaning.

[2]Premix provided per kilogram of complete diet (Diets 1 and 2): Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 70 mg; Mn from manganese oxide, 62 mg; Zn from zinc oxide, 80 mg, I from calcium iodate, 0.6 mg; Se from sodium selenite, 0.2 mg; vitamin A as retinyl acetate, 3.44 mg; vitamin D3 as cholecalciferol, 25 mg; vitamin E as DL-alpha-tocopheryl acetate, 100 mg; vitamin K, 2 mg; vitamin B12, 15 µg; riboflavin, 5 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 500 mg; Biotin, 200µg; folic acid, 5mg; vitamin B1,2 mg; and vitamin B6, 3 mg.

[3]Premix provided per kilogram of complete diet (Diets 3, 4 and 5): Cu from copper sulphate, 100 mg; Fe from ferrous sulphate monohydrate, 90 mg; Mn from manganese oxide, 47 mg; Zn from zinc oxide, 120 mg; I from potassium iodate, 0.6 mg; Se from sodium selenite, 0.3 mg; vitamin A as retinyl acetate, 2.1 mg; vitamin D3 as cholecalciferol, 25 µg; vitamin E as DL-alpha-tocopheryl acetate, 100 mg; vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B1, 2 mg; and vitamin B6, 3 mg.

[4]Premix provided per kilogram of complete diet (Diet 6): Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; vitamin A as retinyl acetate, 0.7 mg; vitamin D3 as cholecalciferol, 12.5 µg; vitamin E as DL-alpha-tocopheryl acetate, 40 mg; vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B1, 2 mg; vitamin B6, 3 mg.

[5]The diet contained 1000 phytase units (FYT) per kg feed RONOZYME HiPhos GT (DSM, Belfast, UK).

[6]Analysed composition.

[7]Calculated composition.

[8] SID lysine = Standardized ileal digestible lysine

Table 3-2. Effect of dietary treatment on piglet weight and growth during the suckling period.

Treatment[1]	**CONTROL**	**DPS**	**LMR**	**LMR+S**	**SEM**	***P*-value**
Number of sows	20	25	23	23		
Litter weight, kg						
Day 2	22.6	22.3	22.8	22.8	0.38	0.56
Day 7	36.6	35.0	36.0	37.3	1.13	0.48
Day 11	50.4	47.5	49.8	50.7	1.40	0.35
Day 19	79.8	76.0	81.9	81.7	2.09	0.15
Day 27	106.3^{B}	$108.0^{A,B}$	114.2^{A}	$111.8^{A,B}$	2.36	0.08
Overall					1.34	0.25
Mean piglet BW, kg[2]						
Day 2	1.49	1.62	1.61	1.56	0.062	0.40
Day 7	2.54	2.59	2.57	2.58	0.063	0.95
Day 11	3.56	3.53	3.61	3.57	0.063	0.78
Day 19	$5.81^{A,B}$	5.70^{B}	5.91^{A}	5.77^{B}	0.063	0.06
Day 27	7.87^{c}	8.10^{b}	8.27^{a}	$7.99^{b,c}$	0.064	<0.001
Overall					0.056	0.27

Treatment[1]	CONTROL	DPS	LMR	LMR+S	SEM	*P*-value
CV, %[3]						
Day 2	22	21	23	21	1.3	0.37
Day 7	21[b]	24[a,b]	25[a]	21[b]	1.3	0.03
Day 11	21[a,b]	24[a]	24[a]	20[b]	1.3	0.01
Day 19	22[a,b]	25[a]	24[a]	21[b]	1.3	0.02
Day 27	22[a,b]	25[a]	23[a]	20[b]	1.3	<0.01
Overall					0.90	<0.001
ADG, g/pig/day[4]						
Day 2 to 7	187[b]	198[a]	193[a,b]	207[a]	4.8	<0.01
Day 7 to 11	243[a,b]	237[b]	263[a]	251[a]	5.5	<0.001
Day 11 to 19	251[b]	263[b]	280[a]	264[b]	5.4	<0.001
Day 19 to 27	223[c]	276[a]	275[a]	253[b]	5.8	<0.001
Overall					4.4	<0.001
Dry matter disappearance[5]						
Pre-weaning creep feed, kg/litter	-	7.8[a]	6.6[a,b]	4.9[b]	0.74	0.03
Pre-weaning creep feed, g/pig	-	565[a]	471[a,b]	353[b]	51.4	0.03

[1]CONTROL, control without supplementation; DPS, Dry pelleted starter diet; LMR, Liquid milk replacer; LMR+S, mixture of liquid milk replacer and liquid starter diet.

[2]BW, body weight.
[3]CV, coefficient of variation.
[4]ADG, average daily gain.
[5]Dry matter disappearance is not applicable for CONTROL at any time point.
[a, b, c] Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).
[A, B] Values within a row that do not share a common superscript tended to differ ($0.05 < P \leq 0.10$).

Table 3-3. Effect of dietary treatment on health parameters and medicinal treatment of sows and piglets during lactation.

Treatment[1]	**CONTROL**	**DPS**	**LMR**	**LMR+S**	**SEM**	***P*-value**
Number of sows	20	25	23	23		
Diarrhoea incidence in pigs, % (day 2-28)[2]	26.0	28.0	33.9	27.8	4.25	0.59
No. of clinical cases of disease per litter[3]	2.0	1.2	1.7	1.6	0.39	0.55
No. of injections per litter	6.6	3.8	4.5	4.7	1.30	0.50
Antibiotic usage per sow, mL[4]	16.9	9.3	4.2	4.9	4.51	0.20
Antibiotic usage per pig, mL[5]	0.2	0.1	0.2	0.2	0.04	0.46
Anti-inflammatory usage per sow, mL[6]	2.1	1.1	0.5	0.5	0.74	0.43
Anti-inflammatory usage per pig, mL[7]	0.04	0.02	0.02	0.03	0.009	0.65

[1]CONTROL, control without supplementation; DPS, Dry pelleted starter diet; LMR, Liquid milk replacer; LMR+S, mixture of liquid milk replacer and liquid starter diet.
[2]A faecal score of 2 or greater was considered indicative of diarrhoea at each time point from day 2 to 28 of age (weaning). The overall incidence was reported for the pre-weaning period.
[3]Number of piglets per litter treated one or more times up to weaning.
[4]Volume of antibiotic administered per sow between day 2 and weaning on day 28.
[5]Volume of antibiotic administered per piglet per litter between day 2 and weaning on day 28.
[6]Volume of anti-inflammatory administered per sow between day 2 and weaning on day 28.
[7]Volume of anti-inflammatory administered per piglet per litter between day 2 and weaning on day 28.

Table 3-4. Effect of dietary treatment on feed intake and growth of pigs from weaning to slaughter at ~120 kg.

Treatment[1]	**CONTROL**	**DPS**	**LMR**	**LMR+S**	**SEM**	***P*-value**
Number of pens	12	12	12	12		
Days from weaning to slaughter	129	130	129	130	3.8	
BW, kg[2]						
Day 0 (weaning)	8.1	8.1	8.0	8.1	0.31	0.99
Day 6 post-weaning	9.2	9.2	8.9	9.2	0.35	0.98
Day 14 post-weaning	12.2	12.2	11.8	11.9	0.43	0.96
Day 21 post-weaning	15.5	15.6	15.0	15.5	0.60	0.93
Day 28 post-weaning	18.8	19.2	18.5	19.3	0.82	0.73
Day 47 post-weaning	32.1	31.9	31.3	32.7	1.72	0.48
Day of slaughter (~157 days of age)	120.0	119.2	119.0	121.8	2.80	0.42
Overall					0.45	0.38
ADFI, g/pig/day[3]						
Day 0 to 6	210	209	197	213	7.8	0.51
Day 6 to 14	382	410	384	412	17.5	0.48
Day 14 to 21	561	594	559	591	26.7	0.68

Treatment[1]	CONTROL	DPS	LMR	LMR+S	SEM	*P*-value
Day 21 to 28	715	735	679	741	36.1	0.62
Day 28 to 47	1095	1074	1064	1111	47.8	0.90
Day 47 to slaughter	2149	2225	2232	2226	92.5	0.91
Overall	847	871	850	882	31.8	0.87
ADG, g/pig/day[4]						
Day 0 to 6	208[a]	198[a]	159[b]	191[a]	12.2	0.03
Day 6 to 14	370	371	357	343	18.7	0.65
Day 14 to 21	472	482	461	507	27.7	0.68
Day 21 to 28	482	526	489	546	24.2	0.19
Day 28 to 47	699	670	676	703	25.4	0.73
Day 47 to slaughter	1095	1076	1087	1092	17.1	0.84
Overall	554	552	537	663	11.8	0.42
G:F[5], g/g						
Day 0 to 6	1.00[a]	0.94[a]	0.83[b]	0.90[a]	0.035	<0.001
Day 6 to 14	0.98[a]	0.92[a]	0.94[a]	0.83[b]	0.033	<0.001
Day 14 to 21	0.83	0.81	0.83	0.86	0.033	0.60

Treatment[1]	CONTROL	DPS	LMR	LMR+S	SEM	*P*-value
Day 21 to 28	0.69	0.73	0.73	0.74	0.033	0.68
Day 28 to 47	0.64	0.63	0.64	0.63	0.033	0.99
Day 47 to slaughter	0.53	0.49	0.49	0.49	0.033	0.74
Overall	0.78	0.75	0.74	0.74	0.020	0.12

[1]CONTROL, control without supplementation; DPS, Dry pelleted starter diet; LMR, Liquid milk replacer; LMR+S, mixture of liquid milk replacer and liquid starter diet.
[2]BW, body weight.
[3]ADFI, average daily feed intake.
[4]ADG, average daily gain.
[5]G:F, gain to feed ratio.
[a, b, c] Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).

Table 3-5. Effect of dietary treatment on pig carcass parameters following slaughter.

Treatment[1]	**CONTROL**	**DPS**	**LMR**	**LMR+S**	**SEM**	***P*-value**
Number of pens (pigs)	12 (128)	12 (143)	12 (141)	12 (140)		
Cold carcass weight, kg	91.7	91.2	91.7	93.1	0.99	0.51
Fat depth, mm	13.5	14.1	13.6	13.2	0.37	0.46
Muscle depth, mm	53.6	54.2	54.0	53.7	0.87	0.96
Lean meat, %	58.5	58.1	58.4	58.7	0.28	0.55
Kill out, %	76.2	76.7	76.8	76.3	0.35	0.59
Carcass ADG finisher, g/d[2,3]	870	861	868	876	13.4	0.87
Carcass G:F finisher, g/g[4,5]	0.44	0.40	0.40	0.40	0.017	0.15
Lean ADG, g/d[6]	653	646	652	664	12.5	0.78

[1]CONTROL, control without supplementation; DPS, Dry pelleted starter diet; LMR, Liquid milk replacer; LMR+S, mixture of liquid milk replacer and liquid starter diet.
[2]ADG, average daily gain.
[3]Carcass ADG (from day 47 post weaning to slaughter) = [(carcass weight in kg – day 47 weight in kg × 0.65) × 1,000]/number of days from 47 to slaughter (Lawlor and Lynch, 2005).
[4]G:F, gain to feed.
[5]Carcass G:F (from day 47 post-weaning to slaughter) was calculated as follows: carcass G:F = carcass ADG (g)/ daily feed intake (g).
[6]Lean ADG (from birth to slaughter) = (carcass weight × carcass lean meat percentage × 10)/number of days to slaughter (Lawlor and Lynch, 2005).

Table 3-6. Effect of dietary treatment on post-weaning diarrhoea incidence and medicinal treatment of pigs.

Treatment[1]	**CONTROL**	**DPS**	**LMR**	**LMR+S**	**SEM**	***P*-value**
Number of pens	12	12	12	12		
Diarrhoea incidence, % (weaning to 28 days post-weaning)[2]	23	17	19	10	5.4	0.45
Weaner period						
Antibiotic usage per pig, mL[3]	0.09	0.02	0.04	0.06	0.039	0.62
Anti-inflammatory usage per pig, mL[4]	0.05	0.01	0.02	0.03	0.020	0.64
Finisher period						
Antibiotic usage per pig, mL[3]	0.17	0.11	0.33	0.33	0.167	0.71
Anti-inflammatory usage per pig, mL[4]	0.05	0.04	0.11	0.11	0.055	0.68

[1]CONTROL, control without supplementation; DPS, Dry pelleted starter diet; LMR, Liquid milk replacer; LMR+S, mixture of liquid milk replacer and liquid starter diet.
[2]A faecal score of 2 or greater was considered indicative of diarrhoea at each time point from weaning to day 28 post-weaning. The overall incidence was reported for the post-weaning period.
[3]Volume of antibiotic administered to each pig on a pen basis.
[4]Volume of anti-inflammatory administered to each pig on a pen basis.

Table 3-7. Effect of dietary treatment on small intestinal morphology of pigs at day 5 post-weaning.

Treatment[1]	**CONTROL**	**DPS**	**LMR**	**LMR+S**	**SEM**	***P*-value**
Number of pigs	10	10	10	10		
Duodenum						
Villus height (μm)	332	363	393	363	21.8	0.29
Crypt depth (μm)	218	226	254	225	20.7	0.61
VH:CD[2] ratio (μm/μm)	1.70	1.74	1.70	1.77	0.216	0.99
Villus width (μm)	120	127	139	128	5.7	0.15
Jejunum						
Villus height (μm)	286	337	319	300	16.8	0.18
Crypt depth (μm)	203	239	256	221	19.9	0.28
VH:CD ratio (μm/μm)	1.53	1.53	1.29	1.48	0.382	0.97
Villus width (μm)	102	111	114	113	4.6	0.25
Ileum						
Villus height (μm)	242^{B}	284^{A}	248^{B}	283^{A}	14.0	0.07
Crypt depth (μm)	232	203	180	211	15.0	0.13
VH:CD ratio (μm/μm)	1.09	1.47	1.43	1.44	0.368	0.19

Treatment[1]	CONTROL	DPS	LMR	LMR+S	SEM	*P*-value
Villus width (μm)	111	119	115	121	3.8	0.31

[1]CONTROL, control without supplementation; DPS, Dry pelleted starter diet; LMR, Liquid milk replacer; LMR+S, mixture of liquid milk replacer and liquid starter diet.
[2]VH:CD = villus height (μm) / crypt depth (μm).
[A, B] Values within a row that do not share a common superscript tended to differ ($0.05<P\leq0.10$).

3.8 Supplementary tables and figures

Table S 3-1. Quantity of milk replacer powder, water and solid starter diet mixed and fed through the milk feeder system for the liquid mixture of milk replacer and liquid starter diet (LMR+S) experimental treatment.

Experimental period	**From 48 h to day 6**	**From day 7 to day 10**	**From day 11 to day 14**	**From day 15 to day 17**	**From day 18 to day 21**	**From day 22 to day 28**
Ingredients, g						
Milk replacer powder	130.43	104.35	78.26	52.17	26.09	0
Water	869.57	862.32	855.07	847.82	840.58	833.33
Starter diet	0	33.33	66.67	100	133.34	166.67

Table S 3-2. pH evolution in the liquid milk replacer (LMR) tank and the liquid mixture of milk replacer and starter diet (LMR+S) tank immediately after feed preparation and just before the last feed of the day.

Experimental day pre-weaning (piglets' birth = day 0)	Day 4	Day 8	Day 12	Day 15	Day 18	Day 25	Mean	SD
Liquid milk replacer (LMR) tank								
Morning pH, after feed preparation (0900 h)	6.12	5.99	6.15	6.13	6.06	6.01	6.08	0.067
Afternoon pH, before the last feed (1530 h)	4.87	4.20	4.25	4.18	4.39	4.23	4.35	0.264
Liquid mixture of milk replacer and starter diet (LMR+S) tank								
Morning pH, after feed preparation (0900 h)	NA[1]	5.12	5.40	5.62	5.64	5.23	5.40	0.230
Afternoon pH, before the last feed (1530 h)	NA	3.95	4.00	4.15	4.22	4.23	4.11	0.128

[1]NA, not applicable, as at day 4 the liquid milk replacer provided in the LMR and LMR+S treatments was prepared from one mixing tank.

Table S 3-3. Effect of pre-weaning creep feed treatment on sow body weight and back-fat depth.

Treatment[1]	**CONTROL**	**DPS**	**LMR**	**LMR+S**	**SEM**	***P*-value**
Number of sows	20	25	23	23		
Lactation length, days	28.4	28.0	28.1	28.0		
BW, kg[2]						
Day 110 of gestation	274	274	274	274	2.4	0.99
Farrowing[3]	239	238	232	235	2.4	0.11
Weaning[4]	240	240	236	238	2.4	0.51
Service[4]	229	230	226	228	2.6	0.68
Overall					1.8	0.38
BF, mm[5]						
Day 110 of gestation	16.8	16.7	16.5	16.3	0.35	0.69
Weaning	13.0	13.0	13.4	13.5	0.35	0.58
Service	12.9	12.6	13.0	13.3	0.37	0.41
Overall					0.26	0.78
Sow BW change, kg						
Day 110 to weaning[6]	-33.2	-34.1	-37.1	-36.4	2.98	0.70
Farrowing to weaning[7]	1.8	2.0	5.3	2.7	2.98	0.78

Treatment[1]	CONTROL	DPS	LMR	LMR+S	SEM	*P*-value
Weaning to service[8]	-7.7	-7.6	-7.1	-8.4	3.29	0.99
Overall					2.14	0.96
Sow BF change, mm						
Day 110 to weaning[9]	-3.7	-3.8	-3.1	-2.9	0.37	0.22
Weaning to service[10]	0.0	-0.3	-0.3	-0.4	0.40	0.87
Overall					0.29	0.70

[1]CONTROL, control without supplementation; DPS, Dry pelleted starter diet; LMR, Liquid milk replacer; LMR+S, mixture of liquid milk replacer and liquid starter diet.
[2]BW, body weight.
[3]Estimated empty farrowing weight = (sow weight at day 110 – (total born × 2.25)). The value of 2.25 kg is an estimate of the increased weight in the gravid uterus and in mammary tissue attributed to each pig in a litter (NRC, 1998).
[4]Weaning = day 28 ± 1 of lactation; service = day 4 ± 1 post-weaning.
[5]BF, back fat.
[6]Sow BW change = (sow BW at weaning – sow BW at day 110 of gestation).
[7]Sow BW change = (sow BW at weaning – sow BW at farrowing).
[8]Sow BW change = (sow BW at service – sow BW at weaning).
[9]Sow BF change = (sow BF at weaning – sow BF at day 110 of gestation).
[10]Sow BF change = (sow BF at service – sow BF at weaning).

Table S 3-4. Effect of treatment on sow litter-size and the number of piglets fostered and died per litter during lactation.

Treatment[1]	CONTROL	DPS	LMR	LMR+S	SEM	*P*-value
Number of sows	20	25	23	23		
Litter						
Total born[2]	15.6^{B}	16.1^{B}	18.8^{A}	$17.4^{A,B}$	0.92	0.06
Live born	13.8^{b}	$15.2^{a,b}$	17.8^{a}	16.9^{a}	0.90	0.01
Litter size at 48 h	14.5	14.5	14.9	14.7	0.20	0.40
Litter size weaning	13.3	13.4	13.9	14.0	0.31	0.29
Deaths and removals per sow						
Cross fostered from 24 h to 48 h[3]	2.2^{A}	$0.6^{A,B}$	-0.9^{B}	-0.5^{B}	0.83	0.05
Deaths total	2.7	2.5	2.9	2.4	0.45	0.86
Deaths after 48 h	1.2	1.2	0.8	0.8	0.23	0.36

[1]CONTROL, control without supplementation; DPS, Dry pelleted starter diet; LMR, Liquid milk replacer; LMR+S, mixture of liquid milk replacer and liquid starter diet.
[2]Total number born = number of piglets born alive, stillborn, and mummified.
[3] Cross fostered from 24 h to 48 h = piglets cross fostered on – piglets cross fostered off; a minus indicates that more were fostered off than fostered on to the sow.
[A, B] Values within a row that do not share a common superscript tended to differ ($0.05<P\leq0.10$).

Table S 3-5. Effect of treatment on percentage of "eaters" within a litter where two or more feeder-directed activities per pig are considered to indicate an "eater".

Treatment[1]	**CONTROL**	**DPS**	**LMR**	**LMR+S**	**SEM**	***P*-value**
Eaters, %[2]						
Day 12 of lactation	-	3	10	13	5.7	0.39
Day 18 of lactation	-	48[a]	18[b]	19[b]	5.7	<0.001
Day 22 of lactation	-	76[a]	17[c]	35[b]	5.7	<0.001
Day 26 of lactation	-	78[a]	24[b]	30[b]	5.7	<0.001
Overall	-	51[a]	17[c]	24[b]	3.3	<0.001

[1]CONTROL, control without supplementation; DPS, Dry pelleted starter diet; LMR, Liquid milk replacer; LMR+S, mixture of liquid milk replacer and liquid starter diet.
[2]The percentage of piglet eaters per pen was calculated on a pen basis for each observation day and for all observation days combined. This was carried out by dividing the number of piglets considered as eaters (having two or more feeder-directed activities) by the total number of piglets in the pen, then multiplying the results by 100 to express as a percentage. The percentage of observations of piglets seen engaging in trough-directed activity is not applicable for CONTROL at any time point.
[a, b, c] Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).

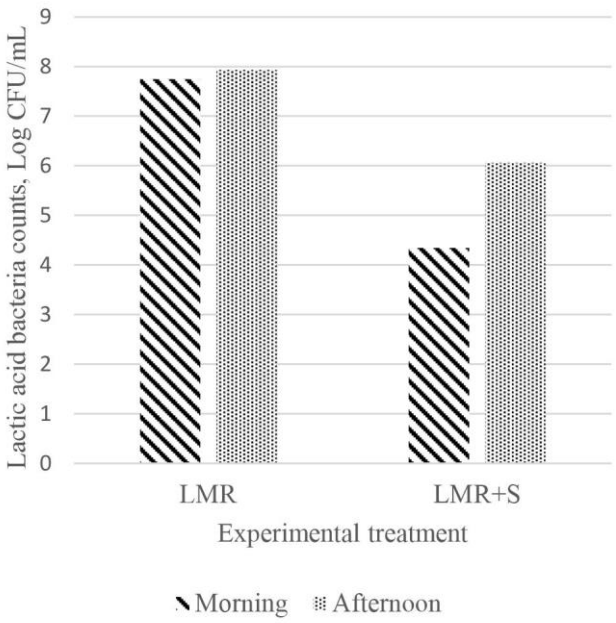

Figure S 3-1. Lactic acid bacteria counts (Log CFU/ml) in the liquid milk replacer (LMR) and the liquid mixture of milk replacer and starter diet (LMR+S) immediately after preparation in the morning (at 0900 h) and just before the last feed in the afternoon (at 1530 h). Counts were taken at day 15 pre-weaning.

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4. Effect of feeding L-glutamine- and enzyme-supplemented liquid feed to suckling piglets on growth, health and intestinal structure

E.A. Arnaud, G.E. Gardiner, J.V. O' Doherty, T. Sweeney, P.G. Lawlor (2024). Effect of feeding L-Glutamine- and enzyme-supplemented liquid feed to suckling piglets on growth, health and intestinal structure. Intended for submission to *Journal of Animal Science*.

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4.1 Abstract

The provision of liquid creep feed to suckling pigs has been shown to increase dry matter intake compared to dry creep feeding. The increased feed intake associated with liquid feeding makes it attractive as a means of delivering feed additives to suckling pigs to optimise growth and health. The objective of this study was to determine the effect of L-glutamine and enzyme supplementation of liquid creep feed to suckling pigs on their growth up to target slaughter weight (~120 kg), intestinal health and structure. Sixty sows and their litters were blocked on parity, previous number of piglets weaned and weight at day 107 of gestation, and randomly assigned to one of 3 dietary treatments: 1) liquid starter diet (control); 2) control diet supplemented with 10 g of L-glutamine per kg of starter diet (glutamine) and 3) control diet supplemented with a cocktail of enzymes (lipase, protease and α-amylase included at 160 Lipase units, 30000 New Feed Protein units and 67.5 Kilo Novozymes units, respectively per kg of starter diet). Dietary treatments were fed from day 8 of age to weaning at day 28. Pig weight and dry matter disappearance (DMd) were recorded during lactation and post-weaning until pigs reached target slaughter weight (~120 kg). At target slaughter weight, carcass weight and quality were recorded. Medication (antibiotic and anti-inflammatory) usage per pig on a litter basis, and the number of injections and clinical cases of disease per litter were recorded from birth to slaughter. At day 5 post-weaning, a subset of pigs (n=30) were sacrificed and intestinal samples were collected for histological analysis. The DMd of creep feed did not differ between treatments (P>0.05). From day 14 to 21 of lactation, glutamine decreased piglet average daily gain (ADG) compared to the control (P<0.05). Glutamine tended to reduced piglet body weight (BW) at day 21 (P=0.09) and 28 (P=0.08) of lactation. Glutamine tended to improve the average daily gain of pigs from day 21 to 28 post-weaning (P=0.07). The volume of antibiotics or anti-inflammatories administered to piglets or sows was not affected by treatment either pre- or post-weaning (P>0.05). However, glutamine tended to increase diarrhoea prevalence between day 8 and 27 of lactation compared to the control (P=0.09). In conclusion, supplementing liquid creep feed with 10kg of glutamine/ tonne of feed tended to reduce pre-weaning growth and to increase diarrhoea prevalence in piglets. Additionally, supplementing liquid creep feed with enzymes had no effect on growth or medication usage in pigs.

