Foraging in the farrowing room to stimulate FEEDING

Getting piglets to eat is bittersweet

Anouschka Middelkoop

Propositions

1. Every piglet can be an eater at 4 weeks of age.
(this thesis)

2. Facilitating foraging and ‘fun’ stimulates feeding in the farrowing room and eases the weaning transition.
(this thesis)

3. The most important part of achieving impact with your research is sharing it.

4. A limit on the number of references and words to be used in scientific papers hampers citation of original research.

5. Top athletes, artists and talents should be protected against the high expectations of fans and management.

6. Everyone needs a best friend at work.

Propositions belonging to the thesis, entitled

Foraging in the farrowing room to stimulate feeding: Getting piglets to eat is bittersweet

Anouschka Middelkoop
Wageningen, 9 October 2020

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Abstract

In common pig production systems, weaning happens early and abruptly, which contrasts the gradual transition from sow's milk to solid feed in nature. A significant proportion of commercially reared piglets is therefore poorly familiarised with the ingestion of solid feed before weaning, which together with concurrent changes in social structure and environment, result in a low feed intake and multiple health and welfare problems initially after weaning. The first aim of this thesis was to investigate the effects of solid feed provision during lactation (creep feed) on piglet development during the weaning transition. Piglets provided with creep feed (including fermentable fibres) during lactation increased their growth towards weaning, tended to be heavier at weaning, and were heavier and more uniform in body weight at two weeks after weaning compared to piglets reared on sow's milk only. This may partly be explained by a more developed gastrointestinal tract and more mature gut microbiota population at weaning of creep feed eaters within litters given creep feed. The second aim of this thesis was to explore whether piglets that were offered more opportunities to forage early in life increased their intake of creep feed before weaning and thereby improved their ability to deal with the weaning transition. Four strategies that could stimulate foraging in the farrowing room were tested: 1) enriching the pen with foraging materials on the floor and extra space, and by alternating chew objects, 2) presenting creep feed in a 'play-feeder' with foraging and playing materials attached, 3) providing diverse feeds in the feeder and 4) hiding feed in the feeder in sand. Enrichment with foraging materials on the floor, more space and alternating chew objects positively influenced feed intake of piglets before and after weaning until the end of the study at 2.5 weeks post-weaning. Presenting the creep feed in a play-feeder stimulated feeder exploration and attracted more piglets than presenting the feed in a conventional feeder. Although the play-feeder did not enhance pre-weaning feed intake, post-weaning feed intake and growth were substantially improved and the incidence of diarrhoea, body lesions and damage were lower. Simultaneous provision of diverse feeds to suckling piglets resulted in more feed exploration and solid feed intake, and in a higher number of good eaters. Hiding feed in sand stimulated the number of good creep feed eaters within the litter, but did not increase exploration towards the feed. With the exception of the first feeding strategy, treatments were not reinforced after weaning and piglets thus experienced loss of these resources, which may have had a negative effect on post-weaning adaptation. This may explain the limited effects of dietary diversity on post-weaning adaption and the negative effects of hiding feed in sand on post-weaning feed intake, growth, manipulation and aggression. Lastly, it was shown that creep feed intake is not only driven by exploration, but also by a low energy intake from milk. Increased creep feed intake by litters with a low milk intake may counteract the growth lag they developed during lactation by improving pre-weaning feed intake and, consequently, initial post-weaning feed intake and growth compared to litters that consume more sow's milk. In conclusion, creep feed provision and consumption accelerated piglet development, and improved opportunities for early foraging consistently enhanced the development of feeding behaviour. If one wants to improve post-weaning adaptation of piglets, a 'mismatch' in environment should be avoided.

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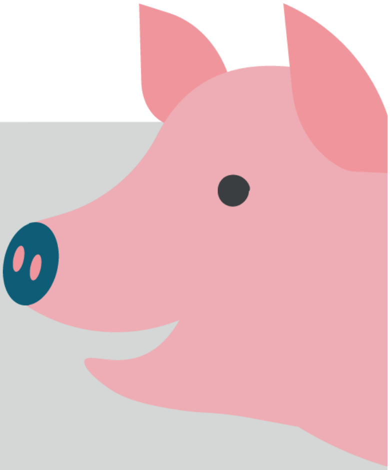
CHAPTER 1



General introduction

Background

Weaning-induced problems are a major issue in pig production, resulting in impaired health, welfare and performance of pigs. Firstly, they contribute substantially to the use of antibiotics in pig farming, which is not only a concern for pig health, but also for public health, because many antibiotics used for humans are also used in pigs (Lekagul et al., 2019). This imposes the risk of transfer of resistant bacteria to humans, endangering the efficacy of antibiotics and, consequently, infection control. Secondly, weaning impairs the welfare of commercial pigs, while citizens are increasingly interested and concerned about farm animal welfare. More than 80% of Europeans believe that the welfare of animals should be better protected than it is now and the majority look for animal welfare-friendly labels when buying products (European Commission, 2016). Public demand for more welfare-friendly pig husbandry has resulted in legislation (European Commission Directive 2001/93/EC) requiring pig farmers in the EU to provide their pigs with permanent access to sufficient amounts of foraging materials. In practise, possibilities of piglets to forage are still very limited to date. Lastly, weaning-induced problems suppress pig performance. However, to meet the growing food demand, it is expected that the global meat production would need to rise by over 200 million tonnes to reach an annual production of 470 million tonnes in 2050 (Alexandratos and Bruinsma, 2012). Moreover, pork is the most consumed meat over the world, accounting for over 36% of the global meat intake (Food and Agriculture Organization of the United Nations, 2014). From both societal and economic perspectives there is therefore an urgent need to reduce weaning-induced problems in pigs. A detailed analysis of the problem may provide additional information to improve the adaptation of pigs to the weaning transition in common production systems. To that aim, this introduction will discuss the differences that exist between the weaning transition of pigs in a natural versus common commercial environment, will summarise the effects of providing solid feed prior to weaning on piglet development around weaning and lastly, will identify environmental factors and piglet characteristics that may contribute to the large variation in solid feed intake by suckling piglets.

Weaning in (semi-)natural versus intensive indoor conditions

Foraging in a (semi-)natural environment

The behaviour of domestic pigs in (semi-)natural conditions (Stolba and Wood-Gush, 1989) still resemble that of their ancestor; the wild boar (Gundlach, 1968; Meynhardt, 1980). They range in groups (Gundlach, 1968; Stolba and Wood-Gush, 1989) in a large area up to tens of kilometres (Saunders and McLeod, 1999). Both young (from about 8 weeks of age) and adult pigs spend more than half of their active time on foraging and eating behaviours (Stolba and Wood-Gush, 1989; Petersen, 1994), like browsing and grazing, foraging on the ground, foraging under the ground by rooting, and preying (Ballari and Barrios-García, 2014). Foraging is a distinct phase of feeding, the ‘appetitive phase’, which brings the pig into contact with feed. It concerns active, flexible, searching behaviours for food. Eating is the ‘consummatory phase’ of feeding and is the achievement (‘consummation’) of the goal and ends the appetitive foraging behaviour (Berridge, 2004; Mills et al., 2010). For example, wild boars shovel and root the ground (foraging), which may be challenging in winter due to snow cover and/or ground frost, before the consumption of roots and worms occurs (eating). Another example on the appetitive and consummatory phase of feeding in pigs, is when wild boars peel off the shells of acorns before they ingest the kernels (Van Wieren, 2000). Pigs are opportunistic omnivores and they generally consume more plant than animal material (reviewed by Ballari and Barrios-García, 2014). The dry matter concentration of the diet therefore contains a high level of crude fibre (Van Wieren, 2000). The diet of wild boar and feral pigs has been well studied and is highly diverse, including roots, stems, bulbs, seeds, grasses, leaves, acorns, fruits, fungi (like truffles and mushrooms), cultivated crops (like maize and sugar beet), insects, earthworms, snails, frogs, crabs, snakes, turtles, voles, eggs and young of ground-nesting birds, new-born lambs and goats, animal carcasses, and more (Ballari and Barrios-García, 2014). The diet and feeding habits are mainly influenced by food availability, energy requirements, season and geographical location (Ballari and Barrios-García, 2014).

Weaning in a (semi-)natural environment

Already from a few days after birth, naturally-reared piglets have been observed foraging for other feed items than sow's milk, by digging soft soil and exploring and sampling leaves, mushrooms, acorns and corn (Gundlach, 1968; Meynhardt, 1980; Petersen, 1994) until they are exclusively feeding on solid feed. This transition from feeding on sow's milk to independent feeding is called weaning and is a slow and gradual process in nature. An example of the development of foraging behaviours in the first weeks of life is shown in **Figure 1**. The exploration of potential feed items starts closely to the farrowing nest during the first week of lactation (Gundlach, 1968; Stangel and Jensen, 1991). In the second week, the sow will take her piglets on short trips away from the nest to forage and feed (Gundlach, 1968; Newberry and Wood-Gush, 1985; Petersen et al., 1989; Stangel and Jensen, 1991) and to visit other sows with litters (Gundlach, 1968; Petersen et al., 1989). The piglets remain in close contact with the sow during such trips to hide, rest and watch her forage (Jensen, 1986; **Supplementary Figure S1**), thereby facilitating learning where, and how to eat and to identify feed items that are safe to ingest. From 10-14 days of age, wild boar piglets were already observed eating acorns and corn (Gundlach, 1968; Meynhardt, 1980). From the fourth week of lactation, piglets start to consume significant amounts of solid feed (Petersen et al., 1989; Damm et al., 2003), which may be correlated with an increase in grazing (Petersen et al., 1989; Petersen, 1994). In turn, grazing seems to correlate negatively with suckling (Jensen, 1995). Suckling bouts will become less frequent, are more often performed while the sow is standing and are more frequently terminated by the sow until suckling activities are fully over (Jensen and Recén, 1989). Weaning can take between 8.5 and 22 weeks of age to be fully completed (Newberry and Wood-Gush 1985; Jensen and Stangel 1992). Apart from large variation between litters, the weaning age of piglets within a litter can also differ (Newberry and Wood-Gush, 1985; Jensen and Recén, 1989; Jensen, 1995) as some piglets are weaned weeks earlier compared to other piglets in the litter (Jensen and Recén, 1989).

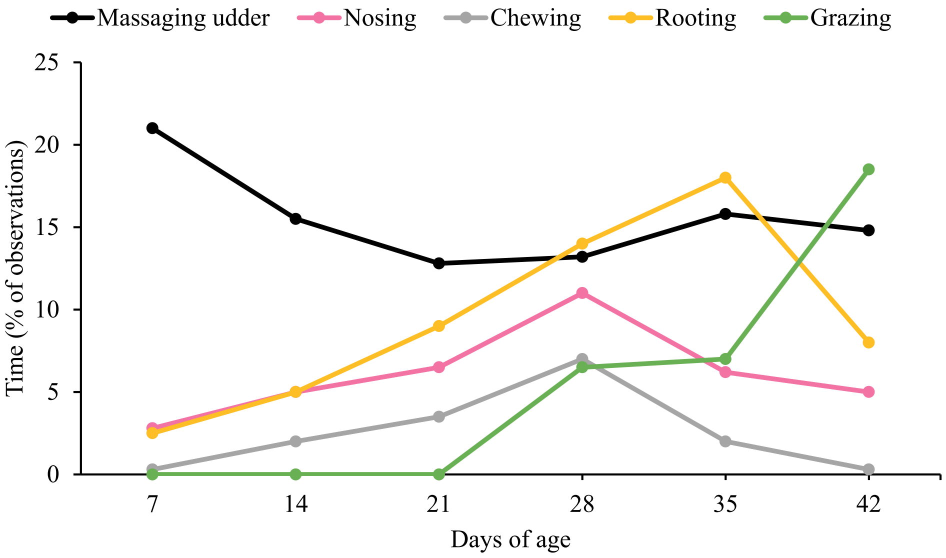


Figure 1. Example of time spent on foraging behaviours during the first 6 weeks of life by free-ranging domestic piglets. Suckling was not reported. Figure adapted from Petersen (1994).

Foraging in an intensive indoor environment

In contrast with (semi-)natural conditions, domestic pigs in intensive indoor housing are generally offered one monotonous pre-mixed diet, that contains a lower crude fibre content. The sow and her litter are fed in separate feeders and provided with a different diet, that is adjusted to their stage of life in terms of nutrient requirements. Moreover the feed is freely available in a feeder, and often provided *ad libitum* (**Figure 2**). However, it seems that domestic pigs in indoor housing are still highly motivated to forage, just like domestic pigs in a (semi-)natural environment, as 4- to 19-week-old pigs were about 60-85% of their active time busy foraging and feeding when reared in an environment with substrates and about 30-55% of their active time when reared in an environment without substrates (Beattie et al., 1996; Bolhuis et al., 2005; Oostindjer et al., 2011d). Foraging in the absence of substrates was mostly reflected by redirected nosing and rooting towards the floor and pen fixtures.

Figure 2. Domestic pigs in an experimental commercial-like farrowing pen (source: A. Middelkoop, 2018) and wild boar in a wildlife park in England (source: D. Pape, 2007).

Weaning in an intensive indoor environment

Rather than a gradual process that ends much later like in nature, weaning in pig husbandry is an early and abrupt event. It generally occurs between 3 and 4 weeks of age in Europe, although weaning occurs earlier outside Europe and occurs later in e.g. organic pig farming. The young weaning age in commercial pig housing as compared with nature is mainly implemented to increase the number of reproductive cycles per sow per year and therefore, to increase the number of piglets produced. Weaning in intensive indoor conditions involves complete separation of piglets from the sow, resulting in a sudden shift in diet from mainly or solely sow's milk before weaning to exclusively (solid) feed after weaning. Usually, weaning also simultaneously involves handling and transportation of piglets to a new environment with a larger group of piglets, leading to development of a new social hierarchy among unfamiliar non-littermate piglets. While piglets encounter non-littermates gradually around 2 weeks of age in (semi-)natural conditions (Gundlach, 1968; Petersen et al., 1989), mixing of piglets in conventional conditions often takes place simultaneously with weaning. Next to separation from their mother, piglets may also be separated from littermates if regrouped by body weight or sex.

As result of the abrupt changes in diet, physical environment and social environment, newly-weaned piglets show an (increased) neophobic reaction towards the weaner diet (Launchbaugh et al., 1997; Bolhuis et al., 2009) and experience stress (reviewed by Weary et al., 2008; **Figure 3**). The innate reluctance of piglets to eat novel feed is a phenomenon known as food neophobia, which is especially relevant after weaning when piglets are heavily stressed (Bolhuis et al., 2009). The distress response is reflected in a delayed, low and variable feed intake of newly-weaned piglets (reviewed by Colson et al., 2006 and Dong and Pluske, 2007), a poor growth performance or even body weight loss (Le Dividich and Sève, 2000, 2001; Dunshea, 2003) and impaired intestinal development (reviewed by Moeser et al., 2017 and Xiong et al., 2019). Weaning is proposed to disturb the absorptive and digestive capacity of the small intestine due to morphological and physiological changes such as villus shortening, crypt deepening and a reduction in brush border enzyme activities. Moreover, it has been suggested that weaning reduces the epithelial barrier function and increases permeability of the small intestine by, for example, reducing mucin production and tight junction protein expression. As a result, weaned piglets are susceptible to transient gut inflammation, gut microbiota dysbiosis and colonization of potential pathogens e.g. *Escherichia coli* and *Salmonella* spp., that can cause post-weaning diarrhoea. The alternations in intestinal morphology, physiology, microbiota, mucosal immunity and oxidative status that occur at weaning can then lead to increased disease susceptibility and mortality rates (reviewed by Moeser et al., 2017 and Xiong et al., 2019).

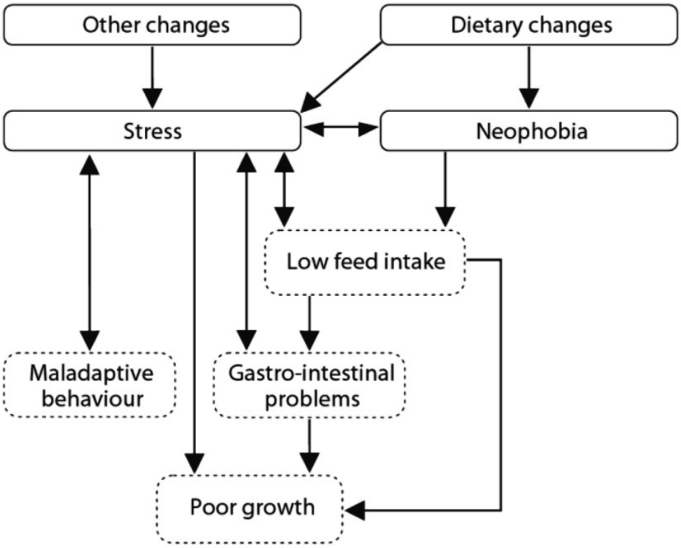


Figure 3. Schematic diagram illustrating the effects of changes in diet, physical and social environment in weaned piglets (reprinted from Bolhuis et al., 2009).

In addition to the impact of weaning stress on feed intake, intestinal functioning and piglet health, weaning also adversely affects piglet behaviour, involving an increase in vocalisations and damaging behaviour directed at their pen mates, including belly nosing (reviewed by Widowski et al., 2008), ear and tail biting (Van Nieuwamerongen et al., 2017). Weaning not only induces transient effects on growth, intestinal development and behaviour, but there is also some evidence that weaning can induce long-lasting changes in the epithelial, immune and enteric nervous system functions of the gastrointestinal tract (reviewed by Moeser et al., 2017) and in penmate-directed behaviour and activity of the hypothalamus-pituitary-adrenal cortex axis (reviewed by Telkänranta and Edwards, 2018).

As summarized in **Table 1**, the rearing environment of pigs in intensive commercial systems differs substantially from that of pigs in a (semi-)natural environment. This may disrupt normal developmental processes and impair pig welfare (reviewed by Telkänranta and Edwards, 2018). An approach to improve adaptation of piglets to the commercial rearing environment is to implement features and processes from the (semi-)natural environment in the commercial environment, of which some have been studied (**Table 1**). Based on the knowledge gained from studies of pigs in (semi-)natural conditions, there seems room for improvement of the diet and foraging and feeding behaviour of commercially housed piglets, particularly in relation to the sensory input provided to the piglets.

Table 1. Differences in rearing environment of piglets in (semi-)natural and commercial settings and studies that applied some of these features from nature to commercial indoor pig housing. Based on Telkänranta and Edwards (2018) and Brooks and Tsourgiannis (2013).

	Natural environment	Commercial environment	Literature
Weaning	Gradual, by sow and piglets, at 3-4 months of age	Early and abrupt, by the farmer, at 3-4 weeks of age	Van Nieuwamerongen et al. (2015): weaning at 9 vs. 4 weeks Van der Meulen et al. (2010): weaning at 7 vs. 4 weeks
Social setting	Family groups of multiple sows with offspring, sow is free to move, social learning of e.g. feeding behaviour	One sow with offspring, sudden contact with non-littermates at weaning, confined sow, limited possibilities for social learning as e.g. sow and piglets are fed separately and a different feed	Oostindjer et al. (2010, 2011d): loose-housed (family-feeder) vs. confined sows (conventional feeder) e.g. Van Nieuwamerongen et al. (2015): multi-litter (loose-housed and floor-fed sows) vs. single-litter housing (sows are crated and fed in elevated feeders)
Space	Unlimited, large home range	Confined to crate or pen	Oostindjer et al. (2010, 2011d): enriched (7 m^2) vs. barren (16 m^2) housing Van Nieuwamerongen et al. (2015): multi-litter (58 m^2) vs. single-litter housing (4 m^2 for piglets and 1 m^2 for the sow)
Behavioural freedom	Highly motivated behaviours such as nest-building, foraging/food seeking, object play and wallowing are expressed	Highly motivated behaviours are not or limitedly expressed, and redirected towards pen mates and pen fixtures	e.g. Oostindjer et al. (2010, 2011d): enriched (substrates and more space) vs. barren housing (no substrates) Ipema et al. (ongoing project at Wageningen University & Research): feeding insect larvae to pigs
Sensory stimuli	Diverse, complex and enriched: multiple substrates and food sources ranging in flavour, texture, taste, size etc., novelty, cognitive challenges	Monotonous, simple and barren: one food source, feed freely available and small in size, no or limited amount of substrates and chew objects	e.g. Van den Brand et al. (2014): large vs. small pellets Adeleye et al. (2014): successive flavour novelty

Creep feed provision

A long-established feeding practise in the farrowing room is the provision of 'creep feed', which means the provision of feed to piglets while they are suckling the sow. The term originates from the fact that this feed was traditionally presented to the piglets in the piglet nest, i.e. 'creep area', of the farrowing pen (Pluske et al., 2018), but nowadays the creep feed is either presented in a piglet feeder close to the head of the sow to facilitate social learning, or in the dunging area to facilitate access by piglets (more space) and the farmer (near corridor). Creep feed provision has been proposed to 1) familiarise piglets to feed prior to weaning, and thereby to reduce neophobia towards the weaner diet and to reduce the weaning-associated dip in feed intake (Bolhuis et al., 2009; Muns and Magowan, 2018; Figueroa et al., 2019), 2) stimulate development of the gastrointestinal tract and gut microbiota by adapting it to nutrients/dietary antigens not found in milk, while piglets are still immunologically protected by the sow (Dong and Pluske, 2007; Yao et al., 2011; Pluske et al., 2018), and 3) to supplement sow milk production, and thereby to improve the growth rate of piglets (Williams, 2003; Pluske et al., 2018). Evidence that any of these benefits might arise if creep feed is provided will be discussed in separate paragraphs below.

Creep feed provision and potential benefits on post-weaning adaptation

Creep feed provision is aimed to reduce the weaning-associated dip in feed intake by creating a more gradual dietary transition at weaning. **Table 2** provides an overview of studies on the effects of creep feed provision on pre- and post-weaning performance. Whilst most studies found no difference in feed intake, growth and feed efficiency in the post-weaning period between piglets provided with (creep-fed piglets) or without creep feed (non-fed piglets), a few studies indicated positive or negative effects on post-weaning performance (**Table 2**). When reviewing the studies on the effects of creep feed intake on pre- and post-weaning performance, which are shown in **Table 3**, it was found that piglets that consumed creep feed before weaning (eaters) had a shorter latency to eat, consumed more feed and gained more weight after weaning compared to piglets within creep-fed litters that did not consume creep feed (non-eaters). Compared to creep feed intake, similar effects on post-weaning adaptation are reported for creep feed intake level and are shown in **Table 4**. These studies indicated that piglets with a regular intake of creep feed (good eaters) had a higher intake of feed, more feeder visits with intake, a higher rate of feed intake and a higher average daily gain within the first few days after weaning versus piglets with an irregular (bad eaters) or no intake of creep feed (non-eaters). The positive effects of creep feed intake and intake level on post-weaning adaptation were found when the same diet or a different diet was fed after weaning, suggesting that proven intake of creep feed familiarises piglets with feed and reduces food neophobia. In addition, the total feed intake of litters before weaning positively correlates with the amount of feed eaten (Kuller et al., 2004; Berkeveld et al., 2007b; Langendijk et al., 2007) and the amount of body weight gain (Berkeveld et al., 2007b; Langendijk et al., 2007) during the initial days after weaning. It should be noted that in these correlation studies only the same feed was given before and after weaning. Taken together, the effects of creep feed provision on post-weaning feed intake are highly dependent on the intake of solid feed (eaters vs. non-eaters within creep-fed litters) and the amount of feed ingested (good vs. bad eaters) and these effects seem most pronounced in the early post-weaning period.

Given the benefits of creep feed intake on initial post-weaning performance, it has been proposed that creep feed intake may reduce stress at weaning (Van der Meulen et al., 2010) and, consequently, may reduce weaning-stress-induced behaviours. However, there are no indications so far that the provision of creep feed affects the stress response of piglets around weaning (Van der Meulen et al., 2010). The impact of creep feed provision on stress has only been looked at in one study, disallowing a general conclusion.

Table 2. Effects of creep feed provision on pre-weaning and post-weaning piglet performance. Litters provided with *ad libitum* creep feed are compared to litters withheld from creep feed unless stated otherwise.

Reference	**SA**	**WA**	**Dietary transition**	**Pre-weaning effects (d0 = birth)**				**Post-weaning effects (d0 = weaning)**			
				Feed intake	**WW**	**ADG**	**Other**	**ADFI**	**ADG**	**FCR**	**Other**
Aherne et al., 1982	14	35	same feed	750 g/piglet[1] 680 g/piglet[2]	↑	↑	↑ hemoglobin level	= d0-21	= d0-21	= d0-21	↑ BW d21 = hemoglobin level = faecal consistency
Hampson et al., 1985	10	21	same feed								↓ stomach pH, SI pH, SI coliform counts = faecal water content
Hampson and Smith, 1986	3	21	same feed		=		= plasma xylose	↑ d0-7			= BW d7 = plasma xylose
Hampson and Kidder, 1986	10	21	same feed	236 g/piglet	=			↓ d0-5	= d0-12		= SI sucrase and lactase activities
Hampson, 1986	10	21	same feed				= SI villus length, crypt depth, villus:crypt ratio, enterocyte counts				= SI villus length, crypt depth, villus:crypt ratio, enterocyte counts
Barnett et al., 1989	10	28	abrupt	13-194 g/piglet	=	=		= d0-35	= d0-35	= d0-35	↗ diarrhoea d0-14
Mathew et al., 1994	15	31	same feed	317-512 g/piglet[3]			= ileal pH = ileal volatile fatty acid, *E. coli* and lactobacilli concentrations		=		
Carstensen et al., 2005	14	29	same feed	behavioural observations as estimate for feed intake[4]				= d0-5			= diarrhoea occurrence d0-5 = shedding of haemolytic *E. coli*

Continue

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Reference	SA	WA	Dietary transition	Pre-weaning effects (d0 = birth)				Post-weaning effects (d0 = weaning)			
				Feed intake	WW	ADG	Other	ADFI	ADG	FCR	Other
Pluske et al., 2005	7	28	not indicated		=	=			↑ d0-7 = d0-28 = d0-slaughter		= BW at slaughter = carcass weight = dressing %
Hedemann et al., 2007	17	31	not indicated	behavioural observations as estimate for feed intake[5]			= villus length, crypt depth, mucosa thickness of SI, caecum and colon	=			= d5 villus length, crypt depth, mucosa thickness of SI, caecum and colon
Guillou and Simongiovanni, 2009	10	28	abrupt	72 g/litter/d	=			= d0-21, 0-41			= BW d41 ↗ % of pigs within 1 standard deviation of the mean in BW at d41
Van der Meulen, 2010	12	28 or 49	same feed	measured on the day before weaning 104 ± 88 g/piglet/d when weaned at d28 580 ± 138 g/piglet/d when weaned at d49	= when weaned at d28 ↑ when weaned at d49		= plasma cortisol, intestinal fatty acid-binding protein ↑ SI villus length when weaned at d28 ↑ SI crypt depth = SI villus:crypt ratio = SI mucosal-to-serosal permeability coefficient of Na-FITC and HRP ↑ SI length ↑ SI circumference when weaned at d49 = SI net absorption	= d1-11 when weaned at d28 ↑ d1-11 when weaned at d49	= when weaned at d28 ↑ d1-11 when weaned at d49		d4 and d7: = plasma cortisol and intestinal fatty acid-binding protein (also on d0, d1, d11) = SI villus length ↑ SI crypt depth = SI villus:crypt ratio = SI mucosal-to-serosal permeability coefficient of Na-FITC and HRP ↑ SI length ↑ SI circumference when weaned at d49 = SI net absorption

Reference	SA	WA	Dietary transition	Pre-weaning effects (d0 = birth)				Post-weaning effects (d0 = weaning)			
				Feed intake	WW	ADG	Other	ADFI	ADG	FCR	Other
Beaulieu et al., 2010	21	28	same feed		=			= d0-14 ↘ d0-1	↓ d0-14	↓ d0-14	↑ BW d0, d1 and d4 for heavy-weaned piglets ↓ feeder visits d0, d1, d4
Sulabo et al., 2010a	3	21	not indicated	1.0 kg/litter	=	=	= litter CV % ↘ mortality %	= d0-28, 0-14, 0-21	= d0-28, 0-14, 0-21	= d0-28, 0-14, 0-21	= BW d14, d21 and d28
Bandara et al., 2012	21	26	abrupt	240 g/litter/d	=	=			= d0-28, 0-3		= BW d3 and d28
Yan et al., 2011	5, 10 or 15	21	same feed	252 g/piglet, 249 g/piglet, 154 g/piglet when given from d5, d10 and d15		=	= mortality % = suckling, sleeping, fighting frequency		=		↓ diarrhoea score d0-7 for piglets given creep feed from d5 or d10 vs. from d15 or piglets without creep feed
Shea et al., 2013	14 or 21	21 or 28	abrupt	57 g/litter/d between d14-21 203 g/litter/d between d21-28	=	↑ when given from d21		↑ d0-56, 0-7, 7-14	↑ d0-56, 0-7, 7-14	=	↗ BW d7 ↑ BW d14 and d56

Continue

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				Feed intake	**WW**	**ADG**	**Other**	**ADFI**	**ADG**	**FCR**	**Other**
Torrallardona et al., 2012	not indicated	26	abrupt					=, ↑ or ↓ depending on weaner diet composition	=	=	d21 jejunum: ↓ crypt depth = villus length and villus:crypt ratio = no. of intraepithelial lymphocytes in villus or crypt = intragroup similarities of ileal microbiota ↓ intragroup similarities of caecal microbiota depending on weaner diet composition
Cabrera et al., 2013	14	21	same feed or with supplement	49 g/piglet	=		= mortality %	= d0-42	= d0-42	= d0-42	= BW d0-42 d7 jejunum: ↑ cell proliferation = villus length and width, crypt depth = maltase activity = absorptive capacity for mannitol and xylose
Park et al., 2014	4	21	abrupt		=	=	= mortality %		= d0-33, 40-141		= BW d33 and d141
Huting et al., 2017	10	28	abrupt	20 g/piglet/d on day before weaning	=	=	= mortality % ↓ litter CV for heavy piglets	=	=		= BW d0-165

Reference	SA	WA	Dietary transition	Pre-weaning effects (d0 = birth)				Post-weaning effects (d0 = weaning)			
				Feed intake	WW	ADG	Other	ADFI	ADG	FCR	Other
Lee and Kim, 2018	7, 14 or 21	24	pigs were studied until weaning	7.49 kg/litter between d7-24 4.82 kg/litter between d14-24 1.56 kg/litter between d21-24	↑ when given from d7 and d14	↑ when given from d7 and d14	= mortality % = faecal score				
Muns and Magowan, 2018	18	28	same feed	6.2 ± 3.3 kg/litter	=	=	= mortality % = SI villus length, crypt depth and their ratio	↑ d0-7 ↗ d0-21	=	=	d7, when fed on a high post-weaning feed allowance: ↓ jejunum villus:crypt ratio ↑ ileum villus:crypt ratio d21: ↓ jejunum villus length = duodenum and ileum villus length and crypt depth

SA = start age of creep feed provision in days
WA = weaning age in days
WW = weaning weight
ADG = average daily gain
ADFI = average daily feed intake
FCR = feed conversion ratio
BW = body weight
CV = coefficient of variation
SI = small intestine
↑ Significant increase, ↗ tendency to increase
↓ Significant decrease, ↘ tendency to decrease
= No difference
Cell left empty means not studied

[1] Piglets within a litter were provided with creep feed or not for two times of 70 minutes a day in isolated compartments

[2] Litters were provided with creep feed are compared to litters withheld from creep feed

[3] Piglets within a litter were provided with creep feed or not for one time of 2 hours a day

[4] Piglets within a litter were provided with creep feed or not for two times of 20 minutes a day

[5] Piglets within a litter were provided with creep feed or not for two times of 1 hour a day

Table 3. Effects of creep feed intake on pre-weaning and post-weaning piglet performance. Eaters are compared with non-eaters within creep-fed litters unless stated otherwise.

Reference	SA	WA	Dietary transition	Pre-weaning effects (d0 = birth)		Post-weaning effects (d0 = weaning)			
				BW	ADG	ADFI	ADG	FCR	Other
Barnett et al., 1989			abrupt						↑ antibody titre response to ovalbumin NE vs. E and NF
Bruininx et al., 2002	11	28	abrupt			↑ d0-8 E vs. NE and NF = d9-34, d0-34 E vs. NE and NF	↑ d0-8, d9-34, d0-34 E vs. NE and NF	↗ d0-8 E vs. NE ↗ d9-34, d0-34 E vs. NF	↑ feeder visits with FI d0-8 for E vs. NE and NF = total feeder visits^1 = initial FI^2 and daily increase in FI^3 ↓ latency to eat E vs. NE ↘ latency to eat E vs. NF
Kuller et al., 2007a	7	25	same feed from d23-31	↓ d14 = d21, 25	↘ d0-6, d7-13 = d14-20 ↑ d21-24		↑ d0-6, d7-13, d14-20, d21-27 = d28-112		↑ BW d7-27 = BW d56-112 = time to slaughter = lean meat %
Kuller et al., 2007b	7	27	same feed from d23						= SI length and width ↑ SI net absorption in uninfected segments = SI net absorption in *E. coli*-infected segments
Callesen et al., 2007a	14	27 or 33	same feed		=		= d0-14 when weaned at d27 ↗ d0-14 when weaned at d33		= faecal consistency d0-14 = antibiotic treatment days d0-14
Callesen et al., 2007b	14	27 or 33	pigs were studied until weaning	= or ↑ at weaning depending on three-way interaction between creep feed intake categorisation, weaning age and diet composition	=				

Reference	SA	WA	Dietary transition	Pre-weaning effects (d0 = birth)		Post-weaning effects (d0 = weaning)			
				BW	ADG	ADFI	ADG	FCR	Other
Sulabo et al., 2010a	3	21	not indicated				↑ d0-28 E vs. NE and NF		↗ BW d21 E vs. NF
Sulabo et al., 2010d	7, 14 or 18	20	abrupt	↓ at d20		↑ d0-3, d0-7 ↗ d0-14 = d0-21, d0-28	↑ d0-3 ↗ d0-7 = d0-14, d0-21, d0-28	= d0-3, d0-7, d0-14, d0-21, d0-28	= BW d3, 7, 14, 21 and 28
Bandara et al., 2012[4]	21	26	abrupt	↓ at d26 E+NuE vs. NE+NuE and NE+NuNE			↑ d0-28 E+NuE vs. NE+NuE and NE+NuNE ↑ d0-3 E+NuE vs. E+NuNE and NE+NuE and NE+NuNE ↑ d4-7 E+NuE and E+NuNE vs. NE+NuE and NE+NuNE (of which NE+NuE higher than NE+NuNE for all three periods mentioned)		= BW d3 ↑ BW d28 E+NuE vs. NE+NuNE
Shea et al., 2013[5]	14 or 21	21 or 28							

SA = start age of creep feed provision in days
WA = weaning age in days
BW = body weight
ADG = average daily gain
ADFI = average daily feed intake
FCR = feed conversion ratio
E = eater, NE = non-eater, NF = not provided with creep feed, Nu = nursery
↑ Significant increase, ↗ tendency to increase
↓ Significant decrease, ↘ tendency to decrease
= No difference
Cell left empty means not studied

[1] Total feeder visits = feeder visits with feed intake (FI) + feeder visits without FI

[2] Amount of feed consumed during the 24 h following the first visit with feed intake to the feeding station

[3] Daily increase in FI between the day of the first visit with feed intake and the day on which energy intake was ≥ 1.5 times maintenance levels for energy intake

[4] In lactation: creep feed eaters (E) vs. non-eaters (NE). In initial nursery period: nursery eaters (NuE) vs. non-eaters (NuNE). Study confounded by weaning weight (see table) and weaning age (E+NuE were older at weaning than E+NuNE and NE+NuNE)

[5] Data are not reported in the table as data were unbalanced and not analysed statistically in the study

Table 4. Effects of creep feed intake level (good, moderate, bad and non-eaters) on pre-weaning and post-weaning piglet performance. Good, moderate, bad and non-eaters are compared within creep-fed litters unless stated otherwise.

Reference	SA	WA	Dietary transition	Pre-weaning effects (d0 = birth)		Post-weaning effects (d0 = weaning)			
				BW	ADG	ADFI	ADG	FCR	Other
Kelly et al., 1990[1]	8	14	same feed		↑ high creep feed level vs. low creep feed level and NF		= d0-12	= d0-12	
Delumeau and Meunier-Salaün, 1995	7	28	not indicated				↗ d1, d2-4, d1-4 GE vs. ME and BE	=	
Bruininx et al., 2004	11	28	abrupt	= d28	↑ GE vs. ME and NE	= SI villus length, crypt depth, villus:crypt ratio at d28 = colonic volatile fatty acid concentration, digesta dry matter content at d28	↑ d0-4 GE vs. NE	↑ d0-4 GE vs. NE	= latency to eat = initial feed intake[2] ↑ feeder visits with intake for GE vs. NE d0-4 ↑ rate of feed intake for GE vs NE d0-4 = total feeder visits[3] d0-4 = time per visit d0-4 = feed intake per visit d0-4 = SI villus length, villus:crypt ratio at d5 ↘ crypt depth NE vs. ME at d5 = colonic volatile fatty acid concentration, digesta dry matter content at d5
Carstensen et al., 2005	14	29	same feed				↓ d0, d1 BE vs. GE and NE = d2, d3, d4		↘ diarrhoea prevalence BE vs. GE and NE ↓ faecal *E. coli* O149 BE vs. GE and NE = haemolytic *E. coli* and *E. coli* O138

Reference	SA	WA	Dietary transition	Pre-weaning effects (d0 = birth)		Post-weaning effects (d0 = weaning)			
				BW	ADG	ADFI	ADG	FCR	Other
Kim et al., 2005	12	31	not indicated	= d19, d23, d27, d31	↑ GE vs. B/NE			↑ GE and ME vs. B/NE d0-4, d4-8 = d8-15, d15-29, d29-79	= BW d0, d4, d8, d15, d29, d79
Pluske et al., 2007	12	31	same feed		↗ GE and ME vs. B/NE			↑ d0-28, d0-3 GE and ME vs. B/NE ↑ d4-7 ME vs. GE and B/NE = d29-78	
Guillou and Simongiovanni, 2009	10	28	abrupt	= d28			↑ d0-21 EE, LE and NF vs. NE ↗ d0-41 EE vs. NE		↑ BW d41 for EE, LE and NF vs. NE

SA = start age of creep feed provision in days
WA = weaning age in days
BW = body weight
ADG = average daily gain
ADFI = average daily feed intake
FCR = feed conversion ratio
GE = good eater, ME = moderate eater, BE = bad eater, NE = non-eater, B/NE = bad/non-eater, EE = early eater, LE = late eater, NF = not provided with creep feed
↑ Significant increase, ↗ tendency to increase
↓ Significant decrease, ↘ tendency to decrease
= No difference
Cell left empty means not studied

[1] Gastric incubation of 10 gram creep feed per day (low creep feed intake) is compared with gastric incubation of an increasing dose of creep feed (high creep feed intake: 60, 80, 90, 100, 110 and 110 g/d until weaning)

[2] Amount of feed consumed during the 24 h following the first visit with feed intake to the feeding station

[3] Total feeder visits = feeder visits with feed intake + feeder visits without feed intake

Creep feed provision and potential benefits on gastrointestinal development

Creep feed provision is hypothesized to prepare the gastrointestinal tract for the post-weaning diet by stimulating development of the digestive and absorptive system. Studies that looked at gut health in relation to creep feed provision and intake vary a lot in the read-out parameters that were used to determine gut health and in the way they were determined. Whilst discussing the results, one should therefore keep in mind that generating one conclusion from these studies is not possible. Activity of several enzymes including pepsin produced by the stomach and amylase produced by the pancreas were only associated with creep feed intake to a limited extent (Lindemann et al., 1986; Chapple et al., 1989; De Passillé et al., 1989). Moreover, creep-fed and non-fed piglets did generally not differ in faecal consistency, gut morphology, permeability and absorption before weaning (e.g. Hedemann et al., 2007; Muns and Magowan, 2018; **Table 2**), except that creep-fed piglets had higher villi and deeper crypts along the small intestine in one of the studies (Van der Meulen et al., 2010). In addition, (good and moderate) eaters did not differ in villus:crypt ratio of the small intestine, and volatile fatty acid concentrations and dry matter content in the colon before weaning from non-eaters (Bruininx et al., 2004). When measurements were taken after weaning some studies reported no effects of creep feed provision (**Table 2**) and creep feed intake (**Table 3**) on faecal consistency and gut development (e.g. Carstensen et al., 2005; Hedemann et al., 2007; Callesen et al., 2007a), while others reported subtle effects on the length, circumference, villus:crypt ratio, cell proliferation and net absorption of the small intestine (e.g. Kuller et al., 2007b; Cabrera et al., 2013; Muns and Magowan, 2018). Moreover, the structure of the small intestine and the concentration of colonic volatile fatty acids in the post-weaning period were not affected by the amount of feed ingested before weaning (Bruininx et al., 2004), with ambiguous results of creep feed intake level on post-weaning diarrhoea. On one hand, litters that consumed more feed during lactation as result of a longer duration of creep feed provision, had lower post-weaning diarrhoea scores than litters with lower amounts of creep feed ingested before weaning (Yan et al., 2011). On the other hand, piglets with a low creep feed contact had less shedding of faecal *E. coli* O149, which was associated with post-weaning diarrhoea in the same study, than piglets with a high or no creep feed contact. Nevertheless, piglets with a high post-weaning feed intake (> 300g) had a lower shedding of *E. coli* O149 than piglets with a lower post-weaning feed intake (200-300g or < 200g), suggesting sufficient feed intake after weaning is important in reducing post-weaning diarrhoea (Carstensen et al., 2005). To date there is a lack of studies that investigated both the incidence and severity of post-weaning diarrhoea, as well as the morphology and function of the gastrointestinal tract in the same study. Although it has become clear that feed intake before and after weaning can influence gut health after weaning, a comprehensive understanding of the complex interplay between feed intake and gut health is needed before more specific conclusions can be drawn.

It has also been suggested, by Yao et al. (2008), that creep feed provision may steer the development and composition of the microbiota in the gut. Such an effect is expected because the gut microbiota are more dynamic, unstable and variable among individuals early in life than later in life. The development of gut microbiota in suckling piglets is affected by host genetics (Bian et al., 2016) and environmental factors, including the sow and her diet and antibiotic exposure, age (Bian et al., 2016; Poulsen et al., 2018), rearing environment (Mulder et al., 2009; Inman et al., 2010; Schmidt et al., 2011; Merrifield et al., 2016), stress (Schokker et al., 2015), antibiotics (Schokker et al., 2014; Schokker et al., 2015; Yu et al., 2017; Poulsen et al., 2018) exposure to soil (Vo et al., 2017), diet (Yeruva et al., 2016; Shi et al., 2018), probiotics (e.g.

Liu et al., 2017; Poulsen et al., 2018) and prebiotics (Alizadeh et al., 2016). It has been shown that 'early life programming' of the gut microbiota by such environmental factors can have a strong influence, including long-term, on gut maturation and (mucosal) immune development (e.g. Alizadeh et al., 2016; Yeruva et al., 2016; Liu et al., 2017) and metabolism (Benis et al., 2015; Merrifield et al., 2016; Shi et al., 2018). In addition, the dietary transition from sow's milk to solid feed at weaning results in a dramatic shift in the gut microbiota population from a 'milk-oriented' to a 'plant-oriented' microbiome (Konstantinov et al., 2006; Su et al., 2008; Frese et al., 2015; Li et al., 2018). An illustration of the abrupt shift in gut microbiota population around weaning is given in **Figure 4**. Creep feed provision may facilitate this dietary change at weaning by stimulating maturation of gut microbiota towards a microbiota composition with an expanded capacity to digest plant-derived nutrients, which may coincide with accelerated intestinal, metabolic and immunological maturation.

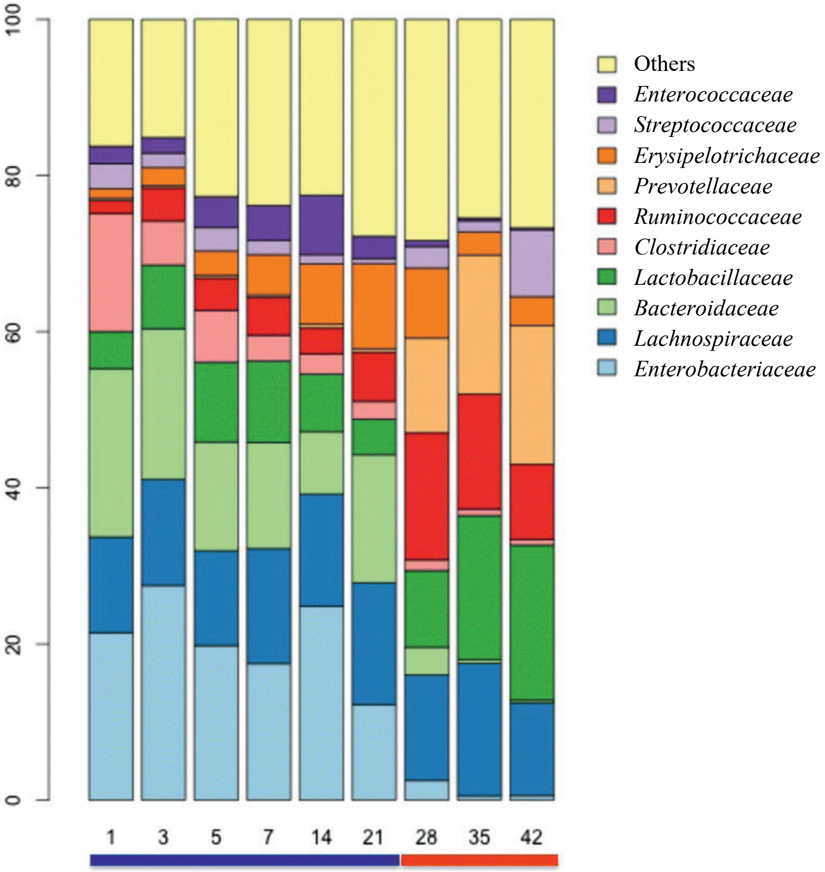


Figure 4. Gut microbiota populations (% of total population) in pig faeces in the first 6 weeks of life. The x-as visualises the days of age. Piglets were suckling the sow without creep feed and fed with an oat-based weaner diet after 21 days of age (i.e. weaning). A significant change in bacterial taxa was observed at weaning, except for *Erysipelotrichaceae* and *Lachnospiraceae*. Reprinted from Frese et al. (2015).

Few studies have looked at the consequences of creep feed provision on gut microbiota development, but there is some evidence that effects on microbiota exist in specific intestinal segments. Creep feed provision increased the heterogeneity of the caecal microbiota of piglets after weaning, but this effect was dependent on the composition of the post-weaning diet that was given, as no effects were reported for other cereal sources in the post-weaning diet than oats and barley (Torrallardona et al., 2012; **Table 2**). Moreover, the succession of faecal microbiota was affected by providing solid feed at one (Yao et al., 2011) and two weeks of age (Bian et al., 2016). Moreover, Yao et al. (2011) found that creep feed provision stabilized the *Lactobacillus* community after weaning and found high similarity coefficients between samples during creep feed provision and after weaning. It should be noted though that the latter two studies did not report the amount of feed ingested and were comparing time points before and after the start of creep feed provision (and were therefore confounded with age) rather than comparing creep-fed and non-fed piglets. In addition, Mathew et al. (1994) failed to find differences between creep-fed and non-fed piglets in digesta pH and concentrations of lactobacilli*, E. coli* and short-chain fatty acids in the ileum before weaning, of which the latter is an indication of the fermentation activity of the gut microbiota (Mathew et al., 1994; **Table 2**). Also no differences in volatile fatty acid concentrations in the colon were apparent at weaning and five days post-weaning as result of the amount of creep feed ingested (Bruininx et al., 2004). Although some indications are present, the role of creep feed provision on gut microbiota development is still ambiguous. The development of gut microbiota along the gastrointestinal tract of creep-fed versus non-fed piglets, and of eaters versus non-eaters and good versus bad eaters, therefore warrants further investigation before conclusions can be drawn.

Creep feed provision and potential benefits on growth before weaning

Creep feed provision may also provide benefits in the pre-weaning period, by supplementing sow milk production (Williams, 2003; Pluske et al., 2018). The milk supply by the sow often becomes limiting for the growth rate of suckling piglets, already during early- to mid-lactation (Hansen et al., 2012). Apart from the amount of milk, also the protein content of the milk may limit piglet growth (Le Dividich and Sève, 2001). The contribution of creep feed to total energy intake is hard to determine, but estimated to be relatively small and variable (7%: Pajor et al., 1991; 1-17%: Pluske et al., 1995; 2.7%: Lawlor et al., 2002b; 37% in the week before weaning: Pajor et al., 1991) and therefore expected to have only a limited contribution to pre-weaning body weight gain (Adeleye et al., 2014). Creep feed provision did mostly not influence pre-weaning average daily gain, weaning weight, litter uniformity and mortality (**Table 2**). Yet, positive effects on pre-weaning growth (Aherne et al., 1982; Shea et al., 2013; Lee and Kim, 2018) and weaning weight (Van der Meulen et al., 2010; Lee and Kim, 2018) were achieved in studies with a relatively high pre-weaning feed intake. This finding corresponds to higher growth rates in the period of creep feed provision for good eaters compared to bad and non-eaters (**Table 4**). Although eaters were consistently reported to have better growth rates after weaning compared to non-eaters (**Table 3**), the effect on pre-weaning growth was inconsistent in these studies with no, positive or negative effects found. Creep feed provision can be beneficial to supplement sow milk production and to increase pre-weaning piglet performance, but only seem to become apparent when high creep feed intake levels are achieved.

Variation in creep feed intake

In the previous section, evidence was found that all three hypotheses (effects of creep feed provision on initial post-weaning feed intake, pre-weaning gut development and pre-weaning growth development) may be true and became particularly visible with actual intake of creep feed and increasing amounts of creep feed ingested (**Table 2** to **4**). It is therefore important that every piglet eats (sufficient amounts of) creep feed for beneficial effects on pre- and post-weaning piglet performance to occur. However, the intake of creep feed is generally low in the farrowing room (e.g. 34-49 g/piglet when weaned at 3 weeks of age: Cabrera et al., 2013; Collins et al., 2013; 78-231 g/piglet when weaned at 4 weeks: Collins et al., 2013; Blavi et al., 2016; Huting et al., 2017). Moreover, it varies greatly between litters (e.g. 24-690 g/piglet: Bruininx et al., 2004; 8-1056 g/piglet: Kuller et al., 2007a) and within litters (e.g. 118-1385 g/piglet: Pajor et al., 1991; 0-674 g/piglet: Delumeau and Meunier-Salaün, 1995b; good, moderate, bad and non-eaters: Huting et al., 2019b) with a significant proportion of piglets in the litter that fail to consume any solid feed up to weaning, ranging from 20 to 96% between studies in which piglets were weaned at 3 weeks of age and from 6 to 66% between studies in which piglets were weaned at 4 weeks of age (**Table 5**). Consequently, there is a need to investigate the factors that explain the variability in creep feed intake and strategies by which creep feed intake and the number of eaters can be increased and made more consistent.

Variation in creep feed intake between litters

Weaning age

The most obvious factors to influence creep feed intake are the age at which the feed is provided, the duration of creep feed provision and the duration of lactation, as creep feed intake and the number of piglets eating increase with piglet age (Pluske et al., 2007; Tucker et al., 2010; Huting et al., 2017) in an exponential manner (**Figure 5A** and **B)**. For example, litters that were given creep feed from 16 and 11 days prior to weaning at 3 weeks of age had a higher feed intake than piglets that were given creep feed from the last 6 days before weaning (Yan et al., 2011). In accordance, litters that were creep-fed from 13 days prior to weaning at 3 weeks of age had a larger number of eaters than litters that were creep-fed from the last 6 or 2 days prior to weaning (Sulabo et al., 2010d). Moreover, creep feed consumption was larger by piglets with a longer duration of lactation, since total creep feed consumption from 9 days of age was larger by piglets weaned at 4 compared to 3 weeks of age, but the percentage of eaters at weaning did not differ between the weaning ages (Collins et al., 2013). Although not statistically tested, creep feed seemed more likely to be consumed in higher total amounts (Callesen et al., 2007b), in higher daily amounts (Van der Meulen et al., 2010), and also, in comparison to Collins et al. (2013), by a higher number of piglets when weaned at a later age (Van der Meulen et al., 2010; Shea et al., 2013; Gauvreau and Beaulieu, 2014).

Table 5. Proportion of piglets that do not consume creep feed, reported across interventions and over time.