4.2 Introduction

Weaning is a challenging event for piglets. They are moved to an unfamiliar environment and the diet changes abruptly from sows' milk, a highly digestible diet in liquid form, to a dry solid diet mainly of vegetable origin. This results in delayed and reduced feed intake and growth, and sometimes diarrhoea in piglets during the early post-weaning period (Collins *et al.*, 2017; Hampson and Smith, 1986; Wolter and Ellis, 2001). Providing dry supplementary feed (i.e. creep feed) to suckling piglets is a good strategy to increase pre-weaning growth and weaning weight. Furthermore, it can familiarise suckling pigs with feed prior to weaning, thereby reducing latency to first feed after weaning and hence increasing post-weaning feed intake and growth (Muns and Magowan, 2018; Pluske *et al.*, 2007). However, dry creep feed consumption varies greatly within and between litters and the response to creep feeding is particularly influenced by the proportion of 'eaters' and 'non-eaters' of creep-feed within the litter (Bruininx *et al.*, 2002; Sulabo *et al.*, 2010). Studies have shown that supplementing pigs with creep feed in liquid form has the potential to increase pre-weaning creep feed intake compared to dry creep feeding (Byrgesen *et al.*, 2021; Martins *et al.*, 2020; Vasa *et al.*, 2023). The high feed intake with liquid creep feeding also means that it could be an efficient means of delivering non-antibiotic feed additives to suckling pigs to ensure optimal growth and health.

At weaning, transition from maternal milk to solid feed leads to a remodelling of the gastrointestinal tract. This includes a switch in enzyme production; for example for the intestinal brush-border disaccharidases this means a switch from lactase to sucrase and maltase (Cunha, 1977). Enzymes with proteolytic activity are also found in low concentrations during the suckling period (Pierzynowski *et al.*, 1990; Pierzynowski *et al.*, 1993). Previous studies suggest that intestinal tract remodelling can be accelerated when an exogenous enzyme blend (amylase, protease and lipase) is fed to suckling mammals (Prykhodko *et al.*, 2015; Prykhodko *et al.*, 2016; Słupecka *et al.*, 2012). In addition, it was demonstrated that supplementing suckling pigs with a complex of microbially-derived amylase, protease and lipase can benefit pig growth and feed efficiency during the grow–finishing period (Prykhodko *et al.*, 2016).

The reduced energy and nutrient intake experienced by pigs early post-weaning often leads to shortening of villus height and increased crypt depth in the small intestine (Miller *et al.*, 1986). This reduces the surface area of the small intestine and the

number of mature enterocytes, thereby limiting nutrient absorption (van Beers-Schreurs *et al.*, 1992). Glutamine is a major fuel for enterocytes in the small intestine and can be metabolised into purines and pyrimidines for the synthesis of nucleotides to support cell proliferation (Watford, 2015). It was previously demonstrated that inclusion of L-glutamine at 1% (i.e. 10 g/kg) in dry creep feed can help to maintain intestinal structure and improve post-weaning feed efficiency (Cabrera *et al.*, 2013). Therefore, supplementing suckling pigs with L-glutamine or a cocktail of enzymes could help to maintain intestinal structure and to hasten relevant enzyme secretory capacity at weaning. The objective of this study was to determine the effect of feeding liquid starter diets with or without a supplementation with L-glutamine or a cocktail of enzymes to suckling pigs from day 8 of age on piglet growth and medicinal usage to weaning. Furthermore, the effect of dietary treatment on post-weaning growth, intestinal structure and medicinal usage to target slaughter weight was also determined.

4.3 Materials and methods

4.3.1 Ethical approval

This study was performed between June 2023 and January 2024, at the Teagasc Pig Development Department, Moorepark, Fermoy, Co. Cork, Ireland. Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. TAEC2020-274) and South East Technological University Ethics Committee (approval no. WIT2021REC011). The project was authorised by the Irish Health Products Regulatory Authority (project authorisation no. AE19132/P129). The experiment was conducted in accordance with the legislation for commercial pig production set out in the European Communities (Welfare of Farmed Animals) Regulations 2010 and in Irish legislation (SI no. 311/2010).

4.3.2 Experimental design and animal housing

Sixty sows (Large White × Landrace; PIC®, Hermitage Genetics, Sion Road, Co. Kilkenny, Ireland) were used in this study, which was conducted over three batches

(with a batch being a group of sows inseminated on the same week). Sows were artificially inseminated at onset of standing oestrus and again 24 h later using pooled semen (Topigs Norsvin Tempo, Premier Pig Genetics Limited, Ireland). Gestating sows were housed in dynamic groups of ~120 animals. Sows were introduced to the dynamic group between 3 and 6 days after service and fed from electronic sow feeders [Schauer Feeding System (Competent 6), Prambachkirchen, Austria]. On day 107 of gestation, sows were blocked within farrowing batch into 20 blocks on the basis of parity group (mean ± SD; 2.5 ± 0.91), number of pigs weaned/sow in the previous cycle (13.7 ± 1.58 for multiparous sows) and body weight (BW) (283 ± 31.7 kg). Sow parity group distribution was as follows: group one, parity 0 (25%); group two, parity 1 to 2 (7%); group three, parity 3 to 5 (65%); and group four, parity 6 to 8 (3%). Within block, sows and suckling litters were randomly assigned to the following pre-weaning dietary treatments: 1) liquid starter diet provided as creep feed from day 8 of age to weaning (control), 2) control supplemented with 10 g of L-glutamine per kg (glutamine) and 3) control supplemented with a cocktail of enzymes (lipase, protease and α-amylase) (enzymes).

Prior to the housing of the sows in farrowing accommodation, each farrowing room was cleaned, disinfected, and allowed sufficient time to dry according to standard practices in the facility. At ~ day 108 of gestation, sows were moved into standard farrowing crates in pens (2.5 m x 1.8 m) with cast-iron slats under the sow and plastic slats with a water-heated floor pad for the piglets (BigDutchman; Vechta, Germany). Farrowing room temperature was maintained at 24 ± 3.0 °C at the time of farrowing and gradually reduced to 21 °C by day 7 of lactation. The temperature of the heat pads was 38-40 °C for the first 2 days after farrowing and was reduced by ~1 °C each day to 30 °C at 10 days after farrowing and it was maintained at this temperature until weaning. Artificial lighting was provided daily from 0800 h to 1630 h. The average number of piglets born alive was 15.7 ± 3.40 piglets. Where possible, litter size was standardized between 24 h and 48 h after parturition. The final number of piglets remaining on each sow at 48 h postpartum was affected by the rearing capacity of each sow (i.e. the number of available functional teats) and the availability of foster sows to take surplus piglets. Piglets' teeth were clipped within 24 h of birth. On day 5 postpartum, tails were docked and all piglets were injected with 1 mL of iron

(Gleptosil, Ceva Santé Animale, Libourne, France). Male pigs remained fully intact and piglets were weaned at day 28 ± 1.0 of lactation.

To study the residual effect of the pre-weaning dietary treatment in progeny, a subsample of 360 pigs (8.5 ± 1.40 kg) was selected at weaning. Within sow treatment groups, pens of 10 pigs of the same sex (entire male or female) of even weight were formed and blocked by sow treatment, sex and BW. Pen groups for control (n=12), glutamine (n=12) and enzymes (n=12) were moved to weaner accommodation at weaning. Pig BW, feed disappearance and health were monitored up to target slaughter weight (~120 kg). Weaner pens were equipped with fully slatted plastic floors (2.5 × 2 m) with automatic environmental control. Each pen had a shelf-type single-space (33 cm) wet-dry feeder (BA19100, Verba, Verbakel, The Netherlands) with inset nipple drinker and a supplementary bowl drinker (SS Drinker, Rotecna, Lleida, Spain). A spiked rubber ball (Easyfix Luna 142, Easyfix, Galway, Ireland) was provided for each pen as environmental enrichment. Temperature in the weaner rooms was maintained at 28 °C during the first week after weaning and reduced by 2 °C each week to 22 °C at the end of 4 weeks. Ventilation was from a punched ceiling with air exhausted via a variable speed fan linked to a thermostat which was controlled by computer (Big Dutchman 135). At day 43 post-weaning, pen groups were moved to finisher accommodation. Finisher pens had fully slatted concrete floors (2.4 × 4.2 m) with automatic environmental control. Each pen had one shelf-type single-space (33 cm) wet-dry feeder (MA19100, Verba) with inset nipple drinker and a supplementary bowl drinker (SS Drinker, Rotecna). A wooden (larch) post was provided for each pen as environmental enrichment. All rooms were equipped with windows for natural light. Temperature in the finisher rooms was maintained at 20 to 22 °C with the same type of ventilation system used as in the weaner house. Pigs in each treatment group were slaughtered over 2 weeks when they reached the target slaughter weight of ~120 kg live weight (LW; average age at slaughter 158 days). The heaviest pigs in each pen group were slaughtered during the first week and the remaining pigs in the pen were slaughtered 7 days later.

4.3.3 Diet preparation and feeding

Diets were formulated to meet or exceed National Research Council (NRC, 2012) recommendations. Diet samples were analysed for dry matter (DM) (oven drying), ash (furnace drying and gravimetry), crude protein (Dumas method), total fat (Weibul acid hydrolysis) and crude fibre (Ankom 200 fibre analyser, Macedon, New York, United States) by Sciantec Analytical Services Ltd, Selby, United Kingdom according to European Union Commission Regulation No 152/2009 of 27 January 2009 (European Commission, 2009). The ingredient composition and chemical composition of the diets are shown in Table 4-1. During gestation, sows were fed a gestation diet (Diet 1) in meal form at a feed allowance of 2.2 kg/day between day 0 and 90 of gestation. From day 90 of gestation to parturition, gestation feed allowance was increased to 2.7 kg/day. In the farrowing room, sows were fed a lactation diet (Diet 2; Table 4-1) in meal form using a computerized feed delivery system (DryExact Pro, Big Dutchman). Sows were fed twice daily from farrowing to day 6 of lactation and three times daily from day 7 to weaning at 28 days. Sows were fed according to a lactation feeding curve which started at 60 MJ digestible energy (DE)/d at day 0 of lactation and gradually increased to 107, 125, 133, and 137 MJ DE/d at days 7, 14, 21, and 26 of lactation, respectively. During lactation, feed allocation for individual sows was adjusted up and down from the curve, as necessary, to ensure that sow feed intake was as close as possible to ad libitum feed allowance and to prevent feed wastage. Between weaning and service, sows were provided with ad libitum access to the lactation diet (Diet 2, Table 4-1) for 4 days followed by the gestation diet (Diet 1; Table 4-1) in meal form. Water was provided on an ad libitum basis to sows from a single-bite drinker in the feed trough and to suckling piglets from a bowl (Big Dutchman) in the farrowing pen.

Each of the starter diets (Diet 3, Table 4-1) was provided as a liquid creep feed to suckling piglets from day 8 to 28 of age. In the liquid creep feed supplemented with L-glutamine, L-glutamine (Ajinomoto, Tokyo, Japan) was included in the starter diet (Diet 3; Table 4-1) at 10 g/kg of diet. In the liquid creep feed supplemented with the enzyme cocktail, the enzyme cocktail containing lipase (Capalase Micro R800, DSM-Firmenich, Heerlen, The Netherlands), protease (ProAct 360, DSM-Firmenich) and α-amylase (RONOZYME HiStarch, DSM-Firmenich) was included in the starter diet (Diet 3, Table 4-1) at 0.725 g/kg of diet so that the finished diet contained 160 lipase

units (PLI)/kg, 30,000 new feed protease units (NFP)/kg and 67.5 Kilo Novo (α-amylase) units (KNU)/kg, respectively. Recovery tests for free L-glutamine, free glutamic acid total glutamic acid protease and α-amylase were conducted on the dry starter diet to confirm correct inclusion of these additives in the feed (see Table S 4-1). Additionally, recovery tests were conducted on the liquid starter diet samples prepared by mixing dry pelleted starter diet with warm water (55 ˚C) at a ratio of 1:5 and incubated for 5 hours at 30°C. Before conducting these analyses, the liquid starter diet was oven dried at 55 ˚C for 3 consecutive days. Free L-glutamine, free glutamic acid, total glutamic acid were analysed according to the International Organization for Standardization (ISO) NF EN ISO13903:2005 standard (International Organization for Standardization, 2005). The enzyme activities were determined using DSM-Firmenich proprietary procedures, with specific assay conditions (pH, temperature, buffers) adapted for the determination of DSM-Firmenich product activities.

The liquid starter diet provided as creep feed was prepared by mixing dry pelleted starter diet with warm water (55 ˚C) at a ratio of 1:5 and provided through an automatic feeding system (Babyfeed, Schauer Agrotronic GmbH, Prambachkirchen, Austria). The liquid feeding trough was positioned to one side of the sow's head at the front of the farrowing pen. Fresh feed was prepared twice daily at 0835 h and at 1645 h. The starter diet was mixed with water at 55 ˚C for ~10 minutes. Ten feeding cycles (each lasting ~ 2 h) were programmed between 0930 h and 0400 h. During each cycle the in-situ trough sensors checked the amount of liquid feed present in the trough 5 times. Whenever the feed level was below the level of the sensor, the trough was detected as empty, and the liquid starter diets were delivered to the trough and the amount delivered to the trough was recorded in the system computer at each re-fill. Therefore, each pen could potentially have been supplied with the liquid starter diets up to 50 times in a 24 h period. Each day after the last feeding, the system was cleaned in closed circuit, which included mixing tanks and all of the pipelines, with a 1% acid solution (Deosan Acidbrite AG313, Diversey Europe Operations BV, Utrecht, The Netherlands). In addition, the system was cleaned once a week with a 0.5% solution of an alkaline detergent (AvalKsan Gold Standard CF, Carbon Group, Ringaskiddy, Ireland), to help remove lime scale from the circuit. The troughs were cleaned each morning with air pressure and rinsed with acidified water and a 0.5% solution of the alkaline detergent was applied to troughs, as for the acid rinse, once weekly.

Following weaning, pigs were fed a sequence of diets in accordance with their growth stage. Starter diet (Diet 3; Table 4-1) was provided from weaning to day 6 post-weaning, link diet (Diet 4; Table 4-1) from day 6 to 17 post-weaning, weaner diet (Diet 5; Table 4-1) from day 17 to 43 post-weaning, and a finisher diet (Diet 6; Table 4-1) from day 43 post-weaning to slaughter (~ day 132 post-weaning). All diets for post-weaning pigs were provided in dry pelleted (3 mm diameter) form and access was on an ad libitum basis. Pigs were inspected daily and any pig demonstrating visual signs of illness were treated appropriately. Assessment of clinical signs of disease and treatment protocols were followed in accordance with farm protocol. All veterinary treatments were recorded including antibiotic and anti-inflammatory treatments.

4.3.4 Data recording and sampling

4.3.4.1 Sow body weight and back fat thickness

Sow BW and back fat (BF) were recorded on day 110 of gestation, at weaning, and at their subsequent service (~day 4 post-weaning). Sow BW was recorded using an electronic sow scales (EziWeigh 7i, O'Donovan Engineering, Co. Cork, Ireland). Empty farrowing weight was calculated using the following equation from the NRC (1998)[1]:

$$^{1}SW_{farr} = [SW_{d110} - (NB \times 2.25)].$$

Where, SW_{farr} = Empty sow farrowing weight, SW_{d110} = Sow weight at day 110 of gestation, NB = total number of piglets born. The 2.25 kg is an estimate of the increased weight in the gravid uterus and in mammary tissue attributed to each pig in a litter (NRC, 1998).

Back fat was measured using a digital BF indicator (Renco LEANMEATER, Renco Corporation, Golden Valley, MN) by placing the probe of the digital indicator on the back of the sow at the level of the last rib, 6 cm to the side of the backbone. A reading was taken from the right and left side of the sow and the average reading was recorded.

4.3.4.2 Farrowing performance and pre-weaning piglet growth performance

The individual weight and sex of each piglet was recorded at birth, when each piglet was tagged for identification purposes, on day 8, 14, 21 and 27 postpartum using an electronic piglet scale (Defender 3000 XtremeW, O'Donovan Engineering). These data were used to determine piglet pre-weaning average daily gain (ADG). The creep feed dry matter disappearance (DMd) was recorded daily.

4.3.4.3 Live observation of trough-directed behaviour per litter

The feeding behaviour of individual piglets within pen groups was observed in batch 1 of the experiment using instantaneous scan sampling at day 13, 16 and 22 after birth. To enable the easy identification of piglets during scan sampling, piglets in each litter were marked with a number from 1 to 17 (linked to their tag number) using black hair dye (Pro Color Plus, Healthpoint, Blackpool, United Kingdom) on the day before scan sampling was conducted. Six 1-hour sessions were conducted between 0900 h and 1600 h on each scan sampling day. During each 1-hour session of live observations, each pen was scanned (i.e. the behaviour of the group was recorded) every 3 minutes, leading to 21 scans/pen/session. A simple ethogram was used for scoring feeding behaviour. At every scan, liquid trough-directed activity was recorded. Trough-directed activity was defined as when a piglet snout was immersed in the liquid feed for at least 2 seconds. Piglets were categorized as eaters using two different thresholds 1) if they were engaged in two or more trough-directed activities at any time during an observation day or 2) if they were engaged in one or more trough-directed activities at any time during an observation day. The percentage of piglet eaters per pen was calculated on a pen basis for each observation day and for all observation days combined. This was carried out by expressing the number of piglets considered as eaters in a pen as a percentage of the total number of piglets present in the pen.

4.3.4.4 Post-weaning pig growth performance and carcass data

Pen groups were weighed on day 0 (weaning), 6, 14, 21, 28, 43 post-weaning and individual pig weights were recorded just prior to slaughter (at ~day 132 post-weaning) using an electronic scale (EziWeigh 7i, O'Donovan Engineering). Pigs were

fasted prior to recording BW before slaughter. Feed disappearance was recorded on a pen basis between weaning and slaughter for the periods for which BWs were recorded. These data were used to determine the average daily feed intake (ADFI), ADG, and feed conversion ratio (FCR).

At day 132 post-weaning, pigs were transported 95 km to the abattoir (Dawn Pork & Bacon, Grannagh, Co. Waterford, Ireland) where they were killed by exsanguination after CO_2 stunning. At the abattoir, carcass cold weight of individual pigs was calculated by multiplying the hot carcass weight, recorded within 45 min of the pig being exsanguinated, by 0.98. Muscle depth and BF measured at 6 cm from the edge of the split back at the level of the third and fourth last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean meat content was calculated according to the following formula(Department of Agriculture and Food and Rural Development, 2001):

$$CL=60.3 - 0.847x + 0.147y$$

Where, CL= carcass lean meat percentage, x = fat depth (mm); y = muscle depth (mm).

The following equations were used to determine parameters of interest relating to carcass growth (Lawlor and Lynch, 2005):

$$C_{ADG} = [(CW - WW \times 0.55) \times 1{,}000] / D1$$

$$C_{FCR} = FI / C_{ADG}$$

$$L_{ADG} = (CW \times CL \times 10) / D2$$

Where, C_{ADG} = carcass ADG (from weaning to slaughter), CW = carcass weight in kg, WW = weaning weight in kg, D1 = number of days from weaning to slaughter, C_{FCR} = Carcass FCR, FI = daily feed intake (g), L_{ADG} = Lean ADG (from birth to slaughter), CL = carcass lean meat percentage and D2 = number of days from birth to slaughter.

4.3.4.5 Faecal scoring and medication usage

Faecal consistency scores on a pen basis were determined weekly at day 8, 14, 21 and 27 before weaning and on days 1, 5, 14, 21 and 28 post-weaning. A 4-point scoring system (Casey et al., 2007) was used and the average score from five pigs was determined as the average score for each litter/pen. In brief: 0 = normal (dry pelleted

faeces), 1 = soft (soft with shape), 2 = mild diarrhoea (very soft or viscous liquid) and 3 = severe watery diarrhoea (watery or with blood). The diarrhoea prevalence at each time-point was determined by considering a faecal score of 2 or greater as indicative of diarrhoea for each litter/pen.

Antibiotic and anti-inflammatory usage was recorded in sows during lactation and in pigs from day 8 of age until they reached their target slaughter weight [separately for the pre-weaning (day 8 to 28), weaner (day 0 to 43 post-weaning) and finisher (day 44 to 158 post-weaning) periods]. Medication was administered when joint-ill, lameness, malaise or diarrhoea were observed by trained farm technicians in piglets and when malaise or vaginal discharge was observed in sows. One antibiotic (Unicillin; Univet Ltd, Cootehill, Co. Cavan, Ireland) and one anti-inflammatory (Loxicom, Norbrook, Newry, United Kingdom) only, were used during this experiment. Animal ID, pen number, product name, product code, dose administered (ml), frequency of administration, date of administration, and reason for use were recorded when an animal was treated. From this, the total number of piglet injections per litter/pen, the average volume of medication (antibiotic / anti-inflammatory) administered per pig on a litter basis and per sow, and the total number of clinical cases of disease (i.e. when an animal was treated one or more times for the same reason) per litter were calculated pre-weaning. The average volume of medication (antibiotic and anti-inflammatory) administered per pig per pen and the total number of clinical cases of disease were also calculated between weaning and target slaughter weight.

4.3.4.6 Euthanasia, blood and tissue sampling

On day 5 post-weaning, thirty pigs (10 female piglets per treatment of even weight), were euthanized using captive bolt followed by immediate exsanguination. Blood was collected into Ethylene Diamine Tetra Acetic acid-coated 10 mL tubes (Becton Dickinson, New Jersey, United States) and stored at room temperature for 7 to 9 h until analysis. After euthanasia, the intestinal tract was removed and whole tissue samples (~2 cm) were collected from the duodenum (15 cm distal to the pyloric junction), jejunum (1.5 m distal to the pyloric junction) and ileum (15 cm proximal to the ileo-caecal junction). The whole tissue samples were carefully immersed in a 50

mL tube containing NOTOXhisto™ fixative (Scientific Device Laboratory, Des Plaines, United States). The tubes was placed on a shaker at room temperature for 48 hours after collection and stored at room temperature until analysis.

4.3.5 Laboratory analyses

4.3.5.1 Haematology analyses

Haematological analysis of the whole blood samples collected from euthanised piglets was performed using a Mythic 5 Vet Pro analyser (Cormay diagnostics, Warsaw, Poland). The following parameters were measured: leukocyte count, lymphocyte count and percentage, monocyte count and percentage, neutrophil count and percentage, eosinophil count and percentage, basophil count and percentage, erythrocyte count, haemoglobin concentration, mean corpuscular volume (fL), mean corpuscular haemoglobin (pg/cell), mean corpuscular haemoglobin (g/dL) and platelet count.

4.3.5.2 Small intestinal histology

The whole tissue samples from the duodenum, jejunum and ileum were sent to Nationwide Laboratories, Devon, UK for histological slide preparation with haemotoxylin-eosin staining to study gross morphological parameters of intestinal structure. For each sample, the villus height (VH) and crypt depth (CD) were measured, as described previously by Crespo-Piazuelo et al. (2022) and the ratio of VH to CD was calculated.

4.3.6 Statistical analysis

All data, except for the prevalence of diarrhoea, were analysed using the PROCMIXED procedure in the Statistical Analysis Systems (SAS) software package version 9.4 (SAS Institute Inc., Cary, North Carolina, United States). The prevalence of diarrhoea per litter in the farrowing accommodation from day 8 to 28 and in the

weaner accommodation per pen from weaning to day 28 post-weaning was analysed using the PROC Glimmix procedure of SAS with a binomial distribution.

For the analysis of pre-weaning litter weight, piglet BW, sow BW and sow BF, the percentage of piglets classified as "eaters", the total number of piglet injections per litter, the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a litter basis and per sow, the total number of clinical cases of disease per litter or per pen, number of deaths and removals per litter, post-weaning growth parameters, carcass quality data and the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a pen basis post-weaning and diarrhoea prevalence; treatment was included in the model as a fixed effect.

For analysis of pre-weaning piglet growth parameters, piglet weight and litter size at day 8 were included as co-variates, when significant in the model. For analysis of sow BW and BF, initial value at day 108 of gestation was included as a covariate in the model.

For the analysis of post-weaning growth and carcass quality parameters, weaning weight was included as a co-variate. For BW at slaughter and cold carcass weight, the number of days from weaning to slaughter was included as a co-variate. Day was included in the above models as a repeated variable when relevant and block was included as a random effect. The litter/sow was the experimental unit for the analysis of all pre-weaning parameters, except piglet weight and growth, where pig nested within sow/litter was the experimental unit. The experimental unit post-weaning was the pen group.

For analysis of intestinal morphology and blood parameters, treatment was included in the model as a fixed effect. Sow was included as a random effect and the pig was the experimental unit.

In all cases, differences in least square means were investigated using the t test after Tukey adjustment for multiple comparisons. Results are presented in the text and tables as the least square means together with their pooled standard error. Differences between treatments were considered significant when $P \leq 0{\cdot}05$, whereas $0.05 < P \leq 0.10$ was considered as a tendency.

4.4 Results

4.4.1 Sow body weight and back fat thickness

The effect of treatment on sow BW and BF depth from farrowing to service (~4 days after weaning) is presented in Table S 4-2. There was no effect of treatment (P>0.05) on any parameter of interest at any time point.

4.4.2 Mortality and removals

The effect of treatment on sow litter-size at day 8 and weaning and the number of piglet deaths per litter from day 8 to weaning is presented in Table S 4-3. There was no effect of treatment on any parameter of interest (P>0.05).

4.4.3 Pre-weaning piglet growth performance

The effect of treatment on piglet creep feed DMd, pig weight and growth during the suckling period is presented in Table 4-2. There was no treatment effect on pre-weaning DMd of creep feed per pig or on litter weight at any time point from day 8 to weaning at day 28 (P>0.05). There was no effect of treatment on mean piglet BW at day 8, 14 and overall (P>0.05) during lactation. L-glutamine supplementation tended to decrease mean piglet BW at day 21 (P=0.09) and day 28 (P=0.08). There was no effect of treatment on ADG from day 8 to 14, day 21 to 27 and overall during lactation (P>0.05). Piglets supplemented with L-glutamine had a lower ADG from day 14 to 21 than control piglets and piglets supplemented with enzymes (P<0.01).

4.4.4 Live observation of trough-directed feeding behaviour per litter

The proportion of piglets having two or more feeder-directed activities on each day of live observation and overall within a litter is presented in Table S 4-4. There was no effect of treatment on the percentage of eaters at day 13, 16 and 22. Overall, piglets supplemented with enzymes tended to have a higher percentage of eaters than control piglets and piglets supplemented with L-glutamine (P=0.07). The proportion of piglets

having one or more feeder-directed activities on each day of live observation and overall within a litter is presented in Table S 4-5. At day 16 of lactation and overall, piglets supplemented with enzymes tended to have a higher percentage of eaters than control piglets and piglets supplemented with L-glutamine (P=0.09 and P=0.06, respectively).

4.4.5 Pre-weaning diarrhoea scores, clinical cases of disease and antibiotic and anti-inflammatory treatment

The effect of treatment on diarrhoea prevalence from day 8 of age to weaning is presented in Table 4-3. Glutamine supplementation tended to increase diarrhoea prevalence in piglets (P=0.09). The effect of treatment on the total number of clinical cases of disease per litter, the total number of injections per litter, and the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a litter basis, and per sow, during the pre-weaning period is presented in Table 4-3. There was no effect of treatment on any of these parameters (P>0.05).

4.4.6 Post-weaning pig growth and carcass quality

The effect of treatment on pig feed intake, growth, and feed efficiency from weaning to slaughter is presented in Table 4-4. There was no effect of treatment on pig ADFI, BW and FCR at any time point post-weaning (P>0.05). Pigs supplemented with glutamine pre-weaning tended to have a better ADG from day 21 to 28 post-weaning than pigs supplemented with enzymes pre-weaning (P=0.07). There was no effect of treatment on ADG at any other time point (P>0.05).

The effect of treatment on carcass parameters is presented in Table 4-5. There was no effect of treatment on any parameter of interest (P>0.05).

4.4.7 Post-weaning diarrhoea scores, antibiotic and anti-inflammatory treatment

The effect of treatment on diarrhoea prevalence from weaning to day 28 post-weaning is presented in Table S 4-6. There was no effect of treatment on diarrhoea prevalence between weaning and day 28 post-weaning (P>0.05).

The effect of treatment on the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a pen basis and the number of clinical cases of disease per pen post-weaning is presented in Table S 4-6. There was no treatment effect on the volume of antibiotics or anti-inflammatories administered per pig on a pen basis during the weaner or finisher periods.

4.4.8 Haematology

The effect of treatment on haematological parameters of pigs at day 5 post-weaning is presented in Table 4-6. Glutamine supplementation pre-weaning increased the red blood cell count (P=0.03) and haemoglobin concentration (P=0.04) in the plasma of pigs at day 5 post-weaning compared to control and enzymes. There was no effect of treatment on any other parameter of interest (P>0.05).

4.4.9 Intestinal morphology

The effect of treatment on small intestinal morphology of pigs at day 5 post-weaning is presented in Table 4-7. There was no effect of treatment on villus height, crypt depth, villus height to crypt depth ratio (VH:CD) or villus width in the duodenum, jejunum and ileum of pigs (P>0.05).

4.5 Discussion

To our knowledge, this study is the first to determine the effect of creep feeding liquid starter diet with or without the inclusion of feed additives (L-glutamine and a cocktail of enzymes) to suckling pigs on pre- and post-weaning growth to target slaughter weight (~120 kg), health and intestinal structure. The results should provide valuable practical information for the design of optimal pre-weaning nutritional and management strategies to maximise pre- and post-weaning pig growth.

4.5.1 L-glutamine

In the current study, supplementing pigs with L-glutamine in liquid creep feed tended to cause a reduction in pig weight at weaning and to increase diarrhoea prevalence of piglets pre-weaning. Glutamine is known to positively influence pig BW when

supplemented orally at 1g/kg of pig BW per day pre-weaning but to be detrimental for piglet BW when supplemented at 2 g/kg of pig BW per day (Haynes *et al.*, 2009). In the present study DMd was 224 g/pig during the supplementation period and L-glutamine was included at 10 g/kg (i.e. 1%) in the diet. Therefore, piglets ingested ~ 2.24 g of L-glutamine in total between day 8 and weaning, which corresponds to 0.11 g of L-glutamine/pig/day, which equates to 0.013 g/kg of pig BW at weaning. This is well below the dose considered as efficacious. In the current study, creep feed DMd was lower than that reported in other studies when liquid feed was supplemented to piglets pre-weaning (Kobek-Kjeldager *et al.*, 2021; Vasa *et al.*, 2023). We would have expected the total creep feed DMd to be closer to 500 g/pig as in our previous study (Vasa *et al.*, 2023), which would have resulted in an L-glutamine intake of closer to 1 g/pig/day during the week before weaning. In the current study, L-glutamine recovery analyses were conducted in order to ensure that L-glutamine was present in the feed at inclusion levels. Results from dry feed analyses showed that the recovery of free L-glutamine was as expected. However, free L-glutamine should have been present at 10 g/kg in the liquid starter diet (after oven drying) and was only found at <0.2 g/kg. It was expected that L-glutamine recovery in the liquid starter diet would be similar to that in dry starter diet since liquid diets were prepared every 6 hours and others had demonstrated that L-glutamine remained stable in water for 24 h (Molfino *et al.*, 2009). In addition, studies demonstrated that L-glutamine is stable after pelletization at 70-88 °C; therefore, oven drying the liquid starter at 55 °C should have not affected its stability (Bampidis *et al.*, 2020). However, in liquid feed, nutrients can be used during microbial fermentation leading to significant gross energy and amino acid losses from feed (O'Meara *et al.*, 2021). Microbial decarboxylation of amino acids in particular can occur under acidic conditions and decarboxylase enzymes can be produced by various microbes, including lactic acid bacteria (Barbieri *et al.*, 2019; Yazgan *et al.*, 2021). In Chapter 3, we demonstrated that lactic acid bacteria grow in liquid feed during the 6-hour period between preparation and the last feed of the day. This could explain the reduced level of free L-glutamine observed in the liquid starter. However, it would be interesting to use a functional metagenomics approach to analyse the metabolic capabilities of the bacterial communities within the liquid starter feed to determine which bacteria are responsible for the degradation of free L-glutamine. Another issue is that microbial decarboxylation of amino acids results in

the production of undesirable metabolites such as biogenic amines (Cullen *et al.*, 2021). Microbial decarboxylation of free glutamine results in the production of ornithine, and two biogenic amines, cadaverine and putrescine, are subsequently formed from the decarboxylation of ornithine (Özogul and Özogul, 2019).These biogenic amines can reduce feed palatability, thereby reducing feed intake in pigs and they are also cytotoxic at high doses (Cullen *et al.*, 2021). This could explain the tendency for L-glutamine to reduce piglet growth and increase diarrhoea prevalence pre-weaning in the current study. It would be interesting to measure the concentration of biogenic amines in the liquid starter diet in future studies.

Weaning is often associated with reduced feed intake in pigs. The lack of nutrient supply at this time can negatively affect the integrity of the intestinal tract, and therefore reduce nutrient absorption and feed efficiency. In the present study, pre-weaning supplementation with L-glutamine tended to improve ADG from day 21 to 28 post-weaning. This could indicate that the pigs supplemented with glutamine pre-weaning recovered faster from weaning than their counterparts. However, there was no improvement in small intestinal morphology at day 5 post-weaning. L-glutamine supplementation pre-weaning increased the red blood cell count and haemoglobin concentration in plasma of pigs on day 5 post-weaning. This increase is in agreement with the literature. Dumaswala *et al.* (1994) demonstrated that glutamine is important for the preservation of red blood cells. It plays an important role in the regulation of oxidative stress which makes its use of interest in the case of red blood cells disorders (such as sickle cell anaemia) (Elenga *et al.*, 2022). In addition, Bhattarai and Nielsen (2015), observed a positive association between haemoglobin concentration and ADG and between red blood cell count and ADG in pigs post-weaning. However, it is important to note that red blood cells counts and haemoglobin concentrations in the plasma of both pigs supplemented with glutamine and unsupplemented pigs were within the range of normal values for weaned pigs. In fact, most of the haematological parameters were within normal reference ranges, with the exception of white blood cell, lymphocyte and platelet counts which were lower and eosinophil count which was higher than the normal ranges (Iowa State University, 2011). The experimental pig facility, where this experiment was performed, had a high health status and observed strict internal and external biosecurity. Consequently, it would be expected to have very low levels of disease compared with commercial farms. This could

explain the low counts of white blood cells and lymphocytes, as these immune cells are solicited and activated during the establishment of an immune response.

4.5.2 Enzymes

Supplementing pigs with a cocktail of enzymes in liquid creep feed did not affect pig weaning weight. This was not surprising as it was expected that improved post-weaning growth and feed efficiency would result from pre-weaning supplementation of the pigs with extraneous enzymes, as previously demonstrated by Prykhodko *et al.* (2016). However, post-weaning growth or feed efficiency were also not affected by pre-weaning treatment. Although not measured in this study, it would therefore seem unlikely that the development of inherent enzyme production capacity in pigs at weaning was increased in response to pre-weaning supplementation with the extraneous enzyme cocktail.

In addition, enzyme supplementation did not affect intestinal morphology at day 5 post-weaning. Contrary to this, Słupecka *et al.* (2012) observed that supplementing suckling piglets twice a day from day 8 to 15 of age by oral gavage with porcine pancreatic enzymes increased villus height and reduced crypt depth in the jejunum. It could be that piglets in the present study did not ingest sufficient amounts of the enzyme cocktail. Feed analyses found that both the protease and the α-amylase were recovered at expected concentrations from the dry starter diet and dried liquid starter diet. In addition, previous studies have demonstrated that these enzymes are stable after feed pelletization at 80-90 °C (Bampidis *et al.*, 2023); therefore, oven drying the liquid starter at 55 °C was not expected to affect their stability It was not possible to determine the recovery of lipase from the diets fed, as the methodology has yet to be developed by the enzyme supplier. However, creep feed DMd (197 g of DM/pig of creep feed) as well as the overall percentage of piglets considered as eaters were both very low (20%). As previously stated, we would have expected a DMd of more than double that found here, based on our previous work with liquid creep feed (Vasa *et al.*, 2023). The lower DMd observed is most likely because liquid feed was prepared with water in the current study as opposed to liquid milk replacer in the case of Vasa *et al.* (2023). Because of the lower DMd observed in the present study, piglets most likely did not ingest a sufficient quantity of each enzyme to positively influence post-

weaning growth and intestinal morphology. If this is the case, then creep feed DMd and consequently enzyme intake could certainly be increased by preparing the liquid feed with a liquid milk replacer, as in our previous studies [Chapter 3, Vasa *et al.* (2023)]. However, feeding liquid milk replacer is particularly expensive and unlikely to be cost-beneficial.

4.6 Conclusions

Piglets that received a liquid starter diet supplemented with L-glutamine tended to have a higher prevalence of pre-weaning diarrhoea. This resulted in a tendency for L-glutamine supplementation to reduce weaning weight but the weight of pigs at slaughter were not affected by treatment. Supplementing liquid creep feed with enzymes had no effect on growth, health or medication usage in pigs. The intestinal morphology of pigs at day 5 post-weaning was not affected by pre-weaning supplementation with L-glutamine or the cocktail of enzymes. It is thought that the lower than expected creep feed intake observed and the degradation of L-glutamine in the liquid starter diet may be in part responsible for the absence of the treatment effect found in this study. Feeding liquid milk replacer or a mixture of milk replacer and starter diet should be considered in future studies in order to ensure high creep feed intakes and as a consequence increased feed additive consumption.

4.7 Tables

Table 4-1. Composition of the experimental diet (on an air-dry basis; kg/tonne).

Diet Number	**1**	**2**	**[1]3**	**4**	**5**	**6**
Diet type	**Dry sow**	**Lactation**	**Starter**	**Link**	**Weaner**	**Finisher**
Ingredients						
Barley	747.5	288.6	50.0	77.0	292.7	589.7
Wheat	0	450.0	0	100.0	450	230.0
Maize	0	0	247	300	0	0
Soybean meal	74.4	173.8	141.1	180.7	150.2	146.1
Full fat soybean meal	0	0	125.0	75.0	50.0	0
Whey permeate	0	0	200	150	0	0
Skim milk powder	0	0	125	50	0	0
Soya hulls	141.9	0	0	0	0	0
Soya oil	10	49.2	77.5	31.0	23.5	10

Premix	1.5[2]	1.5[2]	3.0[3]	3.0[3]	3.0[3]	1.0[4]
L-Lysine HCl	2.18	5.1	6.4	6.8	6.3	4.7
DL-Methionine	0.5	1.6	3.6	3.2	2.1	1.3
L-Threonine	0.9	2.7	3.7	3.3	2.7	2.0
L-Tryptophan	0	0.8	1.4	1.3	0.6	0.3
L-Valine	0	2.6	1.3	1.2	0.7	0
Limestone flour	10	11.1	7.0	7.5	10.0	11.0
Mono dicalcium phosphate	7.2	8.0	5.0	7.0	5.3	1.0
Salt	4	5	3	3	3	3
Phytase[5]	0.1	0.1	0.1	0.1	0.1	0.1
Chemical composition						
Dry matter[6]	883.0	882.0	925.0	904.0	890.5	880.0
Crude protein[6]	117.0	168.0	191.0	174.0	162.5	163.0

Ash[6]	47.0	42.0	57.0	51.0	44.0	46.5
Ether extract[6]	34.8	69.9	115.7	83.4	74.8	36.6
Crude fibre[6]	83.0	30.0	18.0	19.5	31.0	33.5
Lysine[7]	7.8	11.5	16.2	15.0	13.0	10.9
Methionine[7]	2.4	3.9	7.0	6.1	4.7	3.4
Cystine[7]	2.5	3.0	2.7	2.9	3.1	3.1
Threonine[7]	5.6	8.3	10.9	10.1	8.8	7.6
Tryptophan[7]	3.66	3.36	2.66	2.22	1.54	2.79
Digestible energy (MJ/Kg)[7]	13.20	15.20	16.20	15.00	14.27	13.73
Net energy (MJ/Kg)[7]	8.59	10.93	12.06	10.94	10.30	9.80
SID lysine[7,8]	6.6	10.7	15.3	14.1	12.0	10.0
Total calcium[7]	7.2	8.3	8.2	7.5	7.4	6.5
Digestible phosphorus[7]	3.5	3.8	4.6	4.2	3.3	2.5

[1]The starter diet was used to prepare the liquid creep feed supplemented to piglets pre-weaning. In the liquid creep feed supplemented with glutamine, L-Glutamine (Ajinomoto, Tokyo, Japan) was included in the starter diet at 10 g/kg of diet. In the liquid creep feed supplemented with the enzyme cocktail, the enzyme cocktail containing lipase (Capalase Micro R800, DSM-Firmenich, Heerlen, The Netherlands), protease (ProAct 360, DSM-Firmenich) and α-amylase (RONOZYME HiStarch, DSM-Firmenich) was included in the starter diet at 0.725 g/kg of diet so that the finished diet contained 160 lipase units (PLI)/kg, 30,000 new feed protease units (NFP)/kg and 67.5 Kilo Novo (α-amylase) units (KNU)/kg, respectively.

[2]Premix provided per kilogram of complete diet (Diets 1 and 2): Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 70 mg; Mn from manganese oxide, 62 mg; Zn from zinc oxide, 80 mg, I from calcium iodate, 0.6 mg; Se from sodium selenite, 0.2 mg; vitamin A as retinyl acetate, 3.44 mg; vitamin D3 as cholecalciferol, 25 mg; vitamin E as DL-alpha-tocopheryl acetate, 100 mg; vitamin K, 2 mg; vitamin B12, 15 µg; riboflavin, 5 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 500 mg; Biotin, 200µg; folic acid, 5mg; vitamin B1,2 mg; and vitamin B6, 3 mg.

[3]Premix provided per kilogram of complete diet (Diets 3, 4 and 5): Cu from copper sulphate, 100 mg; Fe from ferrous sulphate monohydrate, 90 mg; Mn from manganese oxide, 47 mg; Zn from zinc oxide, 120 mg; I from potassium iodate, 0.6 mg; Se from sodium selenite, 0.3 mg; vitamin A as retinyl acetate, 2.1 mg; vitamin D3 as cholecalciferol, 25 µg; vitamin E as DL-alpha-tocopheryl acetate, 100 mg; vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B1, 2 mg; and vitamin B6, 3 mg.

[4]Premix provided per kilogram of complete diet (Diet 6): Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; vitamin A as retinyl acetate, 0.7 mg; vitamin D3 as cholecalciferol, 12.5 µg; vitamin E as DL-alpha-tocopheryl acetate, 40 mg; vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B1, 2 mg; vitamin B6, 3 mg.

[5]The diet contained 1000 phytase units (FYT) per kg feed RONOZYME HiPhos GT (DSM, Belfast, UK).

[6]Analysed nutrient composition.

[7]Calculated nutrient composition.

[8] SID lysine = Standardized ileal digestible lysine.

Table 4-2. Effect of dietary treatment on creep feed dry matter disappearance, litter weight, piglet weight and growth during the suckling period.

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Number of litters	21	20	19		
Litter weight, kg					
Day 8	42.0	42.3	40.7	1.46	0.42
Day 14	64.0	64.3	62.9	1.49	0.55
Day 21	90.8	88.4	89.1	1.89	0.52
Day 27	114.1	109.5	111.7	2.54	0.35
Overall	77.7	76.1	76.1	1.38	0.22
Mean piglet BW, kg[1]					
Day 8	3.10	3.09	3.12	0.060	0.88
Day 14	4.73	4.72	4.76	0.060	0.88
Day 21	6.70^{A}	6.57^{B}	$6.69^{A,B}$	0.060	0.09
Day 27	8.41^{A}	8.28^{B}	8.41^{A}	0.060	0.08
Overall				0.055	0.32
ADG, g/pig/day[2]					
Day 8 to 14	260	259	260	5.8	0.96

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Day 14 to 21	280[a]	261[b]	277[a]	6.5	<0.01
Day 21 to 27	283	280	288	7.2	0.58
Overall	274	267	275	5.7	0.13
Dry matter disappearance					
Pre-weaning creep feed, g/pig	202	224	197	25.9	0.77

[1]BW, body weight.
[2]ADG, average daily gain.
[a, b, c] Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).
[A, B] Values within a row that do not share a common superscript tended to differ ($0.05 < P \leq 0.10$).
Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

Table 4-3. Effect of dietary treatment on health parameters and medicinal treatment of sows and piglets during lactation.

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Number of sows	21	20	19		
Diarrhoea prevalence, % (day 8-28)[1]	11^{B}	23^{A}	12^{B}	0.4	0.09
No. of clinical cases of disease per litter[2]	1.3	1.1	0.8	0.42	0.76
No. of injections per litter	3.8	3.2	2.4	1.28	0.76
Antibiotic usage per sow, mL[3]	0	7.2	2.5	2.66	0.16
Antibiotic usage per pig, mL[4]	0.13	0.12	0.09	0.045	0.82
Anti-inflammatory usage per sow, mL[5]	0	1.8	0.6	0.66	0.16
Anti-inflammatory usage per pig, mL[6]	0.03	0.02	0.02	0.009	0.79

[1]A faecal score of 2 or greater for a litter was considered indicative of diarrhoea at each time-point between day 2 and 28. The overall prevalence was reported for the pre-weaning period.
[2]Number of piglets per litter treated one or more times.
[3]Volume of antibiotic administered per sow.
[4]Volume of antibiotic administered per piglet.
[5]Volume of anti-inflammatory administered per sow.
[6]Volume of anti-inflammatory administered per piglet.
Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

Table 4-4. Effect of dietary treatment on feed intake and growth of pigs from weaning to slaughter at ~120 kg.

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Days from weaning to slaughter	132	132	132		
BW, kg[1]					
Day 0 (weaning)	8.5	8.3	8.0	1.54	0.98
Day 6 post-weaning	9.9	9.7	9.6	1.54	0.99
Day 14 post-weaning	12.9	12.6	12.6	1.54	0.99
Day 21 post-weaning	16.5	16.2	16.2	1.54	0.99
Day 28 post-weaning	20.9	21.0	20.5	1.54	0.97
Day 43 post-weaning	33.3	33.2	32.3	0.52	0.35
Day of slaughter (~158 days of age)	134.0	136.5	132.6	2.08	0.42
Overall				0.54	0.73
ADFI, g/pig/day[2]					
Day 0 to 6	240	244	252	11.2	0.60
Day 6 to 14	501	491	456	22.5	0.33
Day 14 to 21	638	644	630	34.8	0.96

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Day 21 to 28	906	892	896	37.4	0.96
Day 28 to 47	1258	1284	1262	60.4	0.94
Day 43 to slaughter	2783	2738	2817	36.8	0.33
Overall				25.5	0.94
ADG, g/pig/day[3]					
Day 0 to 6	253	227	250	23.1	0.59
Day 6 to 14	390	371	371	25.0	0.80
Day 14 to 21	529	522	498	22.6	0.54
Day 21 to 28	$643^{A,B}$	689^{A}	598^{B}	28.2	0.07
Day 28 to 43	829	815	800	24.3	0.75
Day 43 to slaughter	1139	1160	1123	27.0	0.61
Overall				17.8	0.44
FCR[4], g/g					
Day 0 to 6	0.98	1.10	0.99	0.069	0.37
Day 6 to 14	1.34	1.34	1.22	0.069	0.38
Day 14 to 21	1.23	1.24	1.24	0.069	0.99

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Day 21 to 28	1.44	1.30	1.49	0.069	0.12
Day 28 to 43	1.55	1.58	1.56	0.069	0.96
Day 43 to slaughter	2.49	2.37	2.51	0.063	0.28
Overall				0.031	0.89

[1]BW, body weight.
[2]ADFI, average daily feed intake.
[3]ADG, average daily gain.
[4]FCR, feed conversion ratio.
[a, b, c] Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).
Only 6 pen replicates by treatment could be used to analyse the growth performance data up to day 43 post-weaning. Data from day 43 post-weaning to slaughter day were analysed using 12 pen replicates by treatment.
Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

Table 4-5. Effect of dietary treatment on pig carcass parameters at slaughter.

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Number of pens	12	12	12		
Cold carcass weight, kg	102.8	104.2	102.6	1.09	0.50
Fat depth, mm	14.7	14.7	14.9	0.35	0.90
Muscle depth, mm	53.3	52.3	54.0	0.74	0.30
Lean meat, %	57.6	57.5	57.5	0.27	0.96
Kill out, %	76.8	76.3	77.7	0.89	0.57
Carcass ADG, g/d[1,2]	741	751	747	10.4	0.80
Carcass FCR, g/g[3,4]	2.82	2.69	2.77	0.070	0.45
Lean ADG, g/d[5]	373	377	376	4.95	0.83

[1]ADG, average daily gain.
[2]Carcass ADG (from weaning to slaughter) = [(carcass weight in kg –weaning weight in kg × 0.55) × 1,000]/number of days from weaning to slaughter (Lawlor and Lynch, 2005).
[3]FCR, feed conversion ratio.
[4]Carcass FCR (from weaning to slaughter) was calculated as follows: carcass FCR = daily feed intake (g)/ carcass ADG (g).
[5]Lean ADG (from birth to slaughter) = (carcass weight × carcass lean meat percentage × 10)/number of days to slaughter (Lawlor and Lynch, 2005).
Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

Table 4-6. Effect of dietary treatment on haematological parameters of weaned pigs at day 5 post-weaning.

Treatment	**Control**	**Glutamine**	**Enzymes**	**SEM**	***P*-value**	**Reference ranges[1]**
Number of pigs	10	10	10			
White blood cells ($\times 10^3$ cells/µl)	8.53	7.23	7.49	0.94	0.60	9.62-25.2
Lymphocytes ($\times 10^3$ cells/µL)	2.99	2.89	2.47	0.459	0.70	4.02-12.5
Monocytes ($\times 10^3$ cells/µL)	0.61	0.56	0.53	0.119	0.88	0.05-2.3
Neutrophils ($\times 10^3$ cells/µL)	4.72	3.62	4.36	0.639	0.47	2.35-11.9
Eosinophils ($\times 10^3$ cells/µL)	0.14	0.12	0.08	0.025	0.17	0-0.05
Basophils ($\times 10^3$ cells/µL)	0.05	0.06	0.06	0.015	0.80	-
Red blood cells ($\times 10^6$ cells/µL)	4.98^b	5.40^a	4.56^b	0.212	0.03	4.87-7.88
Haemoglobin (g/dL)	8.90^b	9.72^a	8.22^b	0.402	0.04	8.08-11.9
Mean corpuscular volume (fL)	61.1	60.6	60.7	1.03	0.94	43.3-64.5
Mean corpuscular haemoglobin (pg/cell)	17.9	18.0	18.0	0.29	0.95	12.4-19.3
Mean corpuscular haemoglobin concentration (g/dL)	29.3	29.7	29.7	0.32	0.58	27.3-31.4
Platelets ($\times 10^3$ cells/µL)	249	317	216	46.9	0.33	374.3-1080.8

[1]Normal reference ranges for pigs 0 – 6 weeks old (Iowa State University, 2011)
[a-b] Values within a row that do not share a common superscript differ significantly at $P<0.05$.
[A-B] Values within a row that do not share a common superscript tended to differ at $0.05<P\leq0.10$.
Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

Table 4-7. Effect of dietary treatment on small intestinal morphology of pigs at day 5 post-weaning.