Reference	Intervention	SA	Categorisation method	Sampling days	Non-eaters, %
Barnett et al., 1989		10	1.0% chromic oxide, faecal loop	two times per day between d10-28	35% at d28 calculated from data in paper
Delumeau and Meunier-Salaün, 1995		7	live behavioural observations, 5-min scan sampling for 90 min	every two days between d5-27	>50% at d27 out of eaters between d14-27: 14% good eaters (>100 g feed intake) 28% moderate eaters (10-100 g) 58% bad eaters (< 10 g)
Bruininx et al., 2002		11	1.0% chromic oxide, faecal loop	d18, 22 and 27	19% at d27
Bruininx et al., 2004		11	1.0% chromic oxide, faecal loop	d18, 22 and 27	24% good eaters (3 times green faeces) 33% moderate eaters (1-2 times) 10% non-eaters (0 times) 33% indistinguishable
Kuller et al., 2007a	intermittent suckling (NS)	7	1.0% chromic oxide, rectal swab	d17, 21, 23 and 24	control: 63% intermittent suckling: 70% at d24
Callesen et al., 2007b	no intervention, but iron was fed on the floor during first 2 weeks	14	5g Indigo carmine per kg of feed added from d24, defaecation after individual separation of piglets	d27	22%
Pluske et al., 2007	piglet age ($P < 0.001$)	12	5g Indigo carmine per kg of feed, defaecation after individual separation of piglets	d19, 23, 27 and 31	51%^{a} at d19 28%^{b} at d23 16%^{b} at d27 20%^{b} at d31 at weaning: 37% good eaters (4 times blue faeces) 48% moderate eaters (2-3 times) 15% bad/non-eaters (0-1 times)
Devillers and Farmer, 2009		21	live behavioural observations, 1-min scan sampling for 60 min	d41	58.6%
Guillou and Simongiovanni, 2009		10	2g chromic oxide per kg of feed, rectal swab	d19 and 26	28% early eaters 61% late eaters 11% non-eaters

Reference	Intervention	SA	Categorisation method	Sampling days	Non-eaters, %
Sulabo et al., 2010a	feed intake of the sow (NS)	3	1.0% chromic oxide, rectal swab	d7, 14 and 21	restrictedly-fed sows: 43% *ad libitum*-fed sows: 38% at d21
Sulabo et al., 2010b	feeder type ($P < 0.001$)	18	1.0% chromic oxide, rectal swab	two times per day at d21 (12 and 3 h before weaning)	with hopper: 31%^{a} without hopper: 53%^{b} pan feeder: 58%^{b} at d21
Sulabo et al., 2010c	feed flavour (NS)	18	1.0% chromic oxide, rectal swab	two times per day at d21 (12 and 3 h before weaning)	enhanced milky flavour: 31% no flavour: 27% at d21
Sulabo et al., 2010d	creep feed duration ($P < 0.03$)	7, 14 or 18	1.0% chromic oxide, rectal swab	two times per day at d14, 18 and 20 for duration of 13 days, d18 and 20 for duration of 6 days and d20 for duration of 2 days	creep feed duration: 13 days: 20%^{a} 6 days: 30%^{b} 2 days: 29%^{b} at d20
Tucker et al., 2010	no intervention, but dentition (premolars erupted and occluded or not) was assessed (NS) piglet age ($P <$ 0.0001 between each day)	5	1.0% chromic oxide, rectal swab	d7, 10, 14, 17, 21, 24 and 27	100% at d6 99% at d0 95% at d14 92% at d17 71% at d21 55% at d24 39% at d27
Van der Meulen et al., 2010	weaning age (not analysed)	12	1.0% chromic oxide, faecal loop	10, 6 and 3 days before weaning	weaned at d28: 95% at d18 59% at d25 weaned at d49: 32% at d39 10% at d46
Bandara et al., 2012		21	0.5g Brilliant blue per kg of feed, rectal swab	d25	63% at d25
Shea et al., 2013		14 or 21	0.5g Brilliant blue per kg of feed, rectal swab	d21 or 28	96% at d21 66% at d28

Continue

Table 5. Proportion of piglets that do not consume creep feed, reported across interventions and over time.

Reference	Intervention	SA	Categorisation method	Sampling days	Non-eaters, %
Gauvreau and Beaulieu, 2014		14 or 21 (7 days before weaning)	0.5g Brilliant blue per kg of feed, added in the last week before weaning, rectal swab	d21 or 28	56-77% at d21 (depending on farm) 18% at d28
Collins et al., 2013	weaning age (NS) diet composition (NS) birth weight (NS) pre-weaning growth (NS)	9	0.2g Ravicol blue per kg of feed, defaecation after placing piglets in individual boxes	d16, 19, 22 or 29	60% at d16 50% at d19 36% at weaning 23-24% at d22 12-19% at d29 good eaters: 22-26% (3 times blue faeces) moderate eaters: 20-29% (2 times) bad eaters: 26-36% (1 time) non-eaters: 12-24% (0 times)
Wu et al., 2014	diet composition ($P < 0.05$)	19	0.5% Brilliant blue added for 3 days, rectal swab	d26	control: 65% bovine colostrum supplemented creep feed: 56%
Van Nieuwamerongen et al., 2015	housing system (NS)	12 (d12-20: creep feed; d21-22: mix creep/weaner feed; d23-27: weaner feed)	faecal colour and consistency, rectal swab. Eaters had brown faeces with a coarse structure. Non-eaters had yellow faeces with a smooth structure.	d26	multi-litter system: 30% single-litter system: 36%
Seddon et al., 2016	feeder type ($P = 0.0002$) enrichment provision (NS)	10	0.5g Brilliant blue per kg of feed at d12, 19, 24, rectal swab	d14, 21 and 26	standard feeder: 48% tray feeder: 27% at d26 enrichment: 42% no enrichment: 33% at d26
Blavi et al., 2015	flavour enhancer (NS)	2	5g Indigo carmine per kg of feed, rectal swab	d7, 14, 21 and 28	basal diet vs. milky derived flavour diet: exact numbers not deducible (4.1 eaters per litter)

Reference	Intervention	SA	Categorisation method	Sampling days	Non-eaters, %
Clark et al., 2015	pellet diameter (NS)	10	1.0% chromic oxide, rectal swab	two times per day at d14, 17 and 21 (07:00 and 16:00 h)	3-mm diameter pellet: 13-mm diameter pellet: 83% 83% at d14 60% 60% at d17 42% 41% at d21
De Ruyter et al., 2017	gradual sow separation ($P = 0.10$)	11	5g Indigo carmine per kg of feed, rectal swab	d16, 21, 27	control: sow separation: 52% 36% at d27
Huting et al., 2017	piglet age ($P < 0.001$) interaction between litter composition and birth weight ($P < 0.01$)	10	1.0% chromic oxide, defaecation after placing piglet on weighing scale for max. 4 min	d13, 16, 19, 21, 23 and 25, of which d19, 21 and 25 were used to classify eaters	97% at d13-16 70% at d21-23 <50% at d25 depending on litter composition and birth weight: good eaters: 5-18% (3 times green faeces) moderate eaters: 9-19% (2 times) bad eaters: 21-39% (1 time) non-eaters: 27-64% (0 times)
Heo et al., 2018	creep feed type: creep, weaner or sow feed (NS)	14	1.0% chromic oxide, rectal swab	d21 and 28	38-52% at d21 6-9% at d28
Muns and Magowan, 2018	no intervention, but piglets were fed on the floor at the heated piglet nest area	18	1.0% chromic oxide, rectal swab	d23, 25 and 27	83% early eaters (2 times green faeces) 11% late eaters (1 time) 6% non-eaters (0 times)

Continue

Table 5. Proportion of piglets that do not consume creep feed, reported across interventions and over time.

Reference	Intervention	SA	Categorisation method	Sampling days	Non-eaters, %
Huting et al., 2019	trend for interaction between foster sow parity and birth weight (*P* = 0.06) foster sow parity (*P* < 0.05 for non, bad and moderate eater) birth weight (*P* < 0.02 for non, moderate and good eaters and *P* = 0.09 for bad eaters)	10	1.0% chromic oxide, defaecation by placing piglet on weighing scale for max. 4 min.	d19, 21 and 25	depending on foster sow parity and birth weight: good eaters: 0-22% (3 times green faeces) moderate eaters: 2-20% (2 times) bad eaters: 13-38% (1 time) non-eaters: 28-80% (0 times)
Van Hees et al., 2019	diet composition (NS)	2 (milk replacer with marker) 14 (creep feed with marker)	0.3% chromic oxide, rectal swab. Eaters had a coloured faecal sample on d13, 19 or 22.	d6, 13, 19 and 22	20-37%

SA = start age of creep feed provision in days
NS = not significant
Within a row means with different superscript letters differ ($P < 0.05$)

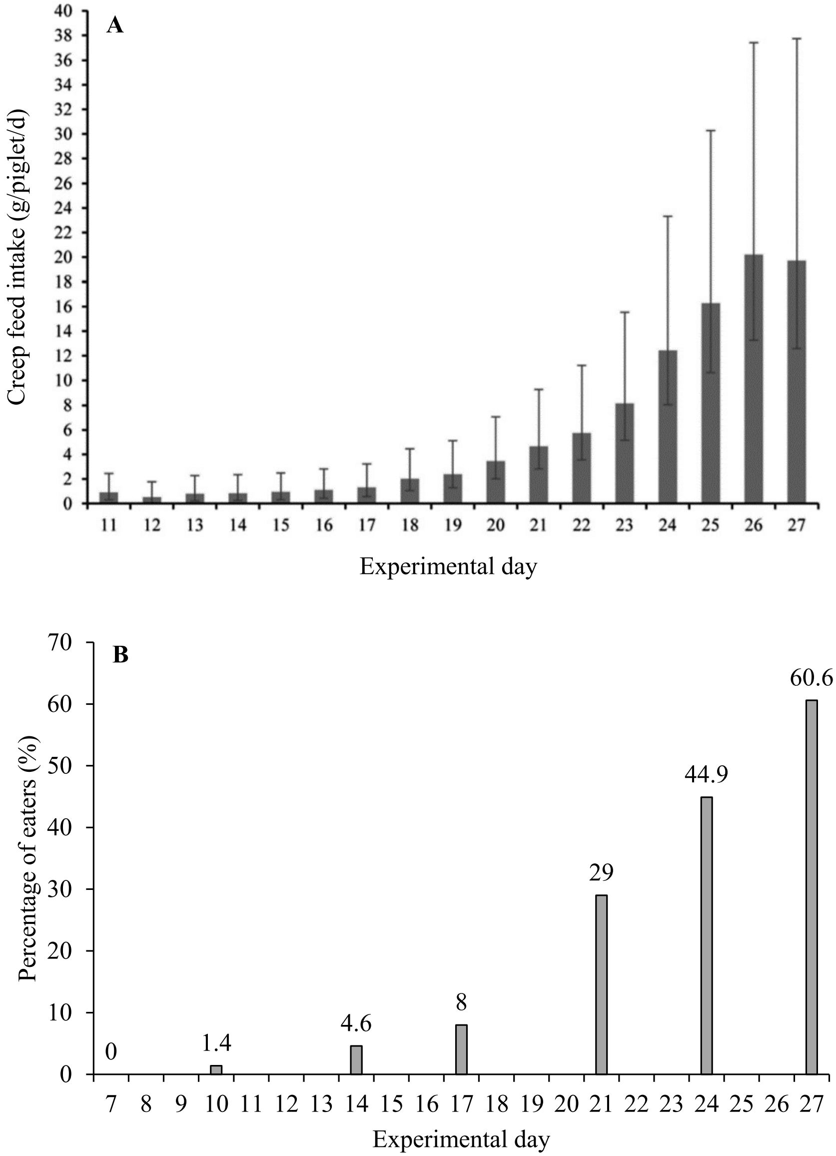


Figure 5. The effect of experimental day on creep feed intake (g/piglet/d) across litters (**A**). Pigs had access to *ad libitum* creep feed from d10 of lactation up to weaning at d27. Data are expressed as least squares means, with error bars representing the 95% confidence interval. Experimental day affected creep feed intake (*P* < 0.001) and from d18 onward, creep feed intake significantly increased over time (*P* < 0.05). Figure reprinted from Huting et al. (2017). The effect of experimental day on the percentage of eaters (**B**), i.e. piglets with green colouring of a rectal swab which indicates prior ingestion of creep feed marked with green feed colourant chromic oxide. The percentage of eaters significantly increased over time (*P* < 0.0001). Data are expressed as raw means based on data from Tucker et al. (2010).

Social learning

Under intensive commercial farming conditions, sows are typically housed with one litter per pen, confined in a crate and fed in an elevated trough. It has been suggested by Wattanakul et al. (2005), however, that creep feed consumption may be higher if piglets have more opportunities for social learning of feeding behaviour, like in natural conditions. Studies suggest that piglets can learn from the sow about novel feed by observation, participation, local and stimulus enhancement (Oostindjer et al., 2011a, 2011c). Local enhancement indicates that piglets are more likely to come to the location where others are eating and are more likely to also start eating there, while stimulus enhancement indicates that piglets are more likely to eat the same food the others are eating (Oostindjer et al., 2011a). Another process, which is defined as 'social facilitation', indicates that piglets are likely to eat more as the result of others being present and eating (Keeling and Hurnik, 1996). However, Wattanakul et al. (2005) and Oostindjer et al. (2010, 2011d) could not demonstrate a clear effect of sow facilitated feeding, in which piglets could eat together with their mother, on piglet creep feed intake and eating behaviour. Yet, in a group-housing system for lactating sows and their litters in which sows were fed on the floor, piglets in the group-housing system spent more time on feed-directed behaviour at two weeks of age compared to piglets in a single-litter system (i.e. one sow with her litter), suggesting earlier initiation of solid feed intake in multi-litter piglets (Van Nieuwamerongen et al., 2015). These studies suggest that the ability to learn about solid feed from the sow may reduce food neophobia and therefore result in earlier initiation of solid feed consumption, but does not result in a greater consumption of solid feed per se.

Stimulating social learning among littermates enhanced the number of feeder visits (with and without intake), the number of piglets at the feeder, and creep feed intake (Appleby et al., 1991, 1992; Delumeau and Meunier-Salaün, 1995; Wattanakul et al., 2005; Sulabo et al., 2010b; Seddon et al., 2015), which may result from a higher intake per piglet (Sulabo et al., 2010b) or a larger number of eaters (Seddon et al., 2016). Stimulating social learning among littermates is mainly done by increasing creep feeder space (e.g. from 2 to 6 feeding places) and/or a change in the creep feeder design to create a more open and/or accessible creep feeder (e.g. from a hopper to a tray feeder). These studies suggest that eating is a social activity for suckling piglets and that local and stimulus enhancement, synchronization and social facilitation of eating occur between littermates (Keeling and Hurnik, 1996). A change in the design of the creep feeder can also affect the eating posture of piglets at the feeder (**Figure 6**), as piglets were observed eating parallel to the feeder at a tray feeder and side by side at a rotary feeder (Sulabo et al., 2010b), which may affect possibilities for social learning, and thereby creep feed intake. The feeders used in the studies mentioned above differed in more aspects than size, and the optimal space allowance at the creep feeder therefore remains unknown.

Figure 6. Different eating postures of suckling piglets at a rectangular feeder (Source: A. Middelkoop, 2016).

There are subtle indications that piglets may not only be stimulated by the sow and littermates to consume creep feed, but also by non-littermates, particularly when piglets are not with the sow during that time. Co-mingling of non-littermates for 8 hours per day led to an increase in ingestive behaviours (eating plus drinking plus eliminating) over time (Turpin et al., 2017b) and when co-mingling of non-littermates was combined with intermittent suckling, these piglets spent more time on ingestive behaviours one week before weaning and ate more creep feed in the last week before weaning than piglets exposed to intermittent suckling only or conventional weaning (Turpin et al., 2017a). In addition, when both sows and piglets could spent time away from each other, by the use of get-away areas for sows and communal areas for the piglets, piglets also consumed more creep feed than piglets from litters that could not mingle with other litters and of which the sows could not get-away (Weary et al., 2002). Eating seems therefore also socially facilitated by non-littermates, although the process is stronger between littermates (Keeling and Hurnik, 1996). It can be concluded that piglets are stimulated by their sow and littermates to develop eating skills, and that non-littermates can also contribute to this process.

A matter of taste and texture

In terms of composition, creep feed is either formulated to supplement the milk production of the sow (by providing extra nutrients in a milk-type feed; high in energy, protein, fat, lactose and pH and low in starch) or formulated to stimulate the development of the gastrointestinal tract and gut microbiota (to prepare for the weaner diet; low in pH and high in starch). To the first aim of supplementing sow milk production, creep feed generally includes ingredients such as whey powder and fish meal to maximize palatability and digestibility. For example, Wu et al. (2014) found that adding powdered bovine colostrum by 6% on top of the creep feed increased the number of eaters in the farrowing room. Moreover, Tran et al. (2014) reported that incorporation of yeast-dried milk, i.e. 50% dried near-dated milk and 50% dried brewer's yeast, by 10% in the creep feed stimulated its consumption compared to a control creep feed that was higher in corn and a whey permeate. Furthermore, piglets that consumed creep feed during lactation consumed more feed in the first week of feed provision than piglets that were provided with a weaner or sow diet during lactation (Heo et al., 2018). In the latter study, the creep feed contained whey protein concentrate, cheese powder and fish meal and was high in whey powder and lactose, while the weaner diet was formulated with high levels of soybean meal and barley, and the sow diet with high levels of soybean meal and corn. On the other hand, a lower creep feed intake, but no difference in the number of good, moderate, bad and non-eaters, was reported for creep feed predominantly based on groats and whey powder compared to creep feed predominantly based on wheat, peas and groats (Collins et al., 2013). Some studies hypothesized that mimicking the taste of sow's milk may increase palatability of creep feed, but inclusion of a milky-derived flavour in the creep feed did not affect pre-weaning feed intake (Sulabo et al., 2008, 2010c; Blavi et al., 2015) and the proportion of eaters (Sulabo et al., 2010c) versus a control creep feed without flavour. In summary, the inclusion of highly palatable and digestible ingredients in the creep feed resulted in ambiguous results on its intake.

To the second aim of stimulating gut (microbiota) development, creep feeds are usually formulated with vegetable protein and fibres. Zhang et al. (2016) investigated, for example, the dietary inclusion of purified cellulose, wheat bran or alfalfa, in creep feed at the expense of corn on the intake of suckling piglets and found similar intakes of the control diet and the three fibrous diets. Comparably, Van Hees et al. (2019) did not report significant differences in feed consumption between a control low-fibre creep feed, a creep feed with largely non-fermentable purified cellulose, a creep feed with fermentable long-chain arabinoxylan or a creep feed with a combination of both fibre sources (corn starch was replaced). However, feed intake was significantly higher for the milk supplements with cellulose and both fibre sources compared to the other milk supplements that were given before the creep feed (fed as meal) was provided. Clouard et al. (2018a) did not report differences in creep feed intake between litters fed high-fibre creep feed, by replacing corn starch with purified cellulose, and litters fed the control low-fibre creep feed in one study. However, in another study by Clouard et al. (2018b), an increased intake of creep feed with cellulose compared to the control creep feed was reported. Clouard et al. (2018b) suggested that the creep feed diet form, which was powder in one study (2018a) versus porridge and pellets in the other study (2018b), may have accounted for the observed differences in creep feed intake between the studies (the effect of diet form will be discussed later on). Another example of a diet composition that aimed to improve gastrointestinal development and influence the gut microbiota is the inclusion of yeast-derived mannan-rich fraction. Creep feed with yeast-derived mannan-rich fraction was consumed in larger amounts in the last week before weaning compared to creep feed without the supplement but with a slightly higher level of the other ingredients (Fouhse et al., 2019). Lastly, examples of additives that are aimed to support the pancreatic function of the suckling pig are protease and amylase, and an example of an additive to inhibit the growth of pathogens is silicon dioxide. The addition of protease, amylase and silicon dioxide to one creep feed reduced pre-weaning feed intake compared to a control creep feed without these additives (Szczurek et al., 2016).

The composition of creep feed can thus play a role in the variation of its intake. It is important to note here, that the composition of the creep feed interacts with the composition of the weaner diet in terms of post-weaning performance (Torrallardona et al., 2012; Heo et al., 2018), and therefore both should be taken into account when aiming to stimulate post-weaning piglet performance.

Flavoured creep feed is often studied in connection with flavour exposure through maternal diets. In such studies, authors hypothesized that flavour supplementation of the sow's diet during late gestation (flavour transmission via amniotic fluid and foetal blood stream) and/or lactation (flavour transmission via the milk and smell from the mouth of the sow) would increase the acceptance of piglets towards creep feed flavoured with the same additive(s) (Oostindjer et al., 2011b). Most studies show, against expectations, that flavour learning does not stimulate creep feed intake when the flavours are also given in the creep feed (e.g. prenatal flavour exposure: Figueroa et al., 2019, 2013; perinatal flavour exposure: Langendijk et al., 2007; postnatal flavour exposure: Millet et al., 2008), with one study that found increased creep feed intake for one out of the three tested flavours (postnatal flavour exposure: Wang et al., 2014) and one study that found increased creep feed intake for the unflavoured creep feed (perinatal flavour learning: Blavi et al., 2016). In a choice setting, piglets also preferred the control creep feed over the creep feed flavoured with the flavour that was added to the sow's feed in late gestation (Figueroa et al., 2013). These inconsistent results indicate that it is hard to predict whether a flavour increases acceptance for the creep feed diet or results in aversion and reduced feed intake. Moreover, the interaction between prenatal and postnatal flavour learning on creep feed intake of piglets remains to be studied. In a study that provided weaned piglets with objects differing in aromas, piglets showed more interest in natural aromas, such as moist soil and grass, compared to synthetic aromas, such as vanilla and strawberry (Nowicki et al., 2015), indicating future research may focus on the supplementation of creep feed with natural flavours instead of synthetic flavours, such as anise and garlic, that were used in previous studies.

When we refer to creep feed, we usually mean a pelleted solid diet to familiarise piglets to solid feed prior to weaning, although milk replacers and wet creep feed (such as porridge) can also be included as they aim to supplement the diet of suckling piglets. Based on studies in nursery pigs that compared the energy intake of liquid and dry feed (Kim et al., 2001; L'Anson et al., 2012), it has been assumed that the provision of liquid creep feed can improve the growth performance of suckling piglets more significantly than the provision of solid creep feed. However, no differences in pre-weaning average daily gain were reported between milk replacer and dry creep feed in Park et al. (2014), and milk replacer only tended to improve pre-weaning average daily gain compared to one creep feed, but not compared to another creep feed (Lynch et al., 1998). Creep feed provided as porridge increased the feed intake of piglets compared to pelleted creep feed, and also stimulated the intake of the pelleted transition feed that was provided the last week before weaning (Clouard et al., 2018b). This suggests that porridge may facilitate the transition from sow's milk (tongue movements) to solid creep and weaner feed (jaw movements). Weaner piglets prefer softer over harder pellets (Jensen and Becker, 1965; Solà-Oriol et al., 2009), but this has not been confirmed in suckling piglets (Clouard et al., 2018b). In agreement, a semi-moist creep feed did not affect creep feed intake compared to a standard dry creep feed (Van Barneveld et al., 2009). Dry creep feed can also be produced in meal/mash, crumbles or (small or large) pellets (**Figure 7**), but piglet preferences for these diet forms have only been reported for small versus large pellets in suckling piglets to my knowledge (will be discussed in the next section).

Figure 7. Different sizes of creep feed (Source: L. Eastwood, OMAFRA, 2018).

Never stop exploring

Variation in possibilities to explore and forage in the pre-weaning environment may explain some of the variation in creep feed intake, as foraging is the appetitive phase of feeding. The most substantial differences in possibilities to explore can likely be found between outdoor and indoor rearing environments (Cox and Cooper, 2001; Hötzel et al., 2004). Outdoor-reared piglets consumed almost twice as much creep feed compared to indoor-reared piglets from 2 weeks of age until weaning at 4 or 6 weeks of age (Miller et al., 2007). In line with this, outdoor-reared piglets spent more time eating (sow feed and vegetation) compared to indoor-reared piglets, although outdoor-reared piglets were not fed creep feed (Cox and Cooper, 2001; Hötzel et al., 2004). Also in a novel environment test, outdoor-reared piglets spent more time eating the feed offered (also after introduction of a food bowl and in presence of a non-sibling), while indoor-reared piglets were more reluctant to eat (Lau et al., 2015). It can therefore be concluded that outdoor-reared piglets are more experienced with solid feed items before weaning compared to indoor-reared piglets. Apart from social learning by the sow and (non-)littermates as previously discussed, one of the reasons for the success of outdoor rearing on solid feed intake may be the presence of diverse substrates that allow exploration and foraging.

To stimulate exploration and foraging in indoor pens, foraging materials are often added in research. For example, adding straw, wood shavings, peat, branches and extra space to indoor housing resulted in less time on exploring and eating creep feed, but more time on chewing substrates compared to barren-housed piglets (Oostindjer et al., 2011d). Added together, enriched-housed piglets spent more time interacting with solid feed resources, i.e. creep feed plus substrates, than barren-housed piglets. Moreover, environmental enrichment by substrates and extra space seemed to have the potential to reduce neophobia for novel feed items (Oostindjer et al., 2011c). In another study, piglets that were provided with a tray of peat showed more manipulation of the peat and creep feed, but were not seen eating creep feed more often compared to piglets without a tray of peat (Vanheukelom et al., 2011).

Also more subtle changes to the environment that induce exploration have been confirmed to be successful in stimulating creep feed intake, although enriching the pen with hanging ropes elicited exploration, but did not stimulate creep feed intake (Seddon et al., 2015). Kuller et al. (2010) designed an 'exploration stimulating feeder', i.e. a feeder with three protrusions, and feed was almost consumed two times more from this exploration stimulating feeder than from the control hopper feeder. Although the feeders differed in multiple characteristics, piglets were frequently observed to chew on the protrusions of the exploration stimulating feeder and this increase in exploratory behaviour towards the feeder may therefore have increased the intake of the creep feed inside. Open creep feeder designs, such as trays and creep feeders without a hopper, and floor feeding may provide greater opportunities for foraging such as rooting through the feed, which could contribute to their success in stimulating creep feed intake (Delumeau and Meunier-Salaün, 1995; Wattanakul et al., 2005; Sulabo et al., 2010b).

Feeding management may also stimulate exploration, as refreshment of the creep feed in the feeder on a daily basis or more often, was found to provoke piglets' interest and stimulated the number of feeder visits by the piglets (Appleby et al., 1991; Wattanakul et al., 2005). Although a higher creep feed intake as result of frequent creep feed refreshment has not yet been demonstrated, this is a quite common feeding strategy.

Also the diet can be adapted to stimulate exploration towards it. A big diameter pellet (5-13 mm) has consistently been shown to increase creep feed intake of suckling pigs compared to a typical pellet diameter of 2-3 mm (A'Ness et al., 1997; Edge et al., 2005; Van den Brand et al., 2014; Clark et al., 2016) and the proportion of eaters did not differ between the pellet diameters, but was only measured in one of the studies (Clark et al., 2016). When piglets were offered both small and large diameter pellets in a choice set-up, piglets had a preference to explore and ingest the large diameter pellets compared to the small diameter pellets during early lactation (Van den Brand et al., 2014). Van den Brand et al. (2014) proposed three reasons that may have encouraged the piglets to sample the big pellets: 1) because they are easier to handle/manipulate, 2) because they may facilitate teething and/or 3) because they stimulate playful exploratory behaviour such as rolling the pellet over the floor. Another way to stimulate exploration and creep feed intake via modifications to the diet is to provide successive variation in flavour. Adeleye et al. (2014) studied flavour novelty by changing the flavours added to the creep feed on a daily sequential basis and thereby increased the number of feeder visits and doubled the intake of creep feed.

Optimal foraging

Jensen and Recén (1989) proposed that the 'optimal foraging theory' may be applicable to suckling piglets. This means that piglets would continuously monitor the costs and benefits of different foraging possibilities, i.e. to suckle the sow or to eat on solid feed, and follow the most beneficial strategy. The cost:benefit ratio of suckling the sow increases over lactation, as the milk yield of sows decreases over lactation (Hansen et al., 2012), and eating on solids would therefore be the most optimal strategy at some point. This may explain why the intake of solid feed commonly starts around two weeks of age (wild boar: Meynhardt, 1980; domestic pig: Sulabo et al., 2010b). Older piglets, however, rarely fail to suckle even though they eat considerable amounts of solid feed (Newberry and Wood-Gush, 1985; Jensen and Recén, 1989; Bøe, 1991), suggesting there may not only be a nutritional satisfaction from suckling, but also a psychological satisfaction (Malm and Jensen, 1996). Indirect indications that the optimal foraging theory may indeed be applicable to suckling piglets will be discussed below for semi-natural and also commercial conditions.

When born in July to September, naturally-reared piglets were weaned more than a week later than piglets born in the other months, which may be because weaning will be completed in winter, when the food is scarce (Jensen and Recén, 1989). Weaning may also occur later in winter due to a lower temperature, because piglets were observed to forage less in an outdoor system on days when the air temperature was lower (average air temperature of 13 °C: Damm et al., 2003). Another explanation may be the shorter photoperiod in winter, as indoor-housed litters exposed to an extended photoperiod of 20 hours of light per day ate more creep feed than litters exposed to eight hours of light per day (Simitzis et al., 2013).

In addition, litters with a lower number of piglets seemed to be weaned later than larger litters (Newberry and Wood-Gush, 1985; Bøe, 1991), as reflected by a higher number of nursing bouts. Possible causes for this may be that maternal investment is lower for smaller than for larger litters (Bøe, 1991) and/or nursing larger litters may be less comfortable for the sow due to a greater extent of udder stimulation (Newberry and Wood-Gush, 1985). The latter statement seems not supported, however, as aggression of the sow towards the piglets often started a long time before weaning was completed and did not correlate in a negative manner to the number of suckling bouts. Though, maternal aggression did increase while piglets got older (Jensen, 1988). Sows in a semi-natural environment tended to spend more time nursing subsequent than previous litters, as weaning age tended to increase with parity, at least from parity 1 to 3 which were studied (Jensen and Recén, 1989). The authors argued that older sows may have increased their level of maternal investment, because their chance of survival decreases. In an outdoor system, however, parity seemed not related to the number of suckling bouts and the feed intake of the piglets, but a low number of sows were used for the older parities (Bøe, 1991).

The intake of creep feed in commercial settings seems to be enhanced by environmental factors and management strategies that can negatively affect the milk yield and/or milk quality produced by the sow. The (milk) nutrient supply of the sow may therefore be inversely related to the intake of creep feed by her piglets. Examples that either support this hypothesis or not will be discussed. Commercially-reared litters that were kept at an ambient temperature of 29 °C had a higher creep feed intake than litters kept at 20 °C (Renaudeau and Noblet, 2001). At 29 °C, sows likely experience heat stress, resulting in a lower feed intake, which in turn can result in a lower milk production and/or change in the quality of the milk (meta-analysis: Vilas Boas Ribeiro et al., 2018). Consequently, this may have driven the piglets to eat more creep feed. The health status of the sow may also be important for creep feed intake, as outdoor-housed piglets reared by sows infected with nematodes ate more creep feed at 4 weeks of age than piglets reared by control sows (Damm et al., 2003), which may indicate a lower milk production by infected sows, but data on nursing, sow feed intake and body weight loss did not support this hypothesis. Primiparous and mid parity sows (parity 3 to 5) had less non-eaters and more bad eaters in their litter than second parity sows, while primiparous sows had more moderate eaters in their litter than second and mid parity sows, suggesting milk production may play a role in the effect of parity on creep feed consumption (Huting et al., 2019).

Cross-fostering (i.e. moving a piglet to another lactating sow) can also impact creep feed intake of the litter by creating more uniform litters, in which litters only consisting of high birth-weight piglets consumed more creep feed (6.5 g/piglet/d) than litters only consisting of low birth-weight piglets (2 g/piglet/d) and litters with an equal number of low and high birth-weight piglets (3.1 g/piglet/d; Huting et al., 2017). In addition, litters consisting of heavy-weight piglets only had the lowest number of non-eaters and the highest number of good/early creep feed eaters compared to the other two litter compositions. This may be the result of increased competition at the udder from similar-sized piglets and thereby insufficient milk intake for their body weight category (Huting et al., 2017).

In line with the optimal foraging theory, one would also expect litter size to influence the amount of creep feed consumed, as the amount of milk available per piglet decreases with larger litters, displayed by lower pre-weaning growth rates (Kobek-Kjeldager et al., 2019). Providing creep feed from 12 days prior to weaning increased pre-weaning growth and resulted in a higher weaning weight of litters with more than 8 piglets compared to smaller litters, suggesting a higher creep feed intake by larger litters, although this could not be confirmed as intake was not recorded (Klindt, 2003). As expected, Barnett et al. (1989) reported that pre-weaning weight gain of piglets tended to be lower when litter size increased from 6 to 13 piglets per litter, but, contrary to expectations, the intake of creep feed per piglet was also lower with increasing litter size. However, piglets in larger litters also had a lower birth weight and the effects of birth weight on creep feed consumption are equivocal, as will be discussed below.

Lastly, litters from intermittent suckling regimes that experienced reduced growth, due to separation from the sow for multiple hours per day, had a higher creep feed intake (Rantzer et al., 1995; Berkeveld et al., 2007b), post-weaning feed intake (Kuller et al., 2004, 2007a) and post-weaning weight gain (Pajor et al., 2002) than faster-growing conventional litters. Because no milk is available during separation, piglets' motivation to search for feed items other than milk is suggested to be higher than regimes where piglets and sows stay together, particularly for piglets that are acquainted with solid feed (Kuller et al., 2004, 2007a), as the proportion of eaters within the litter did not differ between the regimes (Kuller et al., 2007a). A longer consecutive separation interval (one 12 h interval vs. two 6 h intervals) between the sow and her piglets lowered piglet growth and increased creep feed intake and time spent on feeding behaviour by the piglets (Berkeveld et al., 2007a, 2007b). Taken together, the studies indeed suggest a negative correlation between milk intake and creep feed intake on litter level and may explain some of the variation in creep feed intake between litters. The provision of creep feed may become more important with longer lactations (Van der Meulen et al., 2010) and with the current trend in pig production for greater litter sizes (the Netherlands: Kengetallenspiegel, Agrovision), of which the latter results in more lightweight piglets at birth (reviewed by Kemp et al., 2018) and less available milk per piglet (King, 2000; Kobek-Kjeldager et al., 2019).

Variation in creep feed intake within litters

Motivation or maturation?

Milk intake may also explain variation in creep feed intake within litters, since milk production can differ between teats, as reflected by within-litter variation in growth performance. Pigs generally suckle the same teat throughout lactation, which is known as 'teat order' (De Passillé et al., 1988). Teat order may therefore illustrate differences in milk production between teats. There are indications that posterior teats produce a lower milk quantity (e.g. Pedersen et al., 2011; Pluske et al., 2007; Skok et al., 2007) and quality (Kim et al., 2000; Wu et al., 2010) than anterior and middle teats, while teats in the middle part of the udder are more prone to teat disputes than the other teats (Skok et al., 2014), which may compromise milk intake. Such variation in milk yield, quality and accessibility among teats indicates that piglets in the same litter are likely affected differently. Consequently, variability in milk production among teats may explain within-litter variation in solid feed intake, and may for naturally reared piglets also explain variation in weaning age. Pajor et al. (1991) expected a high creep feed consumption by small, slow-growing piglets as these piglets would have a higher motivation to eat compared to large piglets because they need to compensate for inadequate nutrition from the milk (Algers et al., 1990). This hypothesis is referred to as the 'motivation or compensatory feeding hypothesis'.

In a semi-natural environment, lower and middle teats were less often massaged immediately after suckling. Piglets suckling these teats may have turned away from the udder after suckling to eat solid feed and may have weaned themselves earlier (Newberry and Wood-Gush, 1985). Jensen and Recén (1989) observed that piglets that were skipping suckling bouts after the first week of life were often relatively light piglets, suggesting piglets with a low milk intake may indeed wean themselves earlier in a semi-natural environment. In intensive commercial housing, piglets suckling the posterior teats spent more time eating creep feed than piglets suckling the anterior teats (Algers et al., 1990) and piglets suckling the middle and posterior teats had faeces with a higher intensity of the green feed colourant that was in the creep feed than piglets suckling the anterior teats (Huting et al., 2017). Piglets suckling posterior teats also spent more time eating and less time vocalising post-weaning than piglets suckling the other teats (Sommavilla et al., 2015) and teat order negatively correlated with salivary cortisol after weaning, indicating piglets suckling posterior teats had lower cortisol levels (Mason et al., 2003), suggesting a more gradual weaning process which likely resulted from their increased creep feeding behaviour. As result of suckling the less productive posterior teats, piglets suckling posterior teats have lower growth rates than piglets suckling anterior teats (e.g. Pluske et al., 2007; Skok et al., 2007; Pedersen et al., 2011) and piglet growth was therefore hypothesized to be negatively correlated with creep feed intake. This is supported by Appleby et al. (1992) and Fraser et al. (1994) in which in most litters an inverse relationship was found between piglet growth and creep feeding behaviour and Algers et al. (1990) in which an inverse relationship was found between piglet growth and eating behaviour both before and after weaning. Although Pluske et al. (2007) found no difference in birth weight between piglets suckling anterior and posterior teats, posterior teats are also mostly occupied by piglets with a lower birth weight (Scheel et al., 1977; Camerlink et al., 2014). Birth weight was indeed also inversely related to creep feeding behaviour within the litter (De Passillé et al., 1989; Appleby et al., 1992).

In contrast, Pajor et al. (1991) and Bøe and Jensen (1995) found that birth weight and piglet growth were positively correlated with creep feed intake within litters. Moreover, De Passillé et al. (1989) reported that piglet growth, weaning weight, stomach weight, amylase activity and RNA/DNA ratios of the pancreas and stomach were positively correlated with creep feeding behaviour, while pepsin activity was negatively correlated with creep feeding behaviour. These studies suggest that piglets that are of a more mature physiological age have more interest in creep feed, although the reserve could also be true. According to this 'developmental maturation hypothesis', the large, fast-growing piglets, indicated by a greater developmental maturity such as proportionally larger digestive tracts, are expected to consume more creep feed as they can digest nutrients from solid feed at an earlier age compared to their smaller counterparts (Aumaître, 1972).

A large part of the literature supports the motivation/compensatory feeding hypothesis, but there are also studies that did not find relations between birth weight (Bruininx et al., 2004; Collins et al., 2013), teat order (Appleby et al., 1991; Delumeau and Meunier-Salaün, 1995; Pluske et al., 2007) and creep feeding behaviour. Besides, intra-litter variation in weaning age in semi-natural conditions could not be explained by body weight (Newberry and Wood-Gush, 1985). The inconsistency in results between studies may be partly caused by differences in litter size, milk production by the sow, milk production per teat, and in methods to determine teat order and feeding behaviour.

Other piglet factors

Next to birth weight and teat order, other piglet factors may explain part of the variation in creep feed intake. In semi-natural conditions, variation in weaning age between littermates was found to be associated with familial affiliation. Piglets that stayed less close in distance to their mothers and littermates had high frequencies of feeding on solids, while piglets that stayed closer to their family received more milk (Jensen, 1995). Individual coping styles, i.e. diverging behavioural and physiological reaction patterns to stressful stimuli (Koolhaas et al., 1999), have not been related yet to feeding behaviour of suckling piglets. To date, the only evidence for a link between individual 'personality' and feeding behaviour in pigs is that proactive-coping piglets were observed to start eating sooner after weaning than reactive-coping piglets (Ooms et al., 2010).

The motor skills and dentition of piglets are considered important aspects for the development of feeding behaviour (Herring, 1985). Although no associations were found between dental eruption and feed consumption before weaning (determined by a feed colourant), behavioural observations indicated that piglets of 10 days old and younger spent less time at the creep feeder when their premolars were erupted and occluded, while piglets of 21 days old and older spent more time at the creep feeder when their premolars were erupted and occluded compared to piglets without their premolars erupted and occluded. Dental eruption also interacted with parity of the sow, birth weight and sex, as piglets from older sows, heavy piglets at birth and females had earlier eruption of their premolars (Tucker and Widowski, 2009).

Sex could not explain intra-litter variation in weaning age in a semi-natural setting (Newberry and Wood-Gush, 1985) and was not found associated with creep feed intake in commercial settings (Tucker et al., 2010; Muns and Magowan, 2018; Huting et al., 2019). In contrast, just as many studies did find indications that sex can explain some of the variation in individual creep feed consumption. Females spent more time at the creep feeder than uncastrated males and had greener faeces, which indicates a higher intake of creep feed with a green feed colourant. Particularly light-weight females had greener faeces than light-weight uncastrated males (Huting et al., 2017). Furthermore, females tended to consume more creep feed before weaning than castrated males (Delumeau and Meunier-Salaün, 1995). Lastly, a larger number of piglets that had their head in the feeder were of female compared to male sex, both before and after castration (Delumeau and Meunier-Salaün, 1995) and a larger number of females compared to males were designated as eaters (Kuller et al., 2007a). These findings are consistent with observations after weaning, in which females had a higher initial feed intake than males (Delumeau and Meunier-Salaün, 1995; Bruininx et al., 2001b; Dybkjær et al., 2006). It has therefore been suggested that androgens may affect maturation of the central nervous system and thereby the development of feeding behaviour, resulting in females being faster to learn about the novel creep feed than males (Delumeau and Meunier-Salaün, 1995). On the other hand, Tucker and Widowski (2009) reported that females have a faster dental development than males and Sommavilla et al. (2015) found that 70% of the piglets suckling the anterior teats were of male sex, suggesting females may consume more creep feed than males due to a lower milk intake. The above-mentioned piglet characteristics such as teat order, birth weight and sex can be a constraint in reducing variation between piglets in pre-weaning feed intake.

A large number of factors explain variability in creep feed intake between litters including age, milk availability, and possibilities for social learning and exploration. This makes it complex to reduce the between-litter variation in creep feed intake, while piglet characteristics such as birth weight, teat order and sex and their interactions are a constraint in reducing within-litter variation in creep feed intake. These factors influencing creep feed intake should be taken into account when developing new feeding strategies for suckling piglets.

Conclusions and knowledge gaps

Weaning occurs in an abrupt manner under most commercial situations and considerably earlier than weaning in a (semi-)natural environment. This means that a significant proportion of piglets consumes no or only little amounts of solid feed before being weaned, while the intake of (sufficient amounts of) solid feed before weaning has yielded substantial benefits to deal with the weaning process. The interpretation of studies that provide evidence for these benefits at litter level should be taken with caution as the average is taken over the highly variable intake of individual piglets. A part of the large variation in solid feed intake that has been observed between and within litters can be explained by physical factors, such as milk availability and stimuli/materials to explore, social factors such as social learning and piglet factors such as birth weight and sex, as well as potential interactions between them. Most studies so far have focused on testing the effects of creep feed intake on piglet performance, whilst effects on behaviour, stress, gut microbiota and digestive system development largely remain unclear. Further research is therefore needed in order to better understand the impact of creep feed provision on piglet welfare, and to develop feeding strategies that stimulate every piglet to start eating and that increase the amount of solid feed ingested before weaning.

Aim and outline of the thesis

This thesis aimed to facilitate the dietary transition of pigs at weaning and thereby to reduce weaning-induced problems in common pig production systems. Piglet development and the response of piglets to weaning were monitored by measuring performance, behaviour and physiological parameters. The focus was on the first weeks of a piglets' life until the early post-weaning period.

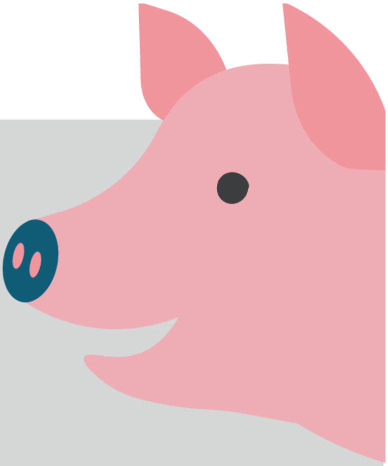
The first aim of this thesis was to investigate the effects of creep feed provision on piglet development during the weaning transition. In **Chapter 2** of this thesis, the effects of creep feed provision on behaviour and performance of piglets were studied up to two weeks post-weaning. Piglets were provided with creep feed or withheld from creep feed before weaning, and were provided a weaner diet or weaner diet supplemented with creep feed after weaning. The effects of creep feed intake on gut microbiota and (macro- and microscopic) digestive system development shortly before weaning were also determined in this study and reported in **Chapter 3**.

The second aim of this thesis was to develop feeding strategies for suckling piglets and to study their effect on piglets' ability to deal with the weaning transition. The feeding strategies in this thesis all aimed to ease weaning by stimulating early foraging behaviour of piglets, as current opportunities to forage for feed are very limited in indoor-housed pigs. In **Chapter 4**, piglets were either housed in an enlarged substrate-enriched environment or in a barren environment and their feed intake around weaning was measured. In **Chapter 5**, it was established whether a low energy intake (homeostatic drive) from milk, by means of maternal feed restriction, and presenting creep feed in an exploratory and playful context (exploratory drive), by means of a foraging-stimulating play-feeder, were important and potentially interacting factors in getting piglets to eat creep feed. Subsequently, the effects of the two pre-weaning factors on post-weaning adaptation in terms of behaviour and performance were studied. In **Chapter 6**, the provision of diverse solid feed types were compared to the provision of solid feed types that vary in flavour only and effects on feeding behaviour up to weaning were studied. The diverse diet was not compared to a monotonous diet and the ability of piglets to deal with the weaning transition remained unknown. This was investigated in **Chapter 7**, where additionally the effects of hiding the pre-weaning diet in substrate was tested. In **Chapter 8**, a subset of piglets from the study described in **Chapter 7** were exposed to behavioural tests to investigate their responsiveness towards a novel environment and novel feed items. The results of the chapters are summarized, integrated and discussed in **Chapter 9** (General discussion), which ends with the implications of this thesis for science, society and industry.

Supplementary data

Supplementary Figure S1. Foraging trips of sows and their litters over time in an outdoor environment created by my grandfather (depicted on the bottom picture). Pictures are made in 1984.

CHAPTER 2



Effects of creep feed provision on behaviour and performance of piglets around weaning

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Abstract

Creep feed provision may ease weaning, hence we determined the impact of providing fibrous creep feed before weaning and adding this feed to the post-weaning diet on piglet behaviour and performance. Litters were given creep feed (CF, n = 12) or not (NF, n = 10) before weaning and a weaner diet (CON) or weaner diet supplemented with creep feed (CS) after weaning (n = 8 pens/treatment). Behaviours were scored in a novelty test at d24 after birth, and in the home pen at d11, 16, 22 and 27 after birth and at week 1 and 2 post-weaning. Feed intake, growth and faecal consistency were measured up to d14 post-weaning. A blood sample was taken at d2, 15 and 29 after birth and d2, 5 and 14 post-weaning. CF-piglets consumed on average 397 ± 71 g creep feed before weaning. CF-piglets grew faster in the last week before weaning than NF-piglets (249 ± 7 vs. 236 ± 11 g/d, $P = 0.02$). CF- and NF-piglets did not differ in behaviours in a novelty test, weaning-stress-induced behaviours and haptoglobin concentrations, but CF-piglets showed more rooting of the environment (2.4 ± 0.3 vs. 1.3 ± 0.2% of observations, $P = 0.03$) and less chewing on faeces plus sham chewing (0.9 ± 0.1 vs. 1.6 ± 0.3%, $P = 0.02$) than NF-piglets at week 2 post-weaning. Creep feed supplementation only enhanced feed exploration (0.28 ± 0.05 vs. 0.09 ± 0.02%, $P < 0.05$). Creep feed provision and supplementation did not affect overall feed intake, growth, feed conversion efficiency and faecal consistency for the first 14 days post-weaning. However, CF-piglets had a greater body weight (13.81 ± 0.19 vs. 13.06 ± 0.15 kg, $P = 0.04$) and lower within-pen coefficient of variation in body weight at d14 post-weaning than NF-piglets (13.6 ± 1.9 vs. 15.1 ± 1.5%, $P = 0.02$), and CS-piglets ate more feed between d9-14 post-weaning than CON-piglets (609 ± 18 vs. 547 ± 17 g/d, $P = 0.01$). In conclusion, creep feed provision showed limited effects on piglet behaviour, but may subtly improve body weight development.

Introduction

Weaning of piglets often results in stress (reviewed by Weary et al., 2008), temporary fasting and in a low feed intake (e.g. Bruininx et al., 2002, 2004). Consequently, piglets are predisposed to maldigestion and malabsorption, colonization by intestinal pathogens, growth stasis, diarrhoea (reviewed by Heo et al., 2013 and Gresse et al., 2017) and maladaptive behaviour (Colson et al., 2006; Van Nieuwamerongen et al., 2017). A major contributor to these problems is the abrupt change in diet from sow's milk to solid feed (Bolhuis et al., 2009). Habituating piglets to solid feed while they are with the sow, by providing creep feed, is therefore considered important for improving the feed intake and hence performance of newly weaned piglets, and may also supplement sow milk, although the impact on performance around weaning seems inconsistent (positive effect: Shea et al., 2013, Muns and Magowan, 2018; negative effect: Beaulieu et al., 2010; lack of effect: Sulabo et al., 2010b, Park et al., 2014). Apart from a potential effect on performance, creep feed provision may also affect piglet behaviour, including explorative activities. To our knowledge, only a single study investigated the impact of creep feed provision on pre-weaning behaviour, focusing on suckling, sleeping and fighting, and found no effects (Yan et al., 2011).

The present study aimed to determine the impact of pre-weaning creep feed provision and adding this creep feed to the weaner diet on piglet behaviour and performance around weaning. It has been suggested that creep feed provision is potentially beneficial for piglet performance by familiarising piglets with a solid diet (Muns and Magowan, 2018), which may reduce neophobia for the weaner diet (Bolhuis et al., 2009; Figueroa et al., 2019) and may reduce stress from maternal separation due to acquaintance with an alternative energy source (Haley et al., 2005). Early familiarisation with feed may thus help piglets to cope with weaning. Besides, creep feed provision may improve the capacity of piglets to cope with novelty via the microbiota-gut-brain axis, as dietary composition has been shown to affect anxiety, exploration, agonistic and activity levels in pigs (Haagensen et al., 2014; Clouard et al., 2016). Therefore, we hypothesized that creep feed provision would stimulate ingestive behaviours, feed intake and growth of weaned piglets, reduce haptoglobin concentrations and weaning-stress-induced behaviours, and attenuate the fear response to novelty. We expected additional beneficial effects on the performance of weaned piglets by post-weaning creep feed supplementation.

Methods

The Animal Care and Use committee of Wageningen University & Research (Wageningen, the Netherlands) approved the protocol of the experiment (AVD104002016515). The protocol is in accordance with the Dutch law on animal experimentation, which complies with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

Animals, housing and management

The study was set up as a 2 × 2 factorial arrangement (see below for treatments). The experiment included a total of 22 sows and their litters and was conducted in two consecutive batches with $n = 10$ and $n = 12$ multiparous Topigs-20 sows respectively of parity 3-5. Sows were housed and inseminated at research facility Carus (Wageningen University & Research, the Netherlands). Sows were fed commercially available diets ('Inno Dracht' during gestation and 'Inno Lac Vital' during lactation, Coppens Diervoeding, Helmond, the Netherlands) twice a day, at 7:30 and 16:00 h. One week before farrowing, the sows were moved to two adjacent farrowing rooms (balanced for treatments) and were individually housed. The pen was equipped with a farrowing area (2.85 x 1.80 m, 80% mats and 20% slatted area) and a free-movement area (1.85 x 1.80 m, 100% mats). The farrowing area consisted of a crate (2.85 x 0.60 m) that included a feed trough, drinking nipple and metal chain with bolts as chew object, that were only accessible by the sow. Around farrowing sows were provided a jute sack as nesting material. The farrowing area had an infrared lamp, drinking nipple and chew object for the piglets. The chew object was a metal chain with bolts given from one week of age. The free-movement area included a drinking nipple for the sows. The free-movement area was accessible by sows and their litters, except near farrowing until four days post-partum. Sows were fed in the feed trough of the farrowing arena, and from d2 after farrowing sows were fed in a removable feed trough in the free-movement area, while being separated for 30 min from their piglets which stayed in the farrowing area during maternal feeding. Unconsumed sow feed was removed after feeding, before piglets were given access to the free-movement area again, to prevent piglets from eating sow feed. Within 24 h after birth, piglets were weighed, ear tagged and injected intramuscularly with iron. Litter size varied from 9 to 14 piglets per litter. Litters were allotted to one of the two treatment groups based on sow's parity, farrowing date and average body weight (BW) of the litter at d0 and d2. From 2 days of age (fixed to birth date) a stainless steel feed trough (100 x 24 x 8 cm), which had approximately eight feeding places for the piglets, was placed in the farrowing area. Room temperature was 25 °C around farrowing and was gradually decreased to 22 °C until weaning.

A subset of 128 piglets was weaned on the same day at four weeks of age (29.8 ± 0.1 days of age) and relocated in two adjacent weaner rooms (balanced for treatments) in which they were housed until six weeks of age. At weaning, piglets were mixed with piglets within pre-weaning treatment and housed in groups of four unfamiliar piglets, of which two males and two females, in a pen of 1.80 x 2.85 m with 80% mats and 20% slatted floor. Piglets were selected based on their sex and their BW at one day before weaning, which was close to the average weight of the litter and treatment group. The selected piglets originated from ten litters per treatment group, of which eight piglets per litter in batch 1 and four to six piglets per litter in batch 2. Piglets with a history of medication and leg/claw problems were excluded from selection. The weaner pen was equipped with a feeder (12 x 50 cm) having three feeding spaces, drinking trough, infrared lamp and chew object, which was a metal chain with bolts. Additional chew objects were provided and replaced daily in a cycle of four objects: a squeaky ball or metal chain with two solid balls, PVC pipe or hose attached. Piglets had *ad libitum* access to a commercial weaner diet (2-mm pellet, 16.4% crude protein, 4.4% crude fibre and 11.9 g standardized ileal digestible lysine/kg dry matter, 'Inno Speen Pro', Coppens Diervoeding, Helmond, the Netherlands). Room temperature was kept at 25 °C in the first week after weaning and decreased to 23 °C in the second week. In both farrowing and weaner rooms, lights and radio were on between 07:00 and 19:00 h and lighting was dimmed during the night.