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Number of pigs					
Duodenum					
Villus height (μm)	357	314	331	24.5	0.46
Crypt depth (μm)	169	184	200	12.4	0.23
VH:CD[1] ratio (μm/μm)	2.54	2.14	2.09	0.188	0.19
Villus width (μm)	150	134	143	5.8	0.18
Jejunum					
Villus height (μm)	269	242	300	18.6	0.11
Crypt depth (μm)	166	161	178	8.2	0.32
VH:CD ratio (μm/μm)	2.00	1.91	2.08	0.177	0.79
Villus width (μm)	118	112	120	5.5	0.57
Ileum					
Villus height (μm)	233	223	219	23.3	0.91
Crypt depth (μm)	176	176	179	9.8	0.96
VH:CD ratio (μm/μm)	1.63	1.48	1.41	0.143	0.55

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Villus width (μm)	127	131	125	5.5	0.76

[1]VH:CD = villus height (μm)/crypt depth (μm).
Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

4.8 Supplementary tables and figures

Table S 4-1. L-glutamine, glutamic acid and enzyme recovery analyses on the dry pelleted starter diet and on oven dried liquid starter diet.

Additives	Dry starter control	Dry starter glutamine	Dry starter enzymes	Dried liquid starter control[1]	Dried liquid starter glutamine[1]	Dried liquid starter enzymes[1]
Amino acids						
L-glutamine (free), g/kg of diet	<0.2	8.58	<0.2	<0.2	<0.2	<0.2
Glutamic acid (free), g/kg of diet	0.4	0.5	0.4	0.4	2.4	1.0
Total glutamic acid, g/kg of diet	32.1	40.9	32.2	32.8	37.9	33.9
Enzymes						
Protease, NFP/kg[2]	<LOD	<LOD	40650	<LOD	<LOD	12860
α-amylase, KNU/kg[3]	<LOD	<LOD	67	<LOD	<LOD	52

[1]The liquid starter diet samples prepared by mixing dry pelleted starter diet with warm water (55 ˚C) at a ratio of 1:5 and incubated for 5 hours at 30°C. Before conducting these analyses, the liquid starter diet was oven dried at 55 ˚C for 3 consecutive days.
[2]ProAct 360 (DSM-Firmenich, Heerlen, The Netherlands); NFP, New Feed Protease units.
[3]RONOZYME HiStarch (DSM-Firmenich); KNU, Kilo Novozymes units.
LOD, Limit of detection.

Table S 4-2. Effect of treatment on sow body weight and back-fat depth.

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Number of sows	21	20	19		
Lactation length, days	27.6	27.7	27.8	0.79	
BW, kg[1]					
Day 108 of gestation	284	284	284	2.4	0.99
Farrowing[2]	248	247	246	2.4	0.88
Weaning[3]	240	241	242	2.4	0.76
Service[3]	232	231	234	3.0	0.79
Overall				1.8	0.92
BF, mm[4]					
Day 108 of gestation	18.4	18.2	18.5	0.29	0.86
Weaning	14.0	14.3	13.7	0.29	0.36
Service	14.3	14.6	13.9	0.36	0.29
Overall				0.22	0.48
Sow BW change, kg					
Day 108 to weaning[5]	-43	-42	-41	3.0	0.81
Farrowing to weaning[6]	-7	-6	-1	3.0	0.25

Treatment	Control	Glutamine	Enzymes	SEM	*P*-value
Weaning to service[7]	-13	-8	-4	4.0	0.20
Overall				2.3	0.06
Sow BF change, mm					
Day 108 to weaning[8]	-4.4	-3.9	-4.8	0.34	0.21
Weaning to service[9]	0.2	0.6	0.3	0.45	0.83
Overall				0.29	0.35

[1]BW, body weight.
[2]Estimated empty farrowing weight = (sow weight at day 108 – (total born × 2.25)). The value of 2.25 kg is an estimate of the increased weight in the gravid uterus and in mammary tissue attributed to each pig in a litter (NRC, 1998).
[3]Weaning = day 28 ± 1 of lactation; service = day 4 ± 1 post-weaning.
[4]BF, back fat.
[5]Sow BW change = (sow BW at weaning – sow BW at day 108 of gestation).
[6]Sow BW change = (sow BW at weaning – sow BW at farrowing).
[7]Sow BW change = (sow BW at service – sow BW at weaning).
[8]Sow BF change = (sow BF at weaning – sow BF at day 108 of gestation).
[9]Sow BF change = (sow BF at service – sow BF at weaning).
Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

Table S 4-3. Effect of treatment on sow litter size and the number of piglets fostered and died per litter during lactation.

Treatment	Control	Glutamine	Enzymes	SEM	P-value
Number of sows	21	20	19		
Litter					
Litter size at day 8	14.0	13.6	13.8	0.40	0.72
Litter size weaning	13.9	13.2	13.6	0.41	0.46
Deaths and removals per sow					
Deaths after day 8	0.1	0.4	0.3	0.13	0.37

Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

Table S 4-4. Effect of treatment on percentage of "eaters" within a litter where two or more feeder-directed activities per pig is considered to indicate an "eater".

Treatment	Control	Glutamine	Enzymes	SEM	P-value
Eaters, %[1]					
Day 13 of lactation	10	15	19	5.8	0.47
Day 16 of lactation	11	7	21	5.8	0.12
Day 22 of lactation	12	10	18	5.8	0.50
Overall	11^{B}	11^{B}	20^{A}	4.0	0.07

[1]An eater was defined as having two or more feeder trough-directed-activity observations on each day. The percentage of piglet eaters per pen was calculated on a pen basis for each observation day and for all observation days combined. This was carried out by expressing the number of piglets considered as eaters in a pen as a percentage of the total number of piglets present in the pen.

[A, B] Values within a row that do not share a common superscript tended to differ ($0.05<P\leq0.10$).

Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

Table S 4-5. Effect of treatment on percentage of "eaters" within a litter where one or more feeder-directed activities per pig is considered to indicate an "eater".

Treatment	Control	Glutamine	Enzymes	SEM	P-value
Eaters, %[1]					
Day 13 of lactation	31	36	49	8.5	0.30
Day 16 of lactation	26[B]	26[B]	49[A]	8.5	0.09
Day 22 of lactation	40	32	41	8.5	0.70
Overall	32[B]	31[B]	46[A]	5.3	0.06

[1]An eater was defined as having one or more feeder trough-directed-activity observations on each day. The percentage of piglet eaters per pen was calculated on a pen basis for each observation day and for all observation days combined. This was carried out by expressing the number of piglets considered as eaters in a pen as a percentage of the total number of piglets present in the pen.

[A, B] Values within a row that do not share a common superscript tended to differ ($0.05<P\leq0.10$).

Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

Table S 4-6. Effect of dietary treatment on post-weaning diarrhoea prevalence and medicinal treatment of pigs.

Treatment	Control	Glutamine	enzymes	SEM	*P*-value
Number of pens	12	12	12		
Diarrhoea prevalence, % (weaning to 28 days post-weaning)[1]	23	22	23	0.1	0.97
Weaner period (weaning to day 43 post-weaning)					
No. of clinical cases of disease per pen[2]	1.1	0.3	0.4	0.37	0.24
Antibiotic usage per pig, mL[3]	0.33	0.08	0.13	0.112	0.24
Anti-inflammatory usage per pig, mL[4]	0.18	0.05	0.08	0.059	0.25
Finisher period (day 43 to day 158 post-weaning)					
No. of clinical cases of disease per pen[2]	0.2	0.3	0.2	0.15	0.91
Antibiotic usage per pig, mL[3]	0.25	0.45	0.27	0.241	0.81
Anti-inflammatory usage per pig, mL[4]	0.05	0.08	0.06	0.043	0.91

[1]A faecal score of 2 or greater was considered indicative of diarrhoea at each time-point (day 1, 5, 14, 21 and 28 post-weaning) between weaning and day 28 post-weaning. The overall prevalence was reported for the post-weaning period.
[2]Number of pigs per pen treated one or more times.
[3]Volume of antibiotic administered to each pig on a pen basis.
[4]Volume of anti-inflammatory administered to each pig on a pen basis.
Control, liquid starter diet provided as creep feed from day 8 of age to weaning; Glutamine, control supplemented with 10 g of L-Glutamine per kg of starter diet; Enzymes, control supplemented with a cocktail of enzymes (lipase, protease and α-amylase).

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5. Effect of post-weaning L-glutamine and/or liquid milk replacer supplementation on pig growth and intestinal development

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5.1 Abstract

This study aimed to determine the effect of providing supplemental liquid milk replacer and/or 1% L-glutamine on intestinal function and growth in weaned pigs. Pigs [12 pens (each with 10 pigs)/treatment] were assigned to one of 4 treatments from day (D) 0-10 post-weaning (pw): (1) control diet; dry pelleted starter diet; (2) control diet plus supplemental liquid milk replacer; (3) control diet with dietary inclusion of 1% L-glutamine and (4) Treatment 3 diet plus supplemental liquid milk replacer with dietary inclusion of 1% L-glutamine. Pig weight and feed disappearance were recorded at intervals up to slaughter (~120 kg). At D7 pw, 40 pigs (n=10/group) were euthanised and intestinal tissues harvested for histology and gene expression analyses. Liquid milk replacer increased average daily feed intake and average daily gain from D0-D10 pw and D20-D47 pw and body weight up to slaughter. It also increased villus height in the small intestine, decreased expression of pro-inflammatory cytokines (IL17, IL18, IL22) in the jejunum and increased faecal abundance of *Rikenellaceae* RC9 and *Oscillaspiraceae* UCG-002 at D11 pw. In conclusion, while glutamine supplementation did not affect pig growth and intestinal integrity, milk replacer increased feed intake and improved intestinal health and lifetime growth in pigs.

5.2 Introduction

Weaning in pigs is often associated with a growth check, due to reduced feed and nutrient intake during the early post-weaning (pw) period (Lawlor *et al.*, 2020). Reducing latency to first feed pw and increasing pw feed intake is key to optimising intestinal health and subsequent growth. One solution to increase feed intake and alleviate nutritional stress at weaning is to provide pigs with supplemental liquid milk replacer during the first days pw. Previous studies found that feeding liquid milk replacer as a supplement to dry starter diet can significantly increase pw feed intake and growth (Zijlstra *et al.*, 1996; Dunshea *et al.*, 1999; Rault *et al.*, 2015). There is currently a focus in the pig industry on finding effective dietary feed additives to replace antibiotics and zinc oxide in pw diets. L-glutamine may be one such additive. Until recently, glutamine was not considered an essential amino acid; however, beneficial effects of pw glutamine administration on pig growth, intestinal morphology, intestinal integrity and oxidative stress have been reported in weaned pigs (Wu *et al.*, 1996; Hsu *et al.*, 2010; Domeneghini *et al.*, 2006; Watford, 2015). Glutamine is a major fuel for the proliferation and survival of intestinal enterocytes (Hsu *et al.*, 2010; Wu *et al.*, 1996). In addition, glutamine enhances mucosal protein synthesis and helps preserve paracellular permeability, which is critical to maintain the epithelial barrier function (Coëffier *et al.*, 2003; Le Bacquer *et al.*, 2003). Maintaining intestinal epithelial integrity and function is critical to ensure optimal nutrient absorption and therefore optimise pw growth. It is also key to preventing the transfer of pathogens and/or toxins across the intestinal epithelium from the lumen to the blood stream (Groschwitz and Hogan, 2009). Previous studies show that dietary supplementation of weaned pigs with L-glutamine at 1% can increase average daily gain (ADG) during the first week pw (Wang *et al.*, 2015) and feed efficiency during the second week pw (Wu *et al.*, 1996). At this dose, L-glutamine was also shown to increase the expression of tight junction proteins (Wang *et al.*, 2015) and improve morphology in the jejunum of pigs at day 7 pw (Wu *et al.*, 1996).

The provision of supplementary liquid milk replacer and/or 1% L-glutamine to weaned pig diets should increase intestinal health, feed intake and growth and may thereby reduce antimicrobial usage (AMU). However, there is a lack of information on the effect of these strategies on pig health and associated AMU. The objective of

this study was to determine the effect of providing supplemental liquid milk replacer in addition to dry pelleted starter diet with or without 1% dietary L-glutamine inclusion to weaned pigs on intestinal structure and function, growth and pw medication usage. The hypothesis was that early pw supplementation with liquid milk replacer and/or L-glutamine inclusion would improve intestinal integrity, increase feed intake and growth and consequently reduce the need for injectable therapeutics in pigs. Furthermore, it was hypothesized that these benefits would increase lifetime growth in pigs.

5.3 Materials and methods

5.3.1 Ethical approval

This study was performed between August 2022 and February 2023, at the Teagasc Pig Development Department, Moorepark, Fermoy, Co. Cork, Ireland. Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. TAEC2020-275) and South East Technological University Ethics Committee (approval no. WIT2021REC011). The project was authorised by the Irish Health Products Regulatory Authority (project authorisation no. AE19132/P129). The experiment was conducted in accordance with the legislation for commercial pig production set out in the European Communities (Welfare of Farmed Animals) Regulations 2010 and laid out in Irish legislation (SI no. 311/2010), and in compliance with the ARRIVE guidelines.

5.3.2 Experimental design and diets

At weaning, 480 pigs (28 ± 0.6 days of age; comprising two batches of 240 pigs) were grouped into 48 single-sex pen groups of 10 pigs of even weight. Within batch, pen groups were blocked on the basis of sex and weight at weaning and treatments were randomly assigned within block so that there were 12 pen replicates per treatment. The trial was a 2x2 factorial arrangement; factors being provision of supplementary liquid milk replacer (no / yes) and dietary inclusion of 1% L-glutamine (no / yes). The dietary treatments were as follows from D0-10 pw: 1) control diet; dry pelleted starter diet

(N-Milk/N-Glu), 2) control diet plus supplemental liquid milk replacer (Milk/N-Glu), 3) control diet with dietary inclusion of 1% L-glutamine (N-Milk/Glu) and 4) Treatment 3 diet plus supplemental liquid milk replacer with dietary inclusion of 1% L-glutamine (Milk/Glu). All pen groups were fed a pelleted starter diet (Table 5-1) and where provided, supplemental liquid milk replacer was fed from weaning to day 10 pw. L-glutamine (Ajinomoto, Tokyo, Japan) was added at 1% inclusion to the starter diet (Table 5-1) and milk replacer powder before reconstitution and fed from weaning to day 10 pw (Table S 5-1). After day 10 pw, all pigs were fed a common sequence of standard diets in pelleted form (Table 5-1).

The dry pelleted starter diet (Table 5-1) was fed dry as a 3 mm pellet to all treatment groups. Pelleted link diet (Table 5-1) was provided from day 11 to 20 pw followed by pelleted weaner diet (Table 5-1) until transfer to the finisher room at day 47 pw. Pelleted finisher diet (Table 5-1) was provided from day 47 pw until slaughter (~day 133 pw). All of the diets were formulated to meet or exceed National Research Council, 2012 recommendations (National Research Council, 2012). Diet samples were analysed for dry matter (DM) (oven drying), ash (furnace drying and gravimetry), crude protein (Dumas method), total fat (Weibul acid hydrolysis) and crude fibre (Ankom 200 fibre analyser, Macedon, New York, United States) by Sciantec Analytical Services Ltd, Selby, United Kingdom according to European Union Commission Regulation No 152/2009 of 27 January 2009 (European Commission, 2009). The ingredient content and chemical composition of all diets are provided in Table 5-1. The feed and water were not medicated and access to both was provided on an *ad-libitum* basis.

5.3.3 Liquid milk replacer feeding

Liquid milk replacer was provided by an automated delivery system (Babyfeed; Schauer Agrotronic GmbH, Prambachkirchen, Austria), to a single liquid feed trough (54 × 11 × 5 cm, length × width ×height) in each pen. The probiotic-free milk replacer powder used was commercially sourced (Opticare milk; Swinco B.V, Helmond, The Netherlands). It contained the following, in descending order of inclusion: sweet whey powder, vegetable oils, porcine dried plasma powder, whey powder, digestible starch, dextrose, hyper-immunised egg powder, soya protein concentrate, hydrolysed wheat

gluten, premix of amino acids, vitamins and trace minerals. The milk replacer powder contained 11.9 MJ/kg net energy, 21.5% crude protein, 9% fat, 0.1% crude fibre, 6.5% crude ash, 1.8% lysine, 0.46% methionine, 0.7% calcium, 0.55% phosphorus, and 0.7% sodium. The liquid milk replacer was reconstituted by mixing 167 g of the milk replacer powder per 1 litre of warm water (55 ˚C).

Fresh liquid milk replacer was prepared twice daily at 0835 h and at 1645 h. Ten feeding cycles (each lasting ~2 hours) were programmed between 0930 h and 0400h. During each cycle the in-situ trough sensors checked the amount of liquid milk replacer present in the feeder trough 5 times. Whenever the liquid milk replacer level was below the level of the sensor, the trough was detected as empty, and liquid milk replacer was delivered to the trough and the amount delivered was recorded in the system computer. Therefore, each pen was potentially supplied with liquid milk replacer up to 50 times each day. Each day after the last feeding, the system was cleaned in closed circuit, which included all of the pipelines, with a 1% acid solution (Deosan Acidbrite AG313, Diversey Europe Operations BV, Utrecht, The Netherlands). In addition, the pipelines were cleaned once a week with a 0.5% solution of an alkaline detergent (AvalKsan Gold Standard CF, Carbon Group, Ringaskiddy, Ireland), to prevent lime-scale accumulation in the circuit. The milk troughs were cleaned with air pressure and rinsed with acidified water once a day and a 0.5% solution of the alkaline detergent was applied to troughs, as for the acid rinse, once weekly.

5.3.4 Housing

Weaner pens (2.5 × 2 m) were equipped with fully slatted plastic floors environmental control was automatic. Each pen had a single-space (33 cm) shelf-type wet-dry feeder (BA19100, Verba, Verbakel, The Netherlands) with inset nipple drinker and a supplementary bowl drinker (SS Drinker, Rotecna, Lleida, Spain). This feeder was used to feed the dry pelleted starter diet. A spiked rubber ball (Easyfix Luna 142, Easyfix, Galway, Ireland) was provided for each pen as environmental enrichment. At day 47 pw, pen groups were moved to finisher accommodation. Finisher pens had fully slatted concrete floors (2.4 × 4.2 m) with automatic environmental control. Each pen had one shelf-type single-space (33 cm) wet-dry feeder (MA19100, Verba) with inset

nipple drinker and a supplementary bowl drinker (SS Drinker, Rotecna). A wooden (larch) post was provided for each pen as environmental enrichment. In the weaner room, temperature was maintained at 28-30 °C in the first week and reduced by 2 °C per week to 22 °C in the 4th week and it remained at that temperature until 47 days pw. In the finisher rooms, temperature was maintained at 20-22°C.

In weaner and finisher rooms, ventilation was from a punched ceiling with air exhausted by a variable speed fan linked to a thermostat and was automatically controlled (Big Dutchman 135, Vetcha, Germany). Weaner and finisher rooms were equipped with windows for natural light. In addition, lighting was provided by tubular fluorescent lights from 0830 h to 1630 h.

5.3.5 Data recording and sampling

5.3.5.1 Pig growth performance and carcass data

Pigs were individually weighed at day 0 (weaning), and at day 5, 10, 20, 28, and 47 pw and prior to sale (~day 133 pw) using an electronic scale (EziWeigh 7i, O'Donovan Engineering, Coachford, Ireland). Pigs in each treatment group were slaughtered over 2 weeks when they reached the target slaughter weight of ~120 kg live weight (average age at slaughter 133 days pw). Pigs were fasted for 15-18 hours before weighing prior to slaughter. Feed disappearance was recorded on a pen basis between weaning and slaughter for the periods between which BWs were recorded (except at day 5 pw). These data were used to determine the average daily feed and milk replacer intake (ADFI) on a dry matter (DM) basis, average daily gain (ADG), and feed conversion ratio (FCR).

On the day of sale, pigs were transported 95 km to the abattoir (Dawn Pork & Bacon, Grannagh, Ireland) where they were killed by exsanguination after CO_2 stunning. At the abattoir, cold carcass weight of individual pigs was calculated by multiplying the hot carcass weight, recorded within 45 min of the pig being exsanguinated, by 0.98. Muscle depth and BF measured at 6 cm from the edge of the split back at the level of the third and fourth last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean meat content was calculated

according to the following formula(Department of Agriculture and Food and Rural Development, 2001):

$$CL=60.3 - 0.847x + 0.147y$$

Where, CL= carcass lean meat percentage, x = fat depth (mm); y = muscle depth (mm).

The following equations were used to determine parameters of interest relating to carcass growth (Lawlor and Lynch, 2005):

$$C_{ADG} = [(CW – WW \times 0.55) \times 1{,}000] / D1$$

$$C_{FCR}= FI/ C_{ADG}$$

$$L_{ADG}= (CW \times CL \times 10) / D2$$

Where, C_{ADG} = carcass ADG (from weaning to slaughter), CW = carcass weight in kg, WW = weaning weight in kg, D1 = number of days from weaning to slaughter, C_{FCR} = Carcass FCR, FI = daily feed intake (g), L_{ADG} = Lean ADG (from birth to slaughter), CL = carcass lean meat percentage and D2 = number of days from birth to slaughter.

5.3.5.2 Faecal scoring and medication usage

Faecal consistency scores on a pen basis were determined at day 1, 2, 5, 6, 10, 20 and 28 pw. A 4-grade score system (Casey *et al.*, 2007) was used and the average score from five pigs was determined as the average score for each pen. In brief: 0 = normal (dry pelleted faeces), 1 = soft (soft with shape), 2 = mild diarrhoea (very soft or viscous liquid) and 3 = severe watery diarrhoea (watery or with blood). The diarrhoea prevalence was estimated at each time point by considering a faecal score of 2 or greater for a pen as indicative of diarrhoea.

Antibiotic and anti-inflammatory usage was recorded in pigs until they reached target slaughter weight. Medication was administered when joint-ill, lameness, malaise or diarrhoea were observed by trained farm technicians. One antibiotic, Unicillin (Univet Limited, Cotehill, Ireland) and one anti-inflammatory, Loxicom (Norbrook, Newry, United Kingdom) only, were used during this experiment. Animal ID, pen number, product name, product code, dose administered (ml), frequency of administration, date

of administration, and reason for use were recorded when an animal was treated. From this, the total number of pig injections per pen, the average volume of medication (antibiotic and anti-inflammatory) administered per pig per pen, and the total number of clinical cases of disease (i.e. when an animal was treated one or more times for the same condition) per pen were calculated.

5.3.5.3 Live observations of feeding behaviour

The feeding behaviour of individual pigs was observed using 3-minute instantaneous scan sampling on day 1 and 4 pw. To enable the easy identification of pigs during scan sampling, pigs in each pen were individually marked with coloured spray markers on their backs (Duramark Long Term Marker Spray, Bandon, Ireland) on the day before scan sampling was conducted. Five 1-hour sessions were conducted between 0900 h and 1600 h on each scan sampling day. During each 1-hour session of live observations, each pen group was scanned every 3 minutes, leading to 21 scans/pen group/session. A simple ethogram was used for scoring feeding behaviour. At every scan, trough-directed activity was recorded (solid or liquid). The trough-directed activity was defined as a piglet snout being inserted into the solid feed trough or immersed in the liquid feed trough for at least 2 s. Observation of pigs sleeping with their snout in the feed trough were excluded from records as the pigs were not actively engaging with the feeder troughs. The percentage of eaters of dry pelleted feed in each pen was determined from the number of piglets in each pen which were observed to engage in dry feeder-directed activity even once during all of the sessions on that day. The percentage of observations that pigs engaged in solid and liquid feeder-directed activities was calculated for each individual piglet on day 1 and 4 and expressed on a per pig per pen basis. In this case, the total number of scans where an individual piglet was observed engaging with either the liquid feed trough or the single spaced wet-dry feeder on each day by the total number of scans on the day, then multiplying the result by 100 to express as a percentage.

5.3.5.4 Faecal sampling, euthanasia, blood and tissue sampling

Freshly voided faecal samples were collected from sixty piglets (15 piglets per treatment, 1 to 2 piglets per pen) on day 11, 47 and 124 pw into 50 mL sterile tubes

(Sarstedt, Nümbrecht, Germany) and transferred into 2 mL sterile tubes (Sarstedt) with sterile hockey loops (Scientific Laboratory Supplies Ltd, Nottingham, United Kingdom). These 2 mL tubes were immediately snap frozen in liquid nitrogen and stored at -80°C until analysis.

On day 7 pw, forty pigs (10 female piglets of even weight per treatment) were euthanized by captive bolt followed by immediate exsanguination. Whole blood was collected into Ethylene Diamine Tetra Acetic acid coated 10 mL tubes (Becton Dickinson, New Jersey, United States) and stored at room temperature for 7 to 9 h until haematological analysis. After euthanasia, the intestinal tract was removed and whole tissue samples (~2 cm) and mucosal strippings were collected from the duodenum (15 cm distal to the pyloric junction), jejunum (1.5 m distal to the pyloric junction) and ileum (15 cm proximal to the ileo-caecal junction). The whole tissue samples were carefully immersed in a 50 mL tube with NOTOXhisto™ fixative (Scientific Device Laboratory, Des Plaines, United States).The tubes was placed on a shaker for 48 hours after collection and stored at room temperature for 1 week until histological analysis. Mucosal strippings were immediately snap-frozen in liquid nitrogen and stored at -80°C for gene expression analyses.

5.3.6 Laboratory analyses

5.3.6.1 Haematology analyses

Haematological analysis was performed on whole blood using a DxH 500 Haematology Analyzer (Beckman Coulter Diagnostics Limited, O'Callaghans Mills, Co. Clare, Ireland) within 7 to 9 h of collection. The following parameters were measured: white blood cell count, red blood cell count, haemoglobin concentration, platelet count, neutrophil count, lymphocyte count, monocyte count, basophil count and eosinophil count.

5.3.6.2 Small intestinal histology

The whole tissue samples from the duodenum, jejunum and ileum were sent to Nationwide Laboratories, Devon, UK for histological slide preparation with

haemotoxylin and eosin staining. The resulting slides were used to study the gross morphological parameters of intestinal structure. For each sample, the villus height (VH) and crypt depth (CD) were measured as described previously by Crespo-Piazuelo et al. 2022 and from this the ratio of VH to CD was calculated.

5.3.6.3 16S rRNA gene sequencing of faecal microbiota

Total DNA was extracted from faecal samples using the Qiagen QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, apart from adding a bead beating step and increasing the lysis temperature to 95 °C, to increase DNA yield (Cullen *et al.*, 2022). The volume of buffer ATE was also reduced from 200 to 50 µl and it was allowed to sit on the filter membrane of the spin column for 5 min prior to centrifugation (both to maximise DNA concentration). Library preparation and sequencing were performed by Macrogen Inc. (Seoul, South Korea) after an initial DNA concentration check with the Invitrogen™ Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States) using a Qubit™ dsDNA Quantification Assay Kit (Thermo Fisher Scientific).

The 16S libraries were prepared according to the Illumina 16S Metagenomic Sequencing Library protocols to amplify the V3-V4 region of the 16S rRNA gene. The initial amplicon PCR mixture contained 5 ng of DNA, 5x reaction buffer, 1 mM dNTPs, 500 nM of each of the forward and reverse primers, and Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA, United States). The conditions for the initial PCR were 3 min at 95°C, followed by 25 cycles of 30 sec at 95°C, 30 sec at 55°C and 30 sec at 72°C, followed by a 5 min final extension at 72°C. The primer pair with Illumina adapter overhang sequences used for the initial PCR was as follows:

V3-F: 5'-
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3', V4-R: 5'-
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'.

The initial PCR products were purified with AMPure beads (Agencourt Bioscience, Beverly, MA, United States). Following purification, an index PCR was performed

with 2 µL of the initial PCR products for final library construction containing Nextera XT indices. The same PCR conditions were used as the initial PCR, except that the number of cycles was reduced to 10. The indexed PCR product was purified with AMPure beads and was quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA Library Quantification kits for Illumina Sequencing platforms) and library sizes were measured using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). Paired-end (2 × 300 bp) sequencing was performed using the MiSeq™ platform (Illumina, San Diego, CA, United States). 16S rRNA sequencing samples were processed in R (version 4.0.2) using DADA2 (version 1.20.0). Quality checks of both forward and reverse reads were carried out using FASTQC and optimal filtering and trimming parameters were identified using FIGARO v3.0. Primer removal and quality filtering and trimming of read pairs was carried out using the filterandTrim function in DADA2. Following dereplication and read pair merging an Amplicon Sequence Variant (ASV) table was constructed. Chimeric sequences from denoised data were subsequently removed. Taxonomic classification of ASVs was carried out using the SILVA taxonomic database (v.139.1). Sample metadata, sequence taxonomy, and ASVs were combined into a phyloseq object (version 1.34.0; McMurdie and Holmes, 2013). Potential contaminants were identified and removed using decontam v.1.12.0.

α-diversity index(Shannon) was estimated on rarefied ASV data using the phyloseq. β-diversity was assessed using the Aitchison distance metric based on clr-transformed compositions and visualized using a Principal Component Analysis (PCA) plot using the microviz package. Taxonomic relative abundances were plotted using the R package ggplot2. Model fitting and likelihood ratio testing for differential abundance between treatments was carried out using Corncob at the genus taxonomic level (available at github.com/bryandmartin/corncob). Significant compositional differences between treatments were investigated by permutational multivariate analysis of variance (PERMANOVA) using the Adonis function from the vegan package.

5.3.6.4 Gene expression analyses

5.3.6.4.1 RNA extraction and cDNA synthesis

Total RNA was extracted from jejunal mucosal strippings using the TRI Reagent (Sigma-Aldrich, St. Louis, MO, United States) according to the manufacturer's instructions. This crude RNA extract was further purified using the E.Z.N.A® Total RNA Kit I (Omega BIO-TEK, Norcross, GA, United States) which incorporated a DNase step using an on-column DNase 1 Digestion set (Sigma-Aldrich). Total RNA was quantified using a Nanodrop-ND1000 spectrophotometer (Thermo Fisher Scientific) and the purity was assessed from the ratio of the absorbance at 260 nm and 280 nm. All samples had a 260:280 ratio > 2.0. The total RNA (2 µg) was reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, United States) and oligo (dT) primers in a final reaction volume of 40 µl, according to the manufacturer's instructions. The cDNA was then adjusted to a volume of 360 µl with nuclease-free water.

5.3.6.4.2 Quantitative real-time Polymerase Chain Reaction (qPCR)

The quantitative PCR (qPCR) reaction mix (20 µl) contained GoTaq qPCR Master Mix (10 µl) (Promega, Madison, WI, United States), forward and reverse primers (1.2 µl) (5 µM), nuclease-free water (3.8 µl) and cDNA (5 µl). All qPCR reactions were performed in duplicate on the 7500 ABI Prism Sequence detection System (Applied Biosystems). The cycling conditions included a denaturation step of 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All primers were designed using the Primer Express Software (Applied Biosystems) and synthesised by MWG Biotech UK Ltd (Milton Keynes, United kingdom) and are listed in Table S 5-2. Normalised relative quantities were obtained using the qbase PLUS software (Biogazelle, Ghent, Belgium) from stable reference genes; *YWHAZ* and *H3F3A*. These genes were selected as reference genes based on their M value (< 1.5) generated by the GeNorm algorithm within GeNorm. The genes analysed in the current study were as follows: *SLC15A1* (previously known as *PEPT1*), *SLC1A4*, *SLC7A1*, *FABP2*, *SLC5A1* (previously known as *SGLT1*), *SLC2A1* (previously known as *GLUT1*),

SCL2A5 (previously known as *GLUT1*), *IL6*, *CXCL8*, *IL17*, *IL18*, *IL22*, *IL23*, *TNF*, *MUC1*, *MUC2*, *OCLN*, *TJP1*, *MMP1*, and *MMP3*.

5.3.7 Statistical analysis

All data except the prevalence of diarrhoea and gene expression were analysed in SAS using the linear mixed models procedure (PROC Mixed) in the Statistical Analysis Systems (SAS) software package version 9.4 (SAS Institute Inc., Cary, North Carolina, United States) for a two-by-two factorial arrangement. The prevalance of diarrhoea from weaning to 28 was analysed using the PROC Glimmix procedure of SAS with a binomial distribution. Gene expression was analysed using the PROC Glimmix procedure of SAS.

For the analysis of pw growth parameters, carcass quality data, live observations of feeding behaviour, diarrhoea prevalence and the average volume of medication (antibiotic and anti-inflammatory) administered per pig on a pen basis pw; milk replacer, glutamine and their associated interactions were included in the model as fixed effects. Block was included as a random effect and weaning weight was included as a co-variate, when significant in the model. Day was included as a repeated variable for the analysis of pw growth parameters. The pen was considered the experimental unit.

For analysis of haematology and intestinal morphology parameters, milk replacer, glutamine and their associated interactions were included in the model as fixed effects. The pen was included as a random effect and the pig was the experimental unit.

For analysis of gene expression, milk replacer was included in the model as a fixed effect and the distribution was adjusted to gaussian or poisson depending on the normality of the residuals. The pig was the experimental unit.

In all cases, differences in least square means were investigated using the t test after Tukey-Kramer adjustment for multiple comparisons. Results are presented in the text and tables as the least square means together with their pooled standard error. Differences between treatments were considered significant when $P \leq 0{\cdot}05$, whereas $0{\cdot}05 < P \leq 0{\cdot}10$ was considered as a tendency.

5.4 Results

5.4.1 Mortality and removals

Three percent of all pigs died or were removed from trial pw. Among these, 60% died or were removed between weaning and day 47 pw and 40% died or were removed after day 47 pw. Post-weaning mortality/removal was 1%, 3%, 5% and 5% for N-Milk/N-Glu, N-Milk/Glu, Milk/N-Glu, and Milk/Glu treatments, respectively. Deaths and removals of pigs were due in all cases to lameness or injury and therefore not considered to be treatment-related.

5.4.2 Growth performance and carcass data

There was no liquid milk replacer x L-glutamine interaction on any growth parameter of interest ($P>0.05$). There was no main effect of L-glutamine on any growth parameter of interest ($P>0.05$). Feed intake was 335 and 356 ± 12.2 g/d/pig ($P=0.26$) from day weaning to day 10 pw, ADG was 325 and 325 ± 11.8 g/d/pig from weaning to day 5 pw ($P=0.99$) and FCR was 0.99 and 0.98 ± 0.028 g/g ($P=0.85$) from weaning to day 10 pw for N-Glu and Glu, respectively. Additionally, L-glutamine did not influence any carcass parameter of interest. Consequently, only the main effect of liquid milk replacer supplementation on growth is presented in Table 5-2 and in Table 5-3 for carcass parameters. Liquid milk replacer supplementation increased pig weight at day 5 ($P=0.04$), 10 ($P<0.01$), 20 ($P<0.001$), 28 ($P<0.001$), and 47 ($P<0.001$) pw, at slaughter (~day 133 pw), and overall ($P<0.001$). Liquid milk replacer supplementation increased ADFI from day 0 to 10 pw ($P<0.001$), day 20 to 28 pw ($P<0.01$) and day 28 to 47 pw ($P<0.01$), and overall ($P<0.001$). It had no effect on ADFI from day 10 to 20 pw and from day 47 pw to slaughter ($P>0.05$). Liquid milk replacer supplementation increased ADG from day 0 to 5 pw ($P<0.001$), day 5 to 10 pw ($P=0.03$), day 20 to 28 pw ($P=0.01$) and overall ($P<0.001$). It had no effect on ADG from day 10 to 20 pw, day 28 to 47 pw, and day 47 pw to slaughter ($P>0.05$). Liquid milk replacer supplementation caused a deterioration in FCR from day 28 to 47 pw ($P=0.03$), and 47 pw to slaughter ($P=0.04$). It did not affect the FCR at any other time period. Liquid milk replacer supplementation increased cold carcass weight of pigs ($P=0.03$). Milk replacer supplementation had no effect on any other carcass parameters ($P>0.05$).

5.4.3 Faecal scoring, medication usage

There was no liquid milk replacer x L-glutamine interaction on diarrhoea prevalence from weaning to day 28 pw or on medication usage during the weaner period (P>0.05). The main effect of milk replacer supplementation on diarrhoea prevalence and medication usage is presented in Supplementary Table S 5-3 and the main effect of L-glutamine on diarrhoea prevalence and medication usage during the weaner period is shown in Supplementary Table S 5-4. Liquid milk replacer or L-glutamine did not influence diarrhoea prevalence from weaning to day 28 pw or medication usage during the weaner period (P>0.05).

5.4.4 Feeding behaviour

There was no liquid milk replacer x L-glutamine interaction on feeding behaviour at day 1 and 4 pw (P>0.05). There was no main effect of L-glutamine on feeding behaviour at day 1 and 4 pw (P>0.05). Consequently, only the main effect of liquid milk replacer supplementation on feeding behaviour is presented in Supplementary Table S 5-5. Liquid milk replacer supplementation reduced the percentage of individual pigs per pen observed engaging in solid feeder trough-directed activity in each pen at day 1 (1.7 vs 8.6 ± 0.46%, P<0.001) and 4 pw (2.8 vs 8.5 ± 0.36, P<0.001). Liquid milk replacer supplementation increased the percentage of individual pigs observed engaging in liquid feeder trough-directed activity in each pen at day 1 (5.1 vs 0 ± 0.21%, P<0.001) and 4 pw (4.8 vs 0 ± 0.32, P<0.001). Liquid milk replacer supplementation reduced the percentage of eaters of solid feed within pens at day 1 (68.3 vs 98.0 ± 3.86, P<0.001) and 4 pw (81.0 vs 99.2 ± 2.71, P<0.001).

5.4.5 Haematology

There was no liquid milk replacer x L-glutamine interaction for any of the haematology parameters measured at day 7 pw (P>0.05). The main effects of liquid milk replacer supplementation and L-glutamine on haematology parameters are shown in Supplementary Tables S 5-6 and S 5-7, respectively. Liquid milk replacer supplementation tended to increase the concentration of thrombocytes in the plasma (P=0.06). Liquid milk replacer supplementation had no effect on any other haematological parameter. L-glutamine increased the erythrocyte count (5.85 *vs* 5.01

± 0.210 $\times 10^6$ cells/μL, reference range: 4.87 - 7.88 $\times 10^6$ cells/μL; $P<0.01$) and haemoglobin concentration (10.62 *vs* 9.43 ± 0.387 g/dL, reference range: 8.08 – 11.9 g/dL; $P=0.04$) in the blood. L-glutamine had no effect on any other haematological parameter.

5.4.6 Small intestinal histology

There was no liquid milk replacer x L-glutamine interaction on small intestinal morphology ($P>0.05$). There was no main effect of L-glutamine on small intestinal morphology ($P>0.05$). The effect of liquid milk replacer supplementation on small intestinal parameters is shown in Table 5-4. Liquid milk replacer supplementation increased VH in the duodenum ($P=0.04$), jejunum ($P<0.001$) and ileum ($P<0.001$) of pigs at day 7 pw. It increased CD in the duodenum ($P=0.03$) and jejunum ($P=0.03$). It increased the VH:CD ratio in the jejunum ($P=0.01$) and ileum ($P<0.01$).

5.4.7 Faecal microbiota

5.4.7.1 Faecal microbial diversity

There was no effect of treatment on bacterial α-diversity in faecal samples at day 11, 49 and 124 pw, with Shannon diversity being similar for each treatment group ($P>0.05$; Fig. 5-1).

The β-diversity of the faecal microbiota is represented using principal component analysis (PCA) (Fig. 5-2). Overall, there were several clusters based on sampling time point/treatment. Faecal samples collected at day 11 pw, those collected at day 49 pw and those collected at day 124 pw clustered separately (Fig 5-2.a). Interestingly, faecal samples collected at day 11 pw from the N-Milk/N-Glu and N-Milk/Glu groups clustered separately to the Milk/N-Glu and Milk/Glu groups (Fig 5-2.b), demonstrating an effect of milk replacer supplementation. Faecal samples collected at day 49 and 124 pw did not appear to cluster by treatment (Fig 5-2.c and 5-2.d). Permutational ANOVA showed an effect of treatment on the overall faecal microbiota composition at day 11 pw ($P<0.01$). A post hoc pairwise Adonis test showed that both N-Milk/N-Glu and N-Milk/Glu treatments differed from Milk/N-Glu and Milk/Glu

treatments (P<0.01). However, no differences were observed between N-Milk/N-Glu and N-Milk/Glu (P>0.05) or Milk/N-Glu and Milk/Glu (P>0.05).

5.4.7.2 Faecal microbiota composition

A total of 15 bacterial phyla, 64 bacterial families and 186 bacterial genera were identified in faecal samples across all sampling time points, with *Bacteroidota* and *Firmicutes* being the predominant phyla. At the family level, *Prevotellaceae* was the most abundant, followed by *Lachnospiraceae* in both N-Milk/N-Glu and N-Milk/Glu groups, and by *Rikenellaceae* in both Milk/N-Glu and Milk/Glu groups. Compositional differences within the faecal microbiota between the N-Milk/N-Glu control group and each of the other treatments were examined at each time point. Only differences at family/genus level are outlined here. The only liquid milk replacer- and/or L-glutamine- mediated effects observed within the faecal microbiota were found at day 11 pw (Fig 5-3). At this time point, 8 genera were differentially abundant between the Milk/N-Glu and the N-Milk/N-Glu control. Nine genera were differentially abundant between the Milk/Glu and the N-Milk/N-Glu control. Seven of these differentially abundant genera were common to both the Milk/N-Glu and Milk/Glu treatments and had the same direction of effect (see Supplementary Tables S 5-8 and S 5-9). Four genera were differentially abundant between the N-Milk/Glu treatment and the N-Milk/N-Glu control (see Supplementary Table S 5-10).

For simplification purposes, only genera that were significantly different in relative abundance compared to the N-Milk/N-Glu control group with a relative abundance > 1% are detailed here. *Rikenellaceae* RC9 (P<0.01), *Paludibacteraceae* (P=0.02), and *Oscillaspiraceae UCG-002* (P=0.02) were more abundant in the Milk/N-Glu group than the N-Milk/N-Glu control group at day 11 pw, while *Agathobacter* (P<0.001) was less abundant. *Rikenellaceae* RC9 (P<0.01), *Oscillaspiraceae UCG-002* (P=0.02), *Paludibacteraceae* (P=0.02) and *Megasphaera* (P=0.01) were more abundant in the Milk/Glu group at day 11 pw, while *Agathobacter* (P<0.001) and *Anaerovibrio* (P=0.04) were less abundant. *Paludibacteraceae* (P=0.02) was more abundant in the N-Milk/Glu treatment at day 11 pw, while *Rikenellaceae* RC9 (P<0.01), *Prevotellaceae NK3B31* (P=0.03) and *Anaerovibrio* (P=0.04) were less abundant. No differentially abundant genera were identified at day 49 or 124 pw.

5.4.8 Gene expression in the jejunum

The expression of targeted genes related to nutrient digestion and absorption, mucosal barrier function and immunity in the jejunum of piglets at day 7 pw are presented in Table 5-5. The expression of Interleukin 17 (*IL17*; P=0.04), Interleukin 18 (*IL18*; P=0.02) and Interleukin 22 (*IL22*; P<0.01) were lower in pigs from the Milk/N-Glu group compared to pigs from the N-Milk/N-Glu control group. The expression of *SLC15A1* (peptide transporter 1; P=0.07) tended to be lower and that of *SLC7A1 (*solute carrier family 7 member 1*; P=0.05)* was lower in pigs from the Milk/N-Glu group compared to pigs from the N-Milk/N-Glu control. There was no treatment effect on the expression of any of the other targeted genes in the jejunum of pigs.

5.5 Discussion

To the authors' knowledge, this is the first study to examine the effect of pw supplemental milk replacer and/or dietary inclusion of 1% L-glutamine on pig intestinal morphology, growth to slaughter, faecal microbiome and the expression of genes related to nutrient digestion and absorption, mucosal barrier function and immunity in the jejunum.

5.5.1 Liquid milk replacer supplementation

In agreement with our hypothesis, supplementing liquid milk replacer for 10 days pw increased the ADFI of pigs by 85% and their ADG by 94% during this period compared to pigs only provided with the dry pelleted starter diet. This is in line with recent findings from our group, where Vasa *et al.* (2023) observed a 48% increase in feed intake and a 57% increase in ADG when pigs were supplemented with liquid milk replacer for 11 days pw. This increase in ADFI was mainly due to an increase in milk replacer intake, as pigs consumed this form of feed preferentially over dry pelleted starter diet when both were offered pw (~25% of the DM intake was from the starter diet and ~75% from the milk replacer powder). Offering liquid milk replacer to weaned pigs on a little and often basis, as was done in the current study, resembles more closely the supply of sow's milk during suckling in terms of nutrient content,

water content and frequency of delivery than a dry pelleted starter diet provided from a single spaced wet-dry feeder. This meant that piglets did not have to learn a new feeding pattern with a new feed form after weaning (Partridge and Gill, 1993; Brooks and Tsourgiannis, 2003) possibly alleviating the feed neophobia commonly experienced by piglets at weaning (Brooks and Tsourgiannis, 2003), thereby increasing early pw feed intake. The composition of the milk replacer powder may also explain the increased feed intake and growth of pigs to whom it was offered. The milk replacer powder fed in the current study contained ~40% lactose, almost twice that of the starter diet (~23%). It has been demonstrated that increasing levels of lactose in the diet leads to increased intake and growth in newly weaned pigs (Pierce *et al.*, 2005). Lactose is highly digestible at weaning as pigs still have high lactase activity (Zhao *et al.*, 2021) and are only developing their ability to secrete other carbohydrate-degrading enzymes. Furthermore, microbial fermentation of lactose to lactic acid in the stomach of newly weaned pigs aids in reducing gastric pH at a time when the piglet's ability to produce hydrochloric acid in the stomach is limited (Cranwell *et al.*, 1976). In addition, the milk replacer powder used contained porcine dried plasma, also well known to increase pw feed intake and growth in pigs(van Dijk *et al.*, 2001). Therefore, the dried plasma and high lactose content in the milk replacer likely contributed somewhat to the increased feed intake and growth observed for pigs supplemented with liquid milk replacer. In the current study, the pw increases in ADFI and ADG led to increased live weight at the end of the supplementation period. This benefit persisted to slaughter at ~day 133 pw when the carcass weight of pigs supplemented with liquid milk replacer was 2.6 kg heavier than those offered only dry pelleted starter diet during the early pw period. Similarly, Vasa *et al.* (2023) observed a numerical increase of 3.2 kg in carcass weight when pigs were supplemented with liquid milk replacer from weaning to day 11 pw.

The abrupt cessation of milk supply at weaning under commercial conditions is normally associated with rapid changes in intestinal structure with a reduction in VH and an increase in CD, due to the death of intestinal enterocytes as a result of reduced nutrient supply (Pluske *et al.*, 1997). In the current study, supplementing pigs with liquid milk replacer increased VH in all small intestinal segments and the VH:CD ratio in the jejunum and ileum. These results are in line with previous studies where piglets fed liquid cow's or ewe's milk on an ad libitum basis for 5 days pw had increased VH

in the small intestine compared to pigs fed dry starter diets (Pluske *et al.*, 1996a; Pluske *et al.*, 1996b) or to pigs where energy intake was restricted (Pluske *et al.*, 1996a).

As growth performance and intestinal morphology were not affected by L-glutamine supplementation, gene expression analysis was conducted for the N-Milk/N-Glu control *vs.* Milk/N-Glu treatment comparison only. Expression of the pro-inflammatory cytokine genes *IL17*, *IL18* and *IL22* was lower in the jejunum of pigs supplemented with liquid milk replacer compared to control pigs. Interleukin 17, IL18 and IL22, are potent mediators of intestinal inflammation and play an important role in protection against enterotoxigenic *E. coli* infections *(*Luo *et al., 2015).* The liquid milk replacer was comprised of fewer plant-based ingredients than the dry pelleted starter diet which possibly lessened associated intake of anti-nutritional factors, thereby lessening the potential damage to intestinal structure and the activation of an inflammatory response. In addition, in the current study, the high feed intakes obtained by feeding the liquid milk replacer were associated with longer villi in the small intestine, suggesting improved intestinal integrity early pw. Consequently, less disruption to the intestinal barrier could be expected with milk replacer feeding. This most likely decreased the passage of antigens from the intestinal lumen to the lamina propria, thereby lowering the production of pro-inflammatory cytokines and other inflammatory mediators produced when immune cells are activated (King *et al.*, 2013). In addition, supplementing pigs with liquid milk replacer pw tended to decrease the expression of the di- and tri-peptide transporter *SLC15A1* and decreased the expression of the amino acid transporter *SLC7A1*. This could suggest that there was decreased availability and transport of small peptides and amino acids in the jejunum of pigs supplemented with liquid milk replacer compared to pigs fed only a dry starter diet. However, studies show that *SLC15A1* is up-regulated during intestinal inflammation (Kovacs-Nolan *et al.*, 2012) and *SLC7A1* is expressed in cases of T-cell activation (Hayashi and Anzai, 2022). Taken together, these results suggest a lower level of intestinal inflammation in liquid milk replacer-supplemented pigs. Intestinal inflammation causes an increase in energy and nutrient demand for maintenance, reducing that available for growth (Williams *et al.*, 1997). Therefore, the reduced intestinal inflammation observed due to liquid milk replacer supplementation likely contributed in some part to the increased growth observed pw.

The abrupt change of substrate available for the microbiota at weaning (e.g. liquid sow's milk *vs.* dry pelleted plant-based diet) often results in a shift in microbiota composition (St-Pierre *et al.*, 2023). The results of the current study show that liquid milk replacer supplementation with or without L-glutamine supplementation for 10 days pw did not influence the microbial richness or evenness of the bacterial communities within the pigs' faeces. However, there was a clear difference in terms of faecal microbial community composition at day 11 pw (i.e. at the end of the supplementation period) between pigs supplemented with liquid milk replacer and those which were not. However, this difference was only transient, as there were no bacterial community differences at day 49 or 124 pw. At day 11, the differences in microbial community composition of liquid milk replacer-supplemented pigs were mainly driven by higher relative abundances of *Rikenellaceae RC9* and *Oscillospiraceae UCG 002*. The family *Rikenellaceae* is known to ferment glucose, melibiose, mannose and lactose with the resultant production of organic acids such as propionic acid and succinic acid (Graf, 2014). The higher content of lactose in the liquid milk replacer compared to the dry pelleted starter diet most likely explains the higher abundance of *Rikenellaceae* in the faeces of liquid milk replacer-supplemented pigs. *Rikenellaceae RC9* was previously found to be positively correlated with the core bacteria of non-diarrheic piglets (Sun *et al.*, 2019). The *Rikenellaceae* family produces hydrogen, which plays a key role in neutralizing cytotoxic reactive oxygen species, thereby protecting intestinal cells from oxidative stress and therefore reducing inflammation (Chen *et al.*, 2011). The *Oscillospiraceae* family is a producer of valeric acid and butyrate (Chen *et al.*, 2021; Yang *et al.*, 2021) and these short chain fatty acids (SCFAs) play an important role in limiting inflammation and improving intestinal health (Liu, 2015).