Treatments

Piglets were assigned to one of four treatment combinations in a 2 × 2 arrangement, with pre-weaning creep feed provision and post-weaning creep feed supplementation as experimental factors.

Pre-weaning creep feed provision

From 2 days of age 12 litters were provided with creep feed in the piglet feed trough (**CF**) and 10 litters were not provided with creep feed before weaning and the trough was kept empty (**NF**). The creep feed (11.8 MJ/kg as-fed net energy, 195 g crude protein, 11.9 g standardized ileal digestible lysine/kg dry matter) was high in dietary non-starch polysaccharides (261 g/kg dry matter), originating from cereal grains, sugarbeet pulp, oat hulls, galactooligosaccharide, inulin and high-amylose starch (**Table 1** and **2**). The amount of creep feed in the feed trough was checked at least twice daily to provide the creep feed *ad libitum* and all creep feed was replaced at each feed weigh-back to maintain freshness of the feed. The creep feed was mixed by Research Diet Services (Wijk bij Duurstede, the Netherlands) and extruded using a co-rotating double screw extruder (M.P.F. 50, Baker Perkins, Peterborough, UK). Extruder settings intendedly varied during production, resulting in differences in pellet diameter (2, 8 and 14 mm), length (10 mm for 2-mm diameter pellet, 8 and 22 mm for 8-mm diameter pellet and 10 and 20 mm for 14-mm diameter pellet respectively), texture and hardness (7.3-17.7 kg) to create dietary diversity. The different pellet types were mixed evenly and provided as one diet. To stimulate early uptake of creep feed (Oostindjer et al., 2011c, 2011d), sows were allowed to eat creep feed together with their piglets during the first week of creep feed provision two times a day for 10-20 min. Immediately after sow feeding, 250 g creep feed/turn/pen was provided in the piglet feed through to which sows were given temporary access. The consumption of creep feed by the sows was not included in the assessment of piglets' creep feed intake. To compensate for the creep feed taken up by sows with CF-litters, sows with NF-litters received 250 g of creep feed additional to their lactation diet. Due to variation in creep feed intake by sows, even after habituation to the creep feed, we decided to stop facilitation of eating behaviour by the sow. Therefore all sows in batch 2 received the creep feed supplement on top of their lactation feed.

Post-weaning creep feed supplementation

After weaning, 16 pens of which 8 with CF- and 8 with NF-piglets had *ad libitum* access to the weaner diet (**CON**), and 16 pens of which 8 with CF- and 8 with NF-piglets received, on top of their weaner diet that was provided *ad libitum*, a limited amount of 80 g of creep feed per pen twice a day as supplementation (**CS**). The creep feed supplement was provided in the same feeder as the weaner diet.

Table 1. Nutrient profile of the creep feed.

Calculated nutrient composition[1]	Creep feed
Net energy	11.8
Dry matter	891
Starch	290
Non-starch polysaccharides[2]	261
Crude protein	195
Crude fat	61
Crude fibre	44
Crude ash	57
Calcium	9.1
Phosphorus	6.1
Sodium	2.2
Standardized ileal digestible lysine	11.9
Standardized ileal digestible methionine	4.8
Standardized ileal digestible threonine	7.1
Standardized ileal digestible tryptophan	2.4

[1] According to CVB (2007). Nutrients are presented in g/kg dry matter, except for dry matter (g/kg) and net energy (MJ/kg)

[2] Calculated as the difference between dry matter and the sum of starch, sugars, crude protein, crude fat and crude ash

Table 2. Ingredient composition of the creep feed.

Ingredient component	%
Wheat	21.9
Barley	15
Maize	15
Soy protein concentrate	7
Soybeans (heat treated)	5
Galacto-oligosaccharides	5
Potato protein	4
Sugarbeet pulp (dehydrated)	4
Oat hulls	4
Inulin	4
High-amylose starch (± 75% amylose)	4
Soybean oil	3
Blood meal (spray dried)	2
Dicalcium phosphate	1.7
Sucrose	1.5
Calcium carbonate	1.0
Sodium chloride	0.5
Premix[1]	0.5
Potassium bicarbonate	0.3
L-lysine hydrochloride	0.3
DL-methionine	0.2
L-threonine	0.04
L-tryptophan	0.04
Total	100

[1] Vitamin and mineral premix (per kg of feed): vitamin A: 10000 IU, vitamin D3: 2000 IU, vitamin E: 40 mg, vitamin K: 1.5 mg, vitamin B1: 1 mg, vitamin B2: 4 mg, vitamin B6: 1.5 mg, vitamin B12: 0.02 mg, niacin: 30 mg, D-pantothenic acid: 15 mg, choline chloride: 150 mg, folate: 0.4 mg, biotin: 0.05 mg, iron: 100 mg, copper: 20 mg, manganese: 30 mg, zinc: 70 mg, iodine: 0.7 mg, selenium: 0.25 mg, anti-oxidant: 125 mg

Measurements

Piglet performance

Piglets were individually weighed on d2 (fixed to birth date, thereafter all measurements were performed on the same day for all piglets), 15, 21, 29 after birth and d2, 5, 9 and 14 post-weaning. Creep feed intake was determined per CF-litter between d2-15, 15-21 and 21-30 after birth. Post-weaning feed intake was determined per pen between d0-2, 2-5, 5-9 and 9-14 after weaning. The intake per feed type (weaner feed or creep feed supplement) was also determined. If any, feed remains on the floor were collected. Feed wastage was kept to a minimum by placing the feeders on the solid floor in the farrowing and weaner pens. Faecal consistency scores of faecal droppings in the pens were taken daily by one observer for the first 14 days post-weaning according to the faecal classification scale with four categories of Pedersen and Toft

(2011). According to this scale, score 1 (firm and shaped) and 2 (soft and shaped) represent normal faeces, and were therefore combined into one score before data analysis. Score 3 (loose) and 4 (watery) represent diarrhoea. The highest faecal consistency score that was observed in a pen was selected on each measurement day and averaged over two weeks post-weaning to calculate the mean faecal consistency score (FCS) per pen. Sow BW and back fat thickness at left and right P2 positions were measured using an ultrasonic Renco Lean Meater (MS Schippers, Bladel, the Netherlands) at one week before farrowing and at weaning.

Piglet behaviour

Piglets were individually numbered using dark permanent hair dye (pre-weaning) or animal marking spray (post-weaning) to allow individual recognition during behavioural observations on all piglets. Live behavioural observations were done in the farrowing rooms at d11, 16, 22 and 27 after birth using 4.5-min instantaneous scan sampling for six sessions of 63 min/d, i.e. 84 scans/piglet/d. Observation sessions started at 8:15, 9:30, 10:45, 13:45, 15:00 and 16:45 h. Behaviour in the weaner rooms was observed at d6 and 13 post-weaning using 2-min instantaneous scan sampling for six sessions of 1h/d, i.e. 180 scans/piglet/d, starting at 8:00, 9:15, 10:30, 14:00, 15:15 and 16:30 h. Behaviours were scored using a Psion hand-held computer with the Pocket Observer 3.1 software package (Noldus Information Technology, Wageningen, the Netherlands) or using a pen and scoring sheets. The ethograms are given in **Supplementary Table S1**. Based on the observations in the farrowing rooms we also discriminated ‘eaters’, i.e. piglets scored eating creep feed from the feed trough or floor at least once, from ‘non-eaters’ per observation day. The percentage of eaters was calculated by dividing the number of eaters per litter by the total number of piglets in the same litter at that observation day.

Combined open field and novel object test

At 3.5 weeks of age (24.1 ± 0.2 days of age), a selection of suckling piglets (n = 72) was subjected to a 10-min combined open field test (OFT) and novel object test (NOT) to investigate piglets’ stress- and fear-related responses, as well as their exploration- and avoidance-related responses. Three to five piglets per litter were selected for the test based on sex (equivalent male to female ratio), BW (close to the average weight of the litter and close to the average weight of the treatment group) and, in addition for CF-litters, eating frequency (littermates with a high eating frequency were selected). The selected piglets originated from ten litters per treatment, i.e. four to five piglets per litter in batch 1 and three piglets per litter in batch 2. Eating frequency per piglet was determined daily from 7:00 to 19:00 h via video observations of CF-piglets that could be identified by back numbers. When a CF-piglet placed its snout into the trough for a minimum of 5 s, the behaviour was scored as eating (Pajor et al., 1991; Adeleye et al., 2014). The eating bout ended when the snout of the piglet was out of the trough for a minimum of 5 s. Testing was carried out on two consecutive days and order was balanced for room, pen, sex and treatment. Individual piglets were transported in a transport cart and placed in an unfamiliar test arena, away from the home pen both visually and auditorily. The arena (3 x 3 x 1.2 m, Hessing et al., 1994; Jensen et al., 1995) with walls of 1.2 m height and a concrete floor was located in a room at the end of the corridor. The OFT started by placing each piglet in the arena in the same start position next to the wall. After 5 min a novel object, i.e. a metal bucket, was dropped from the ceiling onto the floor at the centre of the arena and left for 5 min. Behaviour, postures, locomotion and vocalisations (**Supplementary Table S2 and S3**) were continuously scored live using Psion hand-held computers with the Pocket Observer 3.1 software package (Noldus Information Technology, Wageningen, the Netherlands). Temperature in the test room was kept the same as the room temperature of the farrowing rooms. Before each test, faeces/urine were removed and the arena was cleaned with water and cleanser, and subsequently dried.

Haptoglobin concentrations

Haptoglobin was determined at d2, 15 and 29 after birth and at d2, 5 and 14 after weaning in a selection of male piglets, i.e. n = 14 per treatment before weaning and n = 13 CF-CON-piglets and n = 12 NF-CON-piglets after weaning, from ten litters per treatment. Blood was drawn by puncture of the jugular vein and the order of sampling was balanced for treatment and room. Blood was collected in VACUETTE® K3EDTA tubes (Greiner Bio-One, Alphen aan den Rijn, the Netherlands), subsequently kept on a layer of ice, and centrifuged at 1300 g for 10 minutes at 4 °C to separate plasma. Plasma was stored at -20 °C until analysis of the haptoglobin concentration using a commercial kit (PhaseTM Haptoglobin Assay, Tridelta Development Limited, Maynooth, Ireland). According to the kit test procedure, hemoglobin reagent (100 µL) was added to sera (7.5 µL), gently mixed, followed by addition of chromogen reagent (140 µL). The solution was incubated for 5 min at room temperature and the absorbance was read immediately at 600 nm in a microplate reader. The concentration of haptoglobin (mg/mL plasma) was calculated with a standard linear curve for known concentrations of haptoglobin.

Statistical analyses

Data processing

Behaviours in the OFT and NOT were presented as sum over the two phases of the test. Lying with eyes closed and comfort behaviour did not occur. Sitting, lying with eyes open, chewing and barks hardly occurred and were excluded from analyses. In the analyses of behaviours in the home pen, nosing, rooting, chewing the environment, chewing faeces or sham chewing as well as exploring feeder and exploring drinker were combined into ‘exploring environment’, but also analysed separately, except for exploring drinker during lactation because it occurred <0.1% of the observation time. To investigate ‘nosing pen mates’ after weaning, nosing pen mates and snout contact were merged. Manipulating pen mates, tail biting, ear biting and belly nosing were combined into ‘manipulating pen mates’. Belly nosing did not occur in the farrowing room. Piglet behaviours in the farrowing room at d11, 16, 22 and 27 after birth were averaged per piglet. Piglet behaviours in the weaner room at d6 and 13 after weaning were averaged per piglet per day.

Data analysis

Data were analysed with generalised linear (GLIMMIX) and linear (MIXED) mixed models in the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Model residuals of the MIXED procedure were checked for normality.

Before weaning, average daily creep feed intake (after log transformation) and the percentage of eaters were analysed in a MIXED and GLIMMIX model respectively with fixed main effects for batch (1 vs. 2) and period/day. Piglet average daily gain (ADG) and BW were analysed in a MIXED model with fixed main effects for batch and creep feed provision (CF vs. NF), and a random effect of farrowing pen, nested within treatment and batch. Litter size was used as covariate in the analyses of ADG before weaning and weaning weight. Uniformity in BW expressed as coefficient of variation (CV), as well as sow BW and back fat loss, were analysed in a MIXED with fixed main effects for creep feed provision and batch.

Behavioural states in the farrowing pen and in the combined OFT/NOT were expressed as proportions of time and analysed in a GLIMMIX with a binomial distribution, logit link function and an additional multiplicative overdispersion parameter. Behavioural events in the combined OFT/NOT were analysed in a GLIMMIX with a Poisson distribution, log link function and an additional multiplicative overdispersion parameter. The models included the same fixed main effects and random effect as used for piglet ADG and BW. Latency to explore the bucket was analysed after log transformation in a MIXED procedure.

After weaning, piglet ADG and BW were analysed in a MIXED with fixed main effects for batch (1 vs. 2), main effects and interactions for pre-weaning creep feed provision (CF vs. NF) and post-weaning creep feed supplementation (CS vs. CON) and a random effect of weaner pen, nested within treatments and batch. Average daily feed intake (ADFI), uniformity in BW expressed as CV, feed conversion ratio (FCR) and mean FCS were analysed in a MIXED on pen level and the random effect of weaner pen was therefore omitted from analyses. CV in BW at one day before weaning was used as covariate in the analyses of CV in BW at 14 days post-weaning. For feed intake data between d0-2 post-weaning no batch effect was included as it was recorded in batch 2 only as result of technical difficulties in batch 1. Residuals of creep feed supplement intake between d5-9 after weaning within CS could not be normalized, therefore a Grubb' test was performed to detect possible outliers and one pen was identified as significant outlier. The outlier pen was omitted from analyses of the creep feed supplement intake between d5-9 after weaning within CS, resulting in normally distributed residuals. The duration of diarrhoea (score 3 + 4) was analysed in a GLIMMIX using a Poisson distribution, log link function and an additional multiplicative overdispersion parameter with the same fixed main effects as used for mean FCS. The prevalence of watery diarrhoea (score 4) was analysed as 0-1 variable. A Fisher's exact test was used, because there was a sub-classification category for the interaction effects scoring 0 only, i.e. no pens with watery diarrhoea.

Behavioural states in the weaner pen were expressed as proportions of time and analysed in a GLIMMIX using a binomial distribution, logit link function and an additional multiplicative overdispersion parameter. The model included the same fixed effects and random effect as mentioned for post-weaning ADG and BW.

Haptoglobin concentrations were analysed after log transformation in a repeated MIXED procedure including a spatial power covariance structure. The model had fixed main effects for pre-weaning creep feed provision, day, their interactions, as well as batch.

(Untransformed) Data are presented as means ± SEM (based on pen averages, except for the combined OFT/NOT and haptoglobin data). Differences at $P < 0.05$ were considered statistically significant and differences at $0.05 \leq P < 0.10$ were considered as trend. Significant interaction effects were further analysed using post-hoc pairwise comparisons of least squares means.

Results

Creep feed intake by CF-litters

In CF-litters, creep feed intake increased with age ($P < 0.0001$) with on average 30 ± 5 g/piglet between d2-15, 76 ± 15 g/piglet between d15-21 and 292 ± 52 g/piglet between d21-30. Piglets consumed on average 397 ± 71 g creep feed before weaning (between d2-30), of which 74% was consumed from d21 onwards. Creep feed intake was variable between CF-litters, with cumulative creep feed intake ranging from 96 to 972 g/piglet pre-weaning based on total creep feed consumption per litter.

The percentage of eaters per CF-litter, based on the behavioural observation of eating at least once during scan sampling, increased with age (d11: 30 ± 6%, d16: 67 ± 9%, d22: 78 ± 5%, d27: 91 ± 4%, $P = 0.04$). The minimum and maximum percentage of eaters per litter was 0 and 69.2% at d11, 0 and 100% at d16, 44.4 and 100% at d22 and 58.3 and 100% at d27.

Effects of creep feed provision on piglet behaviour and performance before weaning

Litters with or without creep feed (CF vs. NF) did not differ in litter size, BW loss and back fat loss of their sows during lactation (**Table 3**). ADG of CF-piglets throughout lactation (d2-29) was higher than that of NF-piglets ($P = 0.03$). Analysis per time period revealed that the ADG of CF-piglets was increased as compared with NF-piglets in the last week before weaning ($P = 0.02$). Consequently, CF-piglets tended to have a higher weaning weight than NF-piglets ($P = 0.06$). Litter uniformity in BW was not affected by the provision of creep feed (**Table 3**). CF-piglets spent 2.9 ± 0.5% of their time either exploring or eating the feed (**Table 4**). NF-piglets could not perform these behaviours, but tended to show more chewing faeces plus sham chewing ($P = 0.09$) and tended to explore their empty trough more often ($P = 0.08$). Other behaviours in the farrowing room were unaffected by providing creep feed (**Table 4**). In addition, behaviours in the combined OFT/NOT at 3.5 weeks of age were not affected by creep feed provision (**Table 5**).

Table 3. Effects of creep feed provision from 2 days of age on sow and piglet performance before weaning. Data are means ± SEM based on pen averages. Significant *P*-values are presented in bold.

Item[1]	**NF**	**CF**	***P*-value**
No. of litters	10	12	
Parity of the sow	4.3 ± 0.2	4.3 ± 0.2	
Weaning age, days	29.7 ± 0.5	29.9 ± 0.4	
Piglets / litter			
d 2	11.7 ± 0.6	12.8 ± 0.4	0.13
d 29	11.4 ± 0.5	11.8 ± 0.5	0.49
Sow back fat loss, mm	2.3 ± 1.5	3.0 ± 1.0	0.43
Sow body weight loss, kg	31 ± 4	39 ± 4	0.15
ADG, g/pig/d			
d 2 - 15	218 ± 10	230 ± 8	0.11
d 15 - 21	277 ± 13	272 ± 12	0.71
d 21 - 29	245 ± 12	275 ± 9	**0.02**
d 2 - 29	236 ± 11	249 ± 7	**0.03**
Body weight, kg			
d 0	1.38 ± 0.05	1.37 ± 0.04	0.83
d 2	1.52 ± 0.05	1.53 ± 0.06	0.96
d 29	7.85 ± 0.31	8.27 ± 0.20	0.06
Litter CV, %			
d 2	17.3 ± 1.3	15.6 ± 1.4	0.31
d 29	14.6 ± 2.0	12.3 ± 1.1	0.31

[1] NF = no creep feed during lactation; CF = *ad libitum* creep feed from d2 of lactation

Table 4. Effects of creep feed provision from 2 days of age on piglet behaviour as average over all observation days before weaning. Data are means ± SEM based on pen averages. Trends are presented in bold.

Behaviour, % of observations[1]	NF	CF	*P*-value
'Ingestive behaviour'			
Eating feed	-	2.6 ± 0.5	-
Drinking	0.3 ± 0.1	0.3 ± 0.1	0.94
Suckling	1.6 ± 0.3	1.7 ± 0.2	0.47
Massaging udder	13.9 ± 1.1	11.9 ± 0.6	0.17
'Exploratory behaviour'			
Exploring feed	-	0.3 ± 0.04	-
Exploring environment	14.4 ± 0.8	12.8 ± 1.0	0.18
Nosing environment	9.6 ± 0.6	8.6 ± 0.6	0.23
Rooting environment	2.0 ± 0.2	2.1 ± 0.3	0.59
Chewing environment	1.3 ± 0.1	1.1 ± 0.1	0.22
Chewing faeces or sham chewing	0.8 ± 0.1	0.6 ± 0.1	**0.09**
Exploring trough	0.58 ± 0.12	0.36 ± 0.03	**0.08**
'Postures and locomotion'			
Inactive behaviour	54.1 ± 1.8	55.3 ± 2.1	0.61
Standing and walking	7.1 ± 0.5	6.6 ± 0.3	0.49
'Pig-directed behaviour'			
Nosing pen mates	1.1 ± 0.1	1.1 ± 0.06	0.46
Nosing sow	1.6 ± 0.2	1.3 ± 0.1	0.17
Snout contact	0.3 ± 0.03	0.3 ± 0.04	0.71
Manipulating pen mates	1.0 ± 0.1	0.9 ± 0.07	0.72
Manipulating sow	0.6 ± 0.08	0.4 ± 0.05	0.11
Aggressing pen mates	0.9 ± 0.1	1.0 ± 0.1	0.41
Mounting pen mates	0.2 ± 0.04	0.3 ± 0.03	0.42
'Other behaviour'			
Playing	2.1 ± 0.2	2.3 ± 0.2	0.41
Comfort behaviour	0.4 ± 0.04	0.4 ± 0.03	0.58
Eliminating	0.5 ± 0.04	0.5 ± 0.05	0.91

[1] NF = no creep feed during lactation (114 piglets from 10 litters); CF = *ad libitum* creep feed from d2 of lactation (138 piglets from 12 litters)

Table 5. Effects of creep feed provision on behavioural responses of piglets (n = 36 piglets/treatment) in the combined open field (OF) test/novel object (NO) test (OFT/NOT) at 3.5 weeks of age. Data are means ± SEM.

Behaviour[1]	**NF**	**CF**	***P*-value**
States, % of time			
Walking	41.3 ± 1.9	41.3 ± 1.9	1.00
Standing	54.1 ± 1.7	55.6 ± 1.8	0.66
Standing alert	2.4 ± 0.5	2.9 ± 0.5	0.42
Exploring floor	31.9 ± 1.6	29.0 ± 1.9	0.49
Exploring walls	12.0 ± 1.0	13.3 ± 1.1	0.34
Exploring NO[2]	14.9 ± 1.9	16.1 ± 2.2	0.87
Approaching NO[2]	1.3 ± 0.1	1.8 ± 0.4	0.20
Withdrawing from NO[2]	0.3 ± 0.1	0.4 ± 0.1	0.97
Latency to explore NO[3]	19.3 ± 2.8	16.0 ± 2.3	0.18
Events, frequency			
Low-pitched vocalisations	328.6 ± 19.3	357.5 ± 18.6	0.35
High-pitched vocalisations	103.6 ± 15.5	98.8 ± 15.2	0.94
Escape attempts	5.4 ± 1.3	5.7 ± 1.0	0.65
Eliminating	1.3 ± 0.3	1.1 ± 0.2	0.45

[1]NF = no creep feed during lactation; CF = *ad libitum* creep feed from d2 of lactation

[2]Presented as % of time in the NOT only

[3]Presented in seconds in the NOT only

Effects of creep feed provision and creep feed supplementation on piglet behaviour and performance after weaning

Interactions between pre-weaning creep feed provision (CF vs. NF) and post-weaning creep feed supplementation (CS vs. CON) were found for ADG between one day before and two days after weaning only (**Table 6**). No significant differences were observed using post-hoc pairwise comparisons, but NF-piglets grew numerically faster in this initial period compared to CF-piglets when given a commercial nursery diet without creep feed supplement.

In the first two days post-weaning, NF-piglets tended to eat more than CF-piglets (NF: 177 ± 11 vs. CF: 138 ± 15 g/d, $P = 0.06$), but CF-piglets ate more on subsequent days (**Table 6**; d2-5 post-weaning, CF: 366 ± 16 vs. NF: 296 ± 12 g/d, $P = 0.001$). The latter observation was reflected in a greater ADG of CF-piglets between d2-5 post-weaning compared to NF-piglets (CF: 313 ± 19 vs. NF: 248 ± 15 g/d, $P = 0.01$). In the following days, between d5-9, performance parameters did not differ between the treatment groups. Between d9-14 post-weaning, NF-piglets ate more (NF: 605 ± 16 vs. CF: 550 ± 20 g/d, $P = 0.02$), although this was not reflected in ADG, which was still greater for CF-piglets (CF: 539 ± 16 vs. NF: 486 ± 16 g/d, $P = 0.03$). Creep feed supplementation after weaning stimulated feed intake between d9-14 post-weaning (CS: 609 ± 18 vs. CON: 547 ± 17 g/d, $P = 0.01$), but did not affect ADG in this period and in any of the other time periods studied. Treatments did not affect the total feed intake between d2-14 post-weaning, the growth performance of piglets between d-1-14 post-weaning and FCR between d2-14 post-weaning (**Table 6**). At the end of the measurement period, at d14 post-weaning, the BW of CF-piglets was greater (CF:

13.81 ± 0.19 vs. NF: 13.06 ± 0.15 kg, $P = 0.04$) and less variable (CV in BW, CF: 13.6 ± 1.9 vs. NF: 15.1 ± 1.5%, $P = 0.02$) compared to NF-piglets (**Figure 1**).

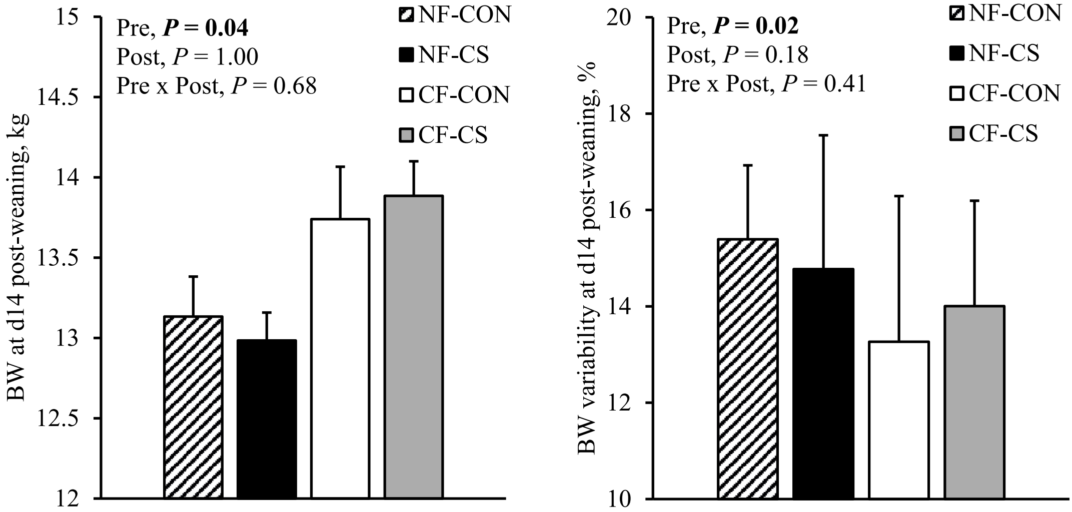

Figure 1. BW and BW variability (coefficient of variation in BW) at 14 days after weaning of piglets that were provided with creep feed from 2 days of age (CF) or not (NF) before weaning and provided with weaner diet (CON, n = 64 piglets in 16 pens) or a creep feed supplement on top of their weaner diet (CS, n = 64 piglets in 16 pens) after weaning. PRE = creep feed yes/no pre-weaning. POST = creep feed supplement yes/no post-weaning. Data are means ± SEM based on pen averages. Significant *P*-values are presented in bold.

Within CS, the creep feed supplement was consumed in greater amounts by NF- than CF-piglets between d2-5 (41 ± 1.2 vs. 22 ± 2.6 g/pig/d, $P < 0.0001$), d5-9 (38 ± 0.6 vs. 36 ± 1.3 g/pig/d, $P = 0.03$), d9-14 (42 ± 0.6 vs. 38 ± 2.3 g/pig/d, $P = 0.04$) and throughout the first two weeks post-weaning (d0-14, 40 ± 0.5 vs. 33 ± 2.0 g/pig/d, $P < 0.01$).

Diarrhoea was first observed in the pens at d2 post-weaning and peaked at d6 post-weaning when half of the pens contained diarrheic faecal pools. Thereafter, the number of pens with diarrhoea partially recovered but diarrhoea remained present and peaked for a second time at the end of the experiment at d14 post-weaning. Diarrheic faecal pools of score 3 were highly prevalent (97% of the pens), whereas pools of score 4 were less prevalent (25% of the pens). Pre-weaning creep feed provision ($P = 0.69$), post-weaning creep feed supplementation ($P = 0.22$) and the pairwise comparisons of their combinations ($P \geq 0.20$ for all) did not affect the prevalence of watery diarrhoea in the first two weeks post-weaning (% of pens with ≥ 1 day watery diarrhoea; CF-CON: 25%, CF-CS: 37.5%, NF-CON: 0%, NF-CS: 37.5%). In addition, the severity and duration of diarrhoea observed in the first two weeks post-weaning did not differ between the treatment groups (**Table 6**).

Table 6. Post-weaning piglet performance of piglets that were provided with creep feed from 2 days of age (CF) or not (NF) before weaning and provided with weaner diet (CON, n = 64 piglets in 16 pens) or a creep feed supplement on top of their weaner diet (CS, n = 64 piglets in 16 pens) post-weaning. PRE = creep feed yes/no pre-weaning. POST = creep feed supplement yes/no post-weaning. Data are means ± SEM based on pen averages. Significant *P*-values and trends are presented in bold.

	NF		**CF**		**Significance**		
	CON	**CS**	**CON**	**CS**	**PRE**	**POST**	**PRE x POST**
ADFI, g/pig/d							
d 0 - 2[1]	182 ± 20	169 ± 11	127 ± 16	150 ± 26	**0.06**	0.84	0.33
d 2 - 5	313 ± 16	279 ± 17	380 ± 23	352 ± 21	**0.001**	0.12	0.88
d 5 - 9	389 ± 13	347 ±13	390 ± 24	396 ± 20	0.18	0.33	0.21
d 9 - 14	575 ± 22	636 ± 18	519 ± 23	582 ± 30	**0.02**	**0.01**	0.97
d 2 - 14	447 ± 6	450 ± 10	441 ± 8	462 ± 9	0.75	0.17	0.30
ADG, g/pig/d							
d -1 - 2	218 ± 19	175 ± 19	167 ± 21	203 ± 21	0.53	0.83	**0.04**[2]
d 2 - 5	236 ± 24	259 ± 19	315 ± 31	310 ± 24	**0.01**	0.71	0.58
d 5 - 9	356 ± 21	339 ± 16	303 ± 22	336 ± 23	0.17	0.69	0.23
d 9 - 14	488 ± 25	484 ± 21	547 ± 28	532 ± 19	**0.03**	0.68	0.81
d -1 - 14	348 ± 13	339 ± 10	359 ± 21	369 ± 12	0.74	0.73	0.21
FCR, d 2 - 14	1.12 ± 0.08	1.15 ± 0.05	1.14 ± 0.06	1.22 ± 0.03	0.39	0.37	0.70
Mean FCS[3]	0.31 ± 0.06	0.42 ± 0.06	0.31 ± 0.06	0.34 ± 0.07	0.53	0.30	0.53
No. of days with diarrhoea	4.38 ± 0.78	5.25 ± 0.56	4.13 ± 0.85	4.25 ± 0.98	0.47	0.57	0.68

[1] ADFI between d 0 - 2 was analysed for batch 2 only as result of technical difficulties in batch 1

[2] No significant differences were observed using post-hoc pairwise comparisons of least squares means

[3] Faecal consistency score

In the first week, however, CF-piglets tended to have a lower pen faecal consistency score (CF: 0.18 ± 0.04 vs. NF: 0.29 ± 0.05, *P* = 0.07) and shorter duration of diarrhoea in the pen compared to NF-piglets (CF: 1.19 ± 0.31 vs. NF: 1.88 ± 0.31, *P* = 0.09) and in the second week, CON-piglets tended to have a lower pen faecal consistency score compared to CS-piglets (CON: 0.37 ± 0.07 vs. CS: 0.54 ± 0.07, *P* = 0.09).

Behaviour in the home pen was observed at week 1 (**Table 7**) and week 2 post-weaning (**Table 8**). CS-piglets spent about three times as much time on exploring feed than CON-piglets at both observation days post-weaning (at week 1, CS: 0.27 ± 0.07 vs. CON: 0.07 ± 0.02%; at week 2, CS: 0.29 ± 0.08 vs. CON: 0.11 ± 0.03%, *P* < 0.05). In addition, CS-piglets tended to explore their pen mates less at week 1 post-weaning (CS: 2.7 ± 0.3 vs. CON: 3.5 ± 0.4%, *P* = 0.09) and tended to explore their environment less at week 2 post-weaning (CS: 24.6 ± 1.7 vs. CON: 29.0 ± 1.2%, *P* = 0.08). Creep feed provision affected post-weaning behaviour of the piglets at week 2 only, at which CF-piglets performed more rooting (CF: 2.4 ± 0.3 vs. NF: 1.3 ± 0.2%, *P* = 0.03), and showed less chewing faeces or sham chewing compared to NF-piglets (CF: 0.9 ± 0.1 vs. NF: 1.6 ± 0.3%, *P* = 0.02). Moreover, CF-piglets tended to perform less chewing on the environment (CF: 8.1 ± 1.1 vs. NF: 11.3 ± 1.4%, *P* < 0.10) and tended to explore their pen mates less (CF: 2.4 ± 0.3 vs. NF: 3.0 ± 0.2%, *P* = 0.07) at week 2 post-weaning versus NF-piglets.

Effects of creep feed provision on plasma haptoglobin concentrations before and after weaning

There was no difference in absolute haptoglobin concentrations between NF- and CF-piglets for any of the time points measured (**Figure 2**). Moreover, creep feed provision did not affect the difference in haptoglobin concentrations between two successive time points (data not shown). Irrespective of treatment however, the concentration of haptoglobin peaked at d2 after weaning and absolute values of haptoglobin at d2 and d5 after weaning were greater than pre-weaning values (*P* ≤ 0.002 for all). In addition, the concentration of haptoglobin at d2 after birth was lower than the concentration of haptoglobin at d14 post-weaning (*P* < 0.001).

Table 7. Behavioural activities (% of total observations) at week 1 after weaning (36 days of age). Piglets were provided with creep feed from 2 days of age (CF) or not (NF) before weaning and provided with weaner diet (CON, n = 64 piglets in 16 pens) or a creep feed supplement on top of their weaner diet (CS, n = 64 piglets in 16 pens) after weaning. PRE = creep feed yes/no pre-weaning. POST = creep feed supplement yes/no post-weaning. Data are means ± SEM based on pen averages. Significant *P*-values and trends are presented in bold.

Behaviour at week 1 after weaning	**NF**		**CF**		**Significance**		
	CON	**CS**	**CON**	**CS**	**PRE**	**POST**	**PRE x POST**
'Ingestive behaviour'							
Eating feed	11.6 ± 0.7	12.7 ± 1.1	10.4 ± 0.9	11.0 ± 1.1	0.14	0.41	0.83
Drinking	1.0 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.2	0.98	0.39	0.78
'Exploratory behaviour'							
Exploring feed	0.07 ± 0.03	0.23 ± 0.10	0.07 ± 0.03	0.31 ± 0.11	0.70	**0.02**	0.71
Exploring environment	22.8 ± 2.4	23.8 ± 2.7	24.0 ± 1.0	21.8 ± 1.4	0.92	0.72	0.42
Nosing environment	11.4 ± 1.8	12.2 ± 1.9	12.5 ± 1.2	10.8 ± 1.0	0.99	0.69	0.32
Rooting environment	1.0 ± 0.2	1.3 ± 0.3	1.6 ± 0.4	1.3 ± 0.3	0.34	0.98	0.38
Chewing environment	7.1 ± 0.8	6.7 ± 1.3	6.9 ± 0.9	6.9 ± 1.2	0.99	0.77	0.84
Chewing faeces or sham chewing	1.7 ± 0.3	1.6 ± 0.5	1.2 ± 0.3	1.2 ± 0.2	0.13	0.78	0.99
Exploring trough	1.3 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.3 ± 0.2	0.50	0.42	0.43
Exploring drinker	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.10	0.25	0.99
'Postures and locomotion'							
Inactive behaviour	46.8 ± 3.1	47.3 ± 3.7	47.4 ± 2.4	50.6 ± 3.4	0.46	0.50	0.61
Standing and walking	7.6 ± 1.0	6.3 ± 0.7	7.1 ± 0.6	7.0 ± 0.8	0.76	0.15	0.26
'Pig-directed behaviour'							
Nosing pen mates	3.5 ± 0.5	2.5 ± 0.4	3.5 ± 0.6	2.9 ± 0.6	0.82	**0.09**	0.70
Manipulating pen mates	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.4	1.0 ± 0.3	0.59	0.54	0.57
Aggressing pen mates	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.93	0.93	0.83
Mounting pen mates	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.2	1.1 ± 0.4	0.98	0.85	0.99
'Other behaviour'							
Playing	3.2 ± 0.6	2.9 ± 0.6	3.3 ± 0.4	2.3 ± 0.3	0.81	0.16	0.54
Comfort behaviour	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.92	0.91	0.16
Eliminating	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	0.49	0.55	0.55

Table 8. Behavioural activities (% of total observations) at week 2 after weaning (44 days of age). Piglets were provided with creep feed from 2 days of age (CF) or not (NF) before weaning and provided with weaner diet (CON, n = 64 piglets in 16 pens) or a creep feed supplement on top of their weaner diet (CS, n = 64 piglets in 16 pens) after weaning. PRE = creep feed yes/no pre-weaning. POST = creep feed supplement yes/no post-weaning. Data are means ± SEM based on pen averages. Significant *P*-values and trends are presented in bold.

Behaviour at week 2 after weaning	**NF**		**CF**		**Significance**		
	CON	**CS**	**CON**	**CS**	**PRE**	**POST**	**PRE x POST**
‘Ingestive behaviour’							
Eating feed	10.4 ± 1.1	10.5 ± 0.7	10.9 ± 1.0	11.5 ± 0.6	0.41	0.62	0.77
Drinking	1.2 ± 0.1	1.2 ± 0.2	1.4 ± 0.2	1.3 ± 0.2	0.34	0.86	0.98
‘Exploratory behaviour’							
Exploring feed	0.07 ± 0.03	0.31 ± 0.14	0.16 ± 0.05	0.26 ± 0.08	0.37	**0.03**	0.32
Exploring environment	33.4 ± 1.5	28.4 ± 3.0	29.5 ± 1.9	26.8 ±1.7	0.22	**0.08**	0.60
Nosing environment	15.1 ± 1.6	15.4 ± 1.4	17.3 ± 1.4	13.2 ± 1.4	0.95	0.17	0.11
Rooting environment	1.6 ± 0.3	1.1 ± 0.3	2.6 ± 0.5	2.1 ± 0.5	**0.03**	0.21	0.62
Chewing environment	14.0 ± 1.9	8.5 ± 1.7	7.3 ± 0.9	8.9 ± 2.1	**< 0.10**	0.26	0.11
Chewing faeces or sham chewing	1.2 ± 1.2	2.1 ± 0.5	0.9 ± 0.1	0.9 ± 0.2	**0.02**	0.19	0.21
Exploring trough	1.3 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.3 ± 0.2	0.88	0.79	0.23
Exploring drinker	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.76	0.76	0.93
‘Postures and locomotion’							
Inactive behaviour	39.9 ± 1.9	45.7 ± 3.4	43.0 ± 2.7	45.5 ± 2.5	0.60	0.14	0.54
Standing and walking	5.7 ± 0.7	4.3 ± 0.4	5.6 ± 0.8	5.8 ± 0.6	0.29	0.31	0.20
‘Pig-directed behaviour’							
Nosing pen mates	2.8 ± 0.2	3.3 ± 0.4	2.4 ± 0.4	2.3 ± 0.3	**0.07**	0.65	0.43
Manipulating pen mates	1.7 ± 0.5	1.7 ± 0.7	1.8 ± 0.5	1.9 ± 0.4	0.52	0.95	0.84
Aggressing pen mates	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.97	0.75	0.58
Mounting pen mates	0.7 ± 0.3	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.86	0.88	0.58
‘Other behaviour’							
Playing	2.8 ± 0.5	2.7 ± 0.4	3.2 ± 0.4	2.5 ± 0.4	0.84	0.33	0.45
Comfort behaviour	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.78	0.94	0.51
Eliminating	0.7 ±0.1	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	0.29	0.58	0.77

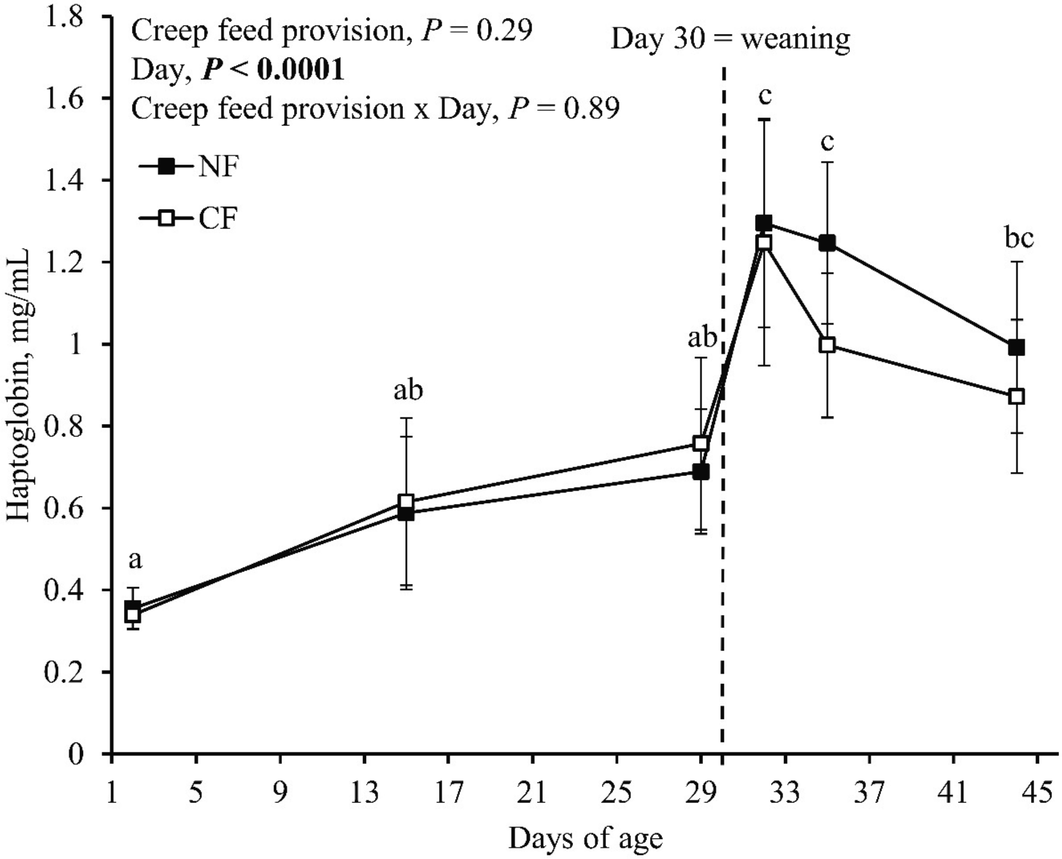

Figure 2. Plasma haptoglobin concentrations of piglets that were provided with creep feed from 2 days of age (CF) or not (NF) before weaning and provided with weaner diet after weaning. Haptoglobin was determined of 14 piglets per treatment before weaning and 13 CF- and 12 NF-piglets after weaning. Data are means ± SEM. Superscripts without a common letter differ over time at $P < 0.05$. Significant P-values are presented in bold.

Discussion

The present study was performed to determine the impact of pre-weaning creep feed provision, post-weaning creep feed supplementation and their interaction on piglet behaviour and performance. We hypothesized that piglets with access to the creep feed both before weaning and, as a supplement, after weaning would experience the lowest level of food neophobia compared to the other treatment groups and therefore ingest the largest amount of feed and perform the best in the first days after weaning. Interactions between pre-weaning creep feed provision and post-weaning creep feed supplementation were only found, however, for ADG between one day before and two days after weaning, but no significant differences were observed using post-hoc pairwise comparisons. Effects of pre-weaning creep feed provision and post-weaning creep feed supplementation will be discussed separately below.

Effects of pre-weaning creep feed provision

Creep feed provision did not significantly affect the behaviour of piglets in the farrowing pen, except that piglets without creep feed (NF) tended to explore the empty feed trough more often and tended to perform more chewing faeces plus sham chewing than piglets with creep feed (CF). NF-piglets also showed more chewing faeces plus sham chewing than CF-piglets after weaning, numerically in the first week and significantly in the second week. This may have represented redirected chewing behaviour due to the absence of (feed) substrates, as free-range piglets occasionally sniffed faeces, but were not observed to eat it (Newberry et al., 1988), while indoor-housed piglets spent a substantial amount of time rooting their faeces (R.C. Newberry, personal observation) and chewing faeces was mainly performed during rooting of faeces (A. Middelkoop, personal observation). We speculate that creep feed intake may therefore partly satisfy piglets' motivation to explore and forage, and may derive from exploratory behaviour (Kuller et al., 2010; Van den Brand et al., 2014; Middelkoop et al., 2018).

Given that piglets provided with creep feed would be more familiarised with solid feed before weaning, we anticipated them to display increased ingestive behaviours and reduced weaning-stress-induced behaviours in the weaner pen, such as belly nosing and manipulation of ears and tails. However, this study did not support this hypothesis, except that CF-piglets tended to chew less on their environment and tended to nose their pen mates less after weaning than NF-piglets, behaviours that were both found to be increased by stress (Dybkjær, 1992; Beattie et al., 1995; Oostindjer et al., 2011d). Creep feed provision did not affect the time spent on exploration in the weaner pen. However, the type of exploratory behaviour performed differed between the groups, where CF-piglets showed more rooting of the environment and NF-piglets performed more chewing behaviour in the second week post-weaning. The latter was particularly observed for NF-piglets that were fed weaner diet without creep feed supplementation after weaning. Both rooting and chewing behaviour towards the environment may be redirected at pen mates when triggered by stress (Beattie et al., 1995; Oostindjer et al., 2011d). The housing conditions of the piglets in this study may have been more favourable in terms of welfare compared to commercial farming, as the density of piglets per pen and the number of piglets that shared one chew object simultaneously (2 piglets/chew object) were considerably lower. Hence, when kept in commercial farming conditions, behaviours like chewing and rooting the environment may become more redirected at pen mates compared to our experiment, where the chewing behaviour mainly targeted the chew object(s).

In the current study, CF-piglets tended to be heavier at weaning and were heavier and more uniform in BW at 14 days post-weaning compared to NF-piglets. This possibly benefits pig production by reaching slaughter age earlier/heavier (Wolter and Ellis, 2001; Collins et al., 2017), although Lawlor et al. (2002) did not find an effect on slaughter weight. In future research, it is therefore interesting to study the effects of creep feed provision on piglet performance up to slaughter. Although we did not observe differences in overall ADFI, ADG and FCR in the first two weeks post-weaning between NF- and CF-piglets, in line with findings of others (Sulabo et al., 2010a; Muns and Magowan, 2018) creep feed provision had consequences for the dynamics in post-weaning performance. In the first two days, NF-piglets tended to eat more than CF-piglets, which is against expectations of greater food neophobia in NF-piglets. In contrast, CF-piglets ate and grew more in the following days from d2-5 post-weaning, indicating a faster recovery in energy intake. Although NF-piglets seemed to 'catch up' by consuming more between d9-14 post-weaning, the CF-piglets again showed a better growth performance. This suggests that the gastrointestinal tract of CF-piglets was better developed in this period. The dynamics in post-weaning performance as result of creep feed provision also implies the difficulty to compare studies that measured performance at different time points and found no effect of creep feed provision (Carstensen et al., 2005; Beaulieu et al., 2010) although similar dynamics have been observed previously. In particular, a tendency for a lower ADFI between d0-1 post-weaning (Beaulieu et al., 2010) and a greater ADFI in the first week post-weaning (Muns and Magowan, 2018) as result of creep feed provision were reported, in accordance to our findings. In addition, diarrhoea in the first week post-weaning was found to be less severe due to creep feed provision (Yan et al., 2011), which corresponds to the trend found in our study. Similar to other studies, creep feed provision in the present study also resulted in a greater ADG in the last week before weaning (Shea et al., 2013), a trend for a greater weaning weight (Makinde et al., 1997), and did not affect litter CV in BW, sow BW loss and back fat loss at weaning (Sulabo et al., 2010a; Yan et al., 2011; Park et al., 2014). Contrary, opposite results to our study have been reported as well, with performance measured in comparable time periods: no difference as result of creep feed provision in pre-weaning ADG and weaning weight (e.g. Sulabo et al., 2010a; Park et al., 2014; Muns and Magowan, 2018), and a lower ADG and FCR (Beaulieu et al., 2010), greater ADFI and ADG (Shea et al., 2013), and a trend for greater diarrhoea scores (Barnett et al., 1989) of CF- vs. NF-piglets in the first two weeks post-weaning. Factors that likely contribute to these inconsistencies regarding the effects of creep feed provision on pre- and post-weaning performance include weaning age (Callesen et al., 2007a; Collins et al., 2013), the duration of creep feed provision (Yan et al., 2011), the composition of the pre- and post-weaning diet as well as their interaction (Torrallardona et al., 2012), the percentage of eaters (Bruininx et al., 2002) and the intake of creep feed (high vs. low intake per piglet; Bruininx et al., 2004). Pre- and post-weaning performance benefits seem more pronounced in piglets with proven intake of creep feed (eaters vs. non-eaters; Bruininx et al., 2002; Sulabo et al., 2010b) and, in particular, in piglets with a high creep feed consumption level (good vs. moderate vs. bad vs. non-eaters; Bruininx et al., 2004; Pluske et al., 2007). Because creep feed intake varies substantially between litters and between littermates, it is difficult to exactly pinpoint the effects of creep feed provision. Therefore the relationship between individual creep feed consumption and piglet behaviour warrants further investigation.

Haptoglobin is an acute phase protein and a biomarker for acute infection, inflammation and stress in pigs, including stress from weaning (De et al., 2017). The observed elevation in haptoglobin after weaning may therefore reflect an increase in stress (but also in e.g. inflammation) associated with weaning (Pomorska-Mól et al., 2012; De et al., 2017), although there was no control of unweaned piglets in the present study. We hypothesized that CF-piglets would experience weaning as a less stressful event than NF-piglets. Firstly because CF-piglets would be habituated to fibrous solid feed before weaning, in terms of behaviour (as suggested by Muns and Magowan, 2018 and Figueroa et al., 2019) and physiology (as suggested by Williams, 2003 and Pluske et al., 2018), and would thereby be better facilitated to the dietary change at weaning. Secondly, this acquaintance with an alternative energy source may also have reduced stress from maternal separation (Haley et al., 2005). However, our data on haptoglobin concentrations and weaning-stress-induced behaviour do not support this hypothesis. Thirdly, providing fibrous creep feed may affect the responsiveness of piglets to novelty via the microbiota-gut-brain axis, because dietary composition has been shown to affect anxiety, exploration, agonistic and activity levels in pigs at various stages of life (Haagensen et al., 2014; Clouard et al., 2016). Indeed, the gut microbiota of CF- and NF-piglets differed in composition from 2 weeks of age (Choudhury, Middelkoop et al., in preparation). Nevertheless, we found no indications that CF-piglets respond differently to novelty than NF-piglets, as no differences were found in exploration- and avoidance-related behaviour in the pre-weaning OFT/NOT. In contrast, Fleming et al. (2017) found that prebiotic supplementation with polydextrose and galacto-oligosaccharides in the milk replacer of artificially reared piglets from 2 days of age increased their exploratory behaviour in a novelty preference test at 27 days of age. Moreover, they found relationships between the exploratory behaviour in the test and volatile fatty acid concentrations, especially in the ascending colon. Notably, the intake of creep feed is relatively low compared to the intake of milk in suckling piglets (approximately 32 g/d creep feed in this study from d21-30 vs. 1 kg milk/d; Quesnel et al., 2015) and therefore is only a small fraction of the piglets' diet. This complicates the comparison of the present study with Fleming et al. (2017). Moreover, the timespan between initiation of creep feed provision and measurement of the behavioural responses in the OFT/NOT was relatively short, and longer study periods may be required to detect effects of nutritional interventions early in life (Prado and Dewey, 2014). Therefore the effects of creep feed provision on behaviour later in life deserve further attention.

Effects of post-weaning creep feed supplementation

We predicted post-weaning supplementation of creep feed to increase feed exploration and intake and to reduce weaning-stress-induced behaviours in the weaner pen by reducing food neophobia of piglets that were given creep feed before weaning. Indeed, creep feed supplementation after weaning increased the time spent on exploratory behaviour towards the feed, both in CF- and in NF-piglets. This might be the result of dietary variety (as suggested by Adeleye et al., 2014; Middelkoop et al., 2018a) established by the two feeds provided, piglets selecting their preferred (size of the) feed item, but may also result from offering larger pellet sizes, which are better suited for (playful) exploration (as suggested by Edge et al., 2005; Van den Brand et al., 2014), within the creep feed supplement. The creep feed supplement was consumed in greater amounts by NF- than CF-piglets after weaning, which indicates that dietary variety and/or larger pellet sizes might mainly help inexperienced piglets to start eating solid feed. This would be in line with studies in which an increased post-weaning feed intake of a large diameter pellet was reported for piglets that were relatively inexperienced with solid feed before weaning (Traylor et al., 1996; Clark et al., 2016), but not in piglets that were, similar to our CF-piglets, more experienced with solid feed pre-weaning (Edge et al., 2005; Van den Brand et al., 2014). Considering creep feed supplementation improved feed intake between d9-14 post-weaning, and we expect piglets to be experienced with solid feed by then, we hypothesize this increase in feed intake to be related to dietary variety mainly. The observed increase in feed intake between d9-14 post-weaning was caused by both the creep feed supplement and a numerically greater intake of the weaner diet. Overall ADFI in the first two weeks post-weaning did not differ, however, between creep-supplemented piglets and control piglets that did not receive creep feed on top of their weaner diet. Post-weaning creep feed supplementation did also not affect behaviours other than feed exploration, except for a trend to reduce exploration towards pen mates in the first week and a trend to reduce exploration towards the environment in the second week post-weaning, which might indicate that piglets' need for oral tactile stimulation was more fulfilled by supplementing the weaner diet with creep feed. Moreover, post-weaning creep feed supplementation did not influence post-weaning ADG and FCR. Creep-supplemented piglets tended to have a greater faecal consistency score compared to piglets in the second week post-weaning, which might be related to the greater variety in feed ingredients and thereby dietary antigens. Post-weaning creep feed provision thus showed positive effects on feed exploration and intake, despite that the creep feed was only provided in a limited amount on top of the weaner diet. We recommend future research to study the effect of dietary variety, for example by offering two feeds *ad libitum* and simultaneously, on the behaviour and performance of weaner piglets.