However, although the positive results for pig growth and intestinal health obtained in the current study demonstrate the potential of supplementing weaned pigs with a liquid diet pw, the use of milk replacer powder is expensive and should be carefully considered. The feed cost during the 10-day supplementation period in the current study was ~€3 per pig for the dry pelleted starter diet compared to ~€12 per pig for the dry pelleted starter diet supplemented with liquid milk replacer. This does not include the higher capital and operating costs associated with the liquid (milk) feeding system. In addition, no reduction in medication usage was observed in this study. In general,

medication usage was low, with very few incidences of antibiotic and/or anti-inflammatory treatment reported. The experimental pig facility, where this experiment was performed, had a high health status and observed strict internal and external biosecurity. Consequently, it would be expected to have very low levels of disease compared with commercial farms

5.5.2 L-glutamine supplementation

Contrary to our hypothesis, dietary inclusion of 1% L-glutamine in the dry pelleted starter diet and/or liquid milk replacer did not improve intestinal morphology or pig growth. In contrast to the results of the present study, it was previously shown that increasing glutamine inclusion in the diet (up to 1%) tended to increase ADG in weaned pigs (Duttlinger *et al.*, 2020; Zou *et al.*, 2006). Supplementing weaned pigs with L-glutamine at 1% in the diet from weaning to day 14 pw in another study was also shown to benefit small intestinal structure at day 7 pw and feed efficiency during the 2nd week of supplementation (Wu *et al.*, 1996).

Inconsistencies between the results of the present study and those from previous studies could be due to differences in diet composition or differences in feed intake. The basal dry pelleted starter diet and milk replacer powder fed in the current study contained high background levels of total glutamic acid (3.3% and 4.2%, respectively) and the ADFI was ~335 g/pig/day from weaning to day 10 pw. Based on the NRC (2012) amino acid values, the background total glutamic acid level of the diet fed in the study conducted by Wu *et al.* (1996) was estimated at ~3.6%. Although this background total glutamic acid level was similar to that in the current study, the ADFI from weaning to day 7 pw (~190 g/pig/day) was much lower in the Wu et al study (National Research Council, 2012). Similarly, the control diet fed in the study of Duttlinger *et al.* (2020) had a background total glutamic acid content (3.5%) similar to that of the current study. However, the pigs in that study also had a much lower post-weaning ADFI (273 g/pig/day from weaning to day 14 pw) than that obtained in the current study. Consequently, pigs in the Wu *et al.* (1996) and Duttlinger *et al.* (2020)studies received 6.8 g and 9.6 g of total glutamic acid per day, respectively from the basal diets. These values are below the 11.1 and 14.0 g of total glutamic acid contained in the basal dry pelleted starter diet and milk replacer powder, respectively

fed in the current study. Therefore, it is likely that the pigs from the present study already received sufficient total glutamic acid from the dry pelleted starter diet and/or milk replacer powder and so did not benefit from the additional L-glutamine added to the diet to help maintain intestinal barrier integrity and function. It is also possible that the proteins contained in the milk-based compounds included in the dry pelleted starter diet fed in our study were more digestible for the pig at this age than proteins from corn and soybean meal, which were the main ingredients of the diet fed in the study conducted by Wu *et al.* (1996).

The age of the pigs at weaning might also help to explain the lack of response to dietary inclusion of L-glutamine in the present study. In the studies conducted by Wu *et al.* (1996) and Duttlinger *et al.* (2020), pigs were weaned at 21 days of age, but in the current study pigs were weaned at 28 days. It has been demonstrated that pigs weaned at 3 weeks of age have a more poorly developed digestive tract compared to those weaned at 4 weeks of age (Colson *et al.*, 2006). It is likely that the benefit of L-glutamine supplementation would be greater when pigs are weaned at 21 days of age compared to 28 days.

In addition, glutamine is used at high rates by immune cells, such as macrophages, neutrophils and lymphocytes under situations of stress, such as weaning (Cruzat *et al.*, 2018). In the current study, sanitary conditions were good, as evidenced by low medication usage and the low mortality rate (i.e. <3%). The immune system may therefore not have been sufficiently challenged to benefit from the L-glutamine added to the dry pelleted starter diet or milk replacer in the current study.

In summary, it is likely that the level of L-glutamine included in pig diets should be considered on a case by case basis, paying particular attention to weaning age, feed intake, diet composition and health status of pigs. We could speculate that the 1% L-glutamine added to the dry pelleted starter diet and/or the milk replacer powder fed in the current study was too high compared to the needs to of the pigs.

Supplementing pigs with 1% L-glutamine in the current study did however, increase erythrocyte counts in the blood, as well as haemoglobin concentrations. Dumaswala *et al.* (1994) previously found that L-glutamine is important for the preservation of red blood cells, as it plays an important role in the regulation of oxidative stress and hence its use is of interest in the case of red blood cell disorders such as sickle cell anemia(Elenga *et al.*, 2022). This increased erythrocyte count and haemoglobin

concentration in the blood demonstrates that the pig used some of the supplemented L-glutamine added to the dry pelleted starter diet/milk replacer. Nonetheless, these haematological parameters were both within the range of normal values for weaned pigs in pigs both supplemented and not supplemented with glutamine.

Dietary inclusion of 1% L-glutamine in the dry pelleted starter diet and/or liquid milk replacer did not have a major impact on the faecal microbial composition of pigs, as no differences in α or β diversity were found between the N-Milk/N-Glu control treatment and the N-Milk/Glu treatment, and between the Milk/N-Glu and Milk/Glu. In vitro studies have shown that up to 40% of dietary glutamine can be utilized by bacteria in the jejunum and ileum (Dai *et al.*, 2010). Although not assessed, bacterial utilisation of L-glutamine in the small intestine could at least in part explain the absence of an intestinal structure and growth response to dietary inclusion of 1% L-glutamine in the current study. Only minor changes in bacterial composition at the genus level were observed in the faeces of pigs supplemented with glutamine, and then only at day 11 post-weaning. Pigs from the N-Milk/Glu group had decreased faecal abundance of *Rikenellaceae RC9* and *Prevotellaceae NK3B31* compared to the N-Milk/N-Glu treatments. As previously stated, bacteria belonging to the *Rikenellaceae* family are carbohydrate fermenters which can result in the production of SCFAs and medium chain fatty acids (MCFAs)(Graf, 2014). *Prevotellaceae* is one of the major fibre-degrading bacterial families in the pig intestine, which has been positively associated with increased concentrations of acetic acid, propionic acid and total SCFAs in the colonic digesta of weaned pigs (Jiang *et al.*, 2020). In addition, the abundance of *Anaerovibrio* was decreased in both L-glutamine treatments (N-Milk/Glu and Milk/Glu) compared to the N-Milk/N-Glu control. *Anaerovibrio* is a lipolytic bacterium, which hydrolyses triglycerides to produce glycerol and fatty acids, and ferments the resultant glycerol to produce SCFAs and MCFAs (Schauder and Schink, 1989). Overall, these data suggest that dietary inclusion of L-glutamine in the current study reduced the abundance of beneficial bacterial groups in the faeces. This may have contributed to the lack of growth and health benefits observed with this strategy. The abundance of *Paludibacteraceae* was however, increased in the N-Milk/Glu compared to the N-Milk/N-Glu control, suggesting that inclusion of glutamine in the dry pelleted starter diet increased its abundance. *Paludibacteraceae* was previously shown to increase in the faeces of pigs fed low protein diets [14%

crude protein (CP)] supplemented with artificial branched chain amino acids (Spring *et al.*, 2020). However, the dry pelleted starter diet fed in the current study was high in protein (~19% CP) and glutamine is an aromatic amino acid. Overall, although some shifts in faecal bacterial composition were observed in the current study as a result of dietary inclusion of glutamine, it is difficult to explain the mechanism(s) by which they occurred.

5.6 Conclusions

Dietary inclusion of 1% L-Glutamine had no effect on the intestinal morphology of pigs at day 7 pw and did not benefit pig growth, although it did have some impact on faecal microbiota composition, which was not sustained. On the other hand, the provision of liquid milk replacer for 10 days post-weaning increased feed intake during the period of supplementation. This increased body weight early pw and this weight advantage was maintained and even increased at d133 pw when the carcasses of liquid milk replacer-supplemented pigs were 2.6 kg heavier. Increased early post-weaning feed intake, improved intestinal morphology, increased faecal abundance of potentially beneficial bacteria and reduced expression of genes linked to intestinal inflammation at day 7 pw all help to explain the observed lifetime benefit of post-weaning liquid milk replacer supplementation. In summary, supplementing weaned pigs with liquid milk replacer for 10 days pw increased early post-weaning growth, culminating in increased carcass weight at slaughter; however, the high cost of milk replacer means that even with these benefits, the practice is not currently cost-beneficial.

5.7 Tables and figures

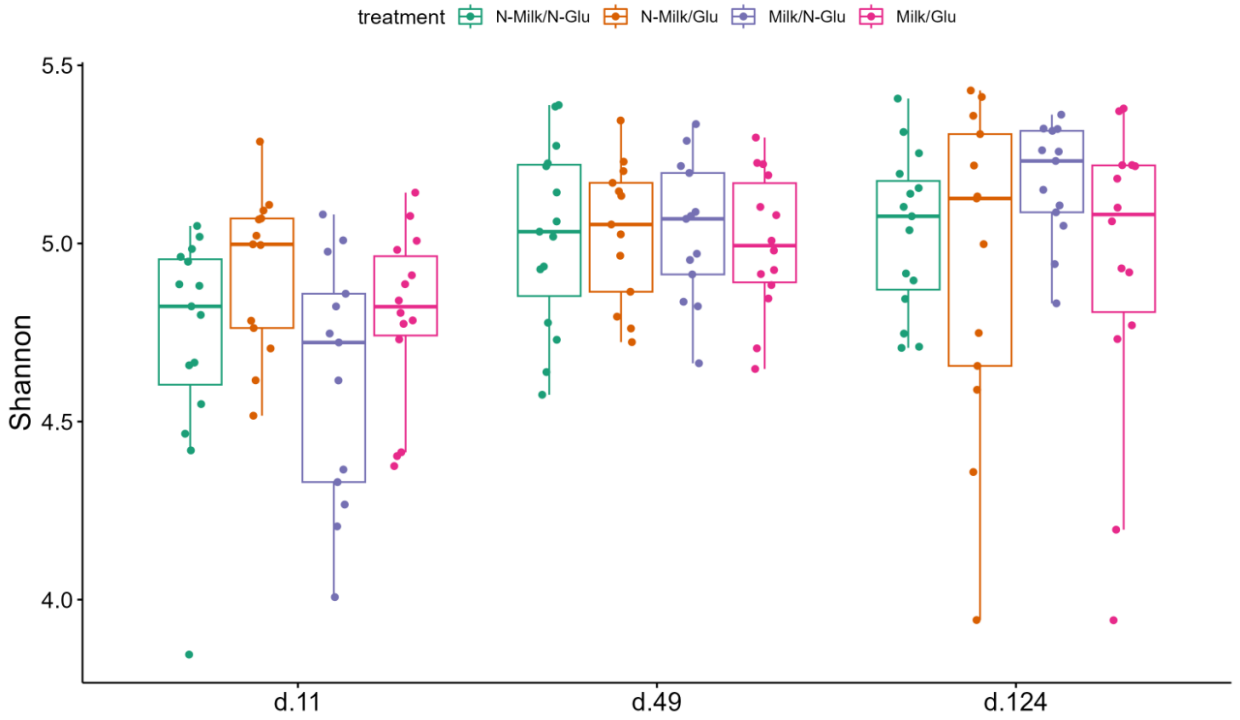

Figure 5-1. Effect of liquid milk replacer supplementation and/or dietary inclusion of 1% L-Glutamine on Shannon α-diversity of the microbiota in pig faeces at all sampling time points. Colours indicate the treatments from D0-10 post-weaning as follows : N-Milk/N-Glu, control diet; dry pelleted starter diet; N-Milk/Glu, control diet with dietary inclusion of 1% L-glutamine; Milk/N-Glu, control diet plus supplemental liquid milk

replacer; Milk/Glu, control diet with dietary inclusion of 1% L-glutamine plus supplemental liquid milk replacer with dietary inclusion of 1% L-glutamine.

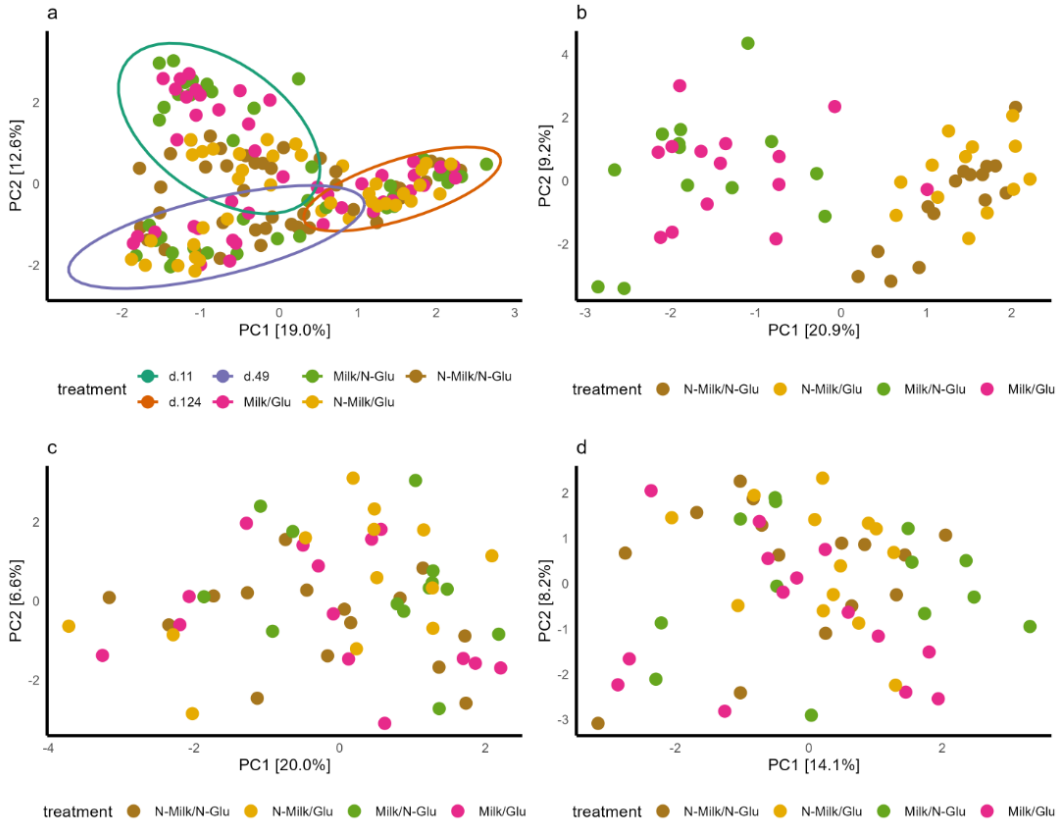

Figure 5-2. (a) Principal component analysis plot of the aitchison distances based on clr-transformed compositions of pig faecal microbiota estimated for all four treatments across all sampling time points. (b-d) PCA plots of the intergroup variation for amplicon sequence variant (ASV) representation at the genus level of faecal microbiota of N-Milk/N-Glu, N-Milk/Glu, Milk/N-Glu and Milk/Glu during the 3 time points post-

weaning (pw): (b) day 11 pw, (c) day 49 pw and (d) day 124 pw. Coloured ellipses indicate the day pw that pigs were sampled. Colours indicate the treatments from D0-10 post-weaning as follows: N-Milk/N-Glu, control diet; dry pelleted starter diet; N-Milk/Glu, control diet with dietary inclusion of 1% L-glutamine; Milk/N-Glu, control diet plus supplemental liquid milk replacer; Milk/Glu, control diet with dietary inclusion of 1% L-glutamine plus supplemental liquid milk replacer with dietary inclusion of 1% L-glutamine.

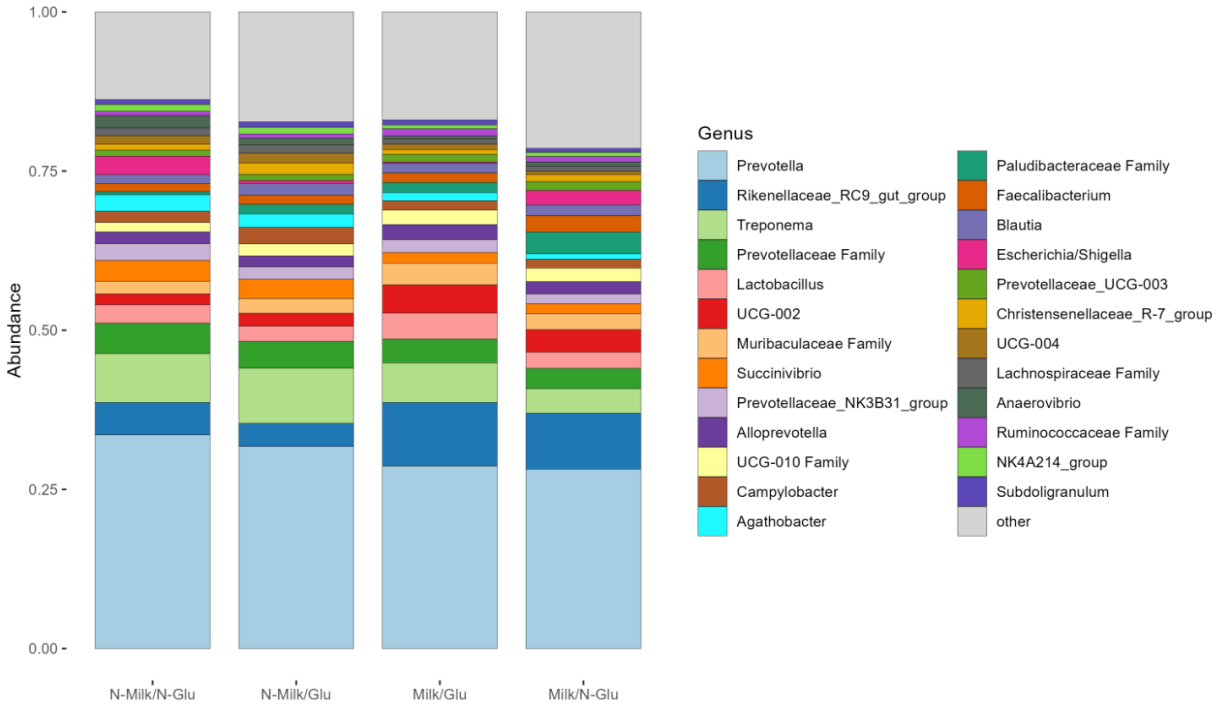

Figure 5-3. The effect of liquid milk replacer supplementation and/or dietary inclusion of 1% L-Glutamine on the mean relative abundance of the 20 most abundant bacterial genera in the faeces of pigs at day 11 post-weaning. Treatments from D0-10 post-weaning are as follows: N-Milk/N-Glu, control diet; dry pelleted starter diet; N-Milk/Glu, control diet with dietary inclusion of 1% L-glutamine; Milk/N-Glu, control diet plus supplemental liquid milk replacer; Milk/Glu, control diet with dietary inclusion of 1% L-glutamine plus supplemental liquid milk replacer with dietary inclusion of 1% L-glutamine.

Table 5-1. Composition of the experimental diet (on an air-dry basis; kg/tonne).

Ingredient	Diet specifications				
	Starter[1]	Starter + L-glutamine[1]	Link[1]	Weaner[1]	Finisher[1]
Barley	50.0	49.5	68.4	495.9	410.5
Wheat	0	0	100.0	216.9	390.0
Maize	231.0	228.7	300.0	0	0
Soybean meal	143.4	142.0	186.9	163.2	165.0
Soya full fat	130.8	129.5	70.0	50.0	0
Skim milk powder	125.0	123.8	50.0	0	0
Whey permeate	200.0	198.0	150.0	0	0
Soya oil	85.0	84.2	38.2	40.0	11.0
Lysine HCl	6.2	6.1	6.7	5.9	4.3
DL-Methionine	3.6	3.6	3.2	2.2	1.0
L-Threonine	3.6	3.6	3.4	2.7	1.9
L-Tryptophan	1.4	1.4	1.3	0.6	0.2
L-Valine	1.3	1.3	1.3	0.6	0
L-Glutamine	0	10.0	0	0	0
Vitamin and mineral mix	3.0[a]	3.0[a]	3.0[a]	3.0[a]	1.0[b]
Ronozyme HiPhos GT2	0.1	0.1	0.1	0.1	0.1
Salt	3.0	3.0	3.0	3.0	3.0
Mono di-calcium phosphate	5.5	5.4	7.0	5.5	1.0
Limestone flour	7.0	6.9	7.5	10.5	11.0
Chemical composition					
Dry matter[3]	914.3	918.0	904.0	890.5	880.0
Crude protein[3]	191.3	194.0	174.0	162.5	163.0
Ash[3]	55.5	53.0	51.0	44.0	46.5
Fat[3]	129.2	120.0	83.4	74.8	36.6
Crude fibre[3]	16.3	16.0	19.5	31.0	33.5

Total glutamic acid[3]	33.4	42.3	32.4	39.1	34.9
Neutral-detergent fibre[4]	60.5	60.5	80.8	140.0	140.2
Lysine[4]	16.2	16.2	15.00	12.99	10.9
Methionine[4]	7.0	7.0	6.1	4.7	3.4
Methionine+Cysteine[4]	9.8	9.8	9.1	7.9	6.6
Threonine[4]	10.9	10.9	10.1	8.8	7.6
Tryptophan[4]	3.7	3.7	3.4	2.7	2.2
Standardised ileal digestible lysine[4]	15.3	15.3	14.1	12.0	10.0
Ca[4]	8.2	8.2	7.5	7.4	6.5
Digestible P[4]	4.6	4.6	4.2	3.3	2.5
Digestible energy[4] (MJ/kg)	16.20	16.20	15.00	14.27	13.76
Net energy[4] (MJ/kg)	12.01	12.01	10.94	10.30	9.80

[1] The starter diet was fed dry as a 3 mm pellet to all treatment groups. Pelleted link diet was provided from day 11 to day 20 pw followed by pelleted weaner diet until transfer to the finisher room at day 47 pw. Pelleted finisher diet was provided from day 47 pw until slaughter.

[2] Ronozyme HiPhos GT (Inform Nutrition, Whites Cross, Ireland) was included to provide 500g phytase units (FYT) in each diet.

[a] Premix provided per kg of complete diet: Cu, 85 mg; Fe, 90 mg; Mn, 47 mg; Zn, 120 mg; I, 0·6 mg; Se, 0·3 mg; vitamin A, 2064 µg; vitamin D3, 25 µg; vitamin E, 100 mg; vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B1, 2 mg; vitamin B6, 3 mg.

[b] Premix provided per kg of complete diet: Cu, 15 mg; Fe, 24 mg; Mn, 31 mg; Zn, 80 mg; I, 0·3 mg; Se, 0·2 mg; vitamin A, 688 µg; vitamin D3, 12·5 µg; vitamin E, 40 mg; vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B1, 2 mg; vitamin B6, 3 mg.

[3] Analysed composition

[4] Calculated composition

Table 5-2. Effect of liquid milk replacer supplementation of weaned pigs on post-weaning pig growth and feed intake from weaning to slaughter.

Milk supplementation	N-Milk	Milk	SEM	*P*-value
Number of pens	24	24		
BW[1], kg				
Day 0 (weaning)	8.7	8.7	0.56	0.99
Day 5 post-weaning	9.6	11.1	0.56	0.04
Day 10 post-weaning	11.4	13.8	0.58	<0.01
Day 20 post-weaning	14.7	17.2	0.58	<0.001
Day 28 post-weaning	19.9	23.0	0.58	<0.001
Day 47 post-weaning	36.2	40.2	0.58	<0.001
Day of slaughter (day 133 post-weaning)	136.7	140.0	1.08	0.04
Overall			0.36	<0.001
ADFI[2], g of DM/pig/day				
Day 0 to 10	242	447	12.2	<0.001
Day 10 to 20	523	539	10.5	0.28
Day 20 to 28	668	747	16.1	<0.01
Day 28 to 47	1010	1105	21.0	<0.01
Day 47 to slaughter	2152	2202	22.3	0.12
Overall			9.5	<0.001
ADG[3], g/pig/day				
Day 0 to 5	176	475	11.9	<0.001
Day 5 to 10	349	410	18.5	0.03
Day 10 to 20	384	402	12.9	0.32
Day 20 to 28	641	702	16.4	0.01
Day 28 to 47	838	861	11.6	0.15
Day 47 to slaughter	1166	1145	16.2	0.38
Overall			8.4	<0.001
FCR[4], g of DM/g				
Day 0 to 10	1.00	0.97	0.028	0.42
Day 10 to 20	1.39	1.35	0.028	0.51
Day 20 to 28	1.05	1.07	0.028	0.61
Day 28 to 47	1.20	1.29	0.028	0.03

Milk supplementation	N-Milk	Milk	SEM	*P*-value
Day 47 to slaughter	1.85	1.93	0.028	0.04
Overall			0.014	0.14

[1]BW, body weight.
[2]ADFI, average daily feed intake.
[3]ADG, average daily gain.
[4]FCR, feed conversion ratio.
Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
Treatments from D0-10 post-weaning were as follows: N-Milk, dry pelleted starter diet; Milk, dry pelleted starter diet plus supplemental liquid milk replacer.

Table 5-3. Effect of liquid milk replacer supplementation of weaned pigs on pig carcass parameters from weaning to slaughter.

Milk supplementation	**N-Milk**	**Milk**	**SEM**	***P*-value**
Number of pens	24	24		
Cold carcass weight, kg	104.4	107.0	0.68	<0.01
Fat depth, mm	15.7	15.6	0.20	0.71
Muscle depth, mm	58.9	60.0	0.89	0.38
Lean meat, %	57.3	57.5	0.13	0.34
Kill out, %	76.4	76.6	0.01	0.48
Carcass ADG, g/d[1,2]	747	762	6.8	0.13
Carcass FCR, g/g[3,4]	2.19	2.17	0.028	0.62
Lean ADG, g/d[5]	694	710	8.3	0.16

[1]ADG, average daily gain.
[2]Carcass ADG (from weaning post weaning to slaughter) = [(carcass weight in kg – day 47 weight in kg × 0.55) × 1,000]/number of days from weaning to slaughter (Lawlor and Lynch, 2005).
[3]FCR, feed conversion ratio.
[4]Carcass FCR (from weaning post-weaning to slaughter) was calculated as follows: carcass FCR = daily feed intake (g)/carcass ADG (g).
[5]Lean ADG (from birth to slaughter) = (carcass weight × carcass lean meat percentage × 10)/number of days to slaughter (Lawlor and Lynch, 2005).
Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
Treatments from D0-10 post-weaning were as follows: N-Milk, dry pelleted starter diet; Milk, dry pelleted starter diet plus supplemental liquid milk replacer.

Table 5-4. Effect of liquid milk replacer supplementation of weaned pigs on morphology of the small intestine of pigs at 7 days post-weaning.

Milk supplementation	N-Milk	Milk	SEM	*P*-value
Number of pigs	20	20		
Duodenum				
Villus height (μm)	326	368	14.3	0.04
Crypt depth (μm)	159	179	6.3	0.03
VH:CD[1] ratio	2.2	2.3	0.10	0.66
Jejunum				
Villus height (μm)	287	396	18.7	<0.001
Crypt depth (μm)	168	187	5.7	0.03
VH:CD ratio	2.0	2.4	0.11	0.01
Ileum				
Villus height (μm)	232	300	11.4	<0.001
Crypt depth (μm)	183	196	5.6	0.11
VH:CD ratio	1.4	1.7	0.07	<0.01

[1]VH:CD, villus height : crypt depth.
Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq .0.10$.
Treatments from D0-10 post-weaning were as follows: N-Milk, dry pelleted starter diet; Milk, dry pelleted starter diet plus supplemental liquid milk replacer.

Table 5-5. The effects of liquid milk replacer supplementation of weaned pigs on the expression of nutrient transporter, immune marker and tight junction protein genes in the jejunum of pigs at 7 days post-weaning.

Milk supplementation	**Gene**[1]	**N-Milk/** N-Glu	**Milk/** N-Glu	**SEM**	***P*-value**
Number of pigs		10	10		
Nutrient transporters	*SCL2A1*	0.80	1.49	0.334	0.17
	SCL2A5	0.84	1.45	0.335	0.23
	SLC5A1	0.99	1.18	0.183	0.38
	FABP2	1.08	0.99	0.112	0.50
	SLC1A4	1.01	1.15	0.177	0.49
	SLC7A1	1.18	0.92	0.117	0.05
	SLC15A1	1.26	0.90	0.169	0.07
Tight junction proteins and immune markers	*TJP1*	0.95	1.07	0.318	0.80
	OCLN	0.96	1.06	0.318	0.83
	MMP1	1.27	0.93	0.330	0.479
	MMP3	1.27	0.96	0.333	0.53
	MUC1	1.14	0.98	0.141	0.32

Milk supplementation	Gene[1]	N-Milk/ N-Glu	Milk/ N-Glu	SEM	*P*-value
	MUC2	0.99	1.08	0.321	0.84
	IL6	1.39	0.85	0.332	0.27
	IL17	2.39	1.08	0.409	0.04
	CXCL8	1.21	1.06	0.337	0.77
	IL18	1.16	0.90	0.090	0.02
	IL22	4.04	1.27	0.496	<0.01
	IL23	1.01	1.20	0.204	0.43
	TNF	1.13	0.98	0.130	0.31

[1]*SLC2A1/GLUT1*, glucose transporter 1; *SCL2A5/GLUT5*, glucose transporter 5; *SLC5A1/SGLT1*, solute carrier family 5 member 1; *FABP2*, fatty acid binding protein; *SLC1A4*; solute carrier family 1 member 4; *SLC7A1*, solute carrier family 7 member 1; *SLC15A1/PEPT1*, peptide transporter 1; *TJP1*; tight junction protein 1; *OCLN*, occludin; *MMP1*, Matrix metalloproteinase 1; *MMP3*, Matrix metalloproteinase 3; *MUC1*, Mucin 1 ; *MUC2*, Mucin 2; *IL6*, interleukin 6; *IL17*, interleukin 17; *CXCL8*, interleukin 8; *IL18*, interleukin 18; *IL22*, interleukin 22; *IL23*, interleukin 23; *TNF*, tumour necrosis factor. Probability values at $P \leq 0{\cdot}05$ are considered significant and as tendencies at $P \leq 0{\cdot}10$. Treatments from D0-10 post-weaning were as follows: N-Milk/N-Glu, control diet; dry pelleted starter diet and Milk/N-Glu, control diet plus supplemental liquid milk replacer.

5.8 Supplementary tables and figures

Table S 5-1. Analysed chemical composition of milk replacer powders (on an air-dry basis; percentage unless otherwise stated).

Analysed chemical composition	Milk replacer powder	Milk replacer powder + L-glutamine
Dry matter	96.30	96.10
Crude protein	22.40	24.80
Ash	6.60	6.50
Fat	8.92	8.59
Crude fibre	1.00	1.00
Total glutamic acid	4.15	5.39

Table S 5-2. Panel of oligonucleotide primers used for qPCR.

Target gene	Accession no.	Forward (F) primer (5'-3') Reverse (R) primer (5'-3')	Amplicon length (bp)
Nutrient transporters			
FABP2	NM_001031780.1	F:CAG CCT CGC AGA CGG AAC TGAA R:GTG TTC TGG GCT GTG CTC CAAGA	102
SLC2A1 (GLUT1)	XM_003482115.1	F:TGC TCA TCA ACC GCA ATG A R:GTT CCG CGC AGC TTC TTC	72
SLC2A5 (GLUT 5)	EU012359	F: CCC AGG AGC CGG TCAA G R: TCA GCG TCG CCA AAG CA	60
SLC5A10 (SGLT1)	NM_001164021.1	F: GGC TGG ACG AAG TAT GGT GT R: ACA ACC ACC CAA ATC AGA GC	153
SLC1A4 (ASCT1)	XM_003125088	F: ACC CTC GCC GAC TTT TAG TCT R: GCC TGT GCC GAG AAG TAA TCC	71
SLC7A1 (CAT1)	NM_001012613	F: TCT CAT CCT AAC GGG ACT TTT AAC TC R: GAC CAG AAC GTT GAT ACA CGT GAA	85
SLC15A1(PEPT1)	NM_214347.1	F:GGA TAG CCT GTA CCC CAA GCT R:CAT CCT CCA CGT GCT TCT TGA	73
Tight junctions and immune system			
MUC1	XM_001926883.1	F: ACA CCC ATG GGC GCT ATG T R: GCC TGC AGA AAC CTG CTC AT	68
MUC2	AK231524	F: CAA CGG CCT CTC CTT CTC TGT R: GCC ACA CTG GCC CTT TGT	70

Target gene	Accession no.	Forward (F) primer (5’-3’) Reverse (R) primer (5’-3’)	Amplicon length (bp)
OCLN	NM_001163647.2	F: GGA CCT CAG GCA GCC TCA T R: CGG GAG CCC GTT TTG AA	65
TJP1	XM_021098896.1	F: TGA GAG CCA ACC ATG TCT TGA A R: CTC AGA CCC GGC TCT CTG TCT	76
MMP1	NM_001166229.1	F: GGA CCG TGC CAT TGA GAA R: CCT CGG AGA CCT TGG TGA AC	74
MMP3	NM_001166308.1	F: GCCCACAGAATCTACACCTCAGA R: CGAAGGACAAAGCAGGATCAC	65
IL6	NM_214399.1	F: GAC AAA GCC ACC ACC CCT AA R: CTC GTT CTG TGA CTG CAG CTT ATC	69
IL17	NM_001005729.1	F: CCC TGT CAC TGC TGC TTC TG R: TCA TGA TTC CCG CCT TCA C	57
IL8	NM_213867.1	F: TGC ACT TAC TCT TGC CAG AAC TG R: CAA ACT GGC TGT TGC CTT CTT	82
IL18	NM_213997.1	F: ACGACCAAGTCCTTTTCATTAACC R: TGAGGTGCATTATCTGAACAGTCA	85
IL22	XM_001926156.1	F: GAT GAG AGA GCG CTG CTA CCT GG R: GAA GGA CGC CAC CTC CTG CAT GT	112
IL23	NM_001130236.1	F: AGG GAC TCA GGG ACA ACA GTC R: GCG AAG GAT CTT GAG GCG GAG AAG GAG	263
TNF	NM_214022.1	F: TGG CCC CTT GAG CATCA R: CGG GCT TAT CTG AGG TTT GAGA	68

Target gene	Accession no.	Forward (F) primer (5’-3’) Reverse (R) primer (5’-3’)	Amplicon length (bp)
Reference genes			
YWHAZ	XM_001927228.1	F: GGA CAT CGG ATA CCC AAG GA R: AAG TTG GAA GGC CGG TTA ATT T	71
H3F3A	NM_213930.1	F: CAT GGC TCG TAC AAA GCA GA R: ACC AGG CCT GTA ACG ATG AG	136

F, Forward; R, Reverse.

Table S 5-3. Effect of liquid milk replacer supplementation of weaned pigs on post-weaning diarrhoea prevalence and antibiotic and anti-inflammatory treatments in pigs.

Milk supplementation	**N-Milk**	**Milk**	**SEM**	***P*-value**
Number of pens	24	24		
Diarrhoea prevalence, % (weaning -28 days post-weaning)[1]	29	34	0.2	0.32
Antibiotic usage per pig, mL[2]	0.14	0.21	0.091	0.57
Anti-inflammatory usage per pig, mL[3]	0.07	0.10	0.064	0.71

[1]A faecal score of 2 or greater for each pen was considered indicative of diarrhoea at each time point from weaning to day 28 post-weaning. The overall prevalence was reported for the post-weaning period.
[2]Volume of antibiotic administered to each pig from weaning to day 43 post-weaning.
[3]Volume of anti-inflammatory administered to each pig from weaning to day 43 post-weaning.
Treatments from D0-10 post-weaning were as follows: N-Milk, dry pelleted starter diet; Milk, dry pelleted starter diet plus supplemental liquid milk replacer.

Table S 5-4. Effect of inclusion of 1% L-glutamine in weaned pig diets on post-weaning diarrhoea prevalence and antibiotic and anti-inflammatory treatments in pigs.

Glutamine supplementation (Glu)	**N-Glu**	**Glu**	**SEM**	***P*-value**
Number of pens	24	24		
Diarrhoea prevalence, % (weaning -28 days post-weaning)[1]	33	31	0.2	0.60
Antibiotic usage per pig, mL[2]	0.18	0.17	0.091	0.92
Anti-inflammatory usage per pig, mL[3]	0.09	0.08	0.064	0.97

[1]A faecal score of 2 or greater for each pen was considered indicative of diarrhoea at each time point from weaning to day 28 post-weaning. The overall prevalence was reported for the post-weaning period.
[2]Volume of antibiotic administered to each pig from weaning to day 43 post-weaning..
[3]Volume of anti-inflammatory administered to each pig from weaning to day 43 post-weaning.
Treatments from D0-10 post-weaning were as follows: N-Glu, no dietary inclusion of 1% L-glutamine; Glu, dietary inclusion of 1% L-glutamine.

Table S 5-5. Effect of liquid milk replacer supplementation of weaned pigs on feeding behaviour at day 1 and 4 post-weaning.

Milk supplementation	N-Milk	Milk	SEM	*P*-value
Number of pigs	240	240		
Observations of individual pigs seen engaging in solid feeder trough-directed activity (%)[1]				
Day post-weaning				
1	8.6	1.7	0.46	<0.001
4	8.5	2.8	0.36	<0.001
Observations of individual pigs seen engaging in liquid feeder trough-directed activity (%)[2]				
Day post-weaning				
1	0	5.1	0.21	<0.001
4	0	4.8	0.32	<0.001
Eaters of solid feed per pen (%)[3]				
Day post-weaning				
1	98.0	68.3	3.86	<0.001
4	99.2	81.0	2.71	<0.001

[1] Observations of individual pigs seen engaging in solid feeder trough-directed activity as a percentage of total number of observations per day.
[2]. Observations of individual pigs seen engaging in liquid feeder trough-directed activity as a percentage of total number of observations per day. Observations of piglets seen engaging in liquid feeder trough-directed activity is not applicable for N-Milk.
[3]. An eater was defined as having one or more feeder trough-directed-activity observations on each day.

Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
Treatments from D0-10 post-weaning were as follows: N-Milk, dry pelleted starter diet; Milk, dry pelleted starter diet plus supplemental liquid milk replacer.

Table S 5-6. Effect of liquid milk replacer supplementation of weaned pigs on haematology parameters of pigs at 7 days post-weaning.

Milk supplementation	**N-Milk**	**Milk**	**SEM**	***P*-value**	Reference ranges[1]
Number of pigs	20	20			
Blood parameter					
Leukocytes ($\times 10^3$ cells/µL)	8.31	9.08	0.639	0.40	9.62 – 25.2
Erythrocytes ($\times 10^6$ cells/µL)	5.34	5.52	0.210	0.55	4.87 – 7.88
Haemoglobin (g/dL)	9.88	10.12	0.387	0.60	8.08 – 11.9
Thrombocytes ($\times 10^3$ cells/µL)	228.2	358.4	47.83	0.06	374.3 – 1080.8
Lymphocytes ($\times 10^3$ cells/µL)	4.38	4.30	0.392	0.87	4.02 – 12.5
Monocytes ($\times 10^3$ cells/µL	0.19	0.15	0.019	0.13	0.05 – 2.3
Neutrophils ($\times 10^3$ cells/µL)	3.69	6.02	1.16	0.17	2.35 – 11.9
Basophils ($\times 10^3$ cells/µL)	0.03	0.03	0.004	0.47	-
Eosinophils ($\times 10^3$ cells/µL)	0.02	0.03	0.005	0.36	0 – 0.5

[1]Normal reference ranges for pigs 0 – 6 weeks old (Iowa State University, 2011).
Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
Treatments from D0-10 post-weaning were as follows: N-Milk, dry pelleted starter diet; Milk, dry pelleted starter diet plus supplemental liquid milk replacer.

Table S 5-7. Effect of inclusion of 1% glutamine in weaned pigs diets on haematology parameters of pigs at 7 days post-weaning.

Glutamine supplementation (Glu)	**N-Glu**	**Glu**	**SEM**	***P*-value**	Reference ranges[1]
Number of pigs	20	20			
Blood parameter					
Leukocytes ($\times 10^3$ cells/µL)	8.08	9.32	0.639	0.18	9.62 – 25.2
Erythrocytes ($\times 10^6$ cells/µL)	5.01	5.85	0.210	<0.01	4.87 – 7.88
Haemoglobin (g/dL)	9.43	10.62	0.387	0.04	8.08 – 11.9
Thrombocytes ($\times 10^3$ cells/µL)	275.0	311.6	47.83	0.59	374.3 – 1080.8
Lymphocytes ($\times 10^3$ cells/µL)	4.14	4.54	0.392	0.49	4.02 – 12.5
Monocytes ($\times 10^3$ cells/µL	0.16	0.18	0.019	0.60	0.05 – 2.3
Neutrophils ($\times 10^3$ cells/µL)	3.73	5.98	1.16	0.18	2.35 – 11.9
Basophils ($\times 10^3$ cells/µL)	0.03	0.03	0.004	0.66	-
Eosinophils ($\times 10^3$ cells/µL)	0.02	0.03	0.005	0.09	0 – 0.5

[1]Normal reference ranges for pigs 0 – 6 weeks old (Iowa State University, 2011)
Probability values at $P \leq 0.05$ are considered significant and as tendencies at $P \leq 0.10$.
Treatments from D0-10 post-weaning were as follows: N-Glu, no dietary inclusion of 1% L-glutamine; Glu, dietary inclusion of 1% L-glutamine.

Table S 5-8. Differentially abundant bacterial genera at a false discovery rate of 0.05 in the faeces of pigs from the N-Milk/N-Glu (control) vs. Milk/N-Glu treatments on day 11 post-weaning.

Treatment	**N-Milk/ N-Glu**	**Milk/ N-Glu**	**SEM**	***P*-value**
Number of pigs	15	15		
Rikenellaceae RC9 gut group	4.96	8.89	0.782	<0.01
Agathobacter	2.68	0.84	0.291	<0.001
Oscillospiraceae UCG-002	1.67	3.46	0.388	0.02
Phascolarctobacterium	0.91	0.56	0.080	0.02
Butyricicoccaceae UCG-008	0.70	0.13	0.106	<0.001
Paludibacteraceae	0.42	3.5	0.962	0.02
Erysipelatoclostridiaceae	0.39	0.22	0.048	0.02
Desulfovibrio	0.14	0.73	0.191	<0.001

Probability values at $P \leq 0.05$ are considered significant.
Treatments from D0-10 post-weaning were as follows: N-Milk/N-Glu, control diet; dry pelleted starter diet; Milk/N-Glu, control diet plus supplemental liquid milk replacer.

Table S 5-9. Differentially abundant bacterial genera at a false discovery rate of 0.05 in the faeces of pigs from the N-Milk/N-Glu (control) vs. Milk/Glu treatments on day 11 post-weaning.

Treatment	**N-Milk/ N-Glu**	**Milk/ Glu**	**SEM**	***P*-value**
Number of pigs	15	15		
Rikenellaceae RC9 gut group	4.96	9.74	1.234	<0.01
Agathobacter	2.68	1.37	0.350	<0.001
Anaerovibrio	2.15	0.44	0.308	0.04
Oscillospiraceae UCG-002	1.67	4.19	0.664	0.02
Phascolarctobacterium	0.91	0.57	0.103	0.02
Butyricicoccaceae UCG-008	0.70	0.21	0.121	<0.001
Megasphaera	0.55	1.16	0.121	0.01
Paludibacteraceae	0.42	1.57	0.265	0.02
Desulfovibrio	0.14	0.46	0.071	<0.001

Probability values at $P \leq 0.05$ are considered significant.
Treatments from D0-10 post-weaning were as follows: N-Milk/N-Glu, control diet; dry pelleted starter diet; Milk/Glu, control diet with dietary inclusion of 1% L-glutamine plus supplemental liquid milk replacer with dietary inclusion of 1% L-glutamine.

Table S 5-10. Differentially abundant bacterial genera at a false discovery rate of 0.05 in the faeces of pigs from the N-Milk/N-Glu (control) vs. N-Milk/Glu treatments on day 11 post-weaning.

Treatment	**N-Milk/ N-Glu**	**N-Milk/ Glu**	**SEM**	***P*-value**
Number of pigs	15	15		
Rikenellaceae RC9 gut group	4.96	3.79	0.382	<0.01
Prevotellaceae NK3B31 group	2.71	1.93	0.394	0.03
Anaerovibrio	2.15	1.04	0.342	0.04
Paludibacteraceae	0.42	1.45	0.476	0.02

Probability values at $P \leq 0.05$ are considered significant.
Treatments from D0-10 post-weaning were as follows: N-Milk/N-Glu, control diet; dry pelleted starter diet; N-Milk/Glu, control diet with dietary inclusion of 1% L-glutamine.

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6. Overall discussion regarding pre- and post-weaning nutritional and management strategies to increase piglet growth and reduce antimicrobial usage

In the pre-and post-weaning period, pigs face a multitude of challenges which can lead to a reduction in post-weaning feed intake and weight gain (commonly referred to as a post-weaning 'growth check') (Lawlor *et al.*, 2020). This also increases the pig's susceptibility to gastrointestinal disease, such as diarrhoea, which is a major concern to the industry. In the past, in-feed antibiotics and pharmacological levels of zinc oxide (ZnO), have been used, specifically around the time of weaning, to reduce the incidence of disease and improve gut health. However, excess zinc is excreted in pig manure, thereby impacting the environment (Hansen *et al.*, 2023). Additionally, the use of ZnO has been linked to increased antimicrobial resistance in pigs (Raro *et al.*, 2023; Bednorz *et al.*, 2013), which is a major risk for public health. As a result, in 2022, the European Union banned the use of pharmacological levels of ZnO in pigs (European Commission, 2017). In addition, all forms of routine antibiotic use in farming was prohibited in 2022, including the use of medicated feed for prophylaxis (European Commission, 2019).

In addition, intensive genetic selection has led to hyperprolific sows giving birth to more piglets than their number of functional teats (Oliviero, 2022). This increase in litter size has led to more heterogeneous litters and a higher proportion of 'light' piglets born alive. Furthermore, the sow has a finite ability to produce milk and colostrum, although the demand increases with increasing litter size (King, 2000). Therefore, the mean volume of colostrum ingested by piglets often does not reach the 200 g necessary for their survival during the first 24 h of life (Devillers *et al.*, 2011) and sow milk availability can limit pre-weaning piglet growth. This is important as increasing weaning weight is key to limiting the growth check at weaning (Lawlor *et al.*, 2020) and increasing lifetime growth (Collins *et al.*, 2017).

Gaps identified in the literature include the fact that, to date, no single alternative nutritional or management strategy has been found that improves growth or reduces the occurrence of diarrhoea to the same extent as antimicrobial usage. Early-life management strategies such as the provision of nonsteroidal anti-inflammatory drugs to the sow postpartum or the application of split-suckling have been shown to increase passive immunity transfer from the sow to the piglets and to increase pre-weaning growth in pigs (Mainau *et al.*, 2016; Navarro *et al.*, 2021). However, there is no

consensus on how to properly apply these strategies and their long-term effects have yet to be determined. Furthermore, data is lacking on their effects on colostrum intake and medication usage in piglets. Although some studies investigated the feeding of dry pelleted creep feed to suckling piglets to increase weaning weight and improve feed intake post-weaning, very few studies were conducted using liquid creep feed. In addition, most of the studies investigating the effect of liquid creep feeding (liquid milk replacer or a liquid diet) in suckling pigs followed pig growth up to weaning only. Data are lacking on the long-term effects of these strategies on pig growth, intestinal health and medication usage. Feeding a liquid creep feed has been demonstrated to increase creep feed dry matter intake (DMI) in piglets compared to dry creep feed (Martins *et al.*, 2020). Therefore, liquid feed could also be an attractive vehicle to supplement piglets with novel feed additives, which are normally added to dry pelleted diets. Lastly, the number of studies investigating the use of liquid milk replacer supplementation early post-weaning in order to reduce the nutritional stress experienced by pigs at weaning is very limited. Consequently, there is an opportunity to develop pre-and post-weaning management and nutritional strategies to increase growth and limit medication usage in pigs.

Considering all of the above, the objectives of this thesis were:

1. To investigate the application of split-suckling with/without postpartum provision of a nonsteroidal anti-inflammatory drug to the sow on colostrum intake in suckling pigs and on lifetime growth, health, and medicinal usage in pigs.
2. To determine the effect of providing supplementary dry pelleted starter diet, liquid milk replacer, and a liquid mixture of milk replacer and starter diet as creep feed to suckling pigs on sow body weight and back fat thickness and lifetime growth, health and medicinal usage in pigs.
3. To assess the effect of L-glutamine or enzyme supplementation of liquid creep feed on sow body weight and back fat thickness and lifetime growth, health and medicinal usage in pigs.

4. To determine the effect of post-weaning supplementary liquid milk replacer and/or dietary inclusion of 1% L-glutamine on lifetime growth, health and medicinal usage in pigs.

Tables 6-1 summarizes the results obtained with all of the nutritional and management strategies implemented throughout this thesis. Chapter 2 aimed to assess if early management strategies (meloxicam provision to sows postpartum and split-suckling application in piglets) could increase colostrum intake and growth in piglets, thereby limiting medication usage. The results showed that a single intramuscular (IM) injection of meloxicam provided to sows as soon as possible after delivery of the placenta increased colostrum intake in piglets. Furthermore, it reduced clinical cases of disease, increased ADG during the first two weeks of life and early post-weaning and increased carcass weight at slaughter in pigs. Providing meloxicam to sows' postpartum also tended to reduce antibiotic and anti-inflammatory usage in piglets during the suckling period. Some Irish farmers have already benefitted from this low-cost intervention by implementing it routinely on their farms. In addition, the farm staff now provide meloxicam to sows at every farrowing in the experimental unit at Teagasc Moorepark where the experiments for this thesis were performed. Therefore, this strategy was applied in the experiments reported in Chapters 3, 4 and 5 of this thesis. Contrary to this, commencing split-suckling 4 h after birth of the first piglet by twice removing the six heaviest piglets from the sow, reduced pig growth prior to weaning and up to slaughter, and had no effect on pre-weaning medication usage. Therefore, as implemented in this thesis, split-suckling was actually detrimental to the growth of the 6 piglets removed. This strategy must be considered carefully when applied on-farm. Perhaps an attempt should be made to determine if individual pigs have suckled colostrum and are full, and then only those pigs that are full should be removed during the split-suckling protocol.