Conclusions

Creep feed provision did not affect behaviour in a novelty test and haptoglobin concentrations around weaning, but improved piglet growth in the last week before weaning by 12%. In addition, pre-weaning creep feed provision affected post-weaning feed intake and growth within specific time periods, but did not influence overall ADFI, ADG, FCR and faecal consistency in the first two weeks post-weaning. Nevertheless, pre-weaning creep feed provision resulted in a 5% greater body weight and in a lower within-pen body weight variability at d14 after weaning. Pre-weaning creep feed provision and post-weaning creep feed supplementation only limitedly affected behaviours in the home pen, with effects on specific exploratory behaviours only. Post-weaning creep feed supplementation resulted in a three-fold increase in feed exploration and a 11% greater feed intake between d9-14 post-weaning, but did not affect ADG and BW after weaning, and did also not affect overall ADFI, FCR and faecal consistency in the first two weeks post-weaning. Results of the present study indicate that providing suckling piglets fibrous creep feed may be effective for subtly increasing body weight development around weaning, but results did not indicate that creep feed provision may reduce stress at weaning.

Acknowledgements

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Supplementary Table S1. Behaviour of piglets before and after weaning.

Behaviour	**Description**
Suckling[1]	Drinking milk from teat of sow (soft suckling noises)
Massaging udder[1]	Massaging udder with head/nose
Drinking	Drinking water from drinking trough/nipple
‘Eating’	
Eating feed	Eating or chewing feed (at the feeder)
Eating feed from floor	Eating or chewing feed from the floor
‘Exploring feed’	
Exploring feed	Sniffing, touching (with snout) or rooting the feed in the feeder
Exploring feed on floor	Sniffing, touching (with snout) feed on floor
Playing with feed	Rolling feed item over floor, walking around with feed item in mouth, (energetically) shaking head with feed item in mouth
‘Exloring environment’	
Nosing environment	Sniffing, touching (with snout) or licking part of the pen (e.g. floor, wall)
Rooting environment	Rooting part of the pen, scraping floor with one the front legs
Chewing environment	Chewing or nibbling part of the pen, including chew object
Chewing faeces or sham chewing	Chewing faeces or sham chewing (not chewing on part of the pen, feed or chew object)
Exploring feeder	Sniffing, touching (with snout), rooting or chewing on feeder
Exploring drinker	Sniffing, touching (with snout) or chewing on drinking trough
‘Inactive behaviour’	
Lying eyes closed	Lying on side or belly with eyes closed without performing any other described behaviour
Lying eyes open	Lying on side or belly with eyes open, sitting or kneeling without performing any other described behaviour
Standing and walking	Piglet is upright, standing or walking without performing any other described behavioir
Snout contact	Mutual snout contact with pen mate or sow
Nosing pen mates	Sniffing, touching (with snout) part of the body of a pen mate excluding snout
Nosing sow[1]	Sniffing, touching (with snout) part of the body of the sow excluding snout
Manipulating sow[1]	Chewing, nibbling or sucking part of the body of the sow including ear, tail, abdominal area and pulling hairs
Mounting pen mates	Standing on hind legs while having front legs on pen mate

[1] Pre-weaning only

Continue

Supplementary Table S1. Behaviour of piglets before and after weaning.

Behaviour	Description
‘Manipulating pen mates’	
Ear biting	Chewing, nibbling or sucking the ear of a pen mate
Tail biting	Chewing, nibbling or sucking the tail of a pen mate
Belly nosing[2]	Rubbing belly of a pen mate with ≥ 3 up and down movements of the snout or sucking the navel or skin of the abdominal area of a pen mate
Manipulating pen mates	Chewing, nibbling or sucking part of the body of a pen mate excluding ear, tail and abdominal area
‘Aggressing pen mates’	
Aggressive behaviour	Aggressively ramming, pushing, head-knocking, lifting or biting a pen mate, including mutual fighting
Feed-related fighting	Aggressively ramming, pushing, head-knocking, lifting or biting a pen mate, including mutual fighting for feed at the feeder
‘Playing’	
Playing individually	Play activities that involve one player; running across pen, rolling, pivoting, tossing head, flopping, sliding, scampering, nudging
Playing socially	Play activities that involve more players; e.g. running, pivoting, scampering, sliding across pen together, play fighting.
Playing with chew object	(Energetically) shaking head with chew object in mouth, flipping chew object over, rolling ball over floor
Comfort behaviour	Rubbing body against objects, the sow or pen mates, scratching body with hind legs or stretching (part of) body
Eliminating	Defaecating or urinating

[2] Post-weaning only

Supplementary Table S2. Behavioural states of piglets during the combined open field and novel object test.

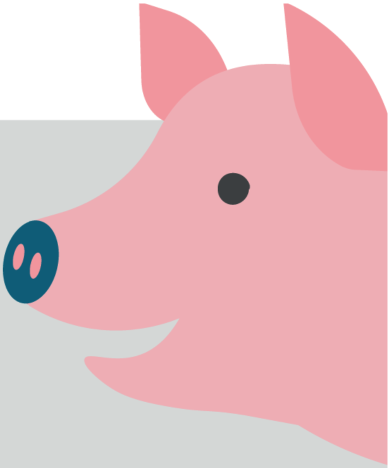
Behaviour	**Definition**
Locomotion class	
Walking	Walking without performing any other described behaviour. All four legs move or the piglet turns around at the same spot without moving all four legs.
Standing	Standing with four paws on the floor without performing any other described behaviour.
Standing alert	Standing motionless with head fixed (up or down) and ears upright, focused on a noise, wall, person, object etc.
Sitting	Sitting or kneeling on the floor without performing any other described behaviour.
Lying eyes closed	Lying on side or belly with eyes closed without performing any other described behaviour.
Lying eyes open	Lying on side or belly with eyes open without performing any other described behaviour.
Behaviour class	
Other behaviour	The piglet is not performing any of the mentioned behaviours below
Draw back[1]	The piglet draws back from the bucket. At first the head is directed to the bucket, during the draw back the head can be either directed to the bucket or turned away from it.
Chewing	Chewing on part of the arena, chewing faeces or sham chewing
'Exploring floor'	
Nosing floor	Sniffing, touching (with snout) or licking the floor. Rooting disc is either in contact or very close to the floor.
Rooting floor	Rooting the floor with the rooting disc that exerts some force.
'Exploring walls'	
Nosing walls	Sniffing, touching (with snout) or licking the walls of arena. Rooting disc is either in contact or very close to the surface.
Rooting walls	Rooting the walls of arena with the rooting disc that exerts some force.
'Approaching bucket'	
Slow approach bucket[1]	The piglet is approaching the bucket slowly (step by step) within 1m distance, but not (yet) exploring it.
Fast approach bucket[1]	The piglet is approaching the bucket quickly (easy walking or running) within 1m distance, but not (yet) exploring it.
'Exploring bucket'	
Touch bucket[1]	Sniffing, touching (with snout), rooting or licking the metal bucket. Rooting disc is in contact with the bucket.
Explore bucket[1]	Sniffing the metal bucket without touching it. Rooting disc is not in contact with the bucket.
Chewing bucket[1]	Trying to chew on the metal bucket by touching it with open mouth and making biting movements.

[1] In novel object test only

Supplementary Table S3. Vocalisations and other behavioural events of piglets during the combined open field and novel object test.

Behaviour	**Description**
Vocalisations	
‘Low-pitched vocalisations’	
Short grunt	A low tone of less than half a second (one note)
Long grunt	A low tone of more than half a second (one note)
Bark	A low tone that sounds like ‘wuff’
‘High-pitched vocalisations’	
Squeal	A high tone (different notes)
Grunt-squeal	A low tone that transforms into a high tone
Scream	A high, long and loud tone, often as long as an expiration
Other behavioural events	
Escape attempt	Jumping in air or against wall of test arena trying to escape
Comfort behaviour	Rubbing body against walls, scratching body with hind legs or stretching (part of) body
‘Eliminating’	
Defaecating	Defaecating
Urinating	Urinating

CHAPTER 3



Impact of early life feeding on local intestinal microbiota and digestive system development in piglets

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Abstract

Early life gut microbial colonisation is known to influence host physiology and development, shaping its phenotype. Suckling piglets with a developing gastrointestinal tract, provide a ‘window of opportunity’ for programming their intestinal microbiota composition and corresponding intestinal development. Here, we investigated the impact of early feeding on local intestinal microbiota composition and intestinal maturation in suckling piglets. From two days of age, early-fed (EF; n = 6 litters) piglets had access to solid feed containing a mixture of fibres until weaning (day 29) in addition to sow’s milk, whereas the control (CON; n = 6 litters) piglets exclusively fed on sow’s milk. Early feeding elicited a significant impact on the colon microbiota, whereas no such effect was seen in the small intestinal microbiota. Quantified eating behavioural scores could significantly explain the variation in microbiota composition of EF piglets and support their classification into good, moderate and bad eaters. Several microbial groups such as *Lachnospiraceae ND3007 group, Eubacterium xylanophilum, Prevotella* and *Ruminococcus* were quantitatively associated with ‘more eating’. Early-fed piglets were found to have a decreased pH in caecum and colon, which coincided with increased short-chain fatty acid concentrations like acetic and butyric acid. Moreover, they also had increased weights and lengths of several intestinal tract segments, as well as a trend for a decreased villus:crypt ratio in jejunal mucosa. The approaches in this study indicate that early (pre-weaning) feeding of a mixed-fibre diet changes the microbiota composition, pH, and fermentation products in the distal gut of piglets, while it also alters both macroscopic and microscopic intestinal measurements. These results emphasize the potential of early feeding to modulate intestinal development in young piglets.

Introduction

In (semi-)natural conditions, piglets begin to forage food items already when a few days old (Gundlach, 1968; Meynhardt, 1980; Petersen, 1994), familiarising with solid feed and preparing for the weaning transition gradually over a period of 8.5-22 weeks of age (Newberry and Wood-Gush, 1985; Jensen and Stangel, 1992). In contrast, weaning in commercial pig farms is a drastic process that commonly takes place between 3-4 weeks of age, where piglets are exposed to various simultaneous stressors, like separation from their sow and littermates, new housing conditions, unknown pen mates, and a sudden change of diet. These abrupt changes are often accompanied by a transient low feed intake, poor growth (Le Dividich and Sève, 2000; Bruininx et al., 2002), intestinal dysbiosis and diarrhoea post-weaning (Heo et al., 2013; Everaert et al., 2017; Gresse et al., 2017; Pluske et al., 2018), thus compromising animal health and welfare, and increasing piglet mortality and economic losses. Creep feeding, a method of supplementing suckling piglets with solid feed, is prevalent in modern pig farming to ease the weaning transition as well as to stimulate post-weaning eating (or feed intake) (Bruininx et al., 2002; Pluske et al., 2005). However, 'traditional' creep feed is highly palatable, easily digestible and mainly based on milk proteins (Okai et al., 1976; Heo et al., 2018), which is distant from fibrous solid feed exposure in both natural and post-weaning conditions.

At the time of weaning, the gastrointestinal tract of a young pig is still developing (McCance, 1974; Everaert et al., 2017) and undergoing rapid changes in gut microbiota colonisation, digestive system and immune development (Pajarillo et al., 2014; Frese et al., 2015; Pluske et al., 2018; Xiong et al., 2019). Importantly, the gut microbiome has been recognised to play a crucial role in overall animal health and development, especially in early life (Mulder et al., 2009; O'Mahony et al., 2009; Schokker et al., 2014; Merrifield et al., 2016). The early life microbial colonisation with potentially beneficial and diverse gut microbes can influence the maintenance of intestinal homeostasis and prevent gut dysbiosis (Pluske et al., 2018; Guevarra et al., 2019; Nowland et al., 2019). Dietary fibres can modulate the gut microbiome, and they are widely recognised as food/feed additives that influences gut health positively (Jha et al., 2019). Notably, dietary fibres have also been implicated in gastrointestinal tract development and mucosal changes in pigs (Bach Knudsen et al., 2012; Van Hees et al., 2019). These fermentable fibres pass through the small intestine undigested and act as a substrate for the distal gut microbiota, stimulating microbial fermentation and short-chain fatty acid (SCFA) production in the colon. The predominant SCFAs formed (approximately 95%) are acetic, propionic and butyric acid, although some other organic acids can be detected as well, such as lactic, succinic, isovaloric, and isobutyric acid (Ríos-Covián et al., 2016). Absorbed SCFAs can provide up to 15% of the maintenance energy requirement of growing pigs and 30% in gestating sows (Varel and Yen, 1997). However, special attention is commonly given to butyric acid since it serves as a major source of energy for colonic epithelial cells, and has been proposed to exert several (additional) effects that are considered pivotal in establishment and maintenance of homeostasis in the colon mucosa, including colonocyte growth and proliferation (Hamer et al., 2008; Berni Canani et al., 2012; Leonel and Alvarez-Leite, 2012; Den Besten et al., 2013; Van der Beek et al., 2017; Xiong et al., 2019). The SCFAs, particularly butyric acid, can modulate the expression of genes involved in gut motility, host defence and inflammatory responses, contributing to formation and protection of the intestinal barrier as well as stimulating differentiation and regulation of T cells (Furusawa et al., 2013; Smith et al., 2013). Although the exact mechanisms by which microbial SCFAs influence mucosal physiology remain to be resolved, a few effects of SCFAs and the underlying mechanisms have been revealed, including the function of SCFAs as ligands for G protein-coupled receptor pair GPR41 and GPR43 in epithelial or immune cells, and their inhibition of histone deacetylases (HDAC) activity (Sun et al., 2017; Xiong et al., 2019).

Although previous studies have characterised porcine gut microbiota in relation to dietary fibre intervention, most of them have focussed on the post-weaning period, assessing the impact on weaned piglets (Dicksved et al., 2015; Umu et al., 2015, 2018; Kraler et al., 2016; Burbach et al., 2017). Currently, a handful of studies have assessed how early life (pre-weaning) feeding might influence the gut microbiome (Shim et al., 2005; Zhang et al., 2016; Shi et al., 2018; Brink et al., 2019) and contribute to the intestinal development of suckling piglets (Alizadeh et al., 2016; Yeruva et al., 2016; Li et al., 2018; Van Hees et al., 2019). In our previous study (Choudhury, Middelkoop et al., in preparation), we established that early feeding with a fibre-enriched pre-weaning feed from 2 days of age accelerates pre-weaning microbiota colonisation patterns towards those that resemble a typical post-weaning microbiome. In the present study, we evaluated the impact of early life feeding on the intestinal microbiota composition in different regions of the intestinal tract, and investigate its consequences for intestinal development and maturation. We hypothesized that the pre-weaning consumption of mixed-fibre solid feed would result in an accumulation of undigested substrate in the colon, and investigated its consequences for local microbiota, SCFA production, macroscopic development of the digestive system, as well as its microscopic consequences on mucosal morphology. Suckling piglets show large variation in solid feed intake before weaning between litters (Kuller et al., 2007a) and within litters (Pajor et al., 1991), and therefore we exploited the quantified variation in eating behaviour of piglets to assess the impact of early feeding at an individual piglet level.

Methods

Animals and treatments

The Animal Care and Use committee of Wageningen University & Research (Wageningen, the Netherlands) approved the protocol of the experiment (AVD104002016515). The protocol is in accordance with the Dutch law on animal experimentation, which complies with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The experiment was conducted with 12 multiparous Topigs-20 sows (range parity: 3-5) and their litters (Tempo x Topigs-20) at research facility Carus (Wageningen University & Research, the Netherlands). The litters were divided into two experimental groups at 2 days of age, early-fed group (**EF**, n = 6 litters) and control group (**CON**, n = 6 litters) based on sow's parity, farrowing date, body weight (of the litter at birth and 2 days of age) and genetic background. Litters belonging to the EF group were provided with solid feed *ad libitum* in addition to suckling sow's milk, whereas the CON group nursed on sow's milk only. Briefly, the diet was high in dietary non-starch polysaccharides (261 g/kg dry matter), originating from cereal grains, sugarbeet pulp, oat hulls, galacto-oligosaccharides, inulin and high-amylose starch (11.8 MJ/kg as-fed net energy, 195 g crude protein, 11.9 g standardized ileal digestible lysine/kg dry matter, **Supplementary Table 1** and **2**). Additional details about housing and management of the animals have been described previously (Middelkoop et al., under revision; Chapter 2 of this thesis). At the end of the suckling period at 29 days of age (just before weaning), a subset of piglets was sacrificed (n = 14 per treatment, seven males and seven females, distributed over two consecutive sampling days). Piglets were euthanised by intravenous injection of 20% sodium pentobarbital (EUTHASOL®, 500 mg/ml, AST Farma B.V., Oudewater, the Netherlands). Sacrificed piglets were selected by the following criteria: no antibiotic treatment, close to the average body weight of the litter, close to the average body weight of the treatment group, equal male to female ratio and one to three piglets per litter, taking into account that from EF litters only those piglets were selected that were seen eating solid feed.

Measurements

Eating behaviour

The eating behaviour of piglets was assessed by means of continuous video recording and sampling. Piglets were individually numbered on their back using dark permanent hair dye to allow individual recognition during behavioural observations. The amount of time spent eating or 'eating time' of individual EF piglets was determined daily via continuous sampling from 07:00 to 19:00 h (lights were on in this period) as an estimate for pre-weaning solid feed intake. When an EF piglet placed its snout into the trough for a minimum of 5 seconds (s), the behaviour was scored as eating (Pajor et al., 1991; Adeleye et al., 2014). The eating time was categorised into short (5-9 s), medium (10-29 s) and long (≥ 30 s) eating bouts. The eating bout ended when the snout of the piglet was out of the trough for a minimum of 5 s. When the snout of the piglet went back into the trough within the interval of ≤ 4 s, the eating bout continued, but these interval seconds were not included to determine eating time. Exploratory behaviour towards the feed trough, such as chewing the trough, was not scored as eating. Daily/weekly eating time per piglet was (semi-)quantified by summing the (minimum) number of seconds spent eating (where short, medium and long bouts counted as 5, 10 and 30 s respectively) from 2 days of age to weaning (at 29 days of age). All the observers were trained and instructed with the evaluating criteria to obtain homogeneous and accurate quantification of eating behaviour.

Intestinal microbiota sampling and microbiota metataxonomic analysis

At sacrifice, the gastrointestinal tract was removed from the abdominal cavity of piglets and dissected immediately to collect 20 cm of intestinal segments from different intestinal locations within 25 min after sacrifice, i.e. jejunum (1.5 metre from duodenal-jejunal flexure), ileum (50 cm upstream from ileocaecal valve) and colon (mid-spiral colon). Luminal contents were collected from the intestinal segments, immediately frozen in liquid nitrogen and stored at -80 °C until further processing.

Approximately 300 mg of luminal content (wet weight) from jejunum, ileum and colon samples was used for microbial DNA extraction. Total genomic DNA was extracted by the repeated bead beating method (Yu and Morrison, 2004) using QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The quality and quantity of extracted DNA was checked by gel electrophoresis and Nanodrop DeNovix DS-11 Spectrophotometer (DeNovix Inc., Wilmington, DE USA) respectively. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified by PCR using primers V3F (5′-CCTACGGGNGGCWGCAG-3′) and V4R (5′-GACTACHVGGGTATCTAATCC-3′), 5′-extended with PCR adapters 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′ and 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′, respectively. PCR reactions were prepared in a total volume of 50 µl containing 5 µl 10× KOD buffer (Toyobo, Japan), 3 µl MgSO4 (25 mM), 5 µl dNTPs (2 mM each), 1.5 µl V3F primer (10 µM, Eurogentec, Luik, Belgium), 1.5 µl V4R primer (10 µM, Eurogentec, Luik, Belgium), 1.0 µl (0.02 U/µl) KOD hot start DNA polymerase (Toyobo, Japan) and 10 ng (minimum) of template DNA. PCR amplification was performed on Bio-Rad C1000 thermal cycler

(Bio-Rad Laboratories, Veenendaal, the Netherlands) with an initiation cycle of 95 °C for 2 min, followed by 25 amplification cycles encompassing denaturation at 95 °C for 20 s, annealing at 55 °C for 10 s, and elongation at 70 °C for 15 s, and was completed by a final extension step at 72 °C for 5 min. Amplicons were purified using MSB Spin PCRapace (STRATEC Molecular, Germany) and were sequenced at BaseClear BV (Leiden, the Netherlands) using (paired-end) Illumina MiSeq system. Purified amplicons were subjected to extension PCR using barcoded Illumina universal index sequencing adapters prior to sequencing. The Illumina MiSeq system generated FASTAQ sequence files using the bcl2fastq2 version 2.18 and these sequences were subjected to quality control based on Illumina Chastity filtering and FASTQC quality control tool version 0.11.5. Subsequently, a BaseClear in-house filtering protocol was applied for removal of reads containing adapters and/or PhiX control signal.

Illumina reads were imported into the CLC Genomics Workbench version 11 and were processed using the CLC Microbial Genomics Module version 2.5 (CLC bio, Arhus, Denmark). The paired-end reads were merged into one high quality representative by CLC Workbench (Mismatch cost = 1, Minimum score = 40, Gap Cost = 4, Maximum unaligned end mismatches = 5). The CLC pipeline was used for primer and quality trimming (Trim using quality scores = 0.05; Trim ambiguous nucleotides: maximum number of ambiguities = 2; Discard reads below length = 5). The remaining high quality sequences were clustered into operational taxonomic units (OTUs) at 97% identity threshold using SILVA database v132 (released on Dec 13, 2017) (Quast et al., 2013). OTUs lower than 2 reads (Minimum combined count = 2) were excluded from the analyses. To evaluate alpha and beta diversity indices, OTUs were rarefied to minimum library size (11,000 reads) attaining even sequencing depth between samples. Alpha diversity was evaluated using microbial species richness (Chao1 bias corrected) and evenness (Shannon) indicators. PERMANOVA test of Bray Curtis distance was performed to assess beta diversity between samples. Relationship between microbial groups and intestinal location or treatment groups was determined by principal component analysis (PCA; unsupervised), partial redundancy analysis (pRDA; supervised) and redundancy analysis (RDA; supervised) using CANOCO 5 (Microcomputer Power, Ithaca, NY, USA), according to accompanying instructions (Ter Braak and Smilauer, 2012). Statistical significance was evaluated by Monte Carlo permutation procedure (MCPP) with 499 permutations. The discriminative microbial families (identified in RDA colon) were visualised in a heat map of microbial relative abundance to assess consistency of the EF treatment at individual level. Heat maps were constructed by hierarchical clustering of microbial groups (selected from Redundancy analysis; microbial genera below 0.01% relative abundance in less than 10% of individual samples were not included) in Perseus software3, where relative abundance values were log2 transformed and subsequently normalized by z-score transformation. Euclidean distance was utilized to measure the distance and clustering was conducted using the average linkage method. The online tool ‘MicrobiomeAnalyst’ (Dhariwal et al., 2017) for comprehensive statistical, visual, and meta-analysis of microbiome data was also used for detecting microbial taxa which were differentially abundant among different locations/treatments. Low abundance OTUs were removed, where OTUs with less than two counts in < 10% of the samples. The OTU table was rarefied to minimum library size and transformed using trimmed mean of M-values (TMM) which was used to evaluate differentially abundant taxa (‘Classical Univariate analysis’ with multiple correction).

pH and SCFA concentrations

After euthanasia, the gastrointestinal tract was removed and the contents of the stomach, small intestine, caecum and colon were collected by gently squeezing the digesta from the different parts of the intestine. Immediately after sampling, the pH was recorded by inserting a pH electrode (pH 300, HANNA Instrument, Padova, Italy) in homogenized digesta.

SCFAs (acetic, propionic and butyric acid) along with succinic acid and lactic acid, were quantified in colon digesta samples using an Ultimate 3000 HPLC equipped with an autosampler, a RI-101 refractive index detector (Shodex, Kawasaki, Japan), and an ion-exclusion Aminex HPX-87 H column (7.8 mm × 300 mm) with a guard column (Bio-Rad, Hercules, CA, USA). Samples weighing ~200 mg were taken in a 2ml eppendorf tube, filled with milliQ water to have a final weight of 1 gram, vortexed followed by centrifugation (10 mins, 30,000*g). The supernatant (10 uL) was injected onto the column and eluted with 5 mM H2SO4 at a flow rate of 0.6 mL/min at 65 °C oven temperature. Calibration curves of each acid were prepared in a range of 0.01 – 1 mg/ml. Chromeleon 7.1 software (Dionex) was used to process HPLC data.

Macroscopic intestinal parameters

During sacrifice, intestinal organ weights and lengths were determined. Weights of the stomach, small intestine, caecum and colon (full and empty), as well as length of the small intestine and large intestine (caecum plus colon) were recorded for each piglet. Empty weights of the intestinal segments were determined after removal of digesta by gently squeezing the intestine, followed by rinsing of the intestine in saline solution and removal of excess rinsing fluids using paper towels. In addition, adrenal gland, gallbladder, pancreas, spleen and liver were also weighed.

Histological morphometric measurements

From 16 (n = 8 piglets per treatment) out of the 28 sacrificed piglets, intestinal sections (about 2 cm) from proximal jejunum and mid colon were fixed with 4% paraformaldehyde (PFA), and then dehydrated and embedded in paraffin blocks. 5 µm sections were cut with a Accu-Cut® SRM™ 200 Rotary Microtome (Sakura Finetek Europe B.V., Alphen aan de Rijn, the Netherlands), deparaffinized, hydrated and stained with Haematoxylin and Eosin (H&E). Slides were examined using a Leica DM6 B microscope (Leica Microsystems Ltd. CH9435 Heerbrugg) and images (5x magnification) were processed with LAS X software (Leica Microsystems Inc., Buffalo Grove, IL, USA). Intestinal (histo-)morphometric parameters like villus length and crypt depth (µm) were measured from jejunal sections and crypt depth was measured from colonic tissue sections. These parameters were measured on 90 well-formed villi and their corresponding crypts per animal (three intestinal sections per piglet, on which 30 villi and crypts were measured per section). In jejunum, villus length was defined from the tip of a villus to its base, and crypt depth was measured as the distance from the base of the villus (i.e. villus-crypt transition) to the muscularis mucosa. Subsequently, the ratio of the villus length to crypt depth (V:C) was calculated.

Statistical analyses

We first investigated the impact of early feeding on microbiota composition, pH, SCFAs, macroscopic and microscopic intestinal measurements. Subsequently, we evaluated whether these parameters are quantitatively associated with individualised quantification of eating behaviour.

Comparative analysis of the diversity indices, pH, SCFA concentrations and histological morphometric measurements were performed by Mann Whitney U-test (for non-parametric statistics) or Student's t-test (for parametric statistics) using GraphPad Prism version 8.1.1 (GraphPad Software, La Jolla California, USA). Normality of data (Shapiro-Wilk test) and statistical differences were checked with a limit of significance set at $P < 0.05$. Macroscopic intestinal parameters were analysed with linear mixed models (MIXED) in the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Organ measurements were compared with a model including treatment (EF vs. CON) as a fixed factor and using body weight at sacrifice as covariate in the model. Model residuals of the MIXED procedure were checked for normality. Data are presented as (untransformed) means ± SEM. Differences at $P < 0.05$ were considered statistically significant and differences at $0.05 \leq P < 0.10$ were considered as trend.

To evaluate the relationship between eating behaviour and other measured parameters, a non-parametric spearman correlation matrix was calculated using GraphPad Prism version 8.1.1 (GraphPad Software, La Jolla California, USA). For correlation analysis with the other measurements in the study, the organ weight and organ size values were converted to body-weight-normalized values by dividing absolute values by the scaled 'body weight factor' (body weight of an animal divided by the smallest body weight in the group; scaled between 1 – 1.9) to obtain 'normalized organ-weight and -size values' per animal.

Results

Gut microbiome composition in different intestinal segments

We assessed the microbiota composition in jejunum, ileum and colon of 28 piglets sacrificed at the end of pre-weaning phase (day 29). Illumina Miseq 16S rRNA gene sequencing of the V3-V4 region generated 1,211,527 number of reads after quality filtering, with a mean sample depth of 16,596 ± 3844 reads.

Prominent (intestinal) location-specific differences in microbiota composition were observed. Principal component analysis showed two distinct clusters (**Figure 1A**), reflecting different early-life microbial colonisers in the small and large intestine. For example, microbial families like *Lactobacillaceae*, *Peptostreptococcaceae* and *Clostridiaceae1* were found to be dominant in the small intestinal (jejunal and ileal) samples, whereas *Rikenellaceae*, *Prevotellaceae*, *Ruminococcaceae* and *Lachnospiraceae* were much more abundant in the large intestine (**Figure 1B** and **Supplementary Figure S1A**). This was further demonstrated by microbiota compositional (redundancy) analysis as a function of 'intestinal location' which identified dominant microbial groups associated with each intestinal segment (**Figure 1B**). The limited difference between jejunum and ileum microbiota was recognised by the second principal component (PC2) with 4.76% explained variation, compared to the first principal component (PC1) of 66.66% which separates small and large intestinal microbiota (**Figure 1A**). The large intestine had microbiota with a higher microbial richness (**Figure 1C**) and evenness (**Supplementary Figure S2A**) compared to the small intestine and clustered separately when assessed by Bray Curtis distance (**Supplementary Figure S2A**; PERMANOVA, $P < 0.0001$). Comparing jejunal and ileal microbiota, *Aerococcaceae*, *Fusobacteriaceae* and *Moraxellaceae* were found to be predominant in jejunum, while only *Pasteurellaceae* was found to be more abundant in ileum (**Supplementary Figure S1B**), which corroborated the redundancy analyses for intestinal location (**Figure 1B**). Remarkably, we observed significant differences in alpha and beta diversity between jejunal and ileal microbiota (**Supplementary**

Figure S2B). Jejunum microbiota was found to have a higher richness (**Figure 1C**) compared to ileum, although there was no difference observed in evenness (**Supplementary Figure S2B**). These findings established prominent differences in microbiota composition in small and large intestinal locations, and underpin the high relatedness between the microbiota of the jejunum and ileum regions of the small intestine, which was also supported by the partial separation of jejunal and ileal samples in hierarchical clustering (**Supplementary Figure S3**).

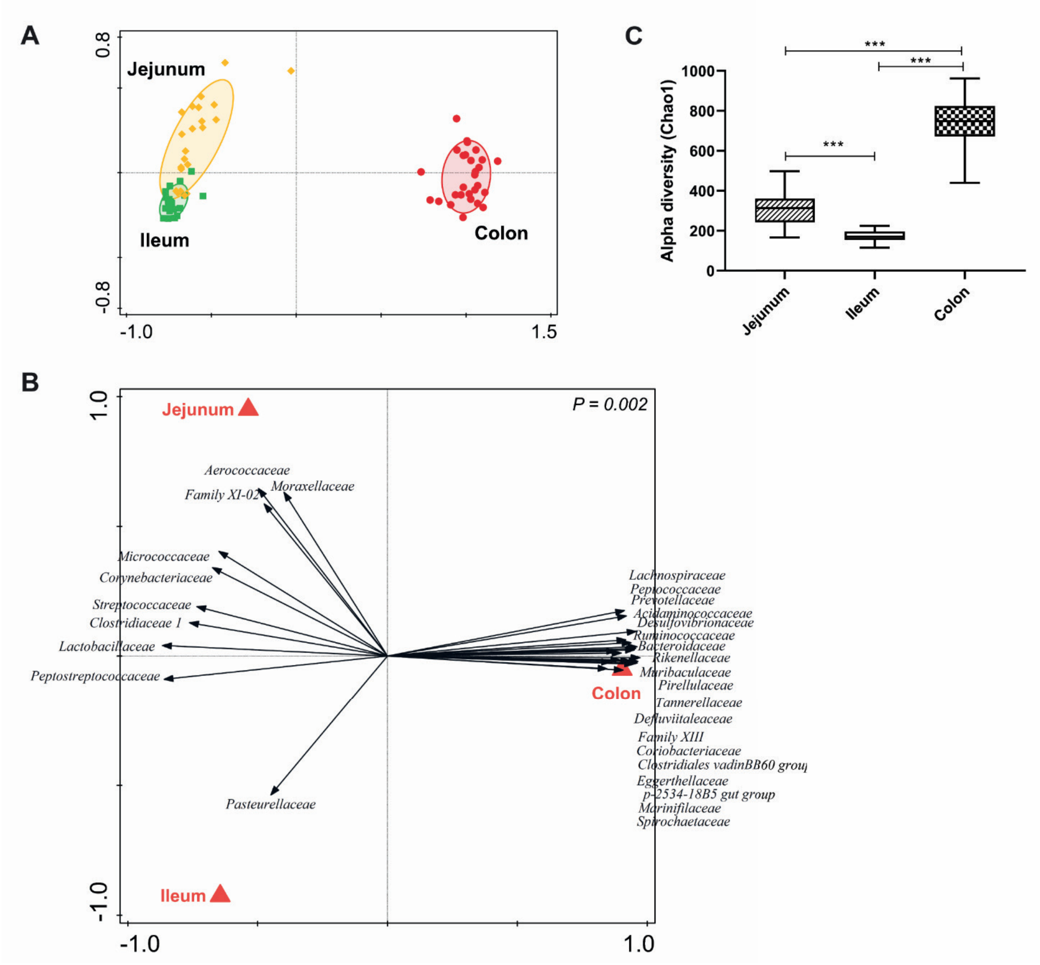


Figure 1. Microbiota composition along the intestinal tract. (**A**) Principal component analysis of jejunum, ileum and colon microbiota (PC1 = 66.66%, PC2 = 4.76%) at genus level. (**B**) Redundancy analysis (explained variation = 67.8%, $P = 0.002$) of intestinal location with associated microbial groups at family level. Microbial groups visualised in this figure have a minimum fit value of at least 40% on the horizontal axis and a response score > 0.63 and > 0.80 for jejunum/ileum and colon, respectively. Specific microbial families which are differentially abundant in jejunum/ileum were enforced in this graph. (**C**) Alpha diversity (Chao1 bias corrected) comparison among jejunum, ileum and colon. Significant differences between groups were assessed by Student's t-test or Mann-Whitney U test (***, $P < 0.001$).

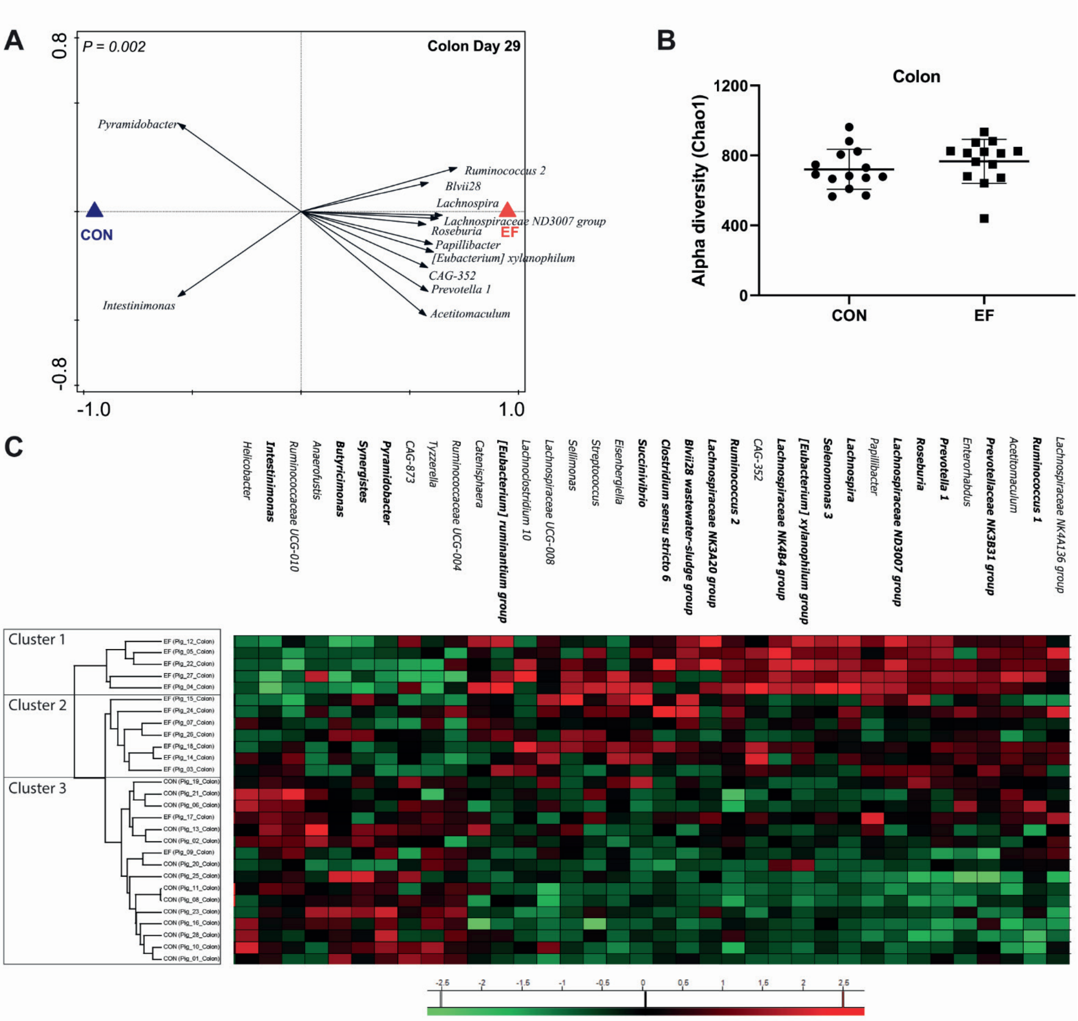


Figure 2. Colonic microbiota composition in the early-fed (EF) and control (CON) group. (**A**) Redundancy analysis at genus level (PC1 = 8.75%, PC2 = 16.12%; $P = 0.002$) with associated microbial groups shown (minimum fit value of at least 30% and > 0.55 response score on horizontal axis). (**B**) Alpha diversity (Chao1 bias corrected) comparison between the two groups. **(C)** Heat map showing relative abundance of discriminative bacterial genera (≥ 0.40 response score in x-axis) as found in redundancy analysis. RDA identified microbes that were also detected in a previous study with similar design (Choudhury, Middelkoop et al., in preparation) are shown in bold.

Effect of early feeding on local intestinal microbiota

One of the main objectives of this study was to gain insight into the impact of early life feeding on jejunal, ileal and colon microbiota. We did not find any impact on small intestinal (jejunal and ileal) microbiome composition or diversity due to early (pre-weaning) feeding of piglets (**Supplementary Figure S4**). In contrast, the colon microbiome was found to be significantly altered due to early feeding, and RDA analyses identified several microbial genera (such as *Ruminococcus2, Lachnospira, Lachnospiraceae ND3007 group, Roseburia, Papillibacter, Eubacterium xylanophilum* and *Prevotella1)* associated with this difference (**Figure 2A**). Notably, some of these microbes (marked in bold; **Figure 2C**) were also detected to be enriched in rectal swab samples taken pre-weaning from early-fed piglets in a previous study (Choudhury, Middelkoop et al., in preparation). Those microbial groups represent typical post-weaning associated microbes, demonstrating that early feeding with a fibrous diet accelerates the 'maturation' of the microbiota towards a post-weaning composition. Due to the absence of post-weaning microbiota samples in this study (piglets sacrificed before weaning), this correlation with the post-weaning microbiota cannot be confirmed, but the association of the same microbial groups with early feeding supports the similarity of microbiota impact. To further evaluate the impact of early feeding on microbiota composition, beta diversity was assessed by Bray curtis distance, which revealed significant dissimilarity between the EF and CON groups (PERMANOVA, $P = 0.04$), although no significant difference was observed in alpha diversity (**Figure 2B**).

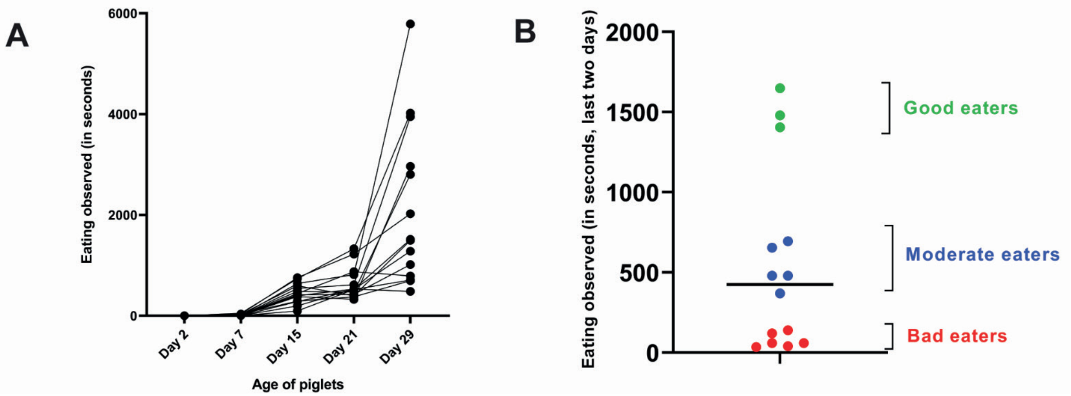
To assess the impact of early feeding at individual animal level, hierarchical clustering of all samples was performed based on EF/CON group-associated microbes (detected in RDA analyses; **Figure 2A**). Partial separation of EF and CON piglets was observed at individual animal level (**Figure 2C**). Overall, the hierarchical clustering divided the piglets into three main clusters: (1) five EF piglets clustering clearly separate, (2) seven EF piglets clustering together but less distant from the next cluster and (3) encompassing all the CON piglets and the remaining two EF piglets. The genera comprising of *Prevotella1, Roseburia, Lachnospiraceae ND3007 group, Lachnospira, Selenomonas3, Roseburia, Eubacterium xylanophilum, CAG-352* and *Ruminococcus2* were found to be most abundant in the first cluster whereas the second cluster had a more variable abundance of these groups. Cluster 3 was characterised by higher abundance of other microbial groups, including *Ruminococcaceae (UGC-004, UGC010), Tyzzerella, CAG-873, Pyramidobacter, Synergistes, Butyricimonas* and *Intestimonas*. Intriguingly, some of these microbes such as *Butyricimonas, Pyramidobacter, Intestimonas* and *Synergistes* were also found associated with the CON group in our previous study (Choudhury, Middelkoop et al., in preparation).

Quantitative estimate of eating behaviour

To estimate the eating time per individual piglet, video recordings of the six EF litters were observed (for 12 hours, 7 days per week) and eating bouts were noted during the four-week pre-weaning period. The eating scores were employed as a quantitative indication of eating. The eating behaviour of the EF piglets gradually increased over time, reaching the highest eating score in the last week pre-weaning (**Figure 3A**), which is similar to our previously reported observations (Choudhury, Middelkoop et al., in preparation). However, the quantification of eating behaviour in the present study was more variable and on average lower as compared to our previously study of a similar design (3204 ± 1810 seconds in the present study versus 8032 ± 3387 seconds in our previous study (Choudhury, Middelkoop et al., in preparation**;**

Supplementary Figure S5). Nevertheless, the substantial variation in the estimated eating among EF piglets in the present study provides the opportunity to evaluate the relation between the individualised quantification of eating behaviour and the piglet-specific microbiome and host physiology.

Previously (Choudhury, Middelkoop et al., in preparation) it was shown that eating scores observed shortly before (rectal swab) microbiota sampling are strongly related to the corresponding microbiota changes observed as a consequence of eating the fibre-enriched feed. Analogously, in this study, we employed the summed eating scores of the last two days prior to sacrifice to investigate their relation with the microbiome signatures observed in individual EF piglets. Based on the eating scores in the last two days, we can classify individual EF piglets into good (> 2 times the median; green), moderate (between 0.8 and 2 times the median; blue) and bad (below 0.8 times the median; red) eaters (**Figure 3B**). The RDA analyses show that these individual eating scores (cumulative eating scores from the 'last two days') could significantly explain the colon microbiota composition (**Figure 3C**). Notably, all periodical eating scores ('Total seconds', 'Last week', 'Last two days' and 'Last day') were significantly and positively correlated with each other (**Supplementary Figure S8**), and were all significantly reflected by the microbiota RDA scores (**Supplementary Figure S8** and **S9**). Moreover, the microbiota composition supported the classification of EF piglets into good, moderate and bad eaters on the basis of discriminatory taxa that were either more abundant in good eaters (e.g. *Lachnospiraceae ND3007 group, Lachnospiraceae NK3A20 group, Kandleria, Eubacterium xylanophilum, Lachnospira, Prevotella1, Faecalibacterium* and *Roseburia*) or more abundant in the bad eaters (e.g. *Odoribacter* and *Butyricimonas*). However, since the overall group of EF piglets in this study is somewhat dominated by bad eaters, significant correlations were observed between only a few individual microbial groups and eating scores (**Figure 3D** and **Supplementary Figure S6**). Nevertheless, the individualised eating behaviour substantiated the partial separation of EF piglets in hierarchical clustering (**Figure 2C**) where some EF piglets (classified as bad-eaters) were clustering closely to the control piglets. Taken together, these findings exemplify the microbiota compositional changes in response to individualised eating behaviour.



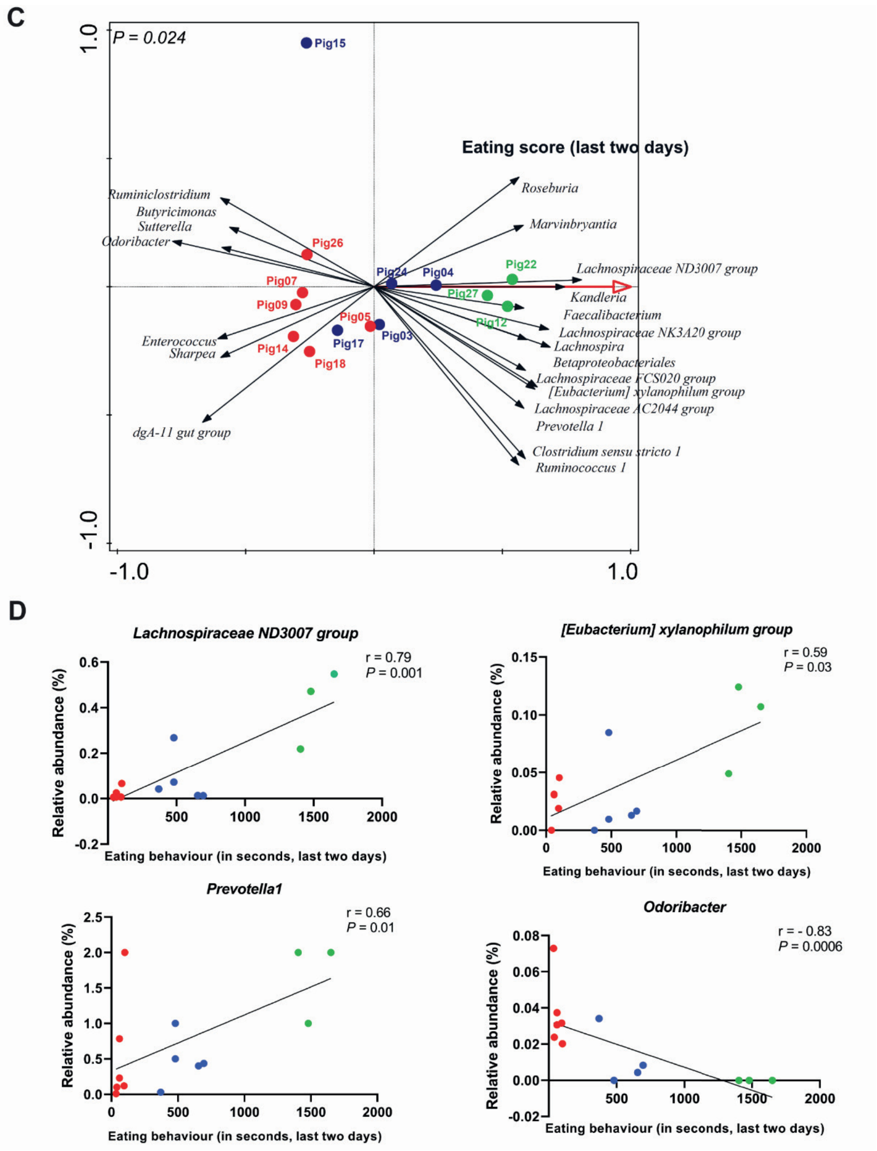


Figure 3. Classification of EF piglets into good (green), moderate (blue) and bad eaters (red). (**A**) Individual piglet's eating behaviour quantification (14 EF piglets; total eating seconds per week) for four weeks pre-weaning by video observation. (**B**) Good (> 2 x median; green), moderate (between 0.8 x and 2 x median; blue) and bad (below 0.8 x median; red) eaters, grouping based on eating observed in the 'last two days' before weaning. (**C**) Redundancy analysis based on eating scores from 'last two days' before weaning (explained variation = 5.42% , *P* = 0.024), establishing the microbiota discrimination between the 'good', 'moderate' and 'bad' classification within the EF piglets (minimum fit value of at least 30% and > 0.55 response score on horizontal axis). (**D**) Spearman correlation of individual microbial genera with the eating score from 'last two days'. *Lachnospiraceae ND3007, Eubacterium xylanophilum, Prevotella1* and *Odoribacter* were identified in redundancy analysis.

Changes in pH and SCFA concentrations

To assess the intestinal fermentation as result of early feeding, the intraluminal pH in different segments of the intestinal tract and SCFA concentrations (i.e. succinic-, lactic-, acetic-, propionic- and butyric acid) in the colon content were determined. The digesta pH of both caecum and colon significantly decreased due to early feeding of piglets ($P < 0.05$), whereas no differences in stomach or small intestinal digesta were detected (**Figure 4A**). Subsequently, the relation between eating scores and digesta pH in individual EF piglets was investigated, revealing that caecum pH tended to be negatively correlated with eating behaviour, whereas no such association was observed for the colon (**Figure 4B).** Nevertheless, the levels of colonic SCFAs were significantly impacted by early feeding, and in comparison to the CON group, concentrations of acetic acid, butyric acid and total SCFAs were significantly higher whereas propionic acid tended to be higher ($P = 0.06$) in the EF group (**Figure 4C**). As anticipated, caecum and colon pH negatively correlated with SCFAs acetic acid, propionic acid and butyric acid (**Supplementary Figure S8**). In contrast, no differences were observed in lactic- and succinic acid between the CON and EF piglets (**Supplementary Figure S7**). Notably, and maybe not unexpected, SCFA (acetic-, propionic- and butyric acid) concentrations in individual piglets were found to be significantly correlating with each other (**Supplementary Figure S8**). However, the distribution of the differential eating classifications (good, moderate and bad eaters) did not appear to be significantly related to the levels of SCFAs observed, although a non-significant but positive correlation was observed between SCFA and eating scores (**Supplementary Figure S8**), which was most apparent for propionic acid that tended to correlate with the 'total seconds' of eating score (**Supplementary Figure S8** and **S9**). Remarkably, a significant correlation was observed between caecal pH and colonic SCFA concentrations (acetic, propionic and butyric acids individually as well as total SCFAs), whereas such generic association was not found within the colon (**Supplementary Figure S8**), where colon pH appeared to be only correlated with colonic butyric acid concentrations.

Digestive organ measurements (macroscopic)

Macroscopic measurements such as digestive organ weight and lengths were determined to check the effect of early feeding on the intestinal development of piglets at weaning **(Table 1)**. EF and CON piglets did not differ in organ weights of the adrenal gland, gallbladder, spleen, liver, stomach and caecum. However, EF piglets tended to have a heavier pancreas ($P = 0.05$) compared to CON piglets. The small intestine ($P = 0.096$) and the total intestinal tract ($P = 0.05$) showed a tendency to be longer in EF piglets, while the weights of the small intestine and the total intestinal tract were higher ($P < 0.05$), both with (full) and without (empty) digesta in EF piglets. Further, the large intestine (caecum plus colon) was significantly longer ($P = 0.046$) and tended to be heavier with digesta ($P = 0.08$).

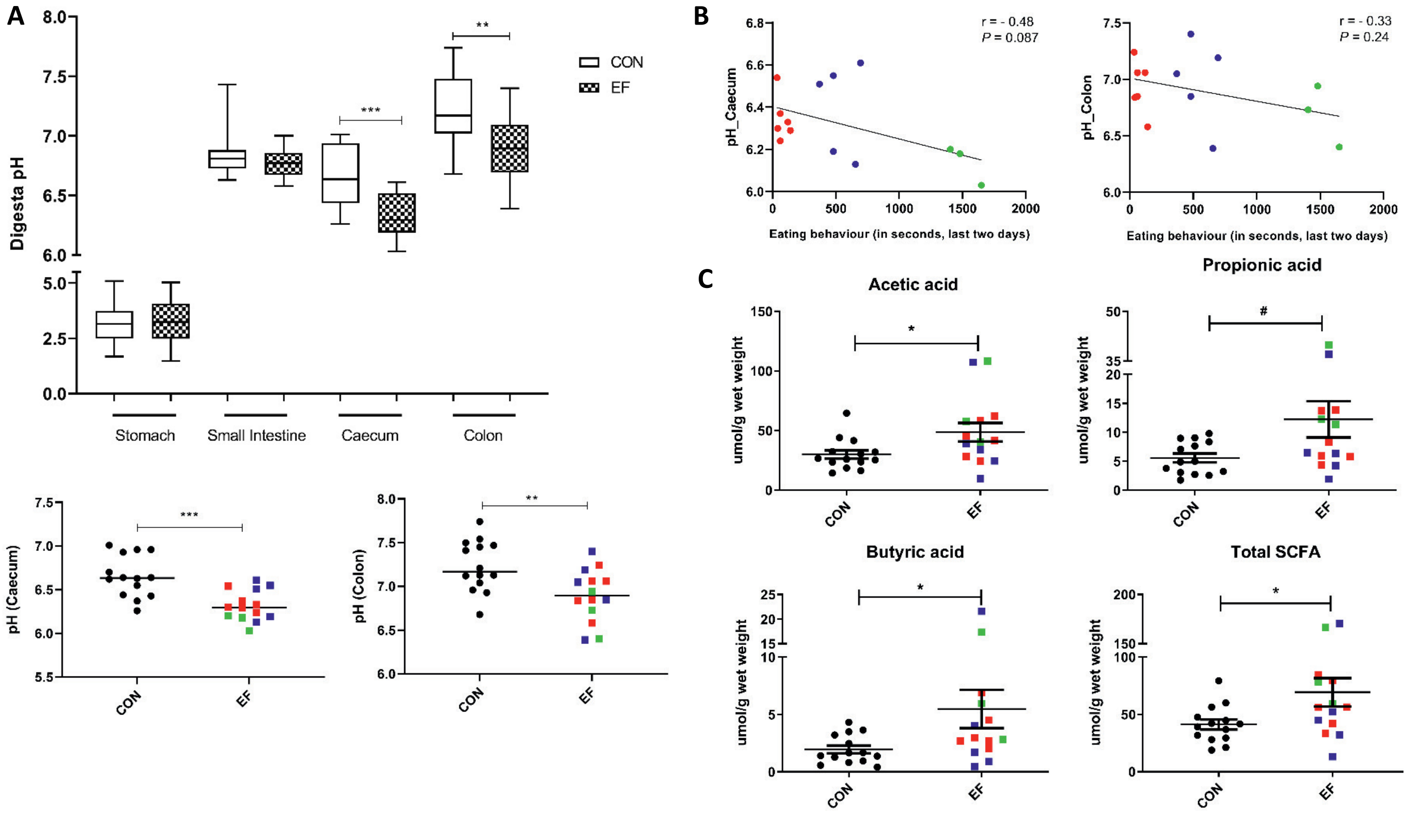


Figure 4. Impact of early feeding on pH and short-chain fatty acid (SCFA) concentrations. (**A**) Digesta pH in different parts of the gastrointestinal tract for EF and CON and an expansion of the two segments where significant differences in pH were detected, illustrating the distribution of individual piglets in both groups (using the classification into good (green), moderate (blue) and bad eaters (red) for the EF group). (**B**) Correlation of pH caecum/colon of individual EF piglets with the quantified eating score from last two days (Caecum: r = - 0.48, *P* = 0.087; Colon: r = - 0.33, *P* = 0.24). (**C**) Group level comparison for colonic SCFA concentration (µmol/g wet weight of digesta) in piglets. Significant differences between groups were assessed by Student's t-test or Mann-Whitney U test (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001). EF = early-fed group; CON = control group.

Table 1. Intestinal weights of early-fed (EF) and control (CON) piglets at weaning (d29). Data are expressed as mean ± SEM and were analysed with linear mixed models using body weight at sacrifice as covariate. Significant *P*-values and trends are presented in bold.

Item	**CON**	**EF**	***P*-value**
Body weight, kg	8.2 ± 0.3	8.5 ± 0.4	0.57
Organ weight, g			
Adrenal gland	0.5 ± 0.02	0.5 ± 0.03	0.70
Pancreas	13.5 ± 0.7	15.5 ± 0.8	**0.05**
Spleen	19.4 ± 1.0	19.4 ± 1.0	0.70
Liver	213.6 ± 7.2	219.4 ± 11.0	0.79
Gallbladder	3.0 ± 0.4	2.5 ± 0.3	0.39
Intestine weight, g			
Stomach, full	146.2 ± 10.7	160.7 ± 11.0	0.47
Stomach, empty	43.2 ± 1.6	44.7 ± 1.7	0.76
Small intestine, full	421.7 ± 17.8	482.5 ± 31.3	**0.02**
Small intestine, empty	328.5 ± 10.5	370.3 ± 20.3	**0.03**
Caecum, full	47.9 ± 2.5	51.0 ± 3.5	0.67
Caecum, empty	18.8 ± 1.1	19.0 ± 0.9	0.89
Colon, full	80.4 ± 4.2	96.8 ± 7.5	**0.08**
Colon, empty	47.2 ± 2.4	50.3 ± 3	0.54
Total gastrointestinal tract, full	696.2 ± 27.3	790.9 ± 45.9	**0.02**
Total gastrointestinal tract, empty	437.7 ± 14.3	484.3 ± 23	**0.03**
Intestine length, m			
Small intestine	7.8 ± 0.2	8.3 ± 0.2	**0.096**
Large intestine[1]	1.2 ± 0.02	1.3 ± 0.03	**0.046**
Total gastrointestinal tract	9.0 ± 0.2	9.6 ± 0.2	**0.05**

[1] Caecum + colon

The intestinal segment lengths showed a significant positive correlation with each other, however, organ and intestinal weights did not show such correlation among each other (**Supplementary Figure S8**). Notably, the weight of the small intestine (without digesta; empty) as well as the whole gastrointestinal tract (with or without digesta; full or empty) significantly correlated ($P = 0.01$; **Supplementary Figure S8** and **S9**) with the eating scores ('total seconds'), whereas small intestine (with digesta; full) tended to show the same correlation ($P = 0.06$; **Supplementary Figure S8**). Taken together, these results indicate that early eating has (moderate) effects on some macroscopic measurements of the digestive system (**Table 1**), but these effects do not appear to significantly correlate with the quantified estimate of eating in all (significantly relevant) parameters.

Mucosal morphometry in jejunum and colon

Intestinal morphometry (microscopic) measurements, such as villus length and crypt depth in jejunum and colon, were assessed using histological staining in a subset of sacrificed piglets (n = 8 per treatment). No significant alteration was observed in villus length and crypt depth of jejunum and in crypt depth of colon in EF compared to CON piglets (**Figure 5**). However, due to a very modest combined increase in jejunal villus length and a decrease in jejunal crypt depth, a tendency for a lower villus length:crypt depth ratio (V:C ratio) was detected in jejunal mucosal tissues obtained from the EF compared to the CON animals (*P* = 0.06, **Figure 5**). Further, no quantitative association was found between these histological microscopic measurements and eating scores (**Supplementary Figure S8)**. However, it should be noted that the selection of the 8 animals that were analysed here does not display an even representation of the eating classification groups recognised in the EF piglets (good, moderate and bad eaters). Only a single piglet of the 'good eater' group appeared to be analysed here and this may have led to an underestimation of the relatedness of early feeding and these mucosal morphometric differences.

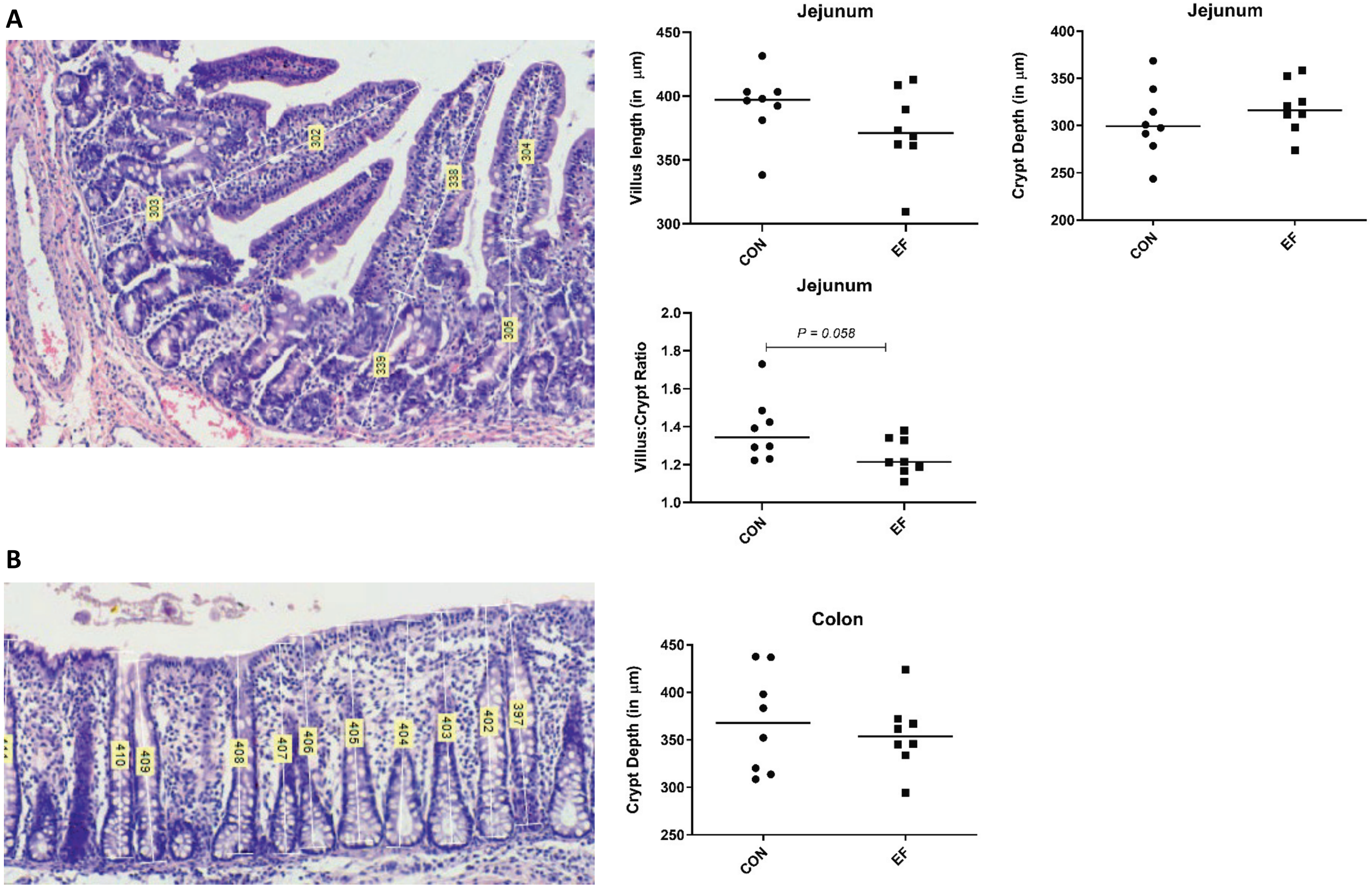


Figure 5. Effects of early feeding on intestinal morphometry at weaning (day 29) in jejunum (**A**) and colon (**B**). EF = early-fed group; CON = control group. Differences between groups were assessed by Student’s t-test or Mann-Whitney U test.

Discussion

Early life conditions are known to have a key influence on the developing gut microbial ecosystem, but also to have long lasting consequences for the microbiota as well as the host (Schokker et al., 2015; Fouhse et al., 2016; Everaert et al., 2017). The present study was aimed to investigate whether early life feeding (pre-weaning access to a mixed-fibre feed) has an impact on the intestinal microbiota composition and digestive system maturation at weaning. The hypothesis was that early feeding (of mixed-fibre feed) would modulate the microbiota composition in the large intestine of piglets and support digestive system development. Our results show that early feeding impacts the intestinal microbiota and its metabolism (SCFAs), as well as digestive system development, determined at both macroscopic and microscopic level. Importantly, the microbiota compositional changes occur in the colon and their magnitude responds proportional to the individual piglet's quantified eating behaviour during the pre-weaning period. In contrast, many of the macro- or microscopic digestive system changes associated with early feeding did not appear to be related with the individualised eating quantification.

Early feeding did not appear to elicit any significant changes in the small intestinal microbiota which was very different in composition and diversity as compared to the colon microbiota that clearly reflected the early eating behaviour. Substantial differences between the microbiota in these different regions are in agreement with earlier studies (Holman et al., 2017; Mu et al., 2017a; Crespo-Piazuelo et al., 2018). Our analyses detected a significant difference in richness (Chao1) between jejunal and ileal microbiota samples. However, previous studies have reported conflicting conclusions related to similar analyses (Kraler et al., 2016; Yang et al., 2016; Holman et al., 2017; Mu et al., 2017a; Crespo-Piazuelo et al., 2018), which may be due to a high dynamics of the small intestinal microbiota that was suggested to be driven by many (nutritional and environmental) factors (Booijink et al., 2010; Zoetendal et al., 2012) that differ within and between studies and thereby intrinsically prohibit consistent conclusions.

The impact of early feeding (of fibrous feed) on the colon microbiota is in accordance with previous studies (Shim et al., 2005; Zhang et al., 2016; Mu et al., 2017b) and corroborate the impact of dietary fibres on the distal regions of the intestine. Various studies have reported the influence of fibres on microbiome, mostly focussing on weaned or growing pigs (Umu et al., 2015, 2018; Kraler et al., 2016; Burbach et al., 2017; Fouhse et al., 2019). In the present study, a customised mixed-fibre feed was formulated especially for suckling piglets, with inclusion of both soluble (GOS, inulin) and insoluble (oat hulls, sugarbeet pulp, resistant starch) dietary fibres. In line with our previously described observations (Choudhury, Middelkoop et al., in preparation), early feeding with this diet elicited higher relative abundances of fibrolytic and/or butyrate-producing bacterial groups, like *Ruminococcus, Lachnospira, Roseburia, Eubacterium,* and *Prevotella*, which reflect the accelerated pre-weaning microbiota development towards a 'post-weaning-resembling' microbial ecosystem.

Consistent with previous studies (Pajor et al., 1991; Delumeau and Meunier-Salaün, 1995; Huting et al., 2019), a large variation in eating behaviour was observed among EF piglets. In addition, in the present study, the eating behaviour of the EF piglets may have been compromised by a diarrhoeic episode during the third week (spreading between day 16-24) in almost all litters, the cause of which is unknown. Bruininx et al. (2004) concluded that there was considerable within-litter variation in creep feed intake, and designated piglets as good, moderate and non-eaters based on the colour of their faeces using chromic oxide as a marker in the feed. Here we used a similar classification system and it should be noted that a relatively large proportion of the dissected piglets was classified as bad eaters (42%), which is substantially more than in our previous study (Choudhury, Middelkoop et al., in preparation) that employed a similar design. The relatively higher degree of variation in feed intake in this study, was exploited to correlate the eating scores with the microbiota changes per individual piglet, demonstrating that the eating time is strongly reflected in the magnitude of the colon microbiota changes. Importantly, this reflection appeared independent of the eating score (time period) employed, supporting the robustness of the observation that the eating time is a key driver of the microbiota adaptation. This is in good agreement with our previous study (Choudhury, Middelkoop et al., in preparation) where it was shown that the microbiota analysed in rectal swabs at different stages during the pre-weaning period accurately reflects the eating behaviour quantification in piglets.

It is well established that dietary fibre reaches the large intestine, escaping the digestion by host endogenous enzymes in the proximal (small) intestine, and is subsequently fermented by the colonic microflora into SCFAs (Jha et al., 2019). Prior studies have shown elevated levels of SCFAs after fibrous diet interventions in piglets, which was associated with lower pH values (Alizadeh et al., 2016; Van Hees et al., 2019). In our study, EF piglets had higher levels of colonic SCFAs such as acetic, propionic and butyric acid as well as a lower pH in caecum and colon. Similarly, inulin supplementation given to suckling piglets (Li et al., 2018) was reported to increase propionic acid, isobutyric acid and total SCFA concentrations in caecum/colon digesta. Notably, although eating time tended to quantitatively correlate with caecal pH in our study, it did not associate with colonic pH or colonic SCFA levels. A possible explanation for this might be that luminal SCFAs production and their absorption by the colonic epithelium is highly dynamic (Von Engelhardt et al., 1989), whereby colonic SCFA levels poorly represent the flux of SCFA production and absorption (Verbeke et al., 2015). Thereby, the caecum, where the fermentative capacity of the microbiota is considered to be at its maximum (Cummings et al., 1987), and where mucosal absorption rates of the luminal SCFAs may be lower as compared to the colon, might have provided a more appropriate intestinal region to assess SCFA concentrations changes in relation to dietary intake, compared to the colon.

Increased weight of digestive organs, including the weight of the intestine itself as well as its length have been associated with solid feed consumption (Jørgensen et al., 1996; Al Masri et al., 2015; Van Hees et al., 2019). Our study reached related conclusions, where early feeding (with mixed-fibre feed) was associated with (relatively small yet significant) changes in macroscopic digestive organ measurements, including a heavier small intestine (empty and full) and complete gastrointestinal tract (empty and full), as well as an extended large intestine and complete gastrointestinal tract length. However, only a few of these changes appeared to have an association with the quantified feed intake estimates within the early-feeding group, although the relatively small number of animals may have obscured such relationships, and to definitely disqualify them would require the analysis of a larger amount of EF animals. For example, eating behaviour was associated with the weights of the small intestine and total gastrointestinal tract (with or without digesta). Increase in gut fill may be due to the typical water-retention activity and 'bulking agent' capacities of the (insoluble) fibres that were present in the pre-weaning diet. These results suggest that early feeding of a fibre-enriched feed stimulates expansion of the intestinal size as well as digesta. This notion was further supported by a significant positive correlation between the changes in microbiota (microbiota scores RDA), colonic SCFA concentrations and the weight of the small intestine, colon and gastrointestinal tract with or without its digesta, indicating their interrelatedness. Intriguingly, some measurements significantly correlated, for example SCFAs were associated with a few macroscopic measurements, such as weight of gall bladder and colon (with digesta). However, the correlations were mainly drawn by two EF (good and moderate) piglets with extreme values compared to other piglets, which is most likely a chance event in the intestinal dynamics of SCFA absorption and production. This probably indicates less reliable biological conclusions derived from correlation plots pulled by a few individuals only.

Presence of luminal nutrients in the gut can cause changes to the structure and function of the intestinal mucosa (Diamond and Karasov, 1983; Pluske et al., 2018). Feed intake has been positively associated with adaptation of the mucosal architecture, i.e. increased villus length or V:C ratio, which has in particular been reported in relation to post-weaning intestinal adaptations (Kelly et al., 1991; Pluske et al., 1996; Dong and Pluske, 2007). However, these effects have not been unambiguously established in the literature, and include contradicting inferences. For example, Bruininx et al. (2004) reported that morphometric measurements (villus length, crypt depth, V:C ratio) were not affected by pre-weaning (commercial) creep feed consumption. On the other hand, a recent study (Alizadeh et al., 2016) reported morphological changes in the intestinal mucosa (thicker and extended villi in the jejunum) of piglets that were separated from their mother after 48 hours and fed with milk replacer, either with or without 0.8% galacto-oligosaccharides, for 26 days. In the present study, we detected moderate changes in the mucosal architecture, which were only apparent in jejunal V:C ratio of early-fed piglets, that is in line with another study (Muns and Magowan, 2018) showing a lower V:C ratio one week after weaning in creep-fed piglets. Remarkably, decreased villus length, increased crypt depth and the corresponding decrease in V:C ratio have been suggested to be indicative of intestinal mucosa maturation in unweaned piglets (Hampson, 1986; Al Masri et al., 2015). Importantly, post-weaning maturation of the intestinal mucosal architecture were observed in a separate study (Choudhury, Middelkoop et al., in preparation), which would imply that the observations made in the present study are indicative for an ‘accelerated maturation’ of the mucosal architecture in EF piglets.

Conclusion

This study illustrates that early feeding with fibre-enriched feed influences the colonic microbiota composition, increases microbial fermentation products in the colon and stimulates intestinal development at weaning. Importantly, the EF-associated changes in colonic microbial signatures were concluded to be strongly driven by the amount of eating, which corresponded with an inferred increased intestinal weight with or without digesta and reduced pH of caecum and colon. Although the estimated feed intake was relatively low and highly variable among EF piglets in this study, eating behaviour quantification and the classification of individual piglets into good, moderate and bad eaters enabled a reliable and consistent evaluation of eating behaviour consequences in piglets at an individual level.

Acknowledgements

This study is part of research programme ‘Genetics, nutrition and health of agricultural animals’ with project number 868.15.010 and is financed by the Netherlands Organization for Scientific Research, Cargill Animal Nutrition and Coppens Diervoeding. The authors thank the technicians and students of the Host-Microbe Interactomics Group and Adaptation Physiology Group (Wageningen University & Research). Special thanks to Pieter Roskam, Soumya Kanti Kar, Sandra Vreman, Anja Taverne-Thiele, Bjorge Laurenssen and Ilona van den Anker-Hensen for assisting in the dissection procedure of the piglets and to Jaqueline Gama de Souza for the microscopic intestinal measurements. We would like to acknowledge the personnel and trainees of the animal research facilities in Wageningen (Carus) for taking care of the animals and for their technical assistance. The authors are grateful to Tamme Zandstra for producing the feed and to FrieslandCampina DOMO for provision of Vivinal GOS. We also want to thank Prof. Henk Schols and Christiane Rösch (Food Chemistry group, Wageningen University & Research) for assisting in analysing the SCFAs in in colon digesta samples.

Supplementary data

Supplementary Table S1. Nutrient profile of the mixed-fibre feed[1].

Calculated nutrient composition[2]	Feed
Dry matter	891
Starch	290
Non-starch polysaccharides[3]	261
Crude protein	195
Crude fat	61
Crude fibre	44
Crude ash	57
Calcium	9.1
Phosphorus	6.1
Sodium	2.2
Standardized ileal digestible lysine	11.9
Standardized ileal digestible methionine	4.8
Standardized ileal digestible threonine	7.1
Standardized ileal digestible tryptophan	2.4
Net energy	11.8

[1] The feed was mixed by Research Diet Services (Wijk bij Duurstede, the Netherlands) and extruded using a co-rotating double screw extruder (M.P.F. 50, Baker Perkins, Peterborough, UK)

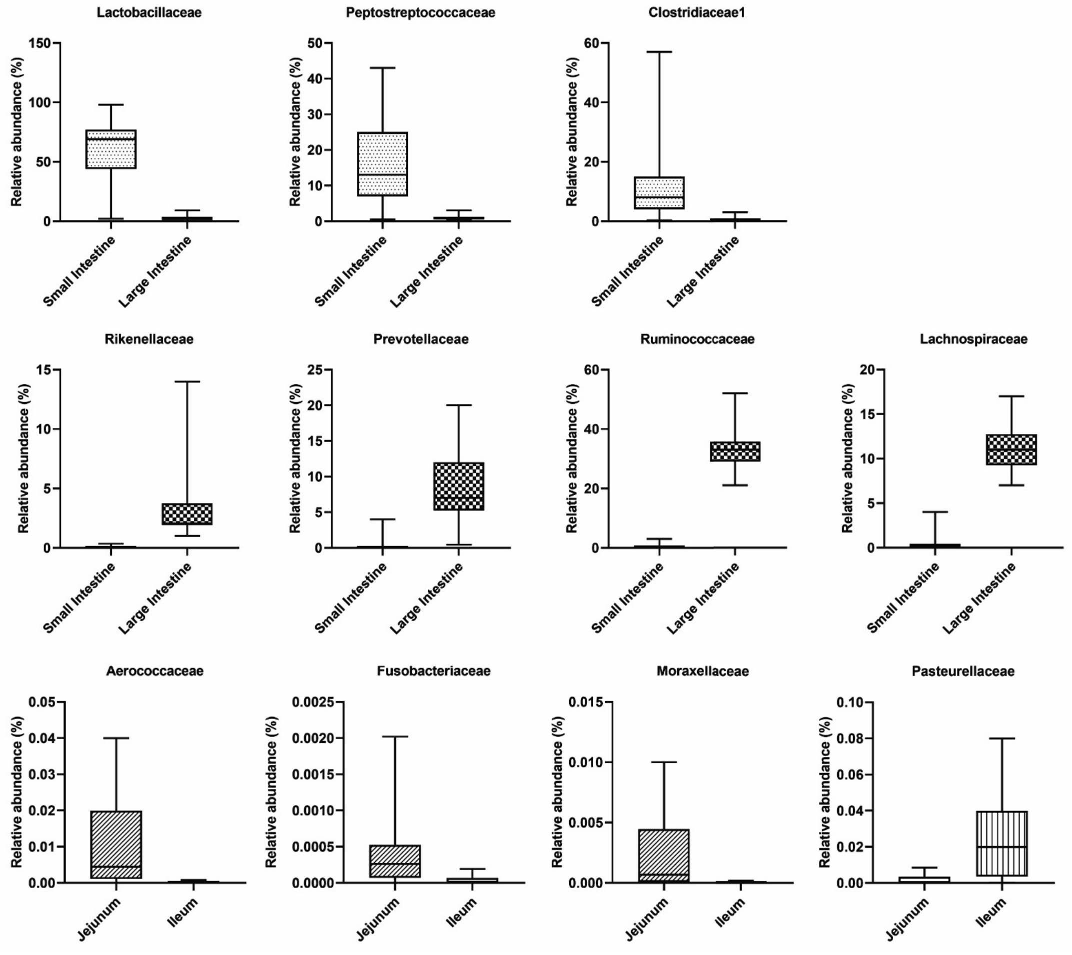
[2] According to CVB (2007). Nutrients are presented in g/kg dry matter, except for dry matter (g/kg) and net energy (MJ/kg)

[3] Calculated as the difference between dry matter and the sum of starch, sugars, crude protein, crude fat and crude ash

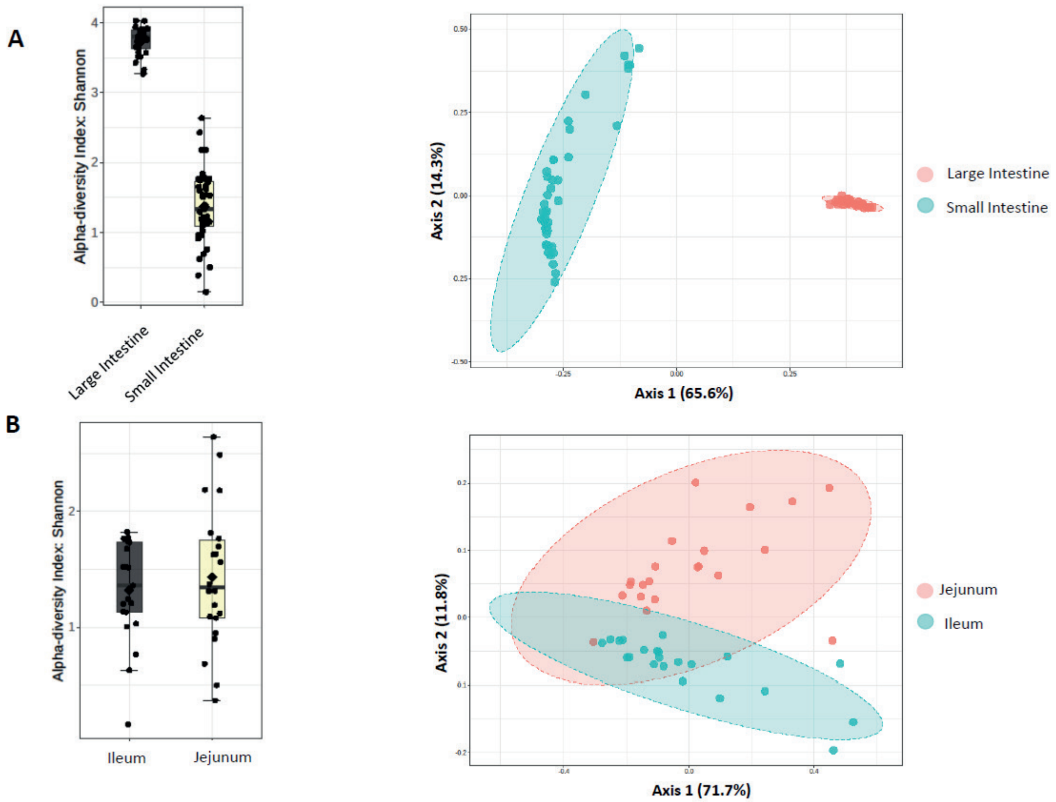
Supplementary Table S2. Ingredient composition of the mixed-fibre feed.

Ingredient component	%
Wheat	21.9
Barley	15
Maize	15
Soy protein concentrate	7
Soybeans (heat treated)	5
Galacto-oligosaccharides	5
Potato protein	4
Sugarbeet pulp (dehydrated)	4
Oat hulls	4
Inulin	4
Resistant starch (± 75% amylose)	4
Soybean oil	3
Blood meal (spray dried)	2
Dicalcium phosphate	1.7
Sucrose	1.5
Calcium carbonate	1.0
Sodium chloride	0.5
Premix[1]	0.5
Potassium bicarbonate	0.3
L-lysine hydrochloride	0.3
DL-methionine	0.2
L-threonine	0.04
L-tryptophan	0.04
Total	100

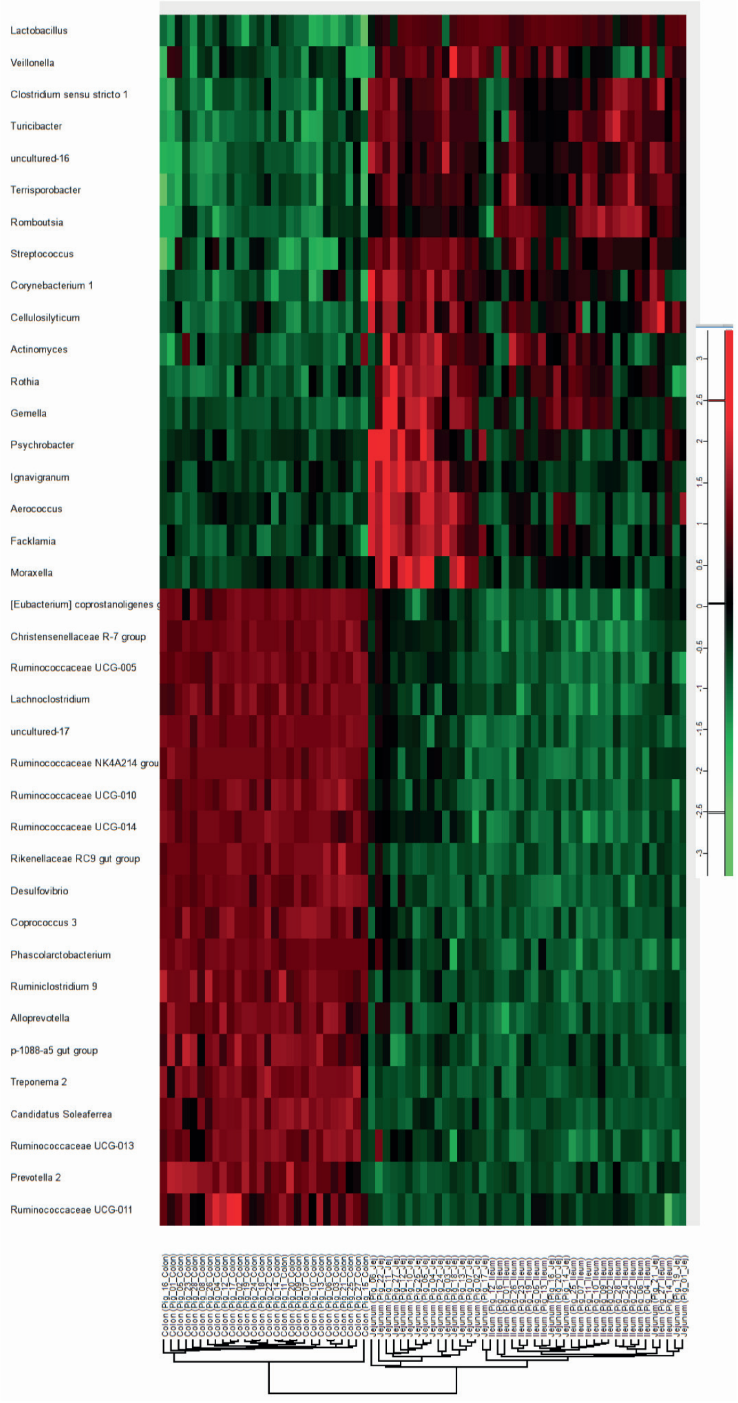
[1] Vitamin and mineral premix (per kg of feed): vitamin A: 10000 IU, vitamin D3: 2000 IU, vitamin E: 40 mg, vitamin K: 1.5 mg, vitamin B1: 1 mg, vitamin B2: 4 mg, vitamin B6: 1.5 mg, vitamin B12: 0.02 mg, niacin: 30 mg, D-pantothenic acid: 15 mg, choline chloride: 150 mg, folate: 0.4 mg, biotin: 0.05 mg, iron: 100 mg, copper: 20 mg, manganese: 30 mg, zinc: 70 mg, iodine: 0.7 mg, selenium: 0.25 mg, anti-oxidant: 125 mg



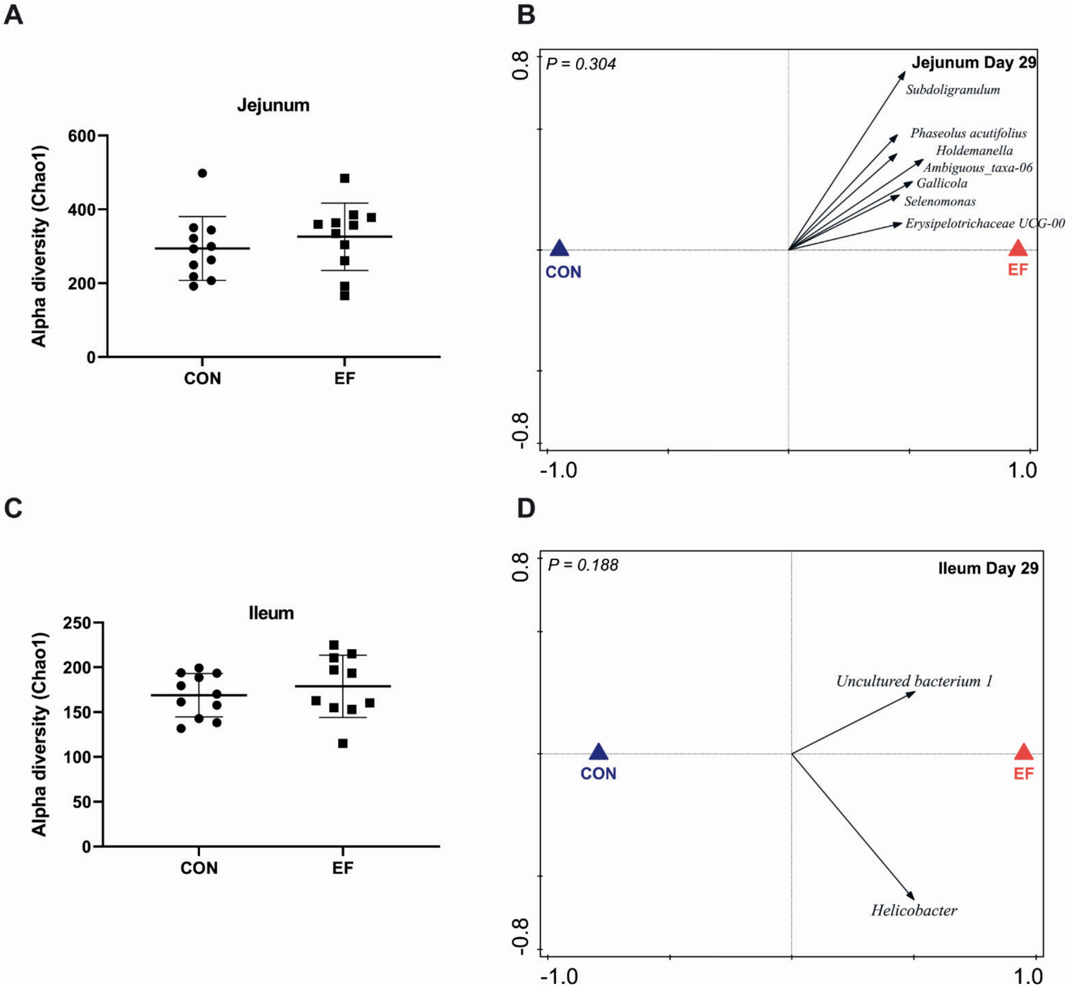
Supplementary Figure S1. Box plots of the relative abundance of microbial families at different intestinal locations. (**A**) Comparing small and large intestine. (**B**) Comparing jejunum and ileum. Representative families which have significant differential relative abundance (false-discovery rate < 0.0001) are shown in this figure.



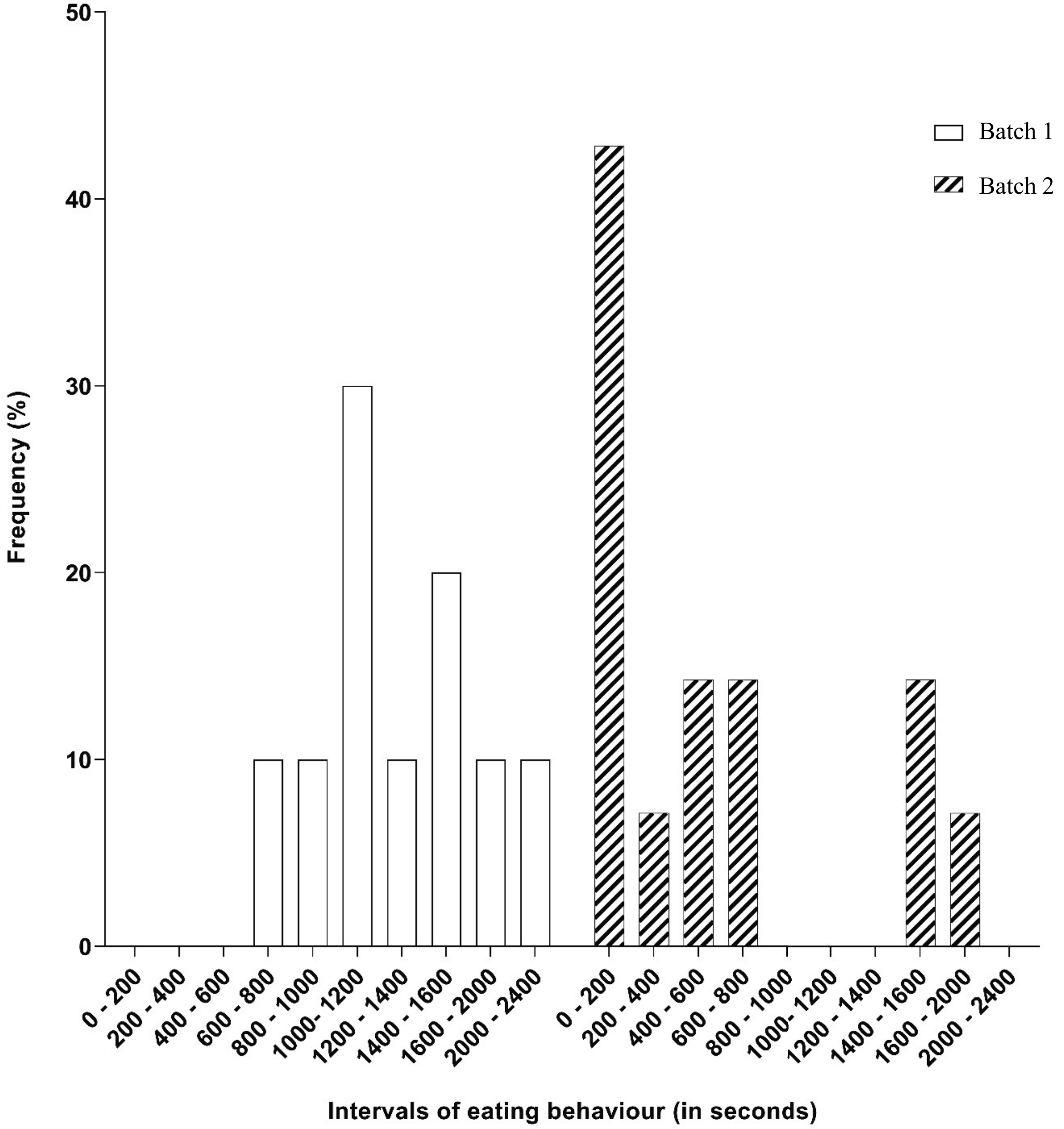
Supplementary Figure S2. Comparison of diversity metrics among different intestinal locations at genus level. (**A**) Comparison between small and large intestine using alpha diversity (Shannon: P =8.1322e-36) and beta diversity (PERMANOVA of Bray Curtis distance as depicted in PCoA 2D plot; $P < 0.001$). (**B**) Comparison between small intestinal locations jejunum and ileum using alpha diversity metrics (Chao1: P = 8.1931e-071; Shannon: $P = 0.89$) and beta diversity (PERMANOVA of Bray Curtis distance as depicted in PCoA 2D plot; $P < 0.002$). All diversity comparisons were performed after rarefying the reads to minimum library size.



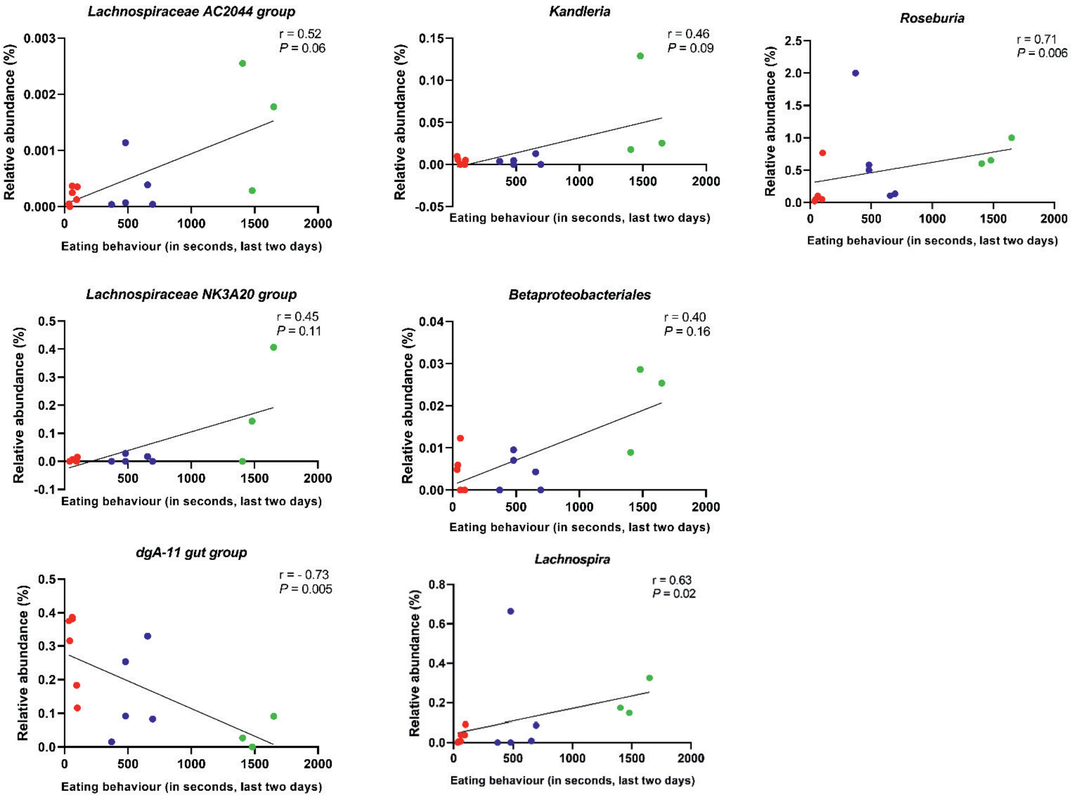
Supplementary Figure S3. Heat map (of individual animals) showing relative abundance of discriminative bacterial groups as identified in redundancy analysis as a function of 'intestinal location' at genus level.



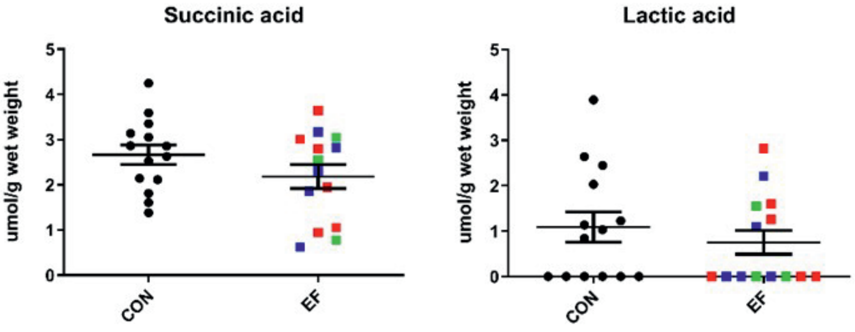
Supplementary Figure S4. Jejunal and ileal microbiota composition in the early-fed (EF) and control (CON) group. (**A, C**) Alpha diversity (Chao1 bias corrected) comparison between the two groups in jejunum and ileum. (**B, D**) Redundancy analysis at genus level in jejunum (Explained variation = 0.65%; PC1 = 5.38%, PC2 = 27.85%; *P* = 0.304) and ileum (Explained variation = 1.27%; PC1 = 5.97%, PC2 = 18.36%; *P* = 0.188) with associated microbial groups shown (20% minimum fit on horizontal axis). EF = early-fed group; CON = control group.



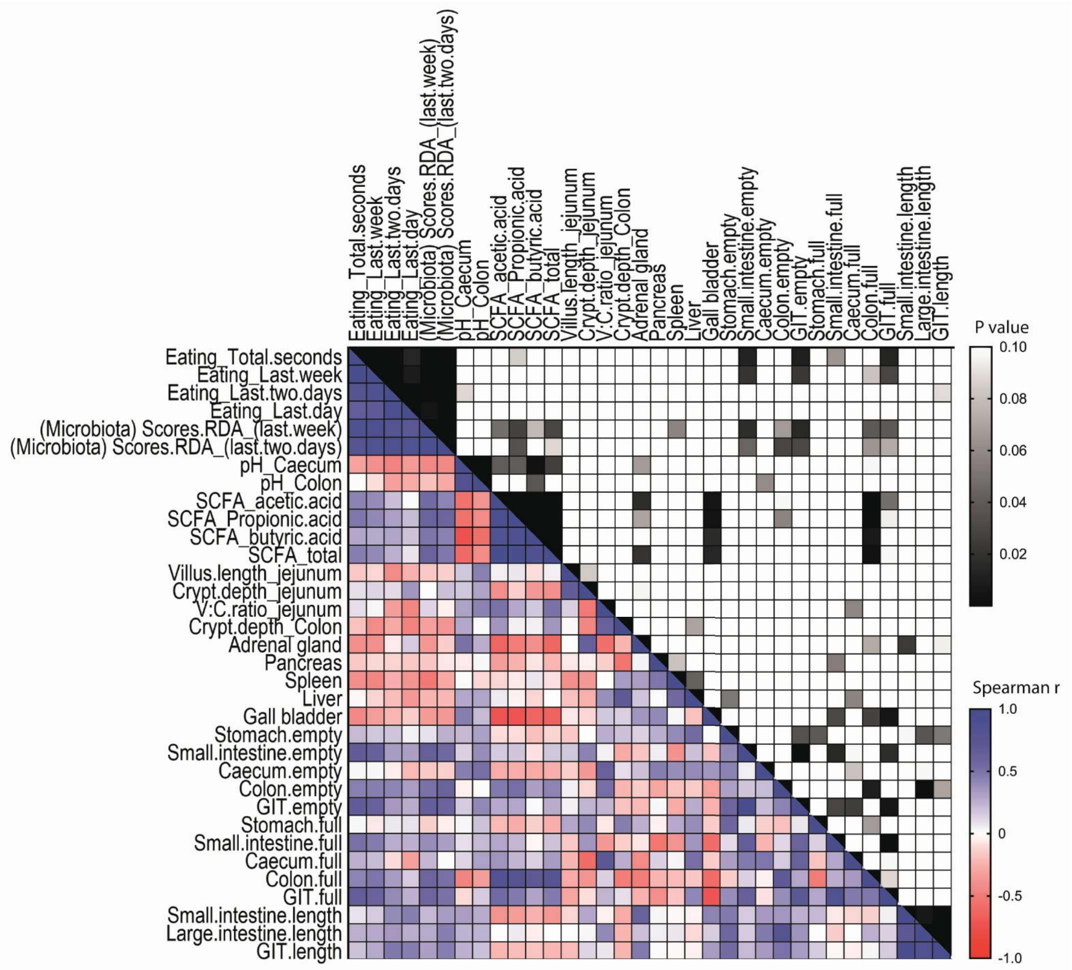
Supplementary Figure S5. Frequency of eating scores in the last two days before weaning, comparing two batches (Batch 1 = Choudhury, Middelkoop et al., in preparation; Batch 2 = this chapter) with respect to eating behaviour. Frequency distribution of early-fed piglets at various intervals (200 seconds considered per interval) of eating behaviour.



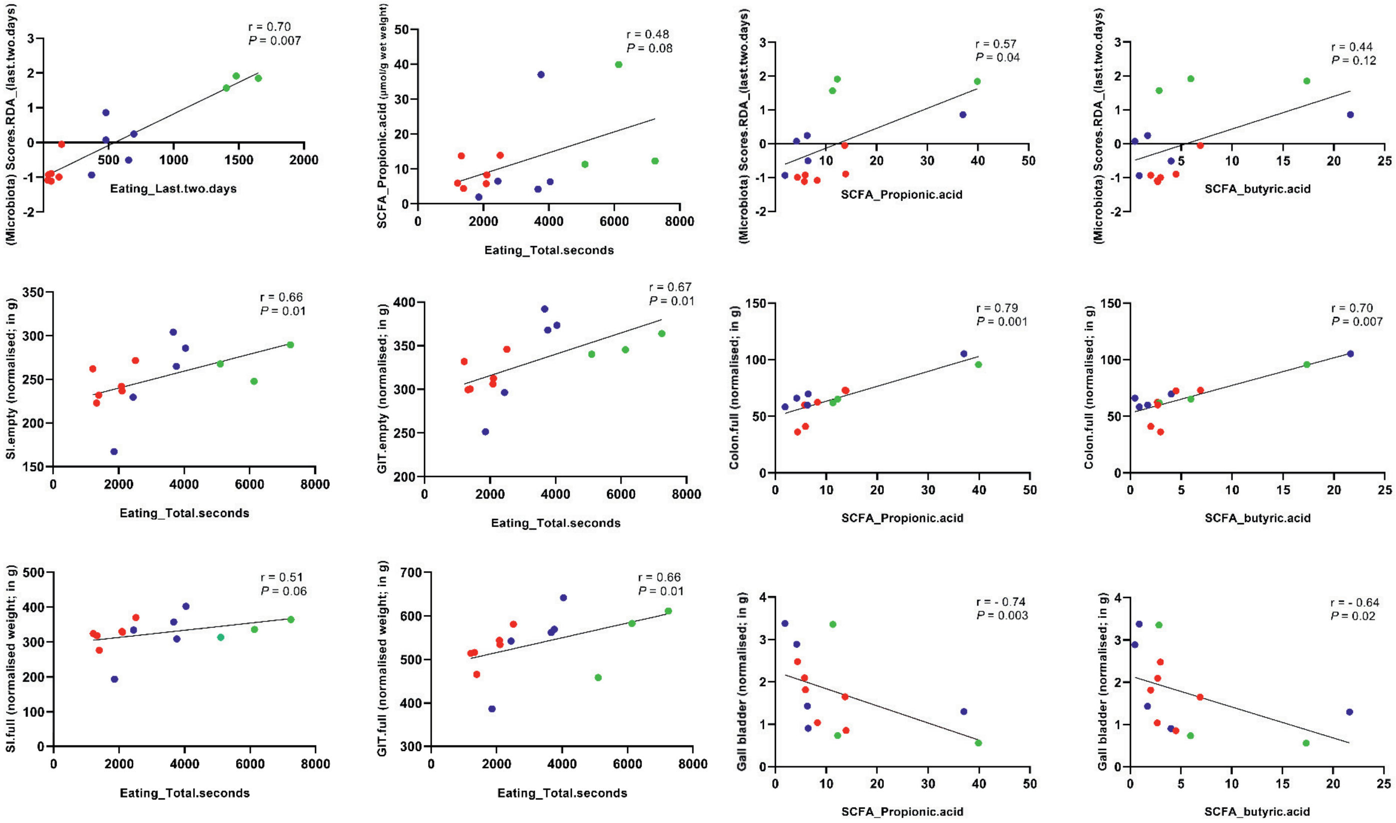
Supplementary Figure S6. Correlation of individual microbial genera with the ‘classified’ eating scores per individual during the last two days before weaning identified in redundancy analysis. The early-fed piglets are classified into good (green), moderate (blue) and bad eaters (red) based on eating time observed in the ‘last two days’ before weaning.



Supplementary Figure S7. Concentrations of succinic acid and lactic acid in CON and EF piglets. The EF piglets are classified into good (green), moderate (blue) and bad eaters (red) based on eating time observed in the ‘last two days’ before weaning. EF = early-fed group; CON = control group.

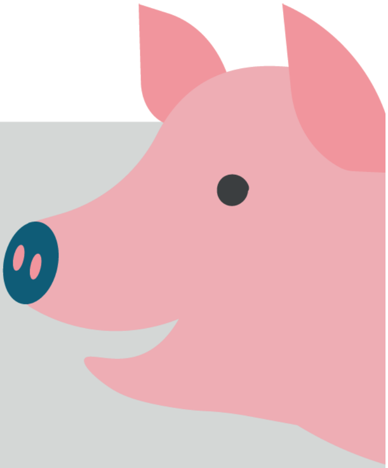


Supplementary Figure S8. Correlation matrix (spearman) of different measured parameters of individual early-fed piglets including eating scores, microbiome RDA scores, pH, SCFA, intestinal morphometry and intestinal macroscopic measurements.



Supplementary Figure S9. Some parameters identified in the correlation matrix visualised in separate (spearman) correlation plots. The early-fed piglets are classified into good (green), moderate (blue) and bad eaters (red) based on eating time observed in the 'last two days' before weaning. The piglets classified as good eaters are also the best eaters when 'total seconds' are considered, but the classification in moderate/bad eaters is less reliable, with two moderate piglets categorised as bad eaters based on 'total seconds'.

CHAPTER 4



The influence of environmental enrichment on early feed intake in pigs

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Abstract

Environmental enrichment may enhance feed intake before weaning and in stressful situations, hence we studied effects of environmental enrichment on the feed intake of piglets before and after weaning. Piglets were reared in an enriched or barren environment from birth (n = 15 litters/treatment before weaning and n = 16 pens/treatment after weaning). The enriched environment contained foraging materials on the floor, and extra space and alternating chew objects compared to the barren environment. Piglets were provided with solid feed from 7 days of age. Pre-weaning feeding behaviour was observed at 22 days of age and feed intake was measured between d7-22, d22-28 and d28-30. At 30 days of age, piglets were weaned and mixed within treatment into groups of 6 unfamiliar piglets. Feed intake was determined between d0-5 and d5-19 after weaning. Time spent eating and the number of eaters, which were determined at 22 days of age only, did not differ between enriched and barren-housed piglets. The intake of feed before weaning tended to be greater as result of environmental enrichment (enriched: 418 ± 119 vs. barren: 274 ± 93 g/piglet, $P < 0.10$) and enriched-housed piglets ate significantly more feed during the last two days prior to weaning (enriched: 174 ± 31 vs. barren: 90 ± 25 g/piglet, $P = 0.03$). After weaning, enriched-housed piglets had a higher feed intake than barren-housed piglets, between d0-5 (enriched: 342 ± 21 vs. barren: 280 ± 15 g/piglet/d, $P < 0.001$), between d5-19 (enriched: 707 ± 15 vs. barren: 650 ± 20 g/piglet/d, $P = 0.02$) as well as in total (611 ± 10 vs. 553 ± 14 g/piglet/d, $P < 0.01$). To conclude, environmental enrichment stimulated feed intake in piglets before and after weaning, implying provision of enrichment is recommended to familiarise piglets with solid feed and to ease the weaning transition.

Introduction

At weaning, piglets are separated from their mother, and additionally often moved to a new pen, mixed with unfamiliar piglets and fed a novel diet, which all result in stress. Environmental enrichment from a few days of age improves piglet adaptation after weaning, shown by reduced levels of stress (Yang et al., 2018) and weaning-stress-induced behaviours (Bolhuis et al., 2005; Van Dixhoorn et al., 2016; Luo, Reimert, Middelkoop et al., under revision), less body damage (Telkänranta et al., 2014) and improved post-weaning feed intake (Munsterhjelm et al., 2009; Oostindjer et al., 2010; Brajon et al., 2017) and growth (Brajon et al., 2017). One of the underlying factors of the success of environmental enrichment on post-weaning adaptation may be a higher intake of solid feed prior to weaning, as a higher feed intake before weaning has been shown to correlate with a higher feed intake after weaning (Berkeveld et al., 2007b; Langendijk et al., 2007). Previous research has determined that creep feed intake can be increased by encouraging exploration (Kuller et al., 2010; Adeleye et al., 2014; Van den Brand et al., 2014). However, piglets reared in barren environments have limited possibilities to explore the environment. Increasing exploratory behaviour in piglets by providing enrichment may therefore stimulate creep feed intake. Munsterhjelm et al. (2009) reported that enriched-housed piglets consumed more solid feed, that was provided from 2 weeks of age, prior to weaning at 29.5 days of age compared to barren-housed piglets, however enriched-housed piglets were also weaned at a significantly higher age (2.6 days older) and reared in a significantly larger litter of one piglet more. Both factors may stimulate feed intake, as pre-weaning feed intake increases exponentially with age (Huting et al., 2017) and is partly driven by a low milk intake (Middelkoop et al., 2019a). Weaning age and litter size can therefore have confounded the results presented in Munsterhjelm et al. (2009). In addition, Oostindjer et al. (2010), showed that enriched-housed piglets did not differ from barren-housed piglets in pre-weaning feed intake, but feed intake was only reported in the last week before weaning at 29 days of age. The present study therefore aimed to investigate the effects of environmental enrichment on the percentage of eaters and the pre- and post-weaning feed intake of pigs. We hypothesized that enriched-housed litters would display a larger number of eaters and a larger feed intake before and after weaning by stimulating exploration of the environment.

Methods

The protocol of the experiment was approved by the Animal Care and Use committee of Wageningen University & Research (Wageningen, the Netherlands) and in accordance with the Dutch law on animal experimentation, which complies with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

Animals and management

The study was set up as a two-group design (see below for housing treatment). The study included a total of 30 sows (Topigs-20) and their litters (Tempo x Topigs-20), which were studied in two equally divided batches. These are the same animals as described by Luo et al. (2019a, 2019b), which studied different aspects of the behaviour of these pigs. Pregnant multiparous sows (range parity: 2-5) were housed at research facility Carus (Wageningen University & Research, the Netherlands) from one month before farrowing and were moved to individual farrowing pens from one week before farrowing. Distribution of sows over the housing treatment for their litters (see below) was balanced for parity (3.6 ± 0.2) and sow body weight and back fat thickness. The farrowing pen (8.6 m^2) was equipped with a farrowing area and a free-movement area (1.85 x 1.80 m). The sows were only crated from shortly before farrowing until the first four days post-partum to minimize piglet crushing. The farrowing area was equipped with a crate for the sow (2.85 x 0.60 m), including a feed trough, a drinking nipple, a jute sack (around farrowing) and a chew object. Sows were fed a standard commercial diet (AgruniekRijnvallei, Wageningen, the Netherlands) twice a day.

The farrowing area included a heating lamp, drinking nipple and feeder for the piglets. Piglets could not access the free-movement area in the first four days post-partum to minimize hypothermia. Within 24 hours after birth, piglets received an ear tag and intramuscular iron injection and their birth weight and sex were determined. No teeth clipping, tail docking or castration were performed. Piglets were cross-fostered within treatment if litter sizes were larger than 14. From 7 days of age, solid feed was provided to the piglets in a semicircular stainless steel piglet feeder of 2.9 L with four feeding places (MS Schippers, Bladel, the Netherlands). Creep feed ('Prestarter Speen Select', AgruniekRijnvallei, Wageningen, the Netherlands) was provided from d7 until d24 after birth, and the creep feed was mixed with the weaner diet ('Speen Uniek', AgruniekRijnvallei, Wageningen, the Netherlands) from d25 until weaning at d30. Birth weight (enriched: 1.48 ± 0.05 vs. barren: 1.51 ± 0.05 kg), litter size at d7 (enriched: 12.7 ± 0.2 vs. barren: 12.6 ± 0.4 piglets/litter), litter size at weaning (enriched: 12.4 ± 0.2 vs. barren: 12.1 ± 0.3 piglets/litter) and weaning age (enriched: 29.7 ± 0.4 vs. barren: 29.6 ± 0.4 days of age) did not differ between treatments (see below).

At 30 days of age, in total 192 piglets (96 per batch) from 30 litters were selected and regrouped in 32 new pens containing 6 non-littermate piglets each. The composition of each new group was balanced for sex (3 males and 3 females), coping style (3 high- and 3 low-resisters as determined by a backtest at 2 weeks of age) and body weight at 28 days of age (see Luo et al., 2019b for details). All weaner pens had a heating lamp, drinking trough and a round feeder with one feeding place (diameter of 30 cm), in which a standard commercial diet was fed *ad libitum* (d0-10 post-weaning: 'Speen Uniek' and d11-19 post-weaning: 'Babybiggen Uniek', AgruniekRijnvallei, Wageningen, the Netherlands).

Piglets were housed in three rooms per batch before weaning and two rooms per batch after weaning. Distribution of treatment groups over the rooms was balanced. In the farrowing rooms, the room temperature was 25 °C around farrowing, and gradually decreased to 21 °C over the course of two weeks. In the weaner rooms, the room temperature was 25 °C at weaning and gradually decreased to 21 °C. Lights and radio were on from 7:00 until 19:00 h. Pens were cleaned daily.

Housing treatment

From birth until weaning, half of the litters were housed in barren farrowing pens (8.6 m^2 as previously described), with a solid floor and a small slatted dunging area, and two fixed chew objects, one metal chain with ball and one metal chain with bolts, that were only accessible by the piglets.

The other half of litters were housed in enriched farrowing pens with a total pen size of 17.1 m^2. A fixed chew object (i.e. a metal chain with bolts) and four chew objects that alternated daily (a squeaky ball or a metal chain with either a ball, PVC pipe or hose attached) were provided to enriched-housed piglets in the farrowing area. These chew objects were also only accessible by the piglets. Enriched-housed piglets had, apart from the 8.6 m^2 farrowing area as described for barren farrowing pens, also access to an enriched area with 1.7 kg straw, 300 L of sawdust, and 270 L of peat on the floor. In addition, 0.8 kg of fresh straw and 40 L of fresh sawdust were added daily, and 30 L of fresh peat was added weekly in the enriched area of the pen. Besides, an extra heating lamp was provided during the first week of life and an extra drinking nipple during lactation. The enriched and barren farrowing pens are depicted in **Figure 1**. The enriched area of the pen was only accessible by the piglets, not by the sows of enriched-housed piglets.

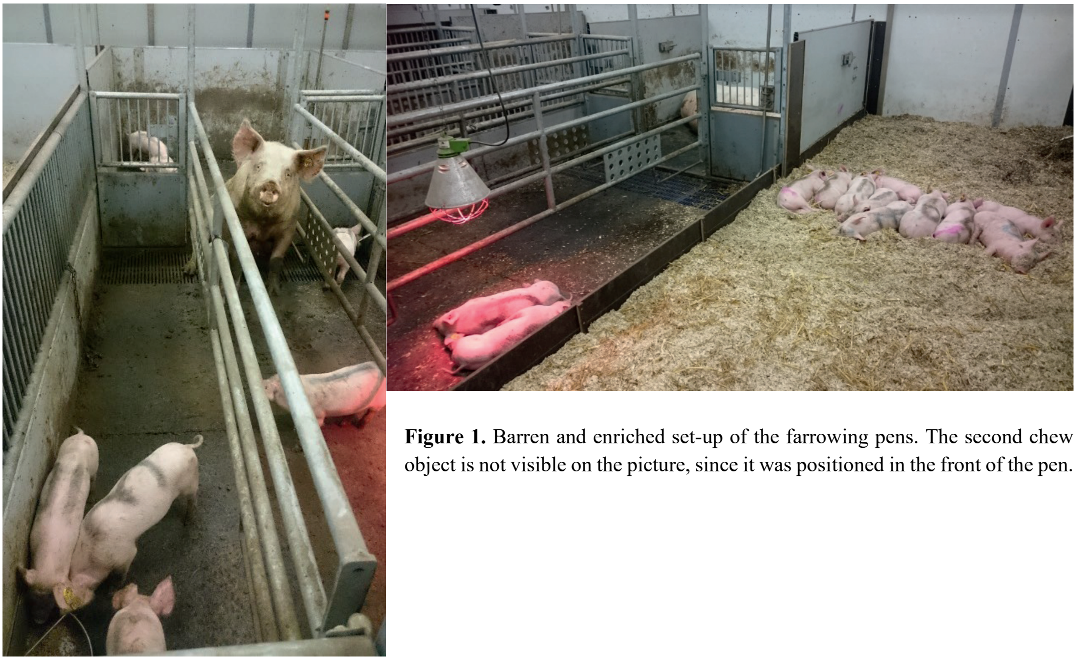

Figure 1. Barren and enriched set-up of the farrowing pens. The second chew object is not visible on the picture, since it was positioned in the front of the pen.

After weaning, a subset of 96 piglets from 15 barren farrowing pens was moved to 16 barren weaner pens (5.6 m^2), with partly solid and slatted floor. A subset of 96 piglets from 15 enriched farrowing pens was relocated in 16 enriched weaner pens (11.2 m^2) with 2.5 kg straw, 400 L of sawdust, and 360 L of peat on the floor. Additionally, 1.25 kg of fresh straw and 60 L of fresh sawdust were added daily, and 45 L of fresh peat was added weekly in the enriched pens. The chew objects in the barren and enriched weaner pens were kept the same as before weaning. Additionally, enriched-housed piglets received extra enrichment from d11 post-weaning on one fixed day a week, such as a jute sack (see Luo et al., 2019b for details). The enriched and barren weaner pens are depicted in **Figure 2.**

Figure 2. Barren and enriched set-up of the weaner pens. The feeder and the second chew object are not visible on the picture since they were positioned in the front of the pen.

Measurements

Sow body weight and back fat thickness at left and right P2 positions were measured using an ultrasonic Renco Lean Meater (MS Schippers, Bladel, the Netherlands) at one month and one week before farrowing. Piglets were individually weighed at d0 (within 24 h after birth), at d28 (to select piglets) and at d30 (at weaning).

Piglets were individually numbered using dark permanent hair dye to allow individual recognition during behavioural observations on eating behaviour. Eating was defined as ‘eating or chewing feed from the feeder or the floor’. Eating by individual piglets was scored live in the farrowing pens using 4-min instantaneous scan sampling for 3 h per day at d22 and 23 after birth (in total, 6 h per pen before weaning). Observation hours started at 8:00, 9:15, 10:30, 14:00, 15:15 and 16:30 h. These procedures resulted in a total of 90 observations per piglet before weaning. Behaviour was scored using a pen and scoring sheets. Eating behaviour in the home pen was averaged per piglet (a total of 368 piglets) and expressed as proportions of the observation time, i.e. time spent on eating. Based on the behavioural observations we also discriminated ‘eaters’, i.e. piglets scored eating at least once, from ‘non-eaters’. The percentage of eaters was calculated by dividing the number of eaters per litter by the total number of piglets in the same litter.

Feed intake was calculated per pen by registering the amount of feed given and weighing residual feed. Before weaning, feed intake was determined between d7-15, d15-22, d22-28 and d28-30. After weaning, feed intake was determined between d0-5 and d5-19 post-weaning.

Statistical analyses

Feed intake was low between d7-15 and d15-22 and was therefore analysed between d7-22. Data were analysed with mixed models in the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Proportions of time spent on eating (% of observations) and the percentage of eaters per litter (%) were analysed using a generalised linear mixed model (GLIMMIX) with a binomial distribution, logit link function and an additional multiplicative overdispersion parameter. Fixed effects of housing (barren vs. enriched) and batch (1 vs. 2) were used, and a random effect of pen nested within housing and batch were included for the proportion of time spent eating. Feed intake was measured at pen level in linear mixed models (MIXED). Model residuals of the MIXED procedure were checked for normality. Feed intake between d7-22, d22-28 and d7-30 after birth were log transformed before analyses. Feed intake before weaning (g/piglet) and average daily feed intake after weaning (g/piglet/d) were analysed with housing and batch as fixed effects. Data are presented as (untransformed) means ± SEM based on pen averages. Differences at $P < 0.05$ were considered statistically significant and differences at $0.05 \leq P < 0.10$ were considered a trend.

Results

Time spent eating at 22 days of age did not differ between enriched- and barren-housed piglets (enriched: 0.95 ± 0.32 vs. barren: 1.44 ± 0.44% of observations, $P = 0.39$). Environmental enrichment did also not affect the number of eaters within the litter at 22 days of age (enriched: 35.0 ± 9.3 vs. barren: 42.7 ± 9.8%, $P = 0.50$). The intake of feed was not affected by environmental enrichment between d7-22 (enriched: 42 ± 18 vs. barren: 28 ± 12 g/piglet) and d22-28 (enriched: 202 ± 74 vs. barren: 156 ± 61 g/piglet), but enriched-housed piglets ate more feed during the last two days prior to weaning than barren-housed piglets (enriched: 174 ± 31 vs. 90 ± 25 g/piglet, $P = 0.03$, **Table 1**). The total intake of solid feed before weaning also tended to be higher in enriched-housed piglets compared to barren-housed piglets (enriched: 418 ± 119 vs. 274 ± 93 g/piglet, $P = 0.07$). After weaning, enriched-housed piglets had a higher ADFI during the first 5 days post-weaning (d0-5, $P < 0.001$), between d5-19 ($P = 0.02$) and in total during the first 19 days post-weaning than barren-housed piglets (d0-19, $P < 0.01$).

Table 1. Feed intake before and after weaning (g/piglet/d) of enriched- and barren-housed piglets. Significant *P*-values and trends are presented in bold.

	Enriched	**Barren**	**Significance**
Pre-weaning (g/piglet/d)			
d7-22	3 ± 1	2 ± 1	0.98
d22-28	34 ± 12	26 ± 10	0.35
d28-30	87 ± 15	45 ± 13	**0.03**
d7-30	18 ± 5	12 ± 4	**0.07**
Post-weaning (g/piglet/d)			
d0-5	342 ± 21	280 ± 15	**< 0.001**
d5-19	707 ± 15	650 ± 20	**0.02**
d0-19	611 ± 10	553 ± 14	**< 0.01**

Discussion

This study investigated the effects of environmental enrichment on the early feed intake of pigs. Environmental enrichment stimulated feed intake before and after weaning. The time spent on eating and the percentage of eaters, which were determined at 3 weeks of age only, were not influenced by environmental enrichment.

Enriched-housed piglets consumed more feed after weaning than barren-housed piglets. This may have been the result of enrichment in the pre-weaning environment (previous housing conditions), enrichment in the post-weaning environment (current housing conditions) or both.

Firstly, the greater post-weaning feed intake in the first days after weaning may have been the result of enrichment in the pre-weaning environment. Enriched-housed piglets had a higher feed intake before weaning than barren-housed piglets, and previous research has determined that this can have a stimulating effect on post-weaning feed intake (Carstensen et al., 2005; Berkeveld et al., 2007a; Middelkoop et al., 2019a). Environmental enrichment may have stimulated pre-weaning feed intake by encouraging exploration of the environment and facilitating the development of foraging behaviours, such as rooting and chewing, which are important precursors of eating in piglets (Petersen, 1994). Besides, environmental enrichment may have affected pre-weaning feed intake by a reduction in food neophobia (Oostindjer et al., 2011c). Irrespective of post-weaning housing, pre-weaning environmental enrichment resulted in a higher feed intake in the first two days after weaning although no differences in feed intake before weaning were reported (Oostindjer et al., 2010) or feed was not provided at all before weaning (Brajon et al., 2017). These findings suggest that previous enrichment during lactation can indeed have implications for feed intake after weaning, also if feed intake was not increased in the pre-weaning period. Environmental enrichment before weaning may also have protective effects after weaning by increasing the ability of piglets to cope with weaning stress. This is suggested since enriched housing during lactation resulted in a lower cortisol concentration one day after weaning compared to barren housing during lactation (Yang et al., 2018) and in a lower frequency of weaning-stress-induced behaviours such as tail biting (Day et al., 2002; Moinard et al., 2003; Telkänranta et al., 2014). It should be noted though that such effects were not observed when the contrast between the pre- and post-weaning environment was too large (Van de Weerd et al., 2005).

Secondly, the greater post-weaning feed intake by enriched-housed piglets may have been the result of enrichment in the post-weaning environment. Post-weaning enriched conditions were also reported to improve growth and feed efficiency and reduce the number of days with diarrhoea irrespective of pre-weaning housing (Oostindjer et al., 2010) and to reduce weaning-stress-induced behaviours (Bolhuis et al., 2005; Oostindjer et al., 2011d). This may be explained by a decrease in (chronic) stress levels in the home pen (Beattie et al., 1995, 1996; Munsterhjelm et al., 2010), for example by stimulating natural foraging behaviours and reducing boredom due to the provision of substrates and by reducing pen density by providing more space (Oostindjer et al., 2010). Stress is known to have an anorexic effect on feed intake (Hötzel et al., 2011). Environmental enrichment may therefore have reduced stress levels and consequently increased feed intake, although a previous study did not find an effect of post-weaning housing on feed intake after weaning (Oostindjer et al., 2010).

Lastly, feed intake after weaning may have been stimulated by both pre- as well as post-weaning enrichment of the environment. Pre- and post-weaning housing conditions, as well as their interaction, did not affect feed intake in the first two weeks post-weaning when the feed before weaning was also given after weaning (Munsterhjelm et al., 2009; Oostindjer et al., 2010). However, piglets reared in pre- and post-weaning enriched conditions had a higher feed intake after a dietary transition than piglets reared in pre- and post-weaning barren conditions and piglets reared in pre-weaning enriched and post-weaning barren conditions, with intermediate feed intake levels of piglets reared in pre-weaning barren and post-weaning enriched conditions (Munsterhjelm et al., 2009). In the present study, piglets were exposed to a dietary transition at 10 days after weaning, suggesting enrichment of the environment may have reduced food neophobia, which may partly explain the higher feed intake by enriched-housed piglets. Measures on the intake of the novel feed in the first hours and days after the dietary transition would have been needed to confirm this.

Although the effects of the pre- and post-weaning housing conditions on the feed intake levels after weaning cannot be separated in this study, we postulate that enrichment of the pre-weaning environment is important to stimulate feed intake in the first days after weaning, while enrichment of the post-weaning environment is important to maintain a higher feed intake level after the weaning transition. Further research is needed to disentangle the effects of pre- and post-weaning housing (in a 2 × 2 arrangement) on feed intake after weaning, including the first days, and to unravel the mechanism by which enrichment stimulates pre-weaning feed intake and facilitates the weaning transition.

Enriched-housed piglets spent a significant amount of time chewing on the substrates (Luo, Reimert, Middelkoop et al., under revision), and piglets therefore likely ingested some substrates next to the feed provided. Although the intake of substrates by enriched-housed piglets was not measured in the present study, the findings of Luo, Reimert, Middelkoop et al. (under revision) suggest that the difference in intake of feed plus substrates between the enriched and barren housing conditions was likely even larger than the observed significant differences in feed intake.

In this study we provided feed to enriched-housed piglets in a feeder separate from the foraging substrates. Before weaning, the feeder was placed in the barren part of their enriched pen, away from the foraging substrates. After weaning, the feeder was placed on top of the substrates. However, pigs seem to prefer a mixture of feed and substrates over feed and substrates offered separately (De Jonge et al., 2008). This suggests that scattering the feed over and/or hiding the feed in substrates may stimulate feed intake more than providing the feed in a feeder separate from the substrates, and is of interest for future research.

Conclusion

Housing in enriched conditions did not affect time spent on eating by piglets and the percentage of eaters, which were determined at 22 days of age only, as compared with housing in barren conditions. Nevertheless, environmental enrichment tended to increase feed intake from feed provision at 7 days of age until weaning at 30 days of age, and significantly increased feed intake in the last two days prior to weaning. Moreover, enriched-housed piglets consumed more feed than barren-housed piglets from weaning until the end of the study at 19 days post-weaning. Taken together, we recommend environmental enrichment as a strategy to familiarise piglets with solid feed and to ease the weaning transition.

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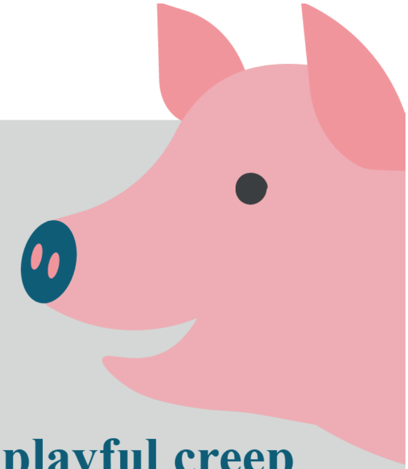
CHAPTER 5



Feed intake of the sow and playful creep feeding of piglets influence piglet behaviour and performance before and after weaning

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Abstract

Creep feed intake is variable and may be partly homeostatically and exploratory driven. We studied effects of maternal feed restriction and a ‘play-feeder’ on piglet behaviour and performance. 37 Litters received creep feed in a conventional (CON) or play-feeder (PL) and their sows were full-fed (FF) or restrictedly-fed (RES). Eaters were determined via rectal swabs. At weaning (d24) four piglets from the same treatment were grouped (n = 36 pens). RES hindered piglet growth by 41 g/d and enhanced time eating, creep feed intake and percentage of eaters at weaning versus FF. RES-PL had the largest proportion of moderate and good eaters. PL stimulated feeder exploration and attracted more piglets to the feeder than CON. Post-weaning, RES increased exploratory behaviours, feed intake between d0-5, and growth between d0-2, and reduced body lesions between d0-2 (within CON), drinking and ear biting. PL increased ingestive behaviours, feed intake and growth between d0-15 and BW at d15 post-weaning by 5%. PL also lowered the prevalence of watery diarrhoea, number of body lesions and piglets with ear (within FF) and tail (within RES) damage at d15 post-weaning. Treatments did not affect FCR. To conclude, RES and particularly PL (broader and for longer) result in less weaning-associated-problems.

Introduction

The transition from feeding on sow's milk to foraging and feeding exclusively on solid feed, i.e. weaning, is a gradual process occurring between 8.5 and 22 weeks of age in free range pigs (Newberry and Wood-Gush, 1985; Jensen and Stangel, 1992). In commercial farms, weaning occurs earlier and sudden by separation of the sows and their piglets, generally at 3 to 4 weeks of age in Europe. Apart from this nutritional challenge, as piglets are abruptly not able to suckle anymore, weaning also simultaneously involves social (maternal separation and mixing with unfamiliar peers) and environmental stressors (handling, transport and housing in a novel pen). These stressors together often lead to a low feed (energy) intake (reviewed by Le Dividich and Sève, 2000), gastrointestinal problems (reviewed by Heo et al., 2013), gut microbiota dysbiosis (reviewed by Gresse et al., 2017), reduced growth and behavioural disturbances (Colson et al., 2006; Van Nieuwamerongen et al., 2017) after weaning, thereby reducing health and welfare. Stimulating the consumption of solid feed prior to weaning, i.e. creep feed, may improve piglet adaptation after weaning (Bruininx et al., 2002, 2004; Kuller et al., 2007a; Sulabo et al., 2010a), but factors influencing creep feed intake in piglets are unclear up to now.

Based on the findings by Algers et al. (1990), it was suggested that energy availability from milk affects individual creep feed intake. The proposed 'compensatory feeding hypothesis' suggests that slower-growing piglets in the litter will compensate for a lower energy intake from milk by consuming more creep feed and therefore undergo a less severe nutritional challenge at weaning and grow faster after weaning. Piglets classified as 'good eaters' of creep feed or with a long time (eating) at the feeder before and after weaning were, indeed, slower-growing piglets before weaning (Algers et al., 1990; Appleby et al., 1992; Sommavilla et al., 2015; Huting et al., 2019). Complementary, other data suggest that the compensatory feeding hypothesis also holds on litter level, which may partly explain the large between-litter variation in creep feed intake (Bruininx et al., 2002, 2004). Firstly, litters from intermittent suckling regimes that experienced reduced growth, due to separation from the sow for multiple hours per day, had a higher creep feed intake (Rantzer et al., 1995; Berkeveld et al., 2007b), post-weaning feed intake (Kuller et al., 2004, 2007a) and post-weaning weight gain (Pajor et al., 2002), than faster-growing conventional litters. However, effects of a low energy intake from milk are confounded in intermittent suckling regimes by the temporal maternal separation before weaning, which may increase separation stress before weaning, but reduce separation stress after weaning (Turpin et al., 2016). Secondly, primiparous sows, that produce less milk (Beyer et al., 2007; Ngo et al., 2012; Strathe et al., 2017) and consequently wean lighter piglets (Bierhals et al., 2011; Huting et al., 2019), have been found to rear more eaters of creep feed than second parity sows, and particularly more moderate eaters of creep feed compared to multiparous sows (Huting et al., 2019). Results of other studies, however, do not support or are even contradictory to the compensatory feeding hypothesis (Pajor et al., 1991; Pluske et al., 2007; Berkeveld et al., 2009).

It is likely that the consumption of creep feed is not solely driven by milk availability, as the milk production by the sow already becomes limiting for piglet growth from one week of lactation (Le Dividich and Sève, 2001) and a substantial proportion of piglets do not consume creep feed before they are weaned at 3 to 4 weeks of lactation (Kuller et al., 2007a; Pluske et al., 2007; Sulabo et al., 2010a). Several studies suggested that creep feed intake may also be driven by intrinsic exploration in piglets towards the feed(er) (Kuller et al., 2010; Van den Brand et al., 2014; Seddon et al., 2015; Middelkoop et al., 2018). Exploratory behaviour is closely related to play behaviour as both are likely involved in ‘object play’ in piglets, which includes holding or carrying an object or material in the mouth, manipulating, shaking and tugging it (Newberry et al., 1988). Object exploration is often hard to distinguish from and precedes object play (reviewed by Power, 1999). In addition, exploratory behaviour, e.g. nosing, rooting, chewing, and object play seem to follow a similar developmental pattern in piglets. Both initiate in the first week of life and peak around four weeks of age, during which ingestion of solid feed items starts to increase (Petersen, 1994; Blackshaw et al., 1997). It is therefore possible that exploratory behaviour and object play are involved in the development of feed intake behaviour in suckling piglets.

Our aim was to establish whether a low energy intake (homeostatic drive) from milk, by means of maternal feed restriction, and presenting creep feed in an exploratory and playful context (exploratory drive), by means of a foraging-stimulating play-feeder, are important and potentially interacting factors in getting piglets to eat creep feed. Subsequently, we studied the effects of the two factors on post-weaning adaptation in terms of behaviour and performance. We hypothesized that both factors would encourage piglets to familiarise themselves with creep feed and thereby reduce the stress from dietary changes at weaning and improve their adaptation after weaning.

Methods

The protocol of the experiment (sows: AVD104002015325, piglets: AVD104002016515) was approved by the Animal Care and Use committee of Wageningen University & Research (Wageningen, the Netherlands) and in accordance with the Dutch law on animal experimentation, which complies with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The use of Indigo carmine as feed colourant was approved by the Medicines Evaluation Board (Utrecht, the Netherlands).

Feeding strategies of sows and piglets during lactation

The study was set up as a 2 × 2 factorial arrangement with sow feeding (SF, lactational feed intake level) and piglet feeding (PF, creep feed presentation strategy) as experimental factors. All sows were fed the same level of commercially available sow diet twice a day (7:30 and 16:00 h) until d9 post-partum (8.9 MJ/kg as-fed net energy, 133 g crude protein, 5.6 standardized ileal digestible lysine/kg dry matter, ‘Standaard zeugenbrok’ during gestation and 9.3 MJ/kg as-fed net energy, 156 g crude protein, 7.7 standardized ileal digestible lysine/kg dry matter, ‘Zeugen Maxima Lacto’ during lactation, AgruniekRijnvallei, Wageningen, the Netherlands). The sows were provided 2.9 kg/d between d102-109 of gestation and the amount was gradually reduced to 2 kg/d at parturition, and increased thereafter with 0.5 kg/d. From d10 post-partum onwards, sows were fed in three feedings (7:00, 13:00 and 19:00 h) and received either 6.5 kg feed per day (full-fed, **FF**, n = 19) or 3.25 kg feed per day (restrictedly-fed, **RES**, n = 18) to create a contrast in energy supply from milk. The milk production of RES-sows was indeed lower compared to FF-sows, at least between d17-24 of lactation, and the milk of RES-sows contained a lower percentage of fat (Costermans et al., under review). Moreover, RES-sows lost more body weight (19.2 ± 2.0 vs. 14.2 ± 2.0 kg) and muscle depth of the longissimus dorsi (1.3 ± 0.1 vs. 0.6 ± 0.1 mm) between d10-24 than FF-sows, but feed restriction of the sow did not affect back fat loss (3.4 ± 0.6 vs. 2.5 ± 0.6 mm) (Costermans et al., 2019). These changes were accompanied by lower plasma insulin-like growth factor 1 levels and higher plasma creatinine levels of RES-sows from d17 onwards, indicating a more severe negative energy balance of RES-sows (Costermans et al., 2019). A more detailed description of the effects of feed restriction during lactation on sow (reproductive) performance, energy mobilisation and milk quality are reported in Costermans et al. (2019) and Costermans et al. (under review). Piglets reared by RES-sows will be named ‘RES-piglets’ and piglets reared by FF-sows will be named ‘FF-piglets’.

The litters were either creep-fed in a round conventional feeder (**CON**, n = 18 of which 9 FF and 9 RES; MS Clickfeeder Mini, MS Schippers, Bladel, the Netherlands) or in a foraging-stimulating ‘play-feeder’ (**PL**, n = 19 of which 10 FF and 9 RES) from d4 after birth. The piglet feeders had a diameter of 26 cm and five feeding places (**Figure 1A**). The play-feeder was created by attaching canvas clothes (purchased at local market), braided natural cotton ropes (10 mm in diameter, MS Schippers, Bladel, the Netherlands) and PVC spiral tubes (13 mm in diameter, Ubbink, Alkmaar, the Netherlands) to the conventional feeder (**Figure 1B** and **C**). These materials were attached on the inside at the bottom of the feeder (4 pieces of canvas clothes, 1 cotton rope, 1 PVC spiral tube). Two canvas clothes and one cotton rope were also tied to the feeder so they were hanging down and a PVC spiral ring was placed around the mounting system of the feeder so that it could be moved up and down. In this way the play-feeder allowed piglets to express object play as well as foraging behaviours, i.e. rooting, nosing, chewing, biting, pushing, pulling and lifting. A video showing interaction of piglets with the play-feeder can be found online as **Supplementary Video S1**. The canvas clothes and cotton ropes were replaced at d18. Feeders were located at the front end of the farrowing area near the feeding corridor.

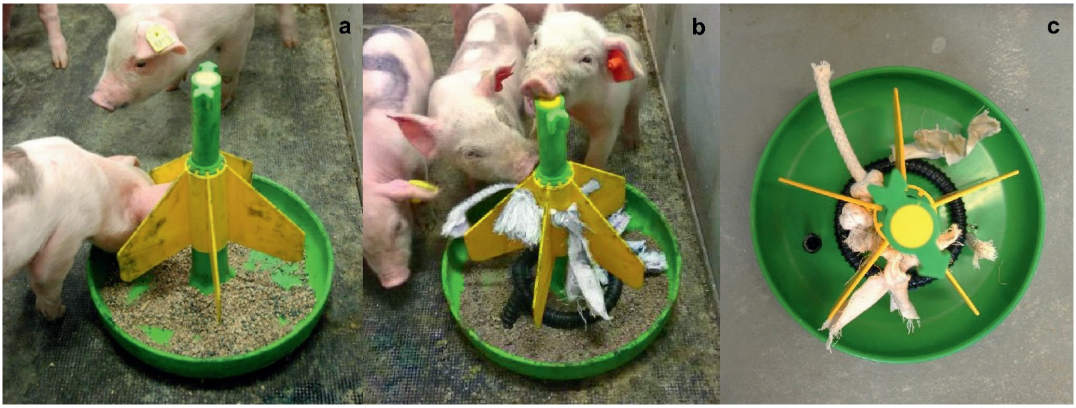

Figure 1. Litters were either creep-fed in a conventional feeder (**A**) or in a foraging-stimulating ‘play-feeder’ (**B, C**) from d4. The play-feeder was created by attaching canvas clothes, cotton ropes and PVC spiral tubes to the conventional feeder.

Piglets were fed an experimental creep feed as crumble (Research Diet Services, Wijk bij Duurstede, the Netherlands). The creep feed (11.8 MJ/kg as-fed net energy, 195 g crude protein, 11.9 g standardized ileal digestible lysine/kg dry matter) was high in dietary non-starch polysaccharides (261 g/kg dry matter), originating from cereal grains, sugarbeet pulp, oat hulls, galacto-oligosaccharide, inulin and high-amylose starch (**Supplementary Table S2** and **S3**). Feed colourant Indigo carmine (E132 Eurocert 311811, Sensient Food Colors, Elburg, the Netherlands) was added manually to the pre-weaning piglet diet (5 g/kg of feed) to allow for identification of eaters (see below). Every morning a small scoop of fresh creep feed was given and feeders were checked at least twice daily (three times a day from d10) to provide the creep feed *ad libitum*. To maintain freshness of the creep feed, all feed was replaced and feed bowls were cleaned using paper towels and water at each feed weigh-back (see below). Two days prior to weaning, the creep feed was mixed with a commercially available crumbled nursery diet to familiarise piglets to the nursery diet before weaning (161 g crude protein, 48 g crude fibre and 11.8 g standardized ileal digestible lysine/kg dry matter, 'Insecto Speen', Coppens Diervoeding, Helmond, the Netherlands).

Litter size was standardized by cross-fostering within 3 days of age, resulting in an average number of 12.7 ± 0.2 piglets per litter at d4. The thirty-seven sows and their litters were allotted to one of four treatments (piglet feeding at d4 after birth and sow feeding at d10 post-partum) based on sow body weight and P2 back fat thickness at parturition and piglet birthdate and body weight at d0 and d4. Litter size at weaning did not differ between treatments (on average 12.4 ± 0.2 piglets per litter at d24).

Animals, housing and management

Pregnant primiparous TN70 sows (Norwegian Landrace x Large White, Topigs Norsvin, Vught, the Netherlands), inseminated with Tempo boar semen, were divided over two farrowing rooms (balanced for treatments) in three consecutive batches. The sows originated from one conventional farm and were housed at research facility Carus (Wageningen University & Research, the Netherlands) from two weeks before farrowing onwards. The pen was equipped with a farrowing area (2.85 x 1.80 m) and a free-movement area (1.85 x 1.80 m). The farrowing area was equipped with a crate (2.85 x 0.60 m) including a feed trough, a drinking nipple, a jute sack (around farrowing), a long-stemmed straw dispenser (from d10 post-partum) and three chew objects that alternated two times a week (metal chain with either one of three attachments: bolts, ball or PVC pipe) for the sow. The farrowing area included a drinking nipple, toy (metal chain with bolts) and heating lamp for the piglets. The floor consisted of slats and rubber mats (2.00 x 1.75 m, in farrowing area) that served as nest for the piglets and provided lying comfort to the sow. The free-movement area was equipped with a drinking nipple for the sow. The sows were loose housed, except from shortly before farrowing until the first four days post-partum to minimize piglet crushing. During this initial period the free-movement area was not accessible to sows and piglets. Within 24 hours after birth, piglets received an ear tag and intramuscular iron injection and their birth weight and sex were determined. No teeth clipping, tail docking or castration were performed. In the farrowing rooms lights and radio were on from 6:00 to 19:30 h and lights were dimmed during the night. The room temperature was 23 °C around farrowing and gradually decreased to 20 °C at weaning.

At weaning (24.3 ± 0.1 days of age) a subset of 144 piglets (n = 9 weaner pens per treatment) was relocated in two weaner rooms (balanced for treatments) in two batches. Piglets were mixed with conspecifics from the same pre-weaning treatment and housed in pens (2.76 x 1.20 m) with four piglets (two males and two females) from three litters. Piglets were selected based on their sex and their body weight at d22 (close to the average weight of the litter and treatment group). Piglets with a history of medication and leg/claw problems were excluded from selection. The weaning weight of the selected piglets was 5.82 ± 0.13 kg, 6.06 ± 0.05 kg, 6.66 ± 0.04 kg and 6.30 ± 0.06 kg for RES-CON, RES-PL, FF-CON and FF-PL respectively. All weaner pens were identical and equipped with a conventional feed trough (three feeding spaces), drinking trough and toy (metal chain with bolts). The floor was party slatted and partly covered with a rubber mat (1.75 x 1.20 m) that provided lying comfort to the piglets and prevented spillage of feed through the slats. Piglets were fed the nursery diet *ad libitum*. In the weaner rooms lights and radio were on from 7:00 to 19:00 h and room temperature gradually decreased from 25 to 23 °C at two weeks post-weaning, when the experiment ended and piglets were 39 days of age.

Measurements

Piglet growth

Piglets were individually weighed at d0 (within 24 h after birth), 4 (before commencing piglet feeding), 10 (before commencing feeding strategy of the sow), 17, 22, 24 (at weaning) before weaning and d1, 2, 5 and 15 post-weaning (at end of experiment).

Piglet behaviours

Piglets were marked using dark permanent hair dye (pre-weaning, Syoss professional performance permanent colouration, 1-1 noir) and animal marking spray (post-weaning, MS marking spray, MS Schippers, Bladel, the Netherlands). Feed-related behaviours in the farrowing pens were observed live at d9, 16 and 23 by 2-min instantaneous scan sampling for six sessions of one hour per day (i.e. 180 scans/piglet/d) using pen and paper. Sniffing, touching with snout or rooting the feed were defined as ‘exploring feed’ and eating or chewing feed was defined as ‘eating feed’. Sniffing, touching with snout, rooting or chewing on (toys of) the feeder, pushing or lifting toys of the feeder or shaking head while having toy of feeder in mouth were classified as ‘exploring feeder’. Feeder exploration and object play with (toys of the) the feeder are grouped as one behaviour in our study, as object exploration is often hard to distinguish from and precedes object play (reviewed by Power, 1999). ‘Suckling’ was recorded on litter level as drinking milk from teat of sow (soft suckling noises). Observations were used to calculate time spent on feed-related behaviours, the proportion of piglets visiting the feeder (piglets that were scored either exploring the feed, exploring the feeder or eating feed at least once) and the proportion of piglets eating (piglets scored eating feed at least once). Behaviours in the weaner pens were recorded live on 30 and 39 days of age (6 and 14 days post-weaning respectively) by 2-min instantaneous scan sampling for six one-hour sessions per day using a Psion hand-held computer with the Pocket Observer 3.1 software package (Noldus Information Technology, Wageningen, the Netherlands). The ethogram with behaviours of interest in the weaner rooms is listed in **Supplementary Table S4**. Observation sessions started at 8:00, 9:15 and 10:30 h in the morning and at 14:00, 15:15 and 16:30 h in the afternoon.

Creep feed eaters and feed intake

Rectal swabs were taken at d10, 17, 22 and 24 to assess the presence of blue colour (Indigo carmine) and thereby to determine qualitative intake of creep feed (yes or no) on piglet level. Blue colour was not detected on the swabs at d10 and therefore this time point was excluded from analyses. Piglets were subsequently classified into different eater classes after Collins et al. (2013). Piglets of which blue colour was present on the swab on three measurement days were classified as ‘good/early eaters’. ‘Moderate eaters’ had blue colour on the swab on two measurement days and ‘bad eaters’ on only one measurement day. This may, by definition, not necessarily concern the last measurement day(s) and therefore these piglets cannot be called ‘late eaters’. Swabs of ‘non-eaters’ did not include blue colour at any of the measurement days. Pre-weaning feed intake was determined per litter between d10-17, d17-22 and d22-24 and post-weaning feed intake was determined per pen between 0-4 h, 4-24 h, d1-2, d2-5 and d5-15 post-weaning.

Body lesions and damage on piglets

We monitored the number of body lesions on the piglets at 4 hours and d1, d2 and d15 post-weaning (as a measure of aggression, according to Turner et al., 2006) and classified bite injuries on ears and tails of the piglets into no damage, bite marks or small wound at d15 post-weaning (as a measure of oral manipulation, according to Van Nieuwamerongen et al., 2015).

Faecal consistency scores of piglets

Faeces in the weaner pens were scored daily for consistency according to Pedersen and Toft (2011). Score 1 (firm and shaped) and 2 (soft and shaped) represent normal faeces, and were combined into one score prior to data analysis. Score 3 (loose) and 4 (watery) represent diarrhoea. The highest faecal consistency score that was observed in a pen was selected on each measurement day and averaged over two weeks post-weaning to calculate the mean faecal consistency score (FCS) per pen. Faeces were removed on a daily basis after scoring to guarantee consistency scoring of fresh faeces.

Statistical analyses

Data processing

Piglet behaviours in the home pen were averaged per piglet per day and expressed as proportions of time. Eating feed was very low at d9 and exploring feed was very low at all ages before weaning (< 0.1% of observation time) and were therefore excluded from analysis. After weaning, exploring feed, feeder and drinking trough were pooled into ‘exploring feed(er) and drinker’. Nosing, rooting and chewing the environment as well as chewing air were combined into ‘exploring environment’. The behavioural element ‘chewing poo’ was excluded from analyses because it was seen very rarely (0.01% of observation time). Lying with eyes closed and lying with eyes open were pooled into ‘inactive behaviour’. Standing and walking were pooled into ‘standing and walking’. Playing individually, socially and with chew object were summed and presented as ‘playing’. To investigate ‘nosing pen mates’, nosing body and snout contact were merged. The ear of the piglets with the highest damage score (either the left or right ear) was used in the analysis of ear damage. The number of body lesions on the piglets at weaning was subtracted from the number of body lesions at 4 hours after weaning.

Data analyses

Data were analysed with mixed models in the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Proportions of time spent on the different behaviours, the percentage of piglets per litter visiting the feeder and eating, the percentage of eaters per litter identified by rectal swab, and uniformity in BW (expressed as coefficient of variation) were analysed using a generalised linear mixed model (GLIMMIX) with a binomial distribution, logit link function and an additional multiplicative overdispersion parameter. The model did not converge with nosing environment at 2 weeks post-weaning as response variable, therefore this variable was analysed in a linear mixed model (MIXED). The number of body lesions and the number days with post-weaning diarrhoea were analysed using a GLIMMIX with a Poisson distribution, log link function and an additional multiplicative overdispersion parameter. The model did not converge with the total number of body lesions at 2 weeks post-weaning as response variable and was therefore analysed in a MIXED after square root transformation. Tail damage of weaner piglets was analysed in a GLIMMIX with a multinomial distribution and a cumulative logit link function. Data on the prevalence of watery diarrhoea in weaner pens were expressed as binary data and analysed with a GLIMMIX with a logit link and binary distribution. Individual creep feed classification of suckling piglets and ear damage on weaner piglets were analysed in a Fisher's exact test, because there was an empty sub-classification category for the interaction effects. Continuous variables creep feed intake, average daily feed intake (ADFI), average daily gain (ADG), body weight (BW), feed conversion ratio (FCR) and mean faecal consistency score (FCS) were analysed in a MIXED procedure. For feed intake and ADG, totals over the pre- (d10-24) and post-weaning period (d0-15 post-weaning) were analysed, as well as effects on separate periods. Model residuals of the MIXED procedure were checked for normality. If model residuals were not normally distributed, data were transformed before analyses.

The model included the fixed effects of sow feeding (RES vs. FF), piglet feeding (PL vs. CON), their interactions, as well as batch (batch 1, 2 or 3). In the analysis of tail damage on weaner piglets, batch was excluded from the multinomial model (not significant). The number of body lesions at 4, 24 and 48 h post-weaning were analysed in a repeated GLIMMIX, therefore also including day and its interactions with the treatment groups as fixed effect. The number of body lesions at 2 weeks post-weaning was analysed separately in a GLIMMIX. Post-weaning ADFI and ADG were analysed in a repeated GLIMMIX and the four periods were also analysed separately. The same results were obtained and the results of the separate analyses are reported to facilitate interpretation. In addition, for behaviour, BW, ADG, the number of body lesions, the model included a random pen effect, nested within treatments and batch (farrowing pen for pre-weaning measurements and weaner pen for post-weaning measurements). Significant fixed effects were further analysed using differences of least squares means. Correlations were calculated at litter level using Pearson's (normally distributed variables) and Spearman's correlation coefficients (not normally distributed variables). Data are presented as (untransformed) means ± SEM based on pen averages. Differences at $P < 0.05$ were considered statistically significant and differences at $0.05 \leq P < 0.10$ were considered a trend.

Results

Feed-related behaviour and percentage of eaters before weaning

The play-feeder increased the time spent exploring the feeder by 3.5 to 6 times, irrespective of feed intake of the sow (PL vs. CON, d9: 0.68 ± 0.16 vs. 0.15 ± 0.04%; d16: 1.87 ± 0.32 vs. 0.54 ± 0.11%; d23: 2.18 ± 0.34 vs. 0.35 ± 0.05%; **Figure 2A**). The treatments did not affect time spent eating creep feed at d16 (**Figure 2B**), however RES-piglets doubled their time spent eating compared to FF-piglets at d23 (RES: 2.23 ± 0.55 vs. FF: 0.94 ± 0.26%). Time spent exploring the feeder correlated with time spent eating ($r = 0.57$; $P < 0.0001$) at all observation days (d9: $r = 0.46$, $P < 0.01$; d16: $r = 0.51$, $P = 0.001$; d23: $r = 0.37$, $P = 0.02$). Irrespective of feed intake of the sow and creep feed presentation method, the percentage of piglets within the litter visiting the feeder was 40, 68 and 83% at d9, 16 and 23 and the percentage observed to be eating was 2, 22 and 60% respectively. The play-feeder stimulated more piglets within a litter to visit the feeder (PL vs. CON, d9: 56.8 ± 5.9 vs. 22.3 ± 4.3%; d16: 80.6 ± 5.4 vs. 55.6 ± 7.3%; d23: 94.5 ± 2.4 vs. 70.4 ± 6.7%; **Figure 2C**), but not to eat (**Figure 2D**).

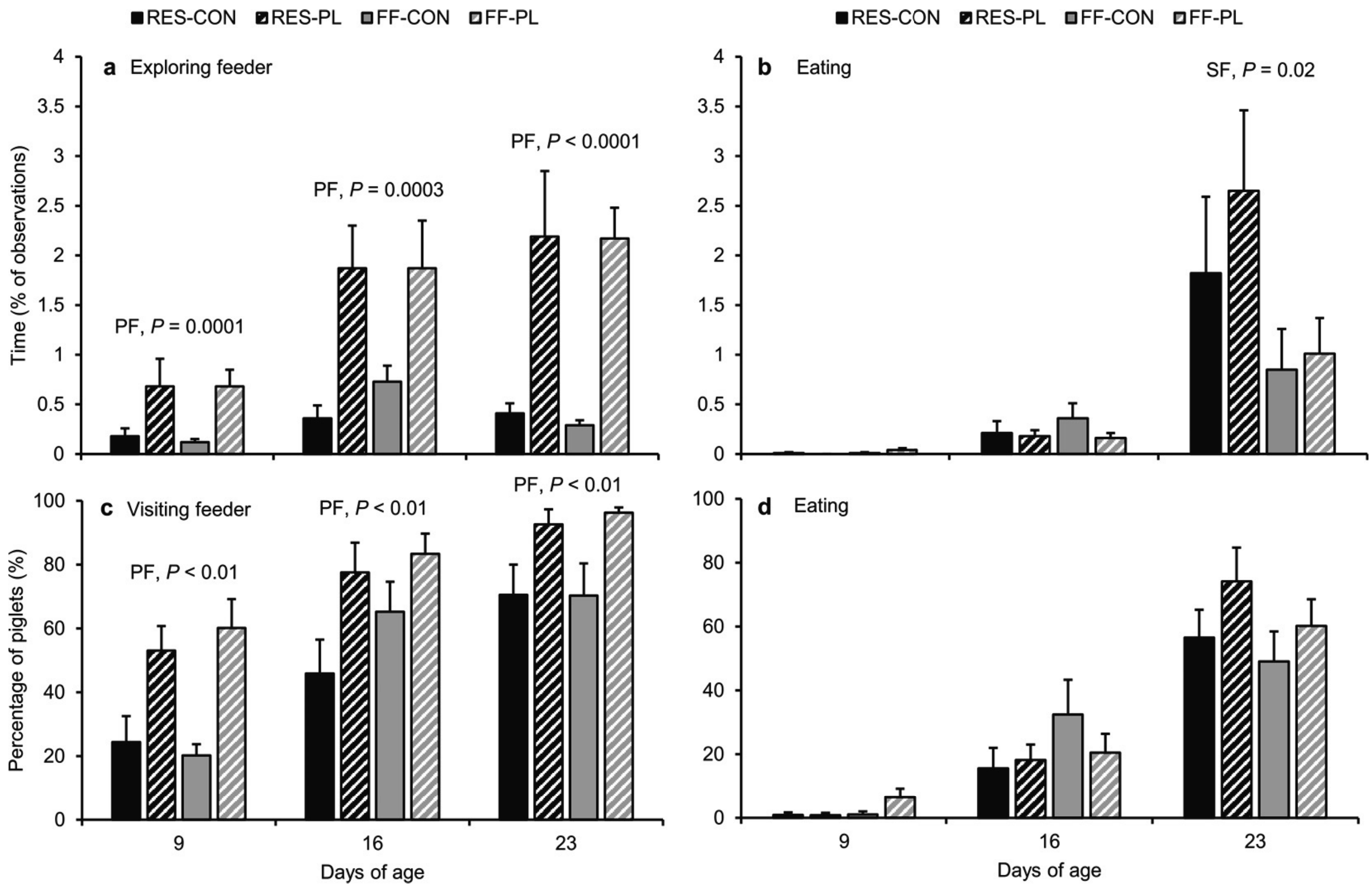

Figure 2. Time spent exploring the feeder (**A**) and eating (**B**) and the percentage of piglets per litter visiting the feeder (**C**) and eating (**D**) based on live home pen observations. Piglets were reared by restrictedly-fed sows (RES) or full-fed sows (FF) and their creep feed presented in a control feeder (CON) or play-feeder (PL) prior to weaning. SF = sow feeding. PF = piglet feeding. Data are expressed as means ± SEM based on pen averages and were analysed with a generalised linear mixed model.

The percentage of creep feed eaters per litter, as identified by blue coloured swabs, was generally low up to d17, but increased with age (d10: 0%, d17: 5%, d22: 31%, d24: 37%). SF, PF and their interaction did not affect the percentage of eaters at d17 and 22. At weaning, litters of RES-sows included double as much eaters than litters of FF-sows (48.6 ± 8.6 vs. 26.5 ± 6.5% eaters/litter; **Figure 3A**). The number of eaters per litter based on observations (seen eating at least once per observation day) correlated with the number of eaters per litter determined by the rectal swabs (blue colour on the swab) that were taken the day after ($r = 0.71$, $P < 0.0001$). Maternal feed restriction stimulated the number of piglets in better eater classes ($P < 0.01$) and the effect was more pronounced in litters that had access to a play-feeder, as shown in **Figure 3B** (SF effect within PL: $P < 0.0001$, particularly moderate and good eaters, and SF effect within CON: $P = 0.07$, particularly bad eaters). The play-feeder also increased the number of piglets in better eater classes ($P < 0.01$, particularly the proportion of moderate and good eaters), but only within RES-piglets (PF effect within RES: $P < 0.0001$ and no effect within FF: $P = 0.78$).

Creep feed intake

Creep feed intake was generally low (especially up to d17 with on average ≤ 8 g/piglet) with on average 62 g/piglet between d4-24, and variable between litters, with total intake ranging from 3 to 399 g/piglet. As feed intake could be measured on litter level only, this was calculated as the intake per litter divided by the number of piglets in the litter and therefore the feed intake of individual piglets could be higher than 399 g. About 50% of the total feed intake was consumed during the last two days prior to weaning. Treatments did not affect feed intake between d17-22, but RES-piglets consumed more feed the last two days before weaning than FF-piglets (49 ± 13.9 vs. 15 ± 4.3 g/piglet; **Table 1**). Overall from the start of maternal feed restriction until weaning, RES-piglets tended to eat double the amount of feed compared to FF-piglets (d10-24: 81 ± 22.6 vs. 41 ± 10.7 g/piglet). Time spent exploring the feeder correlated with feed intake ($r = 0.46$; $P < 0.0001$). Analyses per day showed that time spent exploring the feeder did correlate with feed intake at d9 ($r = 0.45$, $P < 0.01$) and d16 ($r = 0.31$, $P = 0.05$), but not at d23 ($r = 0.07$, $P = 0.66$). Furthermore, time spent eating feed strongly correlated with feed intake ($r = 0.83$, $P < 0.0001$).

Piglet growth, body weight and litter uniformity in body weight before weaning

RES-piglets gained less weight between d10-17 (147 ± 5.3 vs. 188 ± 5.3 g/d), between d17-24 (209 ± 6.0 vs. 262 ± 7.4 g/d) and overall between d10-24 (213 ± 6.3 vs. 254 ± 6.7 g/d) than FF-piglets (**Table 1**). Consequently, RES-piglets tended to weigh less at d17 (4.47 ± 0.14 vs. 4.89 ± 0.22 kg; $P = 0.09$) and weighed less at weaning compared to FF-piglets (5.92 ± 0.16 vs. 6.72 ± 0.26 kg; **Table 1**). PL-piglets did not differ in ADG during lactation or BW at weaning from CON-piglets. SF, PF and their interaction did not affect homogeneity in BW within litters (CV) from d4 to weaning (**Table 1**).

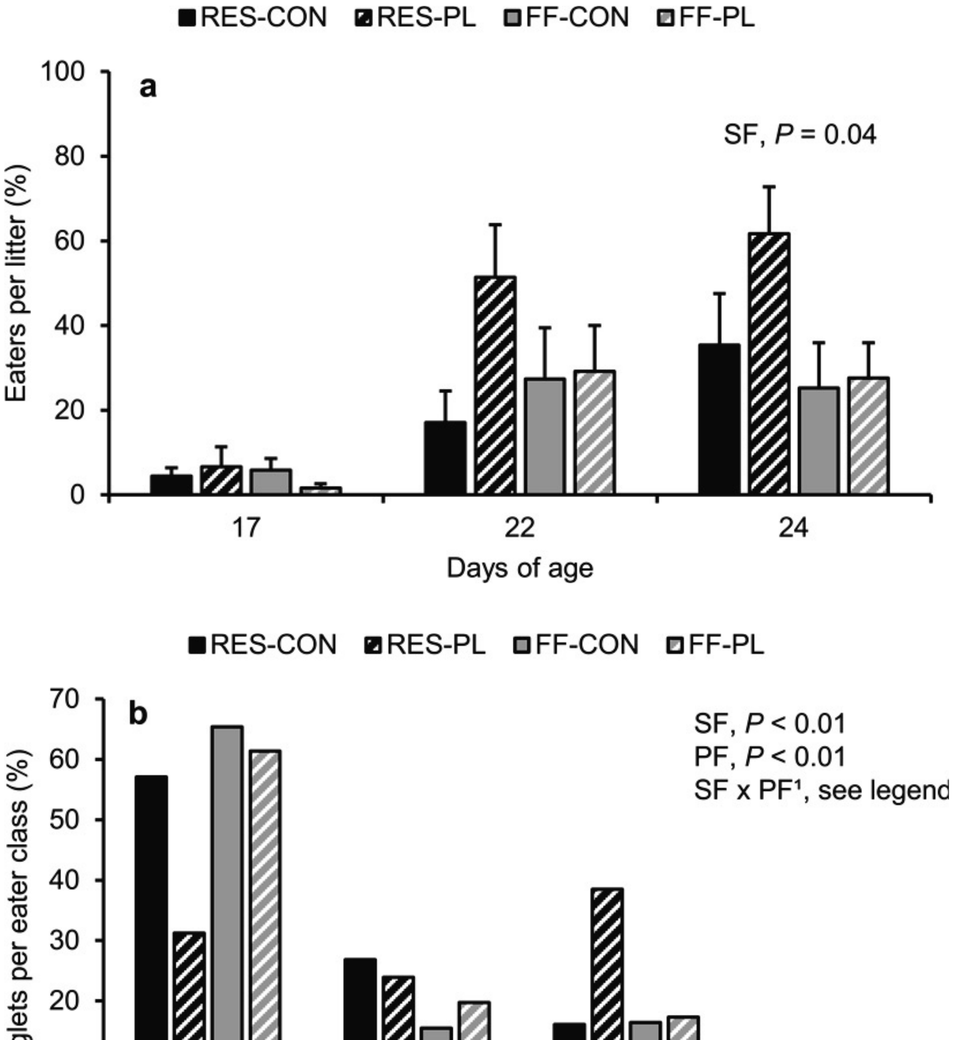

Figure 3. The percentage of eaters per litter over time (**A**) and individual creep feed classification of piglets (**B**) based on the presence of blue colour on rectal swabs. Piglets were reared by restrictedly-fed sows (RES) or full-fed sows (FF) and their creep feed presented in a control feeder (CON) or play-feeder (PL) prior to weaning. SF = sow feeding. PF = piglet feeding. Data are expressed as means ± SEM based on pen averages and were analysed with a generalised linear mixed model (percentage of eaters) and a Fisher's exact test (creep feed classification). ^{1}SF effect within PL: $P < 0.0001$ and within CON: $P = 0.07$. PF effect within RES: $P < 0.0001$ and no effect within FF: $P = 0.78$.

Table 1. Pre-weaning performance of piglets reared by restrictedly-fed sows (RES) or full-fed sows (FF) and their creep feed presented in a control feeder (CON) or play-feeder (PL) prior to weaning. SF = sow feeding. PF = piglet feeding. Data are expressed as means ± SEM based on pen averages and were analysed with mixed models.

	RES		**FF**		**Significance**		
	CON	**PL**	**CON**	**PL**	**SF**	**PF**	**SF x PF**
Creep feed intake, g/piglet							
d 17 - 22	18.0 ± 5.2	35.8 ± 17.1	23.8 ± 11.4	17.9 ± 6.8	0.44	0.54	0.52
d 22 - 24	38.9 ± 13.8	59.4 ± 24.7	13.7 ± 4.5	16.4 ± 7.3	**0.01**	0.74	0.80
d 10 - 24[1]	61.5 ± 18.1	101.1 ± 41.8	44.6 ± 17.5	38.4 ± 13.8	**0.09**	0.58	0.66
ADG, g/piglet/d							
d 10 - 17	145 ± 7	149 ± 8	192 ± 7	185 ± 8	**< 0.0001**	0.97	0.35
d 17 - 24	205 ± 8	212 ± 9	263 ± 10	260 ± 11	**< 0.0001**	0.74	0.50
d 10 - 24	208 ± 9	219 ± 9	259 ± 9	249 ± 10	**< 0.0001**	0.87	0.19
Body weight, kg							
d 0	1.37 ± 0.06	1.41 ± 0.06	1.36 ± 0.04	1.41 ± 0.08	0.95	0.51	0.81
d 4	1.80 ± 0.12	1.87 ± 0.08	1.80 ± 0.12	1.91 ± 0.11	0.55	0.90	0.78
d 24	5.70 ± 0.23	6.15 ± 0.19	6.91 ± 0.42	6.54 ± 0.32	**0.01**	0.87	0.21
Litter CV in BW, %							
d 4	14.6 ± 1.3	15.5 ± 2.1	15.0 ± 1.4	14.6 ± 0.8	0.99	0.65	0.92
d 24	15.7 ± 1.8	17.1 ± 1.7	14.9 ± 1.9	16.4 ± 1.5	0.63	0.42	0.93

[1] Creep feed intake was low from d 10 – 17 and therefore not analysed separately

Piglet behaviour after weaning

Ingestive behaviour

PL-piglets spent more time eating at week 2 after weaning compared to CON-piglets (PL: 11.8 ± 0.5 vs. CON: 10.4 ± 0.4%; **Table 2**). Moreover, PL-piglets showed more drinking than CON-piglets at week 1 (PL: 1.0 ± 0.1 vs. CON: 0.7 ± 0.1%) and week 2 post-weaning (PL: 1.2 ± 0.1 vs. CON: 0.9 ± 0.1%). Drinking was lower for RES-piglets compared to FF-piglets at week 2 post-weaning (RES: 0.8 ± 0.1 vs. FF: 1.2 ± 0.1%).

Exploratory behaviour

RES-piglets had a higher level of exploration towards the feed(er) and drinker compared to FF-piglets at week 1 post-weaning (RES: 2.8 ± 0.3 vs. FF: 2.0 ± 0.2%), but tended to have a lower level of exploration towards the feed(er) and drinker at week 2 post-weaning (RES: 2.1 ± 0.2 vs. FF: 2.6 ± 0.2%). Moreover, RES-piglets spent more time on exploring the environment at week 1 post-weaning compared to FF-piglets (RES: 24.1 ± 1.5 vs. FF: 18.1 ± 1.2%). PL-piglets tended to explore the feed(er) and drinker more at week 2 post-weaning in comparison with CON-piglets (PL: 2.6 ± 0.1 vs. CON: 2.1 ± 0.2%).

Postures and locomotion

RES-piglets tended to show less inactive behaviour compared to FF-piglets at week 1 post-weaning (RES: 42.9 ± 2.3 vs. FF: 48.7 ± 2.3%). The play-feeder did not affect (in)active behaviour after weaning.

Play behaviour

Playing was not affected by treatments.

Pig-directed behaviour

Ear biting was lower for RES-piglets compared to FF-piglets at week 2 post-weaning (RES: 0.4 ± 0.05 vs. FF: 0.7 ± 0.10%). PL-piglets tended to have higher levels of belly nosing compared to CON-piglets at week 1 post-weaning (PL: 0.5 ± 0.2 vs. CON: 0.2 ± 0.1%). The interaction between SF and PF affected aggression at week 1 post-weaning ($P = 0.04$), with less time spent on aggression in FF-CON piglets compared to FF-PL ($P < 0.01$), RES-CON ($P < 0.05$) and RES-PL piglets ($P < 0.05$). No effects were found on nosing pen mates, manipulating pen mates, tail biting and mounting.

Other behaviour

No interaction and main effects of SF and PF were found for the other behaviours after weaning (comfort behaviour: 0.42 ± 0.03%; eliminating: 0.68 ± 0.03%; data not shown).

Body lesions and damage on piglets after weaning

Body lesions in the first two days post-weaning

The number of fresh body lesions was high the first day after weaning, but decreased thereafter with 16.8 ± 1.9, 22.2 ± 2.6 and 11.0 ± 1.8 lesions at 4, 24 and 48 hours post-weaning respectively. Interactions between SF x PF were found on the number of body lesions throughout the first two days after weaning ($P < 0.02$; no interaction with time). RES-piglets had less body lesions than FF-piglets, but only within CON ($P < 0.01$; RES-CON: 10.6 ± 2.2 vs. FF-CON: 23.7 ± 4.8 lesions, with intermediate levels of RES-PL: 10.8 ± 1.8 and FF-PL: 11.1 ± 1.8 lesions).

Body lesions and damage at week 2 post-weaning

On average 4.0 ± 0.4 lesions were found on the body of weaned piglets at two weeks after weaning. PL-piglets had a lower number of body lesions (3.1 ± 0.5 vs. 4.8 ± 0.6 lesions; **Table 3**). Moreover, there were less PL-piglets with wounds on their ears than CON-piglets ($P = 0.02$), but only when reared by FF-sows (PF effect within FF: $P = 0.01$; **Figure 4A**). The percentage of piglets with tail damage was affected by the interaction between SF x PF ($P = 0.02$) and tended to be affected by the play-feeder ($P = 0.05$; **Figure 4B**). RES-PL had less piglets with tail damage than RES-CON ($P < 0.01$, OR = 0.15, 95% CI = 0.04-0.61) and tended to have less piglets with tail damage than FF-PL ($P = 0.06$, OR = 0.26, 95% CI = 0.06-1.05). FF-CON and RES-CON ($P = 0.20$, OR = 1.96, 95% CI = 0.70-5.50) as well as FF-CON and FF-PL ($P = 0.78$, OR = 1.17, 95% CI = 0.40-3.44) did not differ in the proportion of piglets with tail damage.

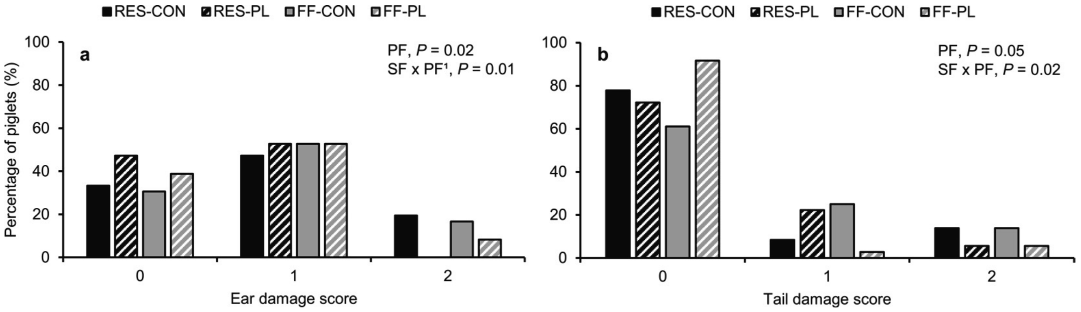

Figure 4. Occurrence of ear (**A**) and tail damage (**B**) (% of piglets with each score) at two weeks post-weaning on piglets reared by restrictedly-fed sows (RES) or full-fed sows (FF) and their creep feed presented in a control feeder (CON) or play-feeder (PL) prior to weaning. SF = sow feeding. PF = piglet feeding. 0: no damage, 1: small bite marks with the size of a pinhead, 2: small wound. Data are expressed as means and were analysed in a Fisher's exact test (ear damage) and generalised linear mixed model with multinomial distribution and cumulative link function (tail damage). ¹Play-feeder effect within FF.

Table 2. Behavioural activities (% of total observations) in the first two weeks after weaning (week 1: 30 days of age, week 2: 38 days of age) of piglets reared by restrictedly-fed sows (RES) or full-fed sows (FF) and their creep feed presented in a control feeder (CON) or play-feeder (PL) prior to weaning. SF = sow feeding. PF = piglet feeding. Data are means ± SEM based on pen averages and were analysed with generalised linear mixed models. Within a row superscripts without a common letter differ at $P < 0.05$.

Behaviour	**RES**		**FF**		**Significance**		
	CON	**PL**	**CON**	**PL**	**SF**	**PF**	**SF x PF**
Week 1 after weaning							
‘Ingestive behaviour’							
Eating feed	13.2 ± 0.7	13.1 ± 0.7	14.2 ± 1.2	13.5 ± 0.9	0.42	0.68	0.74
Drinking	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.2 ± 0.2	0.61	**0.02**	0.54
‘Exploratory behaviour’							
Exploring feed(er) and drinker	2.7 ± 0.3	2.9 ± 0.5	1.9 ± 0.4	2.0 ± 0.3	**0.03**	0.70	0.99
Exploring environment	22.5 ± 2.1	25.6 ± 2.2	18.2 ± 2.4	18.1 ± 1.0	**0.01**	0.43	0.59
‘Postures and locomotion’							
Inactive behaviour	45.0 ± 3.5	40.7 ± 2.9	50.2 ± 4.2	47.2 ± 1.9	**0.08**	0.27	0.84
Standing and walking	6.6 ± 0.6	6.0 ± 0.6	5.9 ± 0.7	7.1 ± 0.8	0.84	0.73	0.21
‘Play behaviour’							
Playing	2.1 ± 0.3	2.3 ± 0.4	2.2 ± 0.4	2.8 ± 0.5	0.68	0.37	0.59
‘Pig-directed behaviour’							
Nosing pen mates	1.4 ± 0.2	1.8 ± 0.3	1.7 ± 0.4	1.7 ± 0.2	0.75	0.36	0.69
Ear biting	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.68	0.48	0.38
Tail biting	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.85	0.85	0.68
Belly nosing	0.1 ± 0.03	0.5 ± 0.3	0.4 ± 0.2	0.5 ± 0.2	0.16	**< 0.10**	0.14
Manipulating pen mates	1.9 ± 0.3	2.2 ± 0.3	1.8 ± 0.4	2.0 ± 0.5	0.53	0.55	0.96
Mounting pen mates	1.2 ± 0.2	1.2 ± 0.3	0.9 ± 0.2	1.2 ± 0.1	0.49	0.52	0.61
Aggression	0.8 ± 0.2^{a}	0.8 ± 0.2^{a}	0.3 ± 0.1^{b}	0.8 ± 0.1^{a}	0.11	**0.04**	**0.04**

Behaviour	RES		FF		Significance		
	CON	PL	CON	PL	SF	PF	SF x PF
Week 2 after weaning							
'Ingestive behaviour'							
Eating feed	10.5 ± 0.6	11.4 ± 0.7	10.4 ± 0.5	12.2 ± 0.8	0.57	**0.01**	0.40
Drinking	0.8 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	1.5 ± 0.2	**0.03**	**0.03**	0.49
'Exploratory behaviour'							
Exploring feed(er) and drinker	2.0 ± 0.3	2.3 ± 0.1	2.3 ± 0.2	3.0 ± 0.2	**0.06**	**0.05**	0.45
Exploring environment	26.0 ± 1.7	25.9 ± 1.9	27.2 ± 1.9	21.7 ± 2.2	0.39	0.13	0.14
'Postures and locomotion'							
Inactive behaviour	46.0 ± 1.7	45.6 ± 2.4	44.0 ± 3.0	45.7 ± 3.9	0.72	0.86	0.76
Standing and walking	4.7 ± 0.4	4.0 ± 0.6	4.5 ± 0.4	5.0 ± 0.6	0.42	0.75	0.26
'Play behaviour'							
Playing	1.8 ± 0.2	1.8 ± 0.2	2.1 ± 0.4	2.4 ± 0.4	0.33	0.53	0.44
'Pig-directed behaviour'							
Nosing pen mates	1.9 ± 0.3	1.3 ± 0.2	1.5 ± 0.2	1.6 ± 0.2	0.84	0.33	0.16
Ear biting	0.4 ± 0.1	0.3 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	**< 0.01**	0.23	0.93
Tail biting	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.93	0.82	0.96
Belly nosing	0.6 ± 0.4	0.4 ± 0.2	0.4 ± 0.2	0.7 ± 0.3	0.66	0.78	0.52
Manipulating pen mates	2.3 ± 0.4	2.7 ± 0.3	2.6 ± 0.4	2.1 ± 0.3	0.67	0.98	0.19
Mounting pen mates	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	0.46	1.00	1.00
Aggression	0.8 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	0.9 ± 0.2	0.88	0.79	0.53

Feed intake, growth and faecal consistency after weaning

Maternal feed restriction and the play-feeder increased feed intake in the first four hours after weaning (RES: 24 ± 4 vs. FF: 17 ± 3 g/piglet, PL: 24 ± 4 vs. CON: 17 ± 3 g/piglet; $P < 0.05$ for both). The treatments tended to interact in their effect on feed intake in this period ($P = 0.08$), showing that RES-PL had a higher intake of feed within the first four hours after weaning compared to the other three treatment groups (RES-CON: 17 ± 3, RES-PL: 31 ± 6, FF-CON: 16 ± 4, FF-PL: 18 ± 4 g/piglet; $P \leq 0.01$ for comparisons). Maternal feed restriction and the play-feeder during lactation increased the ADFI (**Figure 5A**) and ADG (**Figure 5B**) after weaning, with the effect of the play-feeder lasting until the end of the experiment. Maternal feed restriction improved the intake of feed on the first day (RES: 101 ± 11 vs. FF: 32 ± 7 g/day), the second day (RES: 210 ± 10 vs. FF: 114 ± 15 g/day) and between d2-5 post-weaning (RES: 282 ± 9 vs. FF: 237 ± 15 g/day; **Figure 5A**). The play-feeder improved the daily intake of feed on the second day (PL: 188 ± 14 vs. CON: 136 ± 18 g/day), between d2-5 (PL: 286 ± 10 vs. CON: 232 ± 13 g/day), between d5-15 (PL: 405 ± 14 vs. CON: 360 ± 15 g/day) and during the two weeks after weaning (PL: 345 ± 11 vs. CON: 300 ± 12 g/day; **Table 3**).

Interactions between SF x PF were found for ADG on the first and second day after weaning (**Figure 5B**). Maternal feed restriction improved ADG between d0-2 post-weaning (RES: 128 ± 17 vs. FF: -32 ± 21 g/day; $P < 0.0001$) and tended to improve ADG over the two weeks after weaning (RES: 283 ± 9 vs. FF: 263 ± 9 g/day; **Table 3**). RES-piglets did not differ anymore in BW from FF-piglets at d15 post-weaning (RES: 10.18 ± 0.17 vs. FF: 10.43 ± 0.14 kg; **Table 3**). The play-feeder improved ADG between d0-2 (PL: 91 ± 24 vs. CON: 6 ± 27 g/day; $P = 0.001$), d2-5 (PL: 262 ± 12 vs. CON: 222 ± 12 g/day), d5-15 (PL: 342 ± 10 vs. CON: 313 ± 7 g/day; **Figure 5B**) and during the two weeks after weaning (PL: 293 ± 8 vs. CON: 253 ± 7 g/day; **Table 3**). Consequently, PL-piglets had a higher weight than CON-piglets at d15 post-weaning (PL: 10.57 ± 0.13 vs. CON: 10.04 ± 0.16 kg; **Table 3**). The treatments did not affect FCR (**Table 3**). The play-feeder resulted in a shorter duration of diarrhoea (PL: 1.72 ± 0.37 vs. CON: 3.00 ± 0.47 days of diarrhoea during 2 weeks post-weaning), a lower FCS (PL: 0.13 ± 0.03 vs. CON: 0.26 ± 0.04) and a lower prevalence of watery diarrhoea in the weaner pens in the first two weeks post-weaning (PL: 16.7 vs. CON: 61.1% of pens; **Table 3**). SF did not affect the faecal consistency parameters after weaning.

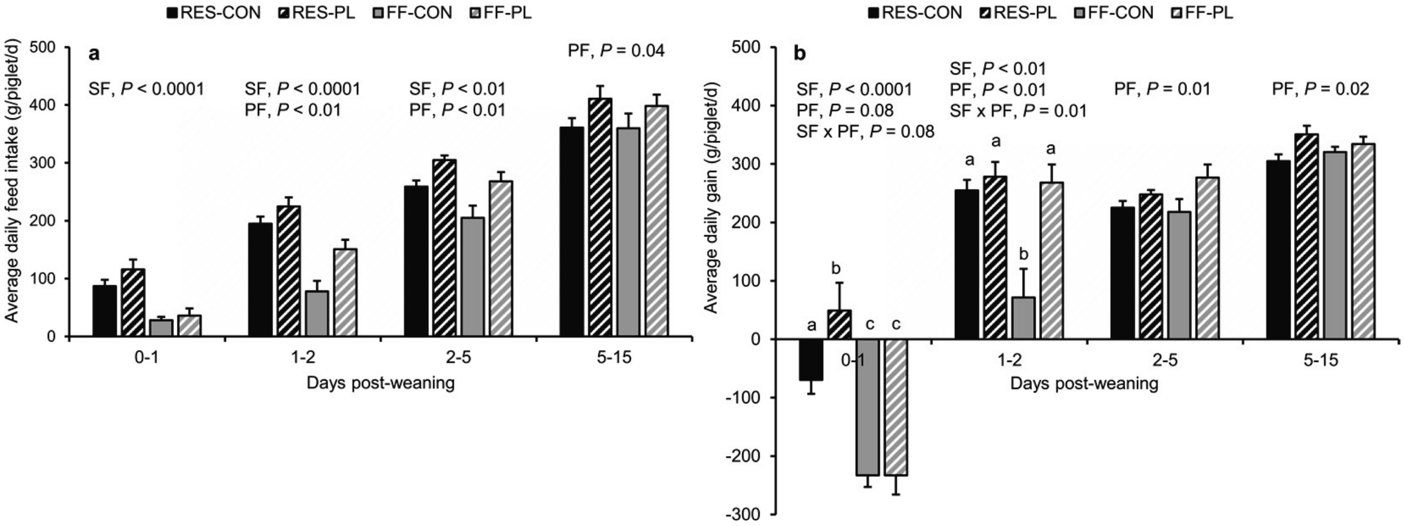

Figure 5. Average daily feed intake (**A**) and average daily gain (**B**) of weaner piglets reared by restrictedly-fed sows (RES) or full-fed sows (FF) and their creep feed presented in a control feeder (CON) or play-feeder (PL) prior to weaning. SF = sow feeding. PF = piglet feeding. Data are expressed as means ± SEM based on pen averages and were analysed with linear mixed models. Within a period superscripts without a common letter differ at $P < 0.05$.

Table 3. Post-weaning performance (d0-15 post-weaning) of piglets reared by restrictedly-fed sows (RES) or full-fed sows (FF) and their creep feed presented in a control feeder (CON) or play-feeder (PL) prior to weaning. SF = sow feeding. PF = piglet feeding. Data are expressed as means ± SEM based on pen averages and were analysed with mixed models. Within a row superscripts without a common letter differ at $P < 0.01$.

	RES		**FF**		**Significance**		
Variable	**CON**	**PL**	**CON**	**PL**	**SF**	**PF**	**SF x PF**
ADFI, d 0 – 15, g/piglet/d	311 ± 12	358 ± 15	288 ± 20	332 ± 16	0.12	**< 0.01**	0.93
ADG, d 0 – 15, g/piglet/d	261 ± 10	305 ± 11	246 ± 11	281 ± 12	**0.06**	**< 0.001**	0.61
FCR, d 0 - 15	1.20 ± 0.05	1.17 ± 0.01	1.16 ± 0.04	1.19 ± 0.04	0.75	0.95	0.46
BW at d 15, kg	9.73 ± 0.22	10.63 ± 0.13	10.35 ± 0.19	10.51 ± 0.23	0.27	**0.02**	0.11
Diarrhoea, days/pen	2.78 ± 0.72	2.00 ± 0.65	3.22 ± 0.64	1.44 ± 0.38	0.74	**0.04**	0.37
% of pens with ≥ 1 day watery diarrhoea	44.4	22.2	77.8	11.1	0.69	**0.01**	0.18
Faecal consistency score	0.23 ± 0.06	0.16 ± 0.06	0.28 ± 0.05	0.10 ± 0.03	0.94	**0.02**	0.28
Body lesions at d 15	4.7 ± 1.2	3.0 ± 0.7	4.8 ± 0.5	3.3 ± 0.8	0.35	**0.02**	0.91

Discussion

A low lactational feeding level of the sow was used to reduce the energy intake of piglets from milk and to investigate whether creep feed intake is homeostatically driven, while a play-feeder was used to present creep feed in an explorative and playful context and to study whether creep feed consumption is exploratory driven. Maternal feed restriction motivated piglets to eat creep feed and thereby eased the weaning transition, as reflected by a higher feed intake and growth and less body lesions, mainly in the first two days after weaning. Although the play-feeder did not improve creep feed intake of piglets, it stimulated feeder exploration before weaning and had a broad and long-term beneficial effect on ingestive behaviours, feed intake, growth, faecal consistency and body lesions and damage in the two weeks after weaning.

Consistent with predictions, maternal feed restriction by 50% resulted in a 16% lower ADG of piglets during lactation and a 12% lower weaning weight. Previous studies showed that a low lactational feeding level of the sow reduced energy supply from milk, due to a lower milk production and changes in milk composition (Verstegen et al., 1985; Van den Brand et al., 2000), resulting in a lower gain of piglets (Verstegen et al., 1985; Van den Brand et al., 2000; Sulabo et al., 2010a). Indeed, RES-sows used in this study had a lower calculated milk production and a lower milk fat percentage, as determined by milk sample analysis (Costermans et al., under review). Litters of RES-sows consumed three times as much feed during the last two days before weaning, doubled their time spent eating one day before weaning and had double as much eaters at weaning compared to litters of FF-sows. These findings disagree with the results of Sulabo et al. (2010a), in which maternal feed restriction stimulated creep feed intake only during the first days after its implementation and also did not stimulate more piglets to eat creep feed; however, in their study RES-sows were fed 25% less than FF-sows and therefore weaning weight was only 6% less. Hunger as homeostatic drive was likely the biggest motivator for RES-piglets to eat creep feed. Alternatively, it cannot be excluded that potential behavioural changes of the sow occurring as result of feed restriction (Appleby and Lawrence, 1987; Terlouw et al., 1991; Spoolder et al., 1995) may have influenced the eating behaviour of RES-piglets. Our results support that the compensatory feeding hypothesis at least holds on litter level, as RES-piglets grew less during lactation and consumed more creep feed towards weaning. Even though creep feed consumption by RES-piglets was only enhanced for a short period (from two days prior to weaning) and by a small amount (34 g/piglet) and creep feed intake was still generally low by then (25 g/piglet/d), RES-piglets did eat 60 g/d more in the first five days post-weaning and did grow 160 g/d faster in the first two days post-weaning (when they ate 83 g/d more) compared to piglets reared by FF-sows. RES-litters had 22% more eaters of creep feed at weaning than FF-litters, which may also have stimulated post-weaning feed intake via social learning, as non-eaters can learn about solid feed from eaters (Morgan et al., 2001). However, as the ADG of RES-piglets in the first two days after weaning was two times larger than the ADFI of RES-piglets in the same period, there seems to be an effect on growth beyond the energy intake from feed. Piglets may have experienced lower levels of stress and/or neophobia due to a more gradual dietary change (Bolhuis et al., 2009). Moreover, RES-piglets had fewer body lesions than FF-piglets within CON after mixing post-weaning and spent less time ear biting at 2 weeks post-weaning than FF-piglets. A lower time spent biting pen mates and a lower number of injuries from aggression might also be related to a reduced level of stress (Schrøder-Petersen and Simonsen, 2001; McGlone and Anderson, 2002; Munsterhjelm et al., 2013), but RES-piglets may also have fought less after mixing to save energy and, instead, engage in exploratory and ingestive behaviour shortly after weaning. Benefits of creep feed intake during lactation on feed intake and growth of piglets in the initial weaner period have been demonstrated previously (Bruininx et al., 2002, 2004; Kuller et al., 2007a; Sulabo et al., 2010a). Getting piglets to eat creep feed is therefore important to reduce the post-weaning dip in performance. We showed that the energy intake of piglets from milk is one of the fundamental factors that influences their creep feed intake, but we do not recommend maternal feed restriction as a feeding strategy to improve creep feed intake and to facilitate the weaning transition of piglets, as feed restriction is detrimental for piglet welfare (Pastorelli et al., 2012), sow welfare (Appleby and Lawrence, 1987) and sow future reproductive performance (Sulabo et al., 2010a; De Bettio et al., 2016; Costermans et al., 2019). This study illustrated that RES-piglets were able to compensate growth once the nutritional restrictions were eliminated at weaning, as RES-piglets attained the BW of FF-piglets at two weeks post-weaning.

In line with our prediction, the play-feeder was successful in eliciting and sustaining exploratory behaviour in suckling piglets, as the time spent on exploration towards the feeder was increased by 3.5 to 6 times, as well as the number of piglets visiting the feeder by 24 to 34%. Thereby, the play-feeder in our study seemed to stimulate exploratory behaviour more elaborately than the exploration-stimulating feeder of Kuller et al. (2010), probably because it allowed object play, object manipulation (e.g. objects were chewable, moveable) and a larger repertoire of foraging behaviours. We found that time spent exploring the feeder correlated with time spent eating (at d9, 16 and 23) and with feed intake (at d9 and 16, but not at d23), suggesting that exploratory behaviour may be mainly important in familiarising piglets with creep feed before actual ingestion starts. Yet, we could not confirm a clear positive effect of the play-feeder on creep feed intake on litter level, as was observed by Kuller et al. (2010), as time eating creep feed, the amount of feed consumed and the percentage of eaters did not significantly differ between the creep feed presentation strategies. However, the play-feeder increased the number of piglets in better eater classes, but only when reared by RES-sows. Moreover, RES-PL ate the most during the first four hours after weaning and grew the fastest during the first day after weaning compared to the other three treatment groups. In addition, FF-CON had more body lesions due to aggressive interactions after mixing than RES-CON and tended to have more lesions than RES-PL and FF-PL. This corresponds to the observation that FF-CON also had significantly the lowest ADG on the second day after weaning compared to the other three groups. The play-feeder thus seemed to attract especially the piglets of sows that had a low lactational feed intake and may therefore be particularly useful in practise to support litters of sows with poor milk production due to e.g. illness or heat stress.

Despite the play-feeder only subtly affected individual creep feed classification compared to feed restriction of the sow, the play-feeder showed greater, broader and longer-term beneficial post-weaning effects, as ingestive behaviours, feed intake and growth of PL-piglets were significantly improved by 15% for (at least) two weeks post-weaning, resulting in a 5% higher weight at d15 post-weaning. The play-feeder also reduced the prevalence (by 44%), duration (by 1.3 days) and severity of diarrhoea (by halving the FCS), and decreased the number of body lesions (by 35%), piglets with ear damage within FF, and piglets with tail damage within RES at d15 post-weaning. A similar effect was observed by Telkänranta et al. (2014) in which growing pigs with pre-weaning experience of ropes and paper had a reduced prevalence of severe tail damage. It has been hypothesized that a higher feed intake and growth, an increase in faecal consistency and a decrease in maladaptive behaviour of weaner piglets are related to each other and may all result from lower levels of stress and/or neophobia at weaning (Fraser, 1978; Bolhuis et al., 2009; Hötzel et al., 2011). Firstly, PL-piglets might have been better acquainted with the sight and smell of the nursery feed (that was mixed with the creep feed during the last two days prior to weaning) compared to CON-piglets, as result of a higher feeder visiting time and more piglets visiting, subsequently resulting in reduced neophobia and a higher intake of that feed after weaning (Nicol and Pope, 1994). Secondly, we speculate that PL-piglets might have developed a positive association between (the smell of) solid feed and object play. Social play has a pleasurable and rewarding nature through opioid, endocannabinoid, dopamine and noradrenaline systems (Vanderschuren et al., 2016) and it has been suggested that object play may also be self-rewarding (Nahallage et al., 2016). Thirdly, we predict that the higher frequency of manipulating behaviours on the toys of the play-feeder, such as chewing, might have facilitated the transition from exploratory to eating behaviour (e.g. via stimulation of mastication muscles (Herring, 1985; Popowics and Herring, 2006), changes in gut hormone secretion (Xu et al., 2015) and reward circuits responses (Ikeda et al., 2018) or habituation to a dry mouthfeel), but only became evident when their need to consume solid feed increased, either by weaning or maternal feed restriction. Lastly, the provision of play-objects before weaning, thereby eliciting an early play experience for piglets, might also improve their ability to emotionally cope with weaning according to the ‘training for the unexpected hypothesis’ (Špinka et al., 2004). However, the latter hypothesis alone seems insufficient to explain the broad effects we found on piglet’s ability to deal with the weaning process, as providing (non-edible) play-objects in the farrowing pen as early life enrichment (of which cotton ropes are mostly studied) only limitedly affected later life performance (Telkänranta et al., 2014; Seddon et al., 2015; Yang et al., 2018). That is why we expect the association between exploration/play and feed to be important in eliciting beneficial effects and therefore recommend play-objects to be located at the feeder instead of else in the farrowing pen. Using play-objects at the creep feeder showed positive effects for the welfare of piglets, as it increased the frequency of normal exploratory and object play behaviour and increased their ability to cope with weaning in a broad sense, despite that the play-feeder was only provided before weaning. We predict the beneficial effect of the play-feeder on post-weaning adaptation to be even more substantial when the play-feeder is also provided after weaning, however this remains to be studied.

Conclusion

In conclusion, litters reared by restrictedly-fed sows grew less, had more eaters and ate more before weaning, suggesting that pre-weaning feed intake is at least partly homeostatically driven. This pre-weaning acquaintance with feed improved feed intake and growth in the first days after weaning. The play-feeder, which was only provided before weaning, encouraged piglets to explore and play with (the toys of) the feeder and sustained piglets’ interest up to weaning. The main finding of this study was that the play-feeder had no effect on creep feed intake in itself, but remarkably eased the weaning transition, as reflected in improved post-weaning feed intake and growth and reduced diarrhoea and body damage. This implies that providing toys at the creep feeder can be considered as an easy applicable enrichment and feeding strategy to reduce weaning stress and improve the health, welfare and productivity of piglets around weaning. The mechanism(s) underlying this beneficial effect are not yet well understood and deserve further attention.

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Supplementary data

Supplementary Video S1. Available online. https://doi.org/10.1038/s41598-019-52530-w

Supplementary Table S2. Nutrient profile of the creep feed.

Calculated nutrient composition[1]	Creep feed
Dry matter	891
Starch	290
Non-starch polysaccharides[2]	261
Crude protein	195
Crude fat	61
Crude fibre	44
Crude ash	57
Calcium	9.1
Phosphorus	6.1
Sodium	2.2
Standardized ileal digestible lysine	11.9
Standardized ileal digestible methionine	4.8
Standardized ileal digestible threonine	7.1
Standardized ileal digestible tryptophan	2.4
Net energy	11.8

[1] According to CVB (2007). Nutrients are presented in g/kg dry matter, except for dry matter (g/kg) and net energy (MJ/kg)

[2] Calculated as the difference between dry matter and the sum of starch, sugars, crude protein, crude fat and crude ash

Supplementary Table S3. Ingredient composition of the creep feed.

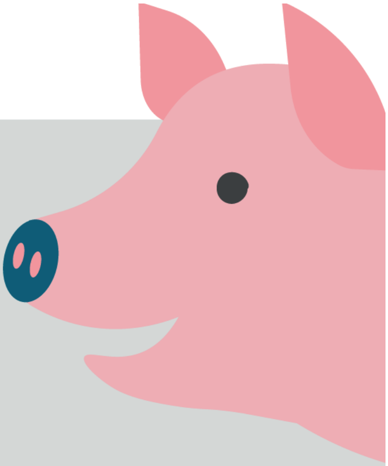
Ingredient component	**%**
Wheat	21.9
Barley	15
Maize	15
Soy protein concentrate	7
Soybeans (heat treated)	5
Galacto-oligosaccharides	5
Potato protein	4
Sugarbeet pulp (dehydrated)	4
Oat hulls	4
Inulin	4
High-amylose starch (± 75% amylose)	4
Soybean oil	3
Blood meal (spray dried)	2
Dicalcium phosphate	1.7
Sucrose	1.5
Calcium carbonate	1.0
Sodium chloride	0.5
Premix[1]	0.5
Potassium bicarbonate	0.3
L-lysine hydrochloride	0.3
DL-methionine	0.2
L-threonine	0.04
L-tryptophan	0.04
Total	**100**

[1] Vitamin and mineral premix (per kg of feed): vitamin A: 10000 IU, vitamin D3: 2000 IU, vitamin E: 40 mg, vitamin K: 1.5 mg, vitamin B1: 1 mg, vitamin B2: 4 mg, vitamin B6: 1.5 mg, vitamin B12: 0.02 mg, niacin: 30 mg, D-pantothenic acid: 15 mg, choline chloride: 150 mg, folate: 0.4 mg, biotin: 0.05 mg, iron: 100 mg, copper: 20 mg, manganese: 30 mg, zinc: 70 mg, iodine: 0.7 mg, selenium: 0.25 mg, anti-oxidant: 125 mg

Supplementary Table S4. Behaviours of piglets after weaning.

Behaviour	Description
'Ingestive behaviour'	
Eating feed	Eating or chewing feed (at the feeder)
Drinking	Drinking water from drinking trough
'Exploratory behaviour'	
Exploring feeder	Sniffing, touching (with snout), rooting or chewing on feeder
Exploring feed	Sniffing, touching (with snout) or rooting the feed in the feeder
Exploring drinking trough	Sniffing, touching (with snout) or chewing on drinking trough
Nosing environment	Sniffing, touching (with snout) part of the pen (e.g. floor, wall)
Rooting environment	Rooting part of the pen, scraping floor with one the front legs
Chewing environment	Chewing or nibbling part of the pen, including toy
Chewing air	Chewing air (not chewing on part of the pen, feed or toy)
'Postures and locomotion'	
Lying eyes closed	Lying on side or belly with eyes closed without performing any other described behaviour
Lying eyes open	Lying on side or belly with eyes open, sitting or kneeling without performing any other described behaviour
Standing	Piglet is upright, standing, without performing any other described behaviour
Walking	Piglet is walking, without performing any other described behaviour
'Play behaviour'	
Playing individually	Play activities that involve one player; running across pen, rolling, pivoting, tossing head, flopping, sliding, scampering, nudging
Playing socially	Play activities that involve more players; e.g. running, pivoting, scampering, sliding across pen together, play fighting
Playing with toy	(Energetically) shaking head with toy in mouth
'Pig-directed behaviour'	
Nosing body	Sniffing, touching (with snout) part of the body of a pen mate excluding snout
Snout contact	Mutual snout contact with pen mate
Ear biting	Chewing, nibbling or sucking the ear of a pen mate
Tail biting	Chewing, nibbling or sucking the tail of a pen mate
Belly nosing	Rubbing belly of a pen mate with ≥ 3 up and down movements of the snout or sucking the navel or skin of the abdominal area of a pen mate
Manipulating pen mates	Chewing, nibbling or sucking part of the body of a pen mate excluding ear, tail and abdominal area
Mounting pen mates	Standing on hind legs while having front legs on pen mate
Aggression	Aggressively ramming, pushing, head-knocking, lifting or biting a pen mate, including mutual fighting
'Other behaviour'	
Comfort	Rubbing body against objects or pen mates, scratching body with hind legs or stretching (part of) body
Eliminating	Defaecating or urinating

CHAPTER 6



Dietary diversity affects feeding behaviour of suckling piglets

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Abstract

Stimulating solid feed intake in suckling piglets is important to facilitate the weaning transition, exemplified by the positive correlation between pre- and post-weaning feed intake. The present study compared the effect of dietary diversity (i.e. offering two feeds simultaneously) and flavour novelty (i.e. regularly changing the flavour of one feed) on the feeding behaviour and performance of suckling piglets until weaning at day 22. It was hypothesized that varying multiple sensory properties of the feed, by presentation of the feed in a more diverse form, stimulates pre-weaning feed intake. Piglets received *ad libitum* feed from 2 days of age in two feeders per pen (choice feeding set-up). One group of piglets (dietary diversity (DD), n = 10 litters) were given feed A and feed B which differed in production method, size, flavour, ingredient composition and nutrient profile, smell, texture and colour. The other group of piglets (flavour novelty (FN), n = 9 litters) received feed A plus feed A to which one of 4 flavours were added from day 6 in a daily sequential order. Feeding behaviour was studied by weighing feed remains (d6, 12, 16, 22) and by live observations (4-min scan sampling, 6h/d; d9, 14, 21; n = 6 litters per treatment). Observations were also used to discriminate ‘eaters’ from ‘non-eaters’. All piglets were weighed at d2, 6 and 22. Piglets did not prefer feed A (d2-22: 1.4 ± 0.16 kg/litter) over B (1.6 ± 0.18 kg/litter) within DD nor had a preference for feed A with (d6-22: 1.1 ± 0.06 kg/litter) or without additional flavours (0.9 ± 0.07 kg/litter) within FN. Nevertheless, DD-litters (d2-22: 3.0 ± 0.32 kg) ate significantly more than FN-litters (2.0 ± 0.12 kg; $P = 0.02$) and explored the feed 2.6 times more at d14 ($P = 0.001$). Furthermore feed A, the common feed provided in DD and FN, was more consumed in DD (d2-22: 1.4 ± 0.16 kg) compared to FN (1.0 ± 0.07 kg; $P = 0.04$). The percentage of eaters within a litter did not differ over time between DD (d9: 26%, d14: 78%, d21: 94%) and FN (20%, 71% and 97%) and no effect was found on pre-weaning weight gain. In conclusion, this study showed that provision of dietary diversity to suckling piglets stimulated their feed exploration and intake more than dietary flavour novelty only, but did not enhance the percentage of piglets within a litter that consume the feed or their growth performance. These data suggest that dietary diversity could be an innovative feeding strategy to stimulate solid feed intake in suckling piglets.

Highlights

- We studied an innovative feeding strategy to increase solid feed intake pre-weaning
- Dietary diversity stimulated feed intake of suckling piglets more than flavour novelty
- The percentage of eaters was not affected, meaning a higher feed intake per piglet
- Our results support that the more diverse the feeds are, the greater their intake
- Intrinsic exploration and sensory-specific satiety may underlie this

Implications

This study indicates that provision of diverse solid feed types (i.e. varying in multiple sensory properties) before weaning can enhance feed exploration and intake by suckling piglets compared to solid feed types that vary in flavour only. Piglets with a high uptake of solid feed before weaning have been shown to outperform piglets with a low pre-weaning uptake of solid feed initially after weaning in terms of feed intake and growth performance (Carstensen et al., 2005; Pluske et al., 2007). As such, pre-weaning dietary diversity may benefit post-weaning piglet (gut) health, welfare and performance.

Introduction

In conventional pig farming, piglets are removed from the sow at 3 to 4 weeks of age. Piglets weaned early and abruptly are challenged with numerous concurrent stress factors such as changes in social structure, environment and diet. The latter includes deprivation of sow's milk and a change to a weaner diet, which usually consists of solid feed. Weaning-related stress is associated with a delayed and low feed intake in the initial post-weaning period (Bruininx et al., 2002, 2004). The combination of stress, acute fasting, shift in diet physical form and subsequent introduction of novel food antigens at weaning results in undesirable changes in gut morphology and microbiota, thereby increasing the risk for maldigestion and absorption, enteric pathogen colonization, post-weaning diarrhoea and growth stasis, as reviewed by Heo et al. (2013). The physical form and composition of the post-weaning diet also play a crucial role on gut health for newly weaned pigs (Sander et al., 2012; Torrallardona et al., 2012).

Creep feed is an optional provision for suckling piglets to familiarise them with solid feed prior to weaning. There is evidence that the consumption of solid feed during lactation has a positive effect on solid feed intake in the initial post-weaning period and growth performance of piglets around weaning (Bruininx et al., 2002, 2004; Kuller et al., 2007a; Sulabo et al., 2010a). Moreover, these effects are especially pronounced in piglets with an early uptake of creep feed (Klindt, 2003; Van den Brand et al., 2014) and/or a high creep feed consumption level (Bruininx et al., 2004; Carstensen et al., 2005; Pluske et al., 2007). The latter is supported by the highly positive correlation between feed intake pre-weaning and feed intake and growth initially after weaning (Kuller et al., 2004; Berkeveld et al., 2007b). However, such studies have also shown that the creep feeding behaviour of conventional suckling piglets is still immature: first, a significant and highly variable proportion of piglets starts to consume creep feed only relatively shortly before weaning or fails to consume any creep feed until weaning (e.g. Pluske et al., 2007; Tucker et al., 2010; Van der Meulen et al., 2010); and second, creep feed consumption by suckling piglets is low, unpredictable and variable between and within litters (e.g. Pajor et al., 1991; Bruininx et al., 2002, 2004; Carstensen et al., 2005). It is therefore important to find strategies that initiate early creep feed intake, stimulate the number of piglets consuming the creep feed, and enhance its consumption level to create more robust piglets around weaning.

In a (semi-)natural environment, the development of feeding behaviour already starts on the first days of a piglet's life by digging soft soil and exploration of feed and non-feed substrates by rooting, nosing, chewing and biting (Gundlach, 1968; Petersen, 1994). Pigs are opportunistic and omnivorous feeders and known to consume an extensive variety of food items, ranging from plant material, like nuts, roots, seeds, tubers and products of animal origin like earthworms (Hanson and Karstad, 1959; Pinna et al., 2007). Suckling piglets thereby encounter a variety of (novel) food items under (semi-)natural conditions and have been observed sampling leaves, mushrooms, acorns and corn (Gundlach, 1968; Meynhardt, 1980; Petersen, 1994). In contrast, conventional suckling piglets are mostly offered a single diet. We hypothesize that presentation of the creep feed in a more diverse and/or novel form stimulates their exploratory and feeding behaviour.

Dietary variety consists of feeds that differ in at least one sensory property (Raynor and Epstein, 2001), of which flavour is mostly studied. Dietary variety, either simultaneous or successive, has been shown to alter feeding behaviour and increase feed intake in humans (Rolls et al., 1981), rats (Rolls et al., 1983; Treit et al., 1983) and sheep (Distel et al., 2007; Villalba et al., 2011). Similar effects have been found recently in suckling piglets as well (Adeleye et al., 2014). These studies indicate that varying one sensory property of the feed (e.g. flavour) can already have a stimulatory effect on feed intake. It is hypothesized however, that the more diverse the feeds are, the more rewarding it is to switch between them and to consume more in total (Rolls et al., 1981). Our study thus aimed to compare the effect of dietary diversity (i.e. offering two feeds simultaneously) and flavour novelty (i.e. regularly changing the flavour of one feed) on the feeding behaviour and performance of suckling piglets.

Materials and methods

Animals and housing

The Animal Care and Use committee of Wageningen University & Research (Wageningen, the Netherlands) approved the protocol of the experiment. Top Pi x Topigs-20 piglets (both sexes) from 19 multiparous sows (range parity: 1 to 7) were used in a two-choice feeding set-up. About one week before farrowing, the sows were moved to two adjacent farrowing rooms and were housed in individual conventional pens (2.2 x 2.0 m) without bedding material. The pen was equipped with a farrowing crate including feed trough, drinking nipple and a metal chain with ball for the sows (not accessible to the piglets) and a drinking nipple for the piglets. Sows were fed a commercially available diet twice a day. The pen consisted of 80% slatted floor and 20% solid floor, with an infrared lamp above it, as a piglet nest area. At one day of age piglets were weighed, received an ear tag, received an intramuscular iron injection of 1 cc, and were tail docked and teeth clipped. Within 2 days after birth, litter size was standardized to 13-15 piglets per litter by cross-fostering. At 5 days of age, male piglets were castrated. Piglets were vaccinated against Mycoplasma, Circo and *E. coli* at 6 days before weaning at 22.3 ± 0.05 days of age. Room temperature was 25 °C around farrowing and was gradually decreased to 22 °C until weaning. Artificial lighting was provided between 07:00 and 18:00 h.

Dietary treatment

Piglets received feed *ad libitum* from 2 days of age in two concrete round creep feed bowls (diameter of 21 cm), each having four feeding places, per pen. The amount of creep feed in the feed bowls was checked at least twice daily to prevent the bowls of getting empty. To minimize spillage of creep feed, the bowls had partitions and were attached to the solid floor of the pen, each positioned at one side of the piglet nest area. The position of the bowls was switched on a daily basis within litters to ensure that feed intake was not affected by feeder position preference.

One group of piglets (dietary diversity, **DD**, n = 10 litters) were given feed A (experimental diet, Animal Nutrition Group, Wageningen University & Research, Wageningen, the Netherlands) and feed B (commercial diet, Baby Big XL, Coppens Diervoeding, Helmond, the Netherlands) which differed in production method, size, flavour, ingredient composition and nutrient profile, smell, texture and colour (**Supplementary Figure S1, Supplementary Table S1** and **S2**). The feeds were provided in separate bowls from 2 days of age onwards. Feed A was an 8-mm diameter pellet mixed by Research Diet Services (Wijk bij Duurstede, the Netherlands) and extruded using a co-rotating double screw extruder (M.P.F. 50, Baker Perkins, Peterborough, UK). Extruder settings intendedly varied during production, resulting in differences in pellet texture, length (8-22 mm) and hardness (7.3-17.7 kg) to create diversity within feed A. Feed B was a 14-mm diameter pellet, with a length of 10-20 mm and a hardness of 6.8 kg. Feed B could not pass the slats in intact form in comparison to feed A. Pellet hardness was measured with a Kahl pellet hardness tester (Amandus Kahl Nachf, Reinbek, Germany) according to Thomas and Van der Poel (1996) using 10 pellets for feed B and 10 pellets per production setting for feed A.

The other group of piglets (flavour novelty, **FN**, n = 9 litters) received feed A only in both bowls from 2 days of age. From day 6 of age flavours (i.e. substances to influence the sensory perception of the feed as related to its taste and smell) were added to feed A in one bowl in a daily sequential order. The flavours were mixed through the feed at a predetermined rate according to the manufacturer's advice and small human flavour tests. The flavours were anise (0.08 g/kg), vanilla (0.35 g/kg), red fruit (0.5 g/kg) and an essential oil mixture (EOM, 0.4 g/kg) (Provimi, Cargill Animal Nutrition, Rotterdam, the Netherlands). The main components of the EOM are essential oil compounds from cinnamon, clove and oregano. Over the lactation period, each flavour was fed four times, once in each of four 4-day blocks.

Litters were allotted to one of two treatment groups by sow's parity (DD: 3.6 ± 0.5, range: 2 to 7; FN: 3.4 ± 0.6, range: 1 to 7) and average weight of the litter at day 1 of age (DD: 1.3 ± 0.06 kg/piglet; FN: 1.4 ± 0.06 kg/piglet) and treatment groups were randomly distributed within farrowing rooms. DD-sows had litters of 14.0 ± 0 piglets and FN-sows had litters of 14.0 ± 0.17 piglets (range: 13 to 15) at the start of dietary treatments. One piglet died after allocation to the treatments. Weaning age did not differ between treatment groups (DD: 22.5 ± 0.06 days of age; FN: 22.2 ± 0.09 days of age).

Measurements

Piglet performance

Piglets were individually weighed at 2 days of age (before commencing creep feeding), at 6 days of age (before commencing flavour novelty in FN) and at 22 days of age (at weaning). Creep feed intake was determined per pen per feed type (in grams) at day 6, 12, 16 and 22 for DD-litters and daily from day 6 onwards for FN-litters. This was done by weighing feed remains in the feed bowl and on the floor. The intake per feed type was also calculated as a percentage of the total feed intake to determine the proportional intake of the feed types.

Behaviours

A subset of litters (n = 11, from one room) was used to study feed-related behaviours. Piglets were marked (from 1 to 14 per litter) the day before observations using dark permanent hair dye. Live behavioural observations were done at 9, 14 and 21 days of age using 4-min instantaneous scan sampling for 6 sessions of one hour per day (i.e. 90 scans per piglet per day). Observations were performed in the morning from 8:15 to 9:15 h, 9:30 to 10:30 h, 10:45 to 11:45 h and in the afternoon from 13:45 to 14:45 h, 15:00 to 16:00 h and 16:15 to 17:15 h. Feeding behaviours were scored by two observers using a Psion hand-held computer with the Pocket Observer 3.1 software package (Noldus Information Technology, Wageningen, the Netherlands). The ethogram is given in **Table 1**. Observations were also used to discriminate ‘eaters’, i.e. piglets scored eating creep feed (from the bowl and/or floor) at least once, from ‘non-eaters’ per observation day. The percentage of eaters was calculated by dividing the number of eaters per litter by the total number of piglets in the same litter at that observation day. In addition, eaters were grouped into different eater classes (i.e. good, moderate and bad) after Collins et al. (2013). Piglets that were observed eating on all 3 observation days (day 9, 14 and 21 of age) were classified as ‘good eaters’. ‘Moderate eaters’ were observed eating on 2 out of 3 observation days and ‘bad eaters’ were observed eating only 1 out of 3 observation days. Piglets that were never seen eating were classed as ‘non-eaters’. If a piglet was scored as eater, it was also investigated which feed types it consumed throughout lactation.

Table 1. Feeding behaviours of piglets during the suckling period.

Behaviour	**Description**
‘Feed-related exploratory behaviour‘	
Exploring feed (bowl)	Sniffing, touching or rooting creep feed in the bowl or sniffing, touching, rooting or chewing on feed bowl
Exploring feed on floor	Sniffing or touching creep feed on the floor
Playing with feed	Rolling creep feed item over floor, walking around the pen with feed item, shaking head while having feed item in mouth
Exploring sow feed	Sniffing or touching feed spilled by the sow on the floor
Exploring sow trough	Sniffing, touching, rooting or chewing on feed trough of sow
‘Ingestive behaviour’	
Eating	Eating or chewing creep feed at the feed bowl
Eating feed from floor	Eating or chewing creep feed from the floor (eaten outside the feed bowl)
Eating sow feed	Eating or chewing feed spilled by the sow on the floor
Drinking	Drinking from drinking nipple
Exploring drinking nipple	Sniffing or touching drinking nipple
‘Suckling behaviour’	
Massaging udder	Massaging udder with head/nose (up-and-down movements)
Suckling	Drinking milk from teat of sow (soft suckling noises)

Statistical analyses

Data were analysed with the statistical software SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Behavioural variables were expressed as proportions of time. Exploring feed (bowl), exploring feed on the floor and playing with feed were pooled into 'exploring creep feed'. Eating and eating feed from the floor were merged into 'eating creep feed'. The behaviours exploring sow feed and eating sow feed were combined into 'interest in sow feed'. Exploring sow trough was excluded from analyses as this behaviour might indicate exploration towards the environment and not exploration towards sow feed per se. To investigate 'interest in water', behaviours drinking and exploring drinking nipple were combined. Model residuals were checked for normal distribution. Feed intake data were square root transformed and behavioural data were arcsine square root transformed if needed to meet the assumption of normality. Correlations between feed intake, time spent eating and time spent exploring the feed were calculated at litter level using Spearman's correlation coefficients (PROC CORR). Performance and behavioural data were analysed using repeated-measure mixed models (PROC MIXED). Differences at $P < 0.05$ were considered statistically significant and differences at $0.05 \leq P < 0.10$ were considered as trend.

Effects of dietary treatment

Models for behaviour included the fixed effects of dietary treatment (DD vs. FN), day and their interaction, with piglet (nested within pen and dietary treatment) as experimental unit and pen (nested within dietary treatment) as random effect. Pen was the experimental unit for analyses of feed intake and the percentage of eaters. To study the effect of dietary treatment on body weight gain (day 2-22) during the suckling period dietary treatment was used as fixed effect and pen (nested within dietary treatment) as random effect. Moreover, a Fisher's exact test (PROC FREQ) was performed to test whether eater classification was affected by dietary treatment.

Effects of feed type within dietary treatment

To study effects on behaviour and feed intake within dietary treatment, feed type (A vs. B in DD and A vs. A + flavours in FN), day and their interaction were used as fixed effects. Furthermore, to test flavour preferences within FN, the daily intake of each of the four flavoured feeds per pen (after correcting for the total feed intake on that day) was analysed including flavour (anise, vanilla, red fruit, EOM), 4-day block (day 6-10, 10-14, 14-18, 18-22 of age) and their interaction as fixed effects.

Significant fixed effects were further analysed using differences of least squares means, with Tukey adjustment for multiple comparisons. Feed intake data were also analysed per period (2-6, 6-12, 12-16 and 16-22 days of age) and over the whole suckling period (2-22 days of age) using mixed models with dietary treatment or feed type as fixed effect. (Untransformed) data are presented as means ± SEM (based on pen averages for body weight (gain) and behavioural variables).

Results

Piglet performance

Irrespective of dietary treatment, feed intake ($P < 0.0001$) increased with age. Feed intake highly correlated with time spent eating at litter level (r = 0.91; $P < 0.0001$), but there was no correlation between feed intake and time spent exploring the feed (r = -0.14; $P = 0.45$).

Effects of dietary treatment

DD-litters (14.0 ± 0 piglets) ate more than FN-litters (14.0 ± 0.17 piglets) in the two weeks before weaning (**Figure 1**). Total feed intake during lactation also differed between DD and FN-litters (DD: 3.0 ± 0.32 kg vs. FN: 2.0 ± 0.12 kg/litter; $P = 0.02$) and varied between individual litters (DD: range 1.9-4.7 kg; FN: 1.6-2.7 kg/litter). Dietary treatment, however, did not affect body weight gain from d2-22 (DD: 4.7 ± 0.11 kg vs. FN: 4.6 ± 0.17 kg/piglet; $P = 0.71$). At weaning, DD-piglets weighed 6.2 ± 0.13 kg and FN-piglets weighed 6.2 ± 0.21 kg.

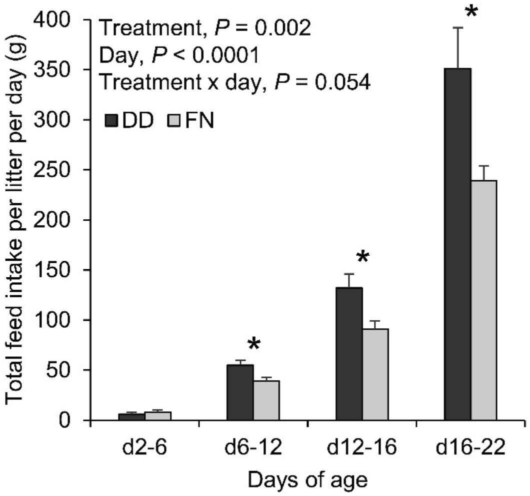

Figure 1. Total feed intake (g) per day of litters offered feed A and B in a choice-test of the dietary diversity treatment (DD, n = 10 pens) and litters offered feed A with and without additional flavours in a choice-test of the flavour novelty treatment (FN, n = 9 pens) from commencing creep feeding (at 2 days of age) until weaning (at 22 days of age). One of 4 flavours were added daily to one bowl of feed A from day 6 onwards in FN. Data are means ± SEM. Asterisks indicate significant ($P < 0.05$) effect of dietary treatment per feed intake period (2-6, 6-12, 12-16 and 16-22 days of age).

Also feed A, the common feed used in DD and FN, was eaten more in DD compared to FN (**Figure 2**). When analysed per period, a significantly higher intake of feed A in DD-litters within d6-12 ($P = 0.02$) and d16-22 ($P = 0.04$) and a numerically higher intake of feed A in DD-litters within d12-16 ($P = 0.17$) relative to FN-litters was found. Total intake of feed A during lactation did also differ between DD and FN-litters (DD: 1.4 ± 0.16 kg vs. FN: 1.0 ± 0.07 kg/litter; $P = 0.04$).

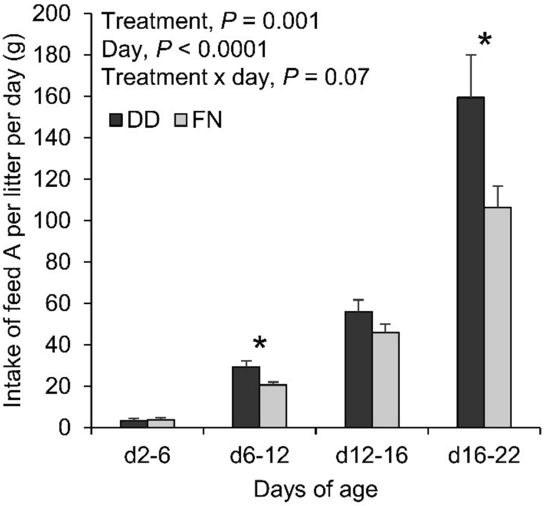

Figure 2. Daily intake (g) of feed A by litters offered feed A and B in a choice-test of the dietary diversity treatment (DD, n = 10 pens) and litters offered feed A with and without additional flavours in a choice-test of the flavour novelty treatment (FN, n = 9 pens) from commencing creep feeding (at 2 days of age) until weaning (at 22 days of age). One of 4 flavours were added to one bowl of feed A from day 6 onwards in FN. The intake of feed A between 2-6 days of age within FN was calculated as the average intake from both bowls of feed A. Data are means ± SEM. Asterisks indicate significant (P < 0.05) effect of dietary treatment per feed intake period (2-6, 6-12, 12-16 and 16-22 days of age).

The percentage of piglets that consumed the feed increased over time and did not differ between DD and FN-litters (**Figure 3**). Once scored as an eater, piglets remained eaters at subsequent observation days, with the exception of five out of 146 piglets. The number of piglets classified as bad eaters was lower for DD (good: 18 (22.2%), moderate: 46 (56.8%), bad: 13 (16.0%) and non-eaters: 4 (4.8%)) in comparison to FN (good: 13 (18.9%), moderate: 35 (50.7%), bad: 21 (30.4%), and non-eaters: 0 (0%); P < 0.05). A small number of eaters was observed sampling only one of the two feed types offered (DD: 8 out of 77 eaters vs. FN: 9 out of 69 eaters), whereas the others sampled both feeds.

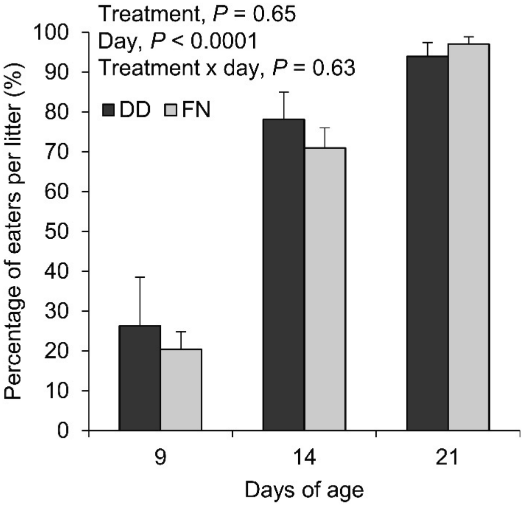

Figure 3. Percentage of piglets that consume creep feed at 9, 14 and 21 days of age in litters offered feed A and B in a choice-test of the dietary diversity treatment (DD, n = 10 pens) and litters offered feed A with and without additional flavours in a choice-test of the flavour novelty treatment (FN, n = 9 pens) from commencing creep feeding (at 2 days of age) until weaning (at 22 days of age). One of 4 flavours were added to one bowl of feed A from day 6 onwards in FN. Data are means ± SEM.

Effects of feed type within dietary treatment

The proportional intake of feed A and B within DD and feed A with and without additional flavours within FN was determined during the suckling period to test feed type preferences (**Figure 4**). Feed type x day (*P* = 0.29) or feed type (*P* = 0.16) did not affect the feed intake of DD-piglets. In accordance, DD-piglets had no preference for feed A (1.4 ± 0.16 kg/litter) or B (1.6 ± 0.18 kg/litter; *P* = 0.31) over the whole suckling period.

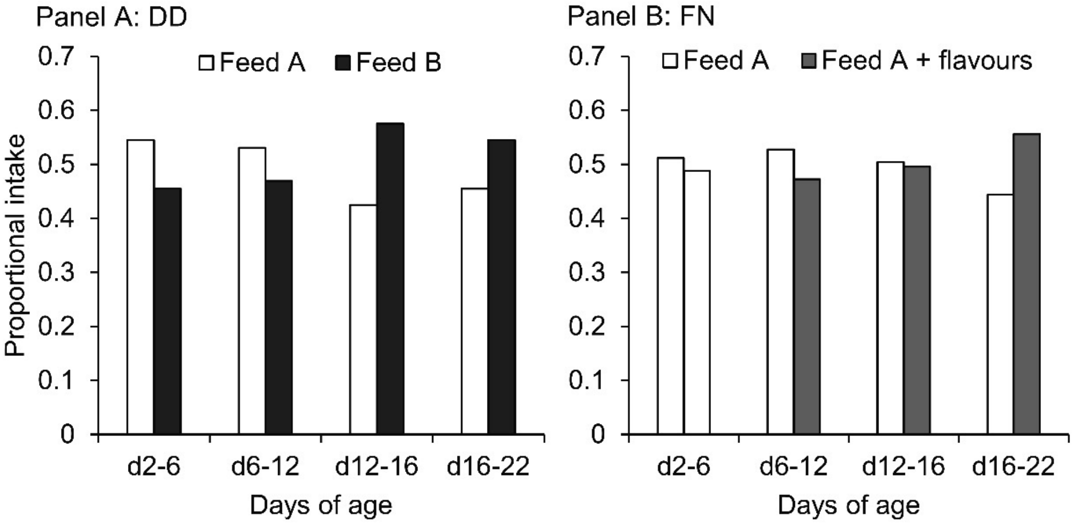

Figure 4. Ratio between feed A and B within a choice-test of the dietary diversity treatment (DD, n = 10 litters, **panel A**) and between feed A with and without additional flavours within a choice-test of the flavour novelty treatment (FN, n = 9 litters**, panel B**). One of 4 flavours were added to one bowl of feed A from day 6 onwards in FN. Data are means ± SEM.

FN-piglets preferred feed A with additional flavours over feed A without additional flavours between d16-22 (*P* = 0.04), but not at earlier time points (Feed type x day: *P* = 0.02; feed type: *P* = 0.104). Specifically, this preference occurred the day that feed A was supplemented with red fruit (i.e. 20 days of age) in the 4-day block from day 18 to 22 (*P* < 0.0001), as red fruit was clearly eaten more within the flavour novelty treatment compared to the other three flavours in this period (Red fruit: 73 ± 3.3%; anise: 54 ± 4.4%; vanilla: 47 ± 3.4%; EOM: 41 ± 4.6% of total intake/pen/day, flavour x 4-day block: *P* = 0.01). No overall preference for feed A with additional flavours (1.1 ± 0.06 kg/litter) was found in FN compared to feed A without additional flavours (0.9 ± 0.07 kg/litter, *P* = 0.14).

Behaviours

Effects of age

Irrespective of dietary treatment, piglets' behavioural activity was affected by age (**Figure 5**), except for exploring the feed (*P* = 0.18).

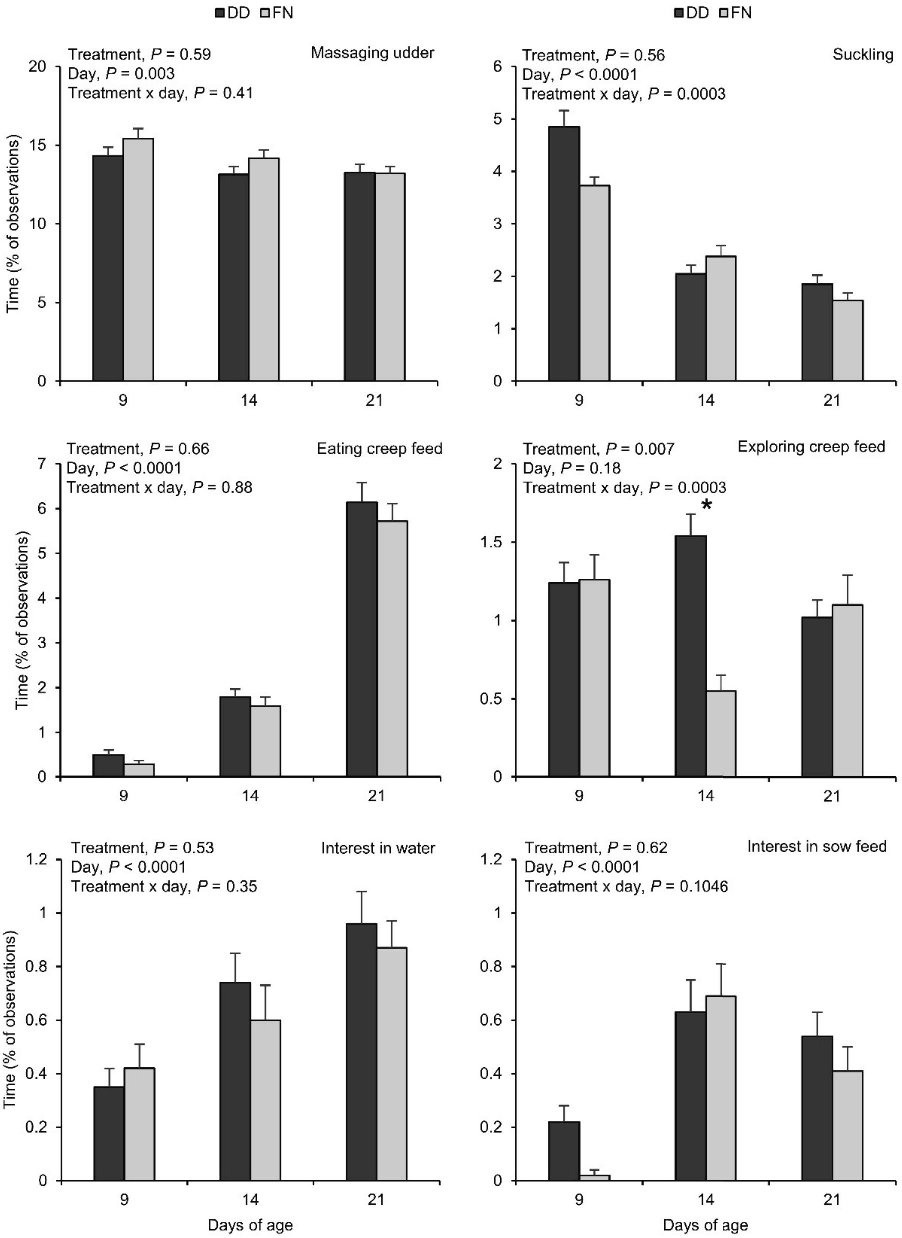

Figure 5. Feed-related behavioural activities (% of total observations) of suckling piglets offered feed A and B in a choice-test of the dietary diversity treatment (DD, n = 83 piglets from 6 litters) and piglets offered feed A with and without additional flavours in a choice-test of the flavour novelty treatment (FN, n = 69 piglets from 5 litters) from commencing creep feeding (at 2 days of age) until weaning (at 22 days of age). One of 4 flavours were added to one bowl of feed A from day 6 onwards in FN. Data are means ± SEM. Asterisks indicate significant ($P < 0.05$) effect of dietary treatment per day (9, 14 and 21 days of age).

Time spent on ‘suckling behaviour’ decreased with time. Nine-day-old piglets spent more time massaging the udder than 14-day-old ($P = 0.05$) and 21-day-old piglets ($P = 0.003$; d9: 14.8 ± 1.15% of observations; d14: 13.6 ± 1.10%; d21: 13.2 ± 0.92%). In addition, suckling significantly differed between day 9, 14 and 21 (d9: 4.3 ± 0.72% of observations; d14: 2.2 ± 0.51%; d21: 1.7 ± 0.43%; $P < 0.01$ for all). On the other hand, time spent on ‘ingestive behaviour’ increased in time. Time spent eating creep feed increased from day 9 to day 14 and 21 (d9: 0.4 ± 0.17% of observations; d14: 1.7 ± 0.23%; d21: 6.0 ± 0.68%; $P < 0.0001$ for all). Moreover, nine-day-old piglets had less interest in sow feed compared to 14-day-old ($P < 0.0001$) and 21-day-old piglets ($P < 0.0001$; d9: 0.13 ± 0.06% of observations; d14: 0.66 ± 0.17%; d21: 0.48 ± 0.11%). Interest in water tended to be less for nine-day-old piglets relative to 14-day-old piglets ($P = 0.052$) and was significantly different between the other time points (d9: 0.38 ± 0.10% of observations; d14: 0.67 ± 0.12%; d21: 0.92 ± 0.09%; $P < 0.01$).

Effects of dietary treatment

A dietary treatment x day interaction was found for suckling ($P = 0.0003$). Although no differences were observed using least squares means, suckling behaviour was numerically higher for DD-piglets at 9 days of age, but numerically lower at 14 days of age compared to FN-piglets respectively. DD-piglets spent more time exploring the feed compared to FN-piglets at 14 days of age (DD: 1.54 ± 0.21% vs. FN: 0.55 ± 0.08%; $P = 0.001$), but no differences were found in time spent eating the feed. In addition, no effects of dietary treatment were found during the suckling period on time spent massaging the udder of the sow, interest in water and interest in sow feed ($P > 0.10$).

Effects of feed type within dietary treatment

DD-piglets explored feed B more compared to feed A (Feed B: 0.83 ± 0.04% vs. Feed A: 0.43 ± 0.07%; feed type: $P = 0.001$). Within DD-litters, no feed type x day interactions were found for exploring the feed ($P = 0.66$) or eating ($P = 0.61$) and no effect of feed type was found on eating ($P = 0.78$). The feed type x day interaction affected the eating behaviour of FN-piglets ($P < 0.0001$), but not their exploratory behaviour towards the feed ($P = 0.17$). Within FN-litters, piglets tended to be more frequently observed eating feed A without additional flavour compared to feed A with additional flavour (i.e. EOM) at 21 days of age (A: 3.80 ± 0.74% vs. A + flavours: 1.92 ± 0.39%; $P = 0.054$), which corresponds to the feed intake measures on that day (EOM: 41 ± 4.6% of total intake/pen at day 21). No effects of feed type were found on eating ($P = 0.12$) and exploring the feed ($P = 0.77$) within FN-litters during the suckling period.

Discussion

In this study we compared the effects of dietary diversity and flavour novelty on the feeding behaviour and performance of suckling piglets. Provision of feed A and B increased pre-weaning feed intake by 50% compared to provision of feed A only (with and without additional flavours). Yet, piglets receiving feed A and B had no overall preference in terms of feed intake for either feed A or B, indicating pre-weaning feed intake increased by an enhanced intake of both feeds. These results support our hypothesis that the more diverse the feeds provided in terms of sensory properties (e.g. ingredient composition, texture), the greater the intake will be. The reason for this is expected to be sensory-specific satiety and/or piglets’ intrinsic motivation to explore. Alternatively, differences in nutrient profiles between the two treatments may have exerted physiological effects that may have influenced feed ingestion.

Sensory-specific satiety involves a rapid and significant decline in pleasantness of taste, smell, appearance and texture of eaten feed in comparison to the pleasantness of non-eaten feed, as reviewed by Rolls (1986). To maintain feed intake at a high level, the feeds provided should therefore vary along as many properties as possible, emphasizing on contrasts, to reduce sensory-specific satieties that impair palatability. Most of the eaters were observed consuming both of the feed types of choice. Therefore it appears that in a choice-condition, piglets prefer a varied diet rather than sampling from just one feed (feed A or B; feed A with or without additional flavours).

Although behavioural observations did not show a significant difference between DD- and FN-piglets in terms of time spent eating creep feed, the exploratory behaviour towards the creep feed was higher for DD-piglets compared to FN-piglets at 14 days of age. Several studies have suggested that feed exploration is beneficial for feed intake in the pre-weaning period (Kuller et al., 2010; Adeleye et al., 2014; Van den Brand et al., 2014). It should be noted, though, that feed intake was not significantly correlated with feed exploration on the same day (at litter level) in this study. In addition, no day effect was found for exploring creep feed, suggesting exploratory foraging behaviour remains important for piglets throughout lactation. Within DD-litters, piglets explored feed B more compared to feed A, which may suggest that increased exploration of one feed, stimulated feed intake of both feeds. Feed B is a large diameter pellet which has been suggested to be easier to pick up, hold or carry in the mouth of young piglets compared to smaller diameter pellets (Van den Brand et al., 2014).

One could hypothesize that feed exploration encourages the development of feed handling skills which are needed for ingestion and thereby increased exploration may increase the percentage of eaters. The percentage of eaters, however, did not differ between DD and FN and the higher feed intake for DD-litters can therefore be explained by a higher intake per piglet, supported by less bad eaters in the DD compared to FN group. One should notice that the percentage of eaters was remarkably high in this study compared to previous studies (e.g. Collins et al., 2013 (d16: 41%, d19: 50%, d21: 77%); Pluske et al., 2007 (d19: 49%, d23: 72%); Sulabo et al., 2010a (d14: 20%, d21: 57%); Tucker et al., 2010 (d10: 1.4%, d14: 4.6%, d21: 29%)), which may have been caused by applying diversity and novelty to the piglets' diet. Nevertheless, this remains to be shown in comparison to a control group (no-variety condition), which was absent in this study. Another possible explanation might be a difference in method, as most studies used a colour marker in the feed, such as chromic oxide, to detect consumption of creep feed. One may not be able to detect the colour marker in the faeces of piglets with a very low creep feed intake as the large amount of sow's milk may mask the colour (Barnett et al., 1989) or of piglets with an irregular feed intake pattern (Kuller et al., 2007a). Two other studies have determined the percentage of eaters using behavioural observations, but used a lower number and distribution of scans per observation day (Delumeau and Meunier-Salaün, 1995: 5-min scan sampling, 90 min/d; Devillers and Farmer, 2009: 1-min scan sampling, 60 min/d), which may have led to false-negative results. On the other hand, these studies used a broader definition of eating in comparison to this study, which may have led to false-positive results.

DD-piglets had a higher feed intake compared to FN-piglets, but weight gain before weaning was not affected. A possible explanation for this result is that pre-weaning weight gain is mainly determined by piglets' milk intake during lactation (Adeleye et al., 2014) and time spent suckling did not differ between DD- and FN-piglets. The relatively short duration of feed provision (i.e. weaning at three weeks of age) would be another possible explanation. Creep feed intake is known to follow an exponential pattern (Pluske et al., 2007). Therefore, a greater pre-weaning feed intake may increase body weight gain only shortly before weaning at a later age (Bruininx et al., 2004; Pluske et al., 2007). Besides, the purpose of a high feed intake before weaning is mainly to facilitate body weight gain after weaning due to its expected benefits for post-weaning feed intake. Even a small improvement in total creep feed intake per piglet (64 g/piglet) has been shown to be advantageous for post-weaning growth (1 kg/piglet in 2 weeks post-weaning; Adeleye et al., 2014).

In the study of Adeleye et al. (2014), litters that were fed creep feed to which different flavours were added in a daily sequential order had a higher hourly frequency of feeder visits and a doubled feed intake compared to control litters which received the same creep feed without additional flavours. By simultaneously providing feed with and without additional successive flavours within a litter by the use of a choice test, we found that feed exploration and intake in general did not differ between feed with or without additional flavours. FN-piglets consumed more of feed A with additional flavours relative to feed A without additional flavours in the last six days before weaning however, which seemed driven by the three times higher intake of red fruit that was observed at day 20. The reason why the piglets chose to consume more of red fruit flavoured feed only at 20 days of age could not be clearly explained. Although it is difficult to compare flavour preferences between studies with a different experimental set-up, red fruit was most preferred compared to vanilla, anise and EOM in this study, but it was least preferred compared to butterscotch, apricot, toffee and apple in Adeleye et al. (2014).

FN (i.e. the flavour novelty treatment) involved both simultaneous (feed A with and without additional flavours) as well as successive variety (novel flavours added over time) in comparison to DD (i.e. the dietary diversity treatment) which only involved simultaneous variety (feed A and feed B). On one hand, the successive exposure to novel flavours on a daily basis likely involved an initial fear response and reluctance by the piglets to try the novel flavoured feed (Oostindjer et al., 2011c) before overcoming neophobia and ingesting the feed. On the other hand, piglets are highly curious animals and were found to seek out for novelty if provided the choice between novelty or familiarity (Wood-Gush and Vestergaard, 1991). No clear evidence for (attenuation of) neophobia toward the novel flavours was found in this study, as the proportional intake of feed A with flavours generally did not increase in time. Dietary diversity seems therefore a more likely cause for the feed intake differences among the treatments, but a possible effect of food neophobia on feed intake cannot be fully excluded. The balance between aversion and acceptance of flavoured creep feed in piglets seems complex, as feed intake of either a familiar or unfamiliar flavour is variable (e.g. Langendijk et al., 2007; Figueroa et al., 2013; Blavi et al., 2016). These inconsistent results indicate that it is hard to predict whether a flavour increases acceptance of the feed or results in aversion and reduced feed intake.

Conclusion

In conclusion, this study showed that provision of dietary diversity to suckling piglets stimulated their feed exploration and intake more than dietary flavour novelty, but did not enhance the percentage of piglets within a litter that consume the feed (at an early age) or their growth performance during the pre-weaning period. Future research will investigate the effect of dietary diversity on the (feeding) behaviour and performance of suckling piglets versus a control group (no-variety condition) and will study the adaptive capacity of these piglets in novelty tests and during the post-weaning period. The amount of solid feed consumed during the suckling period has been shown to correlate positively with the amount of solid feed consumed during the initial weaning period, as well as with the growth performance of newly weaned pigs (Kuller et al., 2004; Berkeveld et al., 2007b). Moreover, dietary variety in early life enhanced the acceptance rate of novel feeds and novel flavours (Catanese et al., 2012; Villalba et al., 2012) and reduced the fear response to a novel environment (Villalba et al., 2012), as shown in weaned lambs. It is therefore hypothesized that early exposure to dietary variety can increase adaptability in novel situations. Inclusion of dietary variety in piglet rearing during lactation may therefore be of particular interest at weaning at which rapid acceptance and high intake of novel feed in a new environment is needed to prevent gastrointestinal dysfunction and associated health problems and production losses.

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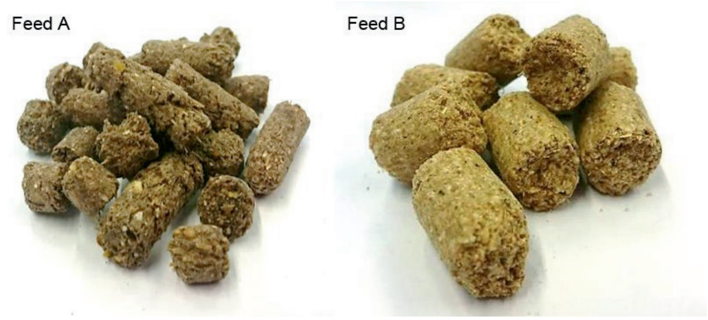
Supplementary data



Supplementary Figure S1. Two feeds provided in separate bowls to suckling piglets, receiving either feed A + B in a choice test or feed A only (with and without additional flavours in a choice test). Feed A (Animal Nutrition Group, Wageningen University & Research, Wageningen, the Netherlands) and B (Baby Big XL, Coppens Diervoeding, Helmond, the Netherlands) differed in production method, size, flavour, ingredient composition and nutrient profile, smell, texture and colour.

Supplementary Table S1. Nutrient profile of feed A and feed B.

Calculated nutrient composition[1]	**Feed A**	**Feed B**
Dry matter	891	880
Starch	290	366
Non-starch polysaccharides[2]	261	175
Crude protein	195	140
Crude fat	61	96
Crude fibre	44	42
Crude ash	57	33
Calcium	9.1	2.8
Phosphorus	6.1	3.6
Sodium	2.2	3.5
Standardized ileal digestible lysine	11.9	7.8
Standardized ileal digestible methionine	4.8	2.6
Standardized ileal digestible threonine	7.1	5.2
Standardized ileal digestible tryptophan	2.4	1.7
Net energy	11.8	11.4

[1] According to CVB (2007). Nutrients are presented in g/kg dry matter, except for dry matter (g/kg) and net energy (MJ/kg)

[2] Calculated as the difference between dry matter and the sum of starch, sugars, crude protein, crude fat and crude ash

Supplementary Table S2. Ingredient composition of the creep feed.

Ingredient component	**%**
Wheat	21.9
Barley	15
Maize	15
Soy protein concentrate	7
Soybeans (heat treated)	5
Galacto-oligosaccharides	5
Potato protein	4
Sugarbeet pulp (dehydrated)	4
Oat hulls	4
Inulin	4
High-amylose starch (± 75% amylose)	4
Soybean oil	3
Blood meal (spray dried)	2
Dicalcium phosphate	1.7
Sucrose	1.5
Calcium carbonate	1.0
Sodium chloride	0.5
Premix[1]	0.5
Potassium bicarbonate	0.3
L-lysine hydrochloride	0.3
DL-methionine	0.2
L-threonine	0.04
L-tryptophan	0.04
Total	100

[1] Vitamin and mineral premix (per kg of feed): vitamin A: 10000 IU, vitamin D3: 2000 IU, vitamin E: 40 mg, vitamin K: 1.5 mg, vitamin B1: 1 mg, vitamin B2: 4 mg, vitamin B6: 1.5 mg, vitamin B12: 0.02 mg, niacin: 30 mg, D-pantothenic acid: 15 mg, choline chloride: 150 mg, folate: 0.4 mg, biotin: 0.05 mg, iron: 100 mg, copper: 20 mg, manganese: 30 mg, zinc: 70 mg, iodine: 0.7 mg, selenium: 0.25 mg, anti-oxidant: 125 mg

CHAPTER 7

Pigs like it varied; Feeding behaviour and pre- and post-weaning performance of piglets exposed to dietary diversity and feed hidden in substrate during lactation

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Abstract

Timely intake of solid feed is essential to ease the nutritional change from sow’s milk to solid feed at weaning and thereby to reduce weaning-related problems. A significant percentage of piglets, however, do not or hardly consume solid feed before weaning. We studied effects of dietary variety and presenting the feed in substrate during lactation on the feeding behaviour and performance of piglets up to two weeks post-weaning. Feed was provided *ad libitum* from d4 in two feeders, with four bowls each. In a 2 × 2 arrangement, forty litters received either creep feed as a monotonous diet (MO) or four feed items simultaneously, i.e. creep feed, celery, cereal honey loops and peanuts, as a diverse diet (DD) and the feed was either provided without (CON) or with substrate (SUB), i.e. sand, in one of the two feeders up to weaning. Dietary diversity highly stimulated feed exploration and eating (≥ 2.5 times), feed intake and the percentage of (good) eaters from early in lactation, and enhanced piglet growth towards weaning (by 29 g/d), although MO-piglets spent more time eating creep feed from d18. Within MO, SUB-litters consisted of more good eaters than CON-litters. At weaning (d28) four piglets from the same treatment were grouped ($n = 40$ pens). DD-CON had the highest post-weaning feed intake and gain between d5-15 and the lowest proportion of pigs with higher tail damage scores. However, effects regarding behaviour remained inconclusive, as DD-piglets had a lower and higher number of body lesions at 4 h and d15 post-weaning respectively, spent less time exploring the feed(er) and drinker and environment, and more time nosing pen mates than MO-piglets. SUB-piglets showed a reduction in total post-weaning feed intake, gain (particularly between d0-2) and inactivity, increased levels of manipulation and aggression at week 1 and a higher number of body lesions at 4 h and d15 post-weaning. In conclusion, dietary diversity seems a promising feeding strategy in getting piglets to eat during lactation. Provision of substrate in the feeder subtly stimulated foraging behaviour, but negatively impacted post-weaning adaptation, probably because treatments were not reinforced after weaning and piglets thus experienced loss of enrichment.

Introduction

In most commercial pig farms, piglets are weaned at 3 to 4 weeks of age by separating them from the sow. This early and abrupt weaning is commonly seen as the most stressful event in a pig's life, as it comprises multiple concurrent stressors, such as changes in environment, social structure and diet. Several studies have shown that stimulating the natural behaviour of pigs benefits their capacity to deal with weaning. Firstly, a stimulus-rich environment has been shown to enhance explorative behaviours and reduce weaning stress and weaning-stress-induced behaviours compared to a barren farrowing and weaner pen (more space and wood shavings, peat, branches and straw on the floor: Oostindjer et al., 2011d; tray of peat: Vanheukelom et al. 2011; box with wood bark: Yang et al. 2018). Secondly, loose housing of sows (Oostindjer et al., 2011d) and group housing of sows and their litters during lactation (Van Nieuwamerongen et al., 2015) were found to reduce damaging oral manipulation of pen mates and increase play behaviour of piglets after weaning. Moreover, group housing of sows and their litters reduced behavioural signs of fear in piglets exposed to a novel environment (Hillmann et al., 2003). In addition, loose housing of sows plus the provision of sawdust on the floor increased average daily gain of piglets in the first two weeks post-weaning and reduced belly nosing in piglets weaned at 3 weeks of age (O'Connell et al., 2005). Loose and group housing of sows resemble the social situation in nature, with pigs living in families (Gundlach, 1968; Meynhardt, 1980), in which information regarding what, where and how to eat is transmitted from sow to piglets (Jensen, 1988; Stangel and Jensen, 1991). Thirdly, outdoor-reared piglets have been reported to consume almost twice as much creep feed from two weeks of age compared to indoor-reared piglets (Miller et al., 2007). Moreover, even when not provided with creep feed, outdoor-reared piglets spent more time eating solid feed before weaning, such as sow feed and vegetation (Cox and Cooper, 2001; Hötzel et al., 2004), spent more time eating creep feed, which was novel to them, in a novel environment on the day of weaning (Lau et al., 2015), and spent more eating solid feed in the first hours after weaning compared to indoor-reared piglets which received creep feed (Webster and Dawkins, 2000; Cox and Cooper, 2001). In addition, outdoor-reared piglets displayed less agonistic behaviour, belly nosing and other oral-nasal interactions than indoor-reared piglets (Cox and Cooper, 2001; Hötzel et al., 2004). It can be concluded that outdoor-reared piglets are more experienced with solid feed before weaning compared to indoor-reared piglets, of which a significant proportion does not or hardly consume solid feed before weaning (Pluske et al., 2007; Collins et al., 2013). This low feed intake before weaning, or even no feed intake at all, often results in a low feed intake after weaning (Bruininx et al., 2002, 2004), accompanied by gastrointestinal problems (reviewed by Heo et al., 2013), gut microbiota dysbiosis (reviewed by Gresse et al., 2017), reduced weight gain and an increase in damaging behaviour (Colson et al., 2006; Van Nieuwamerongen et al., 2017). Since rearing environments for commercial indoor production are different from (semi-)natural conditions, strategies that stimulate the natural feeding behaviour of indoor-reared piglets may improve their weaning transition, as the intake of solid feed before weaning correlates with the intake of feed after weaning (Kuller et al., 2004; Berkeveld et al., 2007b).

In a (semi-)natural environment, pigs spend more than half of their active time on foraging and eating, of which mostly grazing, rooting and nosing (weaned and adult pigs: Stolba and Wood-Gush, 1989; suckling piglets from 8 weeks of age: Petersen, 1994). Their diet is diverse and consists of a variety of feed items such as grasses, fruits, nuts, fungi, leaves, insects, resin and roots (Hanson and Karstad, 1959; Stolba and Wood-Gush, 1989; reviewed by Ballari and Barrios-García, 2014). Already from a few days after birth, piglets have been observed foraging for other feed items than sow's milk, by digging soft soil and exploring and sampling leaves, mushrooms, acorns and corn (Gundlach, 1968; Meynhardt, 1980; Petersen, 1994) until they are exclusively feeding on solid feed between 8.5 and 22 weeks of age (Newberry and Wood-Gush, 1985; Jensen and Stangel, 1992). The foraging behaviour of wild piglets peaks around four weeks of age and therefore precedes the development of ingestive behaviour such as grazing, which mainly develops during week four of age (Petersen, 1994). Foraging is the 'appetitive phase' of feeding, which brings the pig into contact with feed. It concerns active, flexible, searching behaviours for food and indicates the need to satisfy appetite, but also has an important role in information gathering. Eating is the 'consummatory phase' of feeding and is the achievement ('consummation') of the goal and ends the appetitive foraging behaviour (Berridge, 2004; Mills et al., 2010). For example, pigs need to shovel and root the ground, and therefore 'work' for their feed, before roots and worms can be consumed (Stolba and Wood-Gush, 1989).

In contrast with (semi-)natural conditions, indoor-reared pigs are generally offered a monotonous pre-mixed diet, that is freely available and often provided *ad libitum*. However, they still spend more than half of their active time on eating and redirected foraging towards the floor and pen, of which mostly rooting and nosing (weaned piglets of 4-6 weeks of age: Oostindjer et al., 2011d), suggesting indoor-reared pigs are highly motivated to forage, also in absence of hunger and/or nutrient deficiencies. In addition, some pigs are willing to work hard for access to rooting material (Hutson, 1989) and behavioural demand studies have shown a low elasticity in demand for rooting material (Ladewig and Matthews, 1996). These data suggest that the performance of foraging behaviour on its own is rewarding to pigs, but foraging may be more rewarding when linked to feed intake. This is supported by preference tests in which pigs spent more time in an environment with feed hidden in substrate compared to an environment with feed and substrate offered separately (De Jonge et al., 2008) and spent more time with feed hidden in substrate compared to substrate only, although fed *ad libitum* (De Jonge et al., 2008; Holm et al., 2008). Translating these findings to piglet rearing before weaning, supplementing solid feed with substrate may encourage suckling piglets to spend more time at the feeder to perform foraging behaviour and to consume feed. We also suggest that presentation of a more diverse diet stimulates the feeding behaviour of piglets, by increasing exploratory behaviour towards the feed and by reducing 'sensory-specific satiety', which is the decline in liking of eaten feed in comparison to other non-eaten feed (as reviewed by Rolls 1986). Indications have been found that dietary variety, i.e. feeds that differ in at least one sensory domain (Raynor and Epstein, 2001), can indeed increase feed intake in humans (e.g. Rolls et al. 1981), rats (e.g. Rolls et al., 1983) and sheep (e.g. Villalba et al., 2011). This is also shown in pigs by providing feeds differing in flavour successively (Adeleye et al., 2014; Figueroa et al., 2018) or simultaneously (Figueroa et al., 2018).

In this study we therefore aimed to investigate the effect of dietary diversity (versus dietary monotony), the effect of presenting the feed in a foraging-stimulating context (in substrate or not) and their potentially interacting effects on the foraging and ingestive behaviour of suckling piglets, as well as their adaptive capacity to deal with weaning. It is hypothesized that dietary diversity and feed presentation in a foraging-stimulating context would positively affect feed intake prior to weaning and thereby increase post-weaning feed intake and body weight gain and reduce diarrhoea and weaning-stress-induced behaviours.

Methods

The protocol of the experiment (AVD104002016515) was approved by the Animal Care and Use committee of Wageningen University & Research (Wageningen, the Netherlands) and in accordance with the Dutch law on animal experimentation, which complies with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The use of Indigo carmine as colourant in the feed was approved by the Medicines Evaluation Board (Utrecht, the Netherlands).

Animals, housing and management

The study was set up as a 2 × 2 factorial arrangement (see below for treatments). Forty multiparous pregnant sows (range parity: 2-8; inseminated by Tempo boar semen, Topigs Norsvin, Vught, the Netherlands) were divided over two farrowing rooms (balanced for treatments) in three consecutive batches (n = 10 sows per treatment). In total, 24 Topigs-20 sows and 16 Norwegian Landrace x Topigs-20 sows were used, equally assigned to treatment groups. Sows originated from one conventional farm and were housed at research facility Carus (Wageningen University & Research, the Netherlands) from two weeks before farrowing onwards and were crated in farrowing pens from one week before farrowing onwards until weaning. Sows were fed commercially available diets for gestation and lactation (Coppens Diervoeding, Helmond, the Netherlands) twice a day, at 7.30 and 16:00 h. Feed was provided in portions and feed remains were removed within 30 minutes after feeding to prevent piglets from eating sow feed.

The farrowing pen (2.85 x 1.80 m) was equipped with a crate (2.85 x 0.60 m) including a feed trough, drinking nipple, a jute sack around farrowing and three chew objects that alternated two times a week (metal chain with either one of three attachments: bolts, ball or PVC pipe) for the sow. It also included a drinking nipple, chew object (metal chain with bolts) and heating lamp (until day 13 of lactation) for the piglets. The floor consisted of slats and a rubber mat (1.75 x 1.20 m) that served as nest for the piglets and provided lying comfort to the sow. Within 24 h after birth, piglets received an ear tag and intramuscular iron injection and their birth weight and sex were determined. No teeth clipping, tail docking or castration were performed. Litter size was standardized to 13-15 piglets per litter by cross-fostering within 3 days of age, resulting in an average number of 13.5 ± 0.1 piglets per litter at d4.

The forty sows and their litters were allotted to one of four treatments at d4 based on sow breed and parity, and piglet birthdate and body weight at d0 and 4 after birth. From d4, piglets got access to a concrete piglet feeding area (1.37 x 1.80 m), in front of the sow, including two feeders with four bowls/feeding spaces each (17.5 x 13.5 cm per bowl) (**Figure 1**). In the farrowing rooms lights and radio were on from 7:00 to 19:00 h and lights were dimmed during the night. The room temperature was set to gradually decrease from 25 °C around farrowing to 21 °C at weaning. The parity of the sows (on average 5.2 ± 0.3) and litter size at weaning (on average 12.9 ± 0.2 piglets/litter) did not differ between treatments.

Figure 1. Set-up of the farrowing pen. From four days of age piglets had access to a concrete piglet feeding area, in front of the sow, including two feeders with four feeding spaces each.

At weaning, i.e. 28.5 ± 0.2 days of age, a subset of 160 piglets (n = 10 weaner pens per treatment) was relocated in two weaner rooms (balanced for treatments) in two consecutive batches. Piglets were mixed with conspecifics from the same pre-weaning treatment and housed in pens with four piglets, of which two males and two females, which derived from three litters. Piglets were selected based on their sex and their body weight at d26 (close to the average weight of the litter and treatment group). Piglets with a history of leg/claw problems were excluded from selection. All weaner pens were identical and equipped with a feed trough (12 x 50 cm with three feeding places), drinking trough and chew object (metal chain with bolts). The flooring was slatted with a rubber mat (1.75 x 1.20 m) that provided lying comfort and prevented spillage of feed through the slats. Piglets were fed a commercially available nursery diet *ad libitum* (3-mm pellet, 161 g crude protein, 48 g crude fibre and 11.8 g standardized ileal digestible lysine/kg dry matter, Coppens Diervoeding, Helmond, the Netherlands). In the weaner rooms lights and radio were on from 7:00 to 19:00 h and room temperature was set to gradually decrease from 25 °C at weaning to 23 °C at two weeks post-weaning, when the experiment ended (d43). The experiment took place from May until August and the room temperature of the farrowing rooms in batch 3 and weaner rooms in batch 2 exceeded the settings as result of a heat wave from 15th of July until 7th of August 2018. A maximum temperature of 30 and 31 °C were measured in these farrowing and weaner rooms respectively.

Feeding strategies of piglets during lactation

Piglets were assigned to one of four treatment combinations in a 2 × 2 arrangement, with dietary variety (DV) and feed presentation (FP) during lactation as experimental factors. Piglets received either one solid feed item, i.e. creep feed, as a monotonous diet (**MO**) or received four solid feed items simultaneously (creep feed, celery, cereal honey loops and peanuts in shell) as a diverse diet (**DD**) and the feed was either presented without substrate (**CON**) or with substrate (**SUB**) in one of the two feeders to stimulate natural foraging behaviour (**Figure 2**). In the SUB-treatment, the feed in one of the feeders was hidden in the substrate, which was play sand, but a few items were put on top of the substrate from d4 to d12 only. Bowls were checked at least two times a day from d4 (7:30 and 16:00 h) and at least four times a day from d12 (7:30, 12:00, 16:00 and 17:30 h) to provide feed *ad libitum*. Bowls were refilled with feed using cups, in which the volume of the different feed items of DD were equal to each other. Feed in sand was provided in a ratio of one volume of feed in 12 volumes of sand. As feed intake increased with age, the ratio was decreased to one volume of feed in six volumes of sand from d19 onwards. The location of the feeder with sand, provided either left or right, was alternated over pens. The location of the feed items within the feeders, of which each item was provided in one of the four bowls per feeder, was alternated over pens and changed after each feed weigh-back (d12, 19 and 23) in a balanced order to capture all four possible positions during lactation. This was done by repositioning of the bowls. Each feed item was thus fed in the same bowl throughout lactation to prevent possible mixing of flavours.

The feed items were carefully chosen to create a diet as varied as possible in sensory properties (e.g. sweet and bitter taste, small and big sized items, crispy and sticky texture, hard and soft structure, smooth and ribbled surface) and to stimulate foraging such as extraction and chewing. Peanut shells (approximately 3.8 x 1.3 cm) were cracked up to day 12 to ease opening up of the shell by the piglets. Celery leaves were removed and the celery was cut in pieces of approximately 2.5 x 2 x 0.6 cm. To maintain freshness of the feed, feed bowls were cleaned using paper towels and water and all feed items were replaced after each feed weigh-back. In addition, celery in the feed bowls was refreshed every other day. Cereal honey loops (1.5 x 0.5 cm) and creep feed (3 mm in diameter) presented in sand were refreshed daily. Peanuts, celery, cereal honey loops (**Supplementary Table S1**) and sand were purchased from local suppliers. Sand was chosen as substrate, as more edible substrates like wood shavings and peat provide some diversity to the diet and may affect the gut microbiota population of piglets. The creep feed (11.8 MJ/kg as-fed net energy, 195 g crude protein, 11.9 g standardized ileal digestible lysine/kg dry matter) was pelleted by Research Diet Services (Wijk bij Duurstede, the Netherlands). The creep feed was high in dietary non-starch polysaccharides (261 g/kg dry matter), originating from cereal grains, sugarbeet pulp, oat hulls, galacto-oligosaccharides, inulin and high-amylose starch and included 5g/kg of feed colourant Indigo carmine (E132 Eurocert 311811, Sensient Food Colors, Elburg, the Netherlands; **Supplementary Table S2** and **S3**).

Figure 2. Litters were either provided with creep feed as a monotonous diet (MO) or four feed items simultaneously (creep feed, peanuts, celery and cereal honey loops) as a diverse diet (DD). The feed was presented without (CON) or with substrate (SUB), which was sand, in one of two feeders. The feed was hidden in the substrate, but a few items were put on top of the substrate in the first feeding phase from d4 to d12 (pictures were taken in this phase).

Measurements

Piglet behaviour and identification of eaters (based on behavioural observations)

Piglets were individually marked using dark permanent hair dye pre-weaning and animal marking spray post-weaning. Feed-related behaviours in the farrowing pens were observed live at d11, 18 and 27 using 2-min instantaneous scan sampling for six sessions of one hour per day (i.e. 180 scans/piglet/d) using pen and paper. The ethogram with behaviours of interest in the farrowing rooms is given in **Table 1**. Observations in the farrowing rooms were used to calculate time spent on feed-related behaviours