Pre-weaning strategies that effectively increase nutrient intake and growth in piglets up to weaning are important since weaning weight is positively correlated with subsequent health and growth in pigs (Collins *et al.*, 2017). Chapter 3 determined the effect of providing liquid creep feed (liquid milk replacer or a liquid mixture of milk replacer and starter diet) to suckling pigs compared to a dry pelleted starter diet and a

control (no creep feeding) on lifetime growth, health and medicinal usage in pigs and sow body weight and back fat thickness. Contrary to the results of the studies performed by Martins *et al.* (2020) and Byrgesen *et al.* (2021), the results of this chapter rejected the hypothesis that liquid creep feeding would increase creep feed intake in piglets compared to dry creep feeding. They also showed that supplementing piglets with a liquid milk replacer pre-weaning and a dry pelleted starter diet can increase pig growth up to weaning. However, this growth benefit was not maintained throughout the life of the pig. Future studies should optimise the management of the automated milk delivery system to ensure ad-libitum supply of liquid milk/feed which may involve providing more frequent liquid feed deliveries to troughs. This would most likely increase the intake of liquid creep-fed piglets. The results of this work also supported the evidence that supplementing piglets with a starter diet containing plant-based ingredients pre-weaning (either in dry or liquid form) can help to maintain intestinal structure early post-weaning. In order to benefit pig growth pre-weaning and intestinal integrity post-weaning, another strategy would be to supplement suckling pigs with liquid milk replacer along with a dry pelleted starter diet. Finally, the current study failed to demonstrate a positive effect of treatment on medication usage during pre- or post-weaning periods.

Chapter 4 built upon the work performed in Chapter 3 by adding feed additives (L-glutamine or a cocktail of enzymes) to the liquid creep feed (liquid starter diet) provided to suckling piglets and increasing the number of sensor checks per feeding cycle of the automated delivery system to ensure more frequent feed deliveries to troughs. However, although the number of sensor checks was increased in Chapter 4, the total dry matter disappearance (DMd) per pig did not increase compared with that obtained in Chapter 3. It was actually 43% lower in Chapter 4 (i.e. 202 g/pig for the liquid starter diet provided from day 8 to 28 of age versus 353 g/pig for the liquid mixture of milk replacer and starter diet provided from day 3 to 28 of age in Chapter 3). The supplementation period was 5 days shorter in Chapter 4 compared with Chapter 3 as piglets consume very little liquid creep feed during their first week of life (less than 10% of the total amount consumed). Therefore, we believe that the large differences in DMd between Chapter 3 and 4 were not due to the shortening of the

supplementation period. The lower DMd observed is most likely because liquid feed was prepared with water in Chapter 4 as opposed to with liquid milk replacer in Chapter 3. Milk replacer powder contains high lactose levels (~40%) which can lead to higher intakes and is more easily digested compared with a starter diet (containing only ~ 20% of lactose). This emphasized the fact that the composition of the liquid creep feed, specifically the level of milk based-compounds and supplementation with liquid milk replacer (for at least 10 days) before transitioning to a liquid starter diet can have a significant influence on piglet intakes as outlined in Chapter 3. In Chapter 4, the choice of supplementing a liquid starter diet rather than liquid milk replacer, and using water rather than milk replacer to prepare the liquid feed was made in order to reduce feed cost as the milk replacer powder is very expensive (~3 times the price of the starter diet). In addition, the use of liquid feed rather than liquid milk replacer aimed to provide substrates for the enzymes (α-amylase, lipase and protease) included in the experimental treatment. There was no benefit to adding L-glutamine to the liquid creep feed. In fact, it tended to increase the incidence of diarrhoea pre-weaning and to reduce pig weight at weaning, albeit this growth disadvantage did not persist throughout the life of the pig. A low level of L-glutamine (<0.2 g/kg) was found in the oven-dried liquid starter diet, where it should have been present at 10 g/kg. This result, combined with the extremely low DMd helps explain the lack of response to L-glutamine supplementation. In liquid feed, bacteria can decarboxylate amino acids as a result of fermentation, leading to amino acid losses from feed. This could explain the low level of L-glutamine found in the liquid starter diet. In addition, amino acids decarboxylation by bacteria can lead to the production of undesirable metabolites such as biogenic amines (Cullen *et al.*, 2021). These biogenic amines can reduce feed palatability, subsequently reducing feed intake in pigs and they also have cytotoxic activity at high doses (Cullen *et al.*, 2021). Several microbial groups, including lactic acid bacteria, can produce the enzymes responsible for decarboxylation of free amino acids. Interestingly, in Chapter 3, we observed an increase in lactic acid bacteria counts in liquid feed 6 hours after feed preparation. Therefore, bacterial degradation of the dietary L-glutamine could explain the absence of a benefit of L-glutamine supplementation in liquid creep on piglet growth and intestinal health pre-weaning. It would be interesting to use a functional metagenomics approach to analyse the

metabolic capabilities of the bacterial communities within the liquid starter feed to determine which bacteria are responsible for the degradation of free L-glutamine in liquid feed. In addition, adding a cocktail of enzymes to the liquid creep feed did not benefit pig growth pre- or post-weaning. It is likely that, due to the low DMd observed in this Chapter, the pigs did not ingest sufficient quantity of each enzyme to positively influence post-weaning growth and intestinal morphology. With regard to medication usage, Chapter 4 also demonstrated that adding L-glutamine or the chosen cocktail of enzymes to liquid creep feed did not influence antibiotic or anti-inflammatory usage either pre-or post-weaning in pigs.

In Chapters 3 and 4, liquid and/or dry creep feed intakes were monitored for each litter. In order to understand the impact of creep feeding on piglet growth and health, it is important to identify which piglets consume creep feed and in which quantities. Measuring creep feed intake at the individual pig level is very challenging in suckling piglets. This is because individual creep feed intake is very low, it varies between littermates, and there is considerable feed wastage through the pen slats (Middelkoop, 2020). Even it is not always possible to measure creep feed intake at the individual pig level, it is possible to assess which piglets interact with the creep feed. This approach was taken in Chapters 3 and 4, where an attempt was made to determine the percentage of individual piglets interacting with the liquid and/or solid creep feeders per pen/litter. To facilitate this, instantaneous scan sampling of individual piglets in each trial pen was conducted. A piglet was considered an "eater" (of creep feed) if it had one or more feeder-directed activities during the day of live observations. Interestingly, the dry pelleted starter diet supplemented to suckling pigs from day 10 to weaning in Chapter 3 resulted in the highest proportion of piglets within each litter categorized as "eaters" (51% overall) and this treatment also had the highest DMd. Nonetheless, piglets supplemented with dry creep feed did not have the highest weaning weight, which suggests that 1) feed wastage may have been an issue with the dry pelleted starter diet and 2) live observation by scan sampling may not provide sufficiently accurate information to assess the percentage of piglets eating the creep feed. Live observations have the advantage of being non-invasive. However, they also have some limitations: 1) it is difficult to distinguish between piglets eating/chewing and those only playing

with the creep feed, 2) it is time-consuming 3) it requires a lot of manual handling to individually mark piglets. During the live observations, a piglet was scored as "eating" if it had its snout inserted into the creep feeder for at least 2 seconds. Therefore, with this method it is possible that piglets only rooting into the creep feed were scored as "eating". Another method to determine the percentage of eaters is the addition of an inert colour marker (e.g. chromium oxide, indigo carmine or brilliant blue) in the creep feed. With this method, piglets consuming creep feed excrete coloured faeces. Visual inspection of the faeces or the faecal swabs is then necessary to determine which piglets ate the creep feed (Muns and Magowan, 2018). Combining both methodologies, i.e. live observations and the use of a coloured marker, could be performed in future studies to obtain more accurate results regarding the proportion of eaters per litter. However, it is important to note that none of these methodologies provide information on the amount of creep consumed by individual pigs, which can vary a lot from one piglet to another.

In order to alleviate the nutritional stress experienced by pigs at weaning, and following on from the strategies used in suckling piglets in Chapters 3 and 4, a post-weaning nutritional strategy was investigated in Chapter 5. This involved supplementation of pigs with a liquid milk replacer for 10 days post-weaning with or without dietary inclusion of 1% L-glutamine. The results demonstrated the huge potential of supplementing liquid milk replacer to pigs post-weaning in terms of increasing lifetime growth and improving intestinal health. Supplementing a liquid milk replacer to pigs from weaning to day 10 post-weaning increased the DMI and growth of pigs early post-weaning. This led to an increase in carcass weight at slaughter. It increased villus height in the small intestine and reduced the expression of genes encoding pro-inflammatory cytokines (*IL-17*, *IL18* and *IL22*) in the small intestine of pigs at day 7 post-weaning. It also increased the relative abundance of beneficial bacteria (*Rikenellaceae RC9* and *Oscillaspiraceae UCG-002*), producers of short chain fatty acids (SCFAs), in the faeces of pigs at day 11 post-weaning. Although SCFAs were not measured as part of this work, it would be interesting to determine whether liquid milk replacer supplementation post-weaning can influence SCFA production in the pig large intestine. This would confirm whether or not the increase

in beneficial bacteria also leads to a higher production of their metabolites. However, contrary to the findings of previous studies (Wu *et al.*, 1996; Duttlinger *et al.*, 2020), including 1% L-glutamine in the dry pelleted starter diet and/or in the milk replacer powder did not influence the growth or intestinal structure of pigs post-weaning. It also did not influence medication usage in pigs. Furthermore, this strategy only had minor effects on the bacterial composition of pig faeces at day 11 post-weaning. As reported in Chapter 5, the lack of effects of L-glutamine supplementation post-weaning could be due to the high level of background total glutamic acid contained in the basal diets, the high DMI observed early post-weaning and the high health status of pigs in the Teagasc Moorepark Pig facility. In order to re-assess the benefit of L-glutamine, future studies should reduce the dietary inclusion dose of L-glutamine and create a more challenging environment, closer to that of commercial farms (e.g. different hygiene protocol).

All of the strategies applied in this thesis aimed to increase early feed intake post-weaning, as low post-weaning feed intake can lead to reduced growth and intestinal dysbiosis at weaning. The highest post-weaning feed intake was obtained in Chapter 5 by supplementing weaned pigs with a liquid milk replacer for 10 days post-weaning (i.e. 447 g of DMI per day per pig from weaning to day 10 post-weaning). This finding is critical, as pig feed intakes usually vary between 100 and 200 g of DMI/day during the first and second week post-weaning (Brooks and Tsourgiannis, 2003). Pig DMI was < 230 g/day from weaning to day 6 post-weaning with all of the other experimental treatments applied in this thesis. It is also important to note that none of the strategies applied in this thesis reduced post-weaning feed intakes. All of the strategies applied also aimed to limit medication usage in pigs. However, administration of meloxicam to sows postpartum (Chapter 2) was the only strategy that reduced antibiotic and anti-inflammatory usage in piglets (and then only a tendency) and reduced clinical cases of disease in litters. Meloxicam administration to sows postpartum increased piglet colostrum intake. It likely increased transfer of passive immunity from the sow to her piglets. Although not measured in this study, it would be interesting to assess the level of immunoglobulin G in the colostrum of the sows and in the plasma of the piglets 24 hours after birth in order to confirm the

increase in passive immunity transfer. It is also important to note that all of the experiments were conducted in the Teagasc Moorepark Pig facility which had a high health status and observed strict internal and external biosecurity. Therefore, the immune system of the pigs used in these studies may have been less challenged than pigs on commercial farms. This might also explain the lack of effect of the other strategies on medication usage. Additionally, it is important to note that the improvements obtained with the strategies highlighted in this paragraph (i.e. supplementing weaned pigs with liquid milk replacer for 10 days and meloxicam provision to sows postpartum) led to increases in carcass weight at slaughter. No improvement in carcass weight at slaughter was observed with the other strategies implemented in this thesis.

Despite the benefits for pig growth and health, farmers will only implement these strategies if they are cost-effective. Therefore, based on the results of these experiments, some cost-benefit analyses were conducted for the strategies investigated in Chapters 2, 3 and 5. In Chapter 2, a cost benefit analysis of the provision of meloxicam to sows showed that there is potential to increase the value of each pig sold by €5.10. This represents an increased sale value of €139 per sow per year and a return on investment of €53 for every €1 spent on the intervention. This return on investment was calculated assuming 27.4 piglets born per sow per year and 2.24 litters produced per sow per year (Teagasc, 2022). This is a very inexpensive strategy, which can be easily implemented on farms. In comparison, the milk replacer powder used in Chapters 3 and 5 is expensive (3 times the price of dry pelleted starter diet). As mentioned in Chapter 3, the feed cost per pig during lactation was €0.59, €0.71 and €1.41 per pig for the dry pelleted starter diet, the mixture of liquid milk replacer and liquid starter and the liquid milk replacer, respectively. No positive returns on investment were obtained with these strategies, as there was no benefits with respect to carcass weight or mortality. Similarly, in Chapter 5, a cost-benefit analysis demonstrated that feeding liquid milk replacer to weaned pigs for 10 days post-weaning would lead to an additional feed cost of ~€4 per pig for its lifetime, despite the huge benefit obtained in carcass weight (+2.6 kg) with this feeding strategy. It is also important to note that the calculations performed for Chapters 3, 4 and 5 do not

include the capital and running costs of the liquid feeding system. Therefore, milk replacer powder is expensive and its use must be economically justified. The milk replacer powder used in this thesis was mainly composed of whey powder, a highly nutritious by-product from the cheese industry. Whey powder can also be used by the food industry for human consumption (e.g. in infant formula, whey protein-enriched drinks and pastry products). The growing demand for this raw material partly explains the high cost of milk replacer powders. In order to reduce the cost associated with the use of milk replacer powders, future studies should investigate the effects of supplementing a mixture of liquid milk replacer and liquid starter diet post-weaning on pig growth, intestinal health and medication usage. Costs could also be reduced by including alternative by- and co-products from the food industry in the liquid feed supplemented post-weaning. Finally, the post-weaning supplementation period could be reduced in future studies in order to save cost.

Overall, this thesis provides essential information regarding early-life management strategies to improve colostrum intake in piglets. It has shown that injecting meloxicam to sows as soon as possible after the delivery of placenta is an effective and economically viable means of improving colostrum intake and growth in piglets and reducing clinical cases of diseases. Moreover, this thesis provides valuable information to pig producers and the feed industry regarding the use of liquid feed in pigs pre- and post-weaning. Specifically, it provides critical information about the management of the automatic liquid feeding system in farrowing houses. In addition, it demonstrates the potential of feeding a liquid feed to pigs early post-weaning on their growth and intestinal health and the limitations of adding feed additives, specifically L-glutamine, to liquid feed.

Table 6-1. Summary of the results obtained in this thesis from the implementation of nutritional and management strategies to improve lifetime growth and health and reduce medication usage in pigs.

Chapter number	Strategy	Period of application	Pre-weaning		Post-weaning (d.0 = weaning)					Others
			ADG	Weaning weight	ADFI	ADG	FCR	Carcass BW	Intestinal structure	
2	Meloxicam provision to sows	After release of placenta	↑	↑	=	↑ from d.0-6 ↗ overall	↘ d.47 to slaughter	↑	NA	↗ colostrum intake in piglets ↘ medication usage
2	Split-suckling the 6 heaviest piglets	2 periods of 1.5 h, 4 h after the birth of the first piglet	↓	↓	↘ d.6 - 14	=	↘ overall	=	NA	= medication ↘ colostrum intake in heavy piglets
3	Dry pelleted starter diet as creep feed	From d.10 of age to 28 (weaning)	↑ 11-27	↑	=	=	=	=	↑ VH in ileum	= medication
3	Liquid milk replacer as creep feed	From d.3 of age to 28 (weaning)	↑ 7-27	↑	=	↓ d.0-6	↑ d.0-6	=	=	= medication
3	Mixture of liquid milk replacer and liquid starter diet as creep feed	From d.3 of age to 28 (weaning)	↑ d.2-7 & 19-27	=	=	=	↑d.6-14	=	↑ VH in ileum	= medication
4	Liquid starter supplemented with	From d.8 of age to 28 (weaning)	↓ d.14-21	↘	=	=	=	=	=	= medication ↗ diarrhoea

	1% L-glutamine as creep feed									
4	Liquid starter supplemented with enzymes as creep feed	From d.8 of age to 28 (weaning)	=	=	=	=	=	=	=	= medication
5	Supplementation with liquid milk replacer	From weaning to d.10 post-weaning	NA	NA	↑ d.0-10 & d.20-47	↑ d.0-10 & d.20-47	↑ d.80 to slaughter	↑	↑ VH in duodenum, jejunum and ileum ↑ VH:CD ratio in jejunum and ileum	= medication ↑ abundance of *Rikenellaceae RC9* and *Oscillaspiraceae UCG-002* ↑ expression of interleukin 17, 18 & 22
5	Dietary inclusion of 1% L-glutamine	From weaning to d.10 post-weaning	NA	NA	=	=	=	=	=	= medication

↑Significant increase, ↗ tendency to increase
↓ Significant decrease, ↘ tendency to decrease
= No difference
d., day; NA, not analysed; BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; FCR: feed conversion ration; VH, villus height; CD, crypt depth.

Based on the findings of this thesis, future work should include:

- Assessing if the application of split suckling by removing piglets that look “full” (e.g. already suckled colostrum) can improve colostrum intake, improve growth and reduce medication usage in pigs.
- Determining the effect of a supplementation with a mixture of liquid milk replacer and liquid starter diet to suckling pigs, with liquid milk replacer provided for at least 10 days before switching to a liquid starter diet on pig growth, intestinal health and medication usage. The economical sustainability of this solution should also be assessed.
- Determining which bacteria are responsible for amino acid fermentations in the liquid creep feed using a functional metagenomics approach to analyse the metabolic capabilities of the bacterial communities.
- Determining the effect of Babyfeed system sanitisation on bacterial fermentation in liquid creep feed, on pig growth, intestinal health and medication usage.
- Determining the effect of providing a liquid mixture of liquid milk replacer and liquid starter diet to pigs for 10 days (or less) post-weaning on their growth, intestinal health and medication usage. The economical sustainability of this solution should also be assessed.

6.1 References

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Appendix

Research Dissemination

Peer-reviewed publications:

E.A. Arnaud, G.E. Gardiner, P.G. Lawlor (2023). Selected Nutrition and Management Strategies in Suckling Pigs to Improve Post-Weaning Outcomes. *Animals*. 13(12): 1998. https://doi.org/10.3390/ani13121998

E.A. Arnaud, G.E. Gardiner, K.M. Halpin, C. Ribas, J.V. O'Doherty, T. Sweeney, P.G. Lawlor (2023). Post-partum meloxicam administration to sows but not split-suckling increased piglet growth and reduced medicinal treatment of piglets. *Journal of Animal Science*. 101: skad275. https://doi.org/10.1093/jas/skad275

E.A. Arnaud, G.E. Gardiner, M. Chombart, J.V. O'Doherty, T. Sweeney, P.G. Lawlor (2024). Effect of creep feeding pelleted starter diet, liquid milk replacer and a liquid mixture of starter diet and milk replacer to suckling pigs on their growth and medication usage. *Translational Animal Science*. 8: txae04. https://doi.org/10.1093/tas/txae041

S.R. Vasa, G.E. Gardiner, **E.A. Arnaud**, K. O'Driscoll, G. Bee, P.G. Lawlor (2024). Effect of supplemental milk replacer and liquid starter diet for 4 and 11 days post-weaning on intestinal parameters of weaned piglets and growth to slaughter. *Animal (in press).*

K.M Halpin, P.G. Lawlor, **E.A. Arnaud**, J. Teixé-Roig, J.V. O'Doherty, T. Sweeney, T.M. O' Brien, G.E. Gardiner (2024). Effect of implementing an effective farrowing accommodation hygiene routine on clinical cases of disease, medication usage and growth in suckling and weaned pigs. *Translational Animal Science (in press).*

Conference abstracts & oral presentations:

E.A. Arnaud, G.E. Gardiner, K.M. Halpin, C. Ribas, J.V. O'Doherty, T. Sweeney, P.G. Lawlor (2022). Effect of post-partum analgesia administration to sows and/or split-suckling on growth and medicinal treatment of piglets. In proceedings of *British*

Society of Animal Science Annual Conference, 12-15th of April 2022, Nottingham, United Kingdom. (Oral presentation).

K.M. Halpin, P.G. Lawlor, J. Teixé-Roig, **E.A. Arnaud**, J.V. O'Doherty, T. Sweeney, G.E. Gardiner (2022). Optimal farrowing accommodation hygiene reduces pre-weaning antibiotic and anti-inflammatory usage. In proceedings of the *European Federation of Animal Science Annual meeting*, 4-9th of September 2022, Porto, Portugal.

S.R. Vasa, **E.A. Arnaud**, G.E. Gardiner, K. O'Driscoll, G. Bee, P.G. Lawlor (2023). Effect of providing supplemental milk and liquid starter diet for 4 and 11 days post-weaning on feed intake, growth and intestinal structure and function of newly weaned piglets. In proceedings of the *American Society of Animal Science Midwest Meeting,* 13-15th of March 2023, Madison, United States.

E.A. Arnaud, G.E. Gardiner, M. Chombart, J.V. O'Doherty, T. Sweeney, P.G. Lawlor (2023). Effect of creep feeding (liquid milk, dry and liquid diet) on pig growth and intestinal structure. In proceedings of the *South East Technological University Postgraduate Conference,* 31st of May 2023, South East Technological University, Carlow, Ireland (page 25). (Oral presentation).

E.A. Arnaud, G.E. Gardiner, M. Chombart, J.V. O'Doherty, T. Sweeney, P.G. Lawlor (2023). Effect of creep feeding (liquid milk, dry and liquid diet) on pig growth and intestinal structure. In proceedings of the *European Federation of Animal Science Annual Meeting*, 26th of August - 1st of September 2023, Lyon, France. (Oral presentation).

E.A. Arnaud, G.E. Gardiner, S.R. Vasa, J.V. O'Doherty, T. Sweeney, P.G. Lawlor (2023). Effect of glutamine and/or milk supplementation post-weaning on pig growth and intestinal structure. In proceedings of the *European Federation of Animal Science Annual Meeting*, 26th of August - 1st of September 2023, Lyon, France. (Oral presentation).

E.A. Arnaud, G.E. Gardiner, J.V. O'Doherty, T. Sweeney, P.G. Lawlor (2024). Effect of L-Glutamine and enzyme supplementation of liquid creep feed from day 8 to 28 of

age on pre-weaning growth and health of pigs. *American Society of Animal Science Midwest Meeting*, 11-13th of March 2024, Madison, United States. (Oral presentation).

P.G. Lawlor, S.R. Vasa, **E.A. Arnaud**, G.E. Gardiner (2024). Pre- and post-weaning liquid feeding of pigs. Accepted for oral presentation at the *European Federation of Animal Science Annual Meeting*, 1st-5th of September 2023, Florence, Italy. (Oral presentation).

Posters presented:

E.A. Arnaud, P.G. Lawlor (2023). Pain relief provision in sows post-partum. *Teagasc Pig Open Days*, 9th and 11th of May 2023, Fermoy, Ireland.

E.A. Arnaud, P.G. Lawlor (2023). Split-suckling. *Teagasc Pig Open Days*, 9th and 11th of May 2023, Fermoy, Ireland.

S.R. Vasa, **E.A. Arnaud,** P.G. Lawlor (2023). Liquid supplementation of suckling piglets. *Teagasc Pig Open Days*, 9th and 11th of May 2023, Fermoy, Ireland.

S.R. Vasa, **E.A. Arnaud,** P.G. Lawlor (2023). Liquid supplementation of newly weaned piglets. *Teagasc Pig Open Days*, 9th and 11th of May 2023, Fermoy, Ireland.

E.A. Arnaud, S.R. Vasa, P.G. Lawlor (2024). Economics of liquid creep feeding suckling piglets. *Teagasc Pig Open Days*, 22nd and 24th of May 2024, Fermoy, Ireland.

E.A. Arnaud, S.R. Vasa, P.G. Lawlor (2024). Economics of providing supplementary liquid milk replacer to weaned pigs. *Teagasc Pig Open Days*, 22nd and 24th of May 2024, Fermoy, Ireland.

Popular press:

P.G. Lawlor and **E.A. Arnaud** (2022). Administering pain relief to sows after farrowing increased piglet weaning weight and reduced the need for medicinal treatment of suckling piglets. Teagasc Pig Newsletter, April 2022.

P.G. Lawlor and **E.A. Arnaud** (2022). Reducing antibiotic usage in pigs. Teagasc Research Impact Highlights in 2022.

Awards:

- 2022: British Society of Animal Science Industry Prize Award
- 2023: South East Technological University Postgraduate Conference best oral presentation of the session award

Modules:

- Online, LAST Ireland (Ireland) - LAST course large animals. November 2020. (5 ECTs)
- South East Technological University (Ireland) - Research Integrity and Ethics. April 2021. (5 ECTs)
- University of Science and Technology in Bydgoszcz (Poland) - Monogastric nutrition course. October 2021 (5 ECTs)
- University College Cork (Ireland) – SAS course. Autumn/winter 2021 (not accredited)
- Aarhus University (Denmark) - Gut Biology and Health course. June 2022 (5 ECTs)
- South East Technological University (Ireland) – Statistics and Quantitative Data Analysis. April 2022.
- South East Technological University (Ireland) – Academic writing. March 2023. (5 ECTs)
- Teagasc (Ireland) – Monogut Health, industry training school. February 2024 (not accredited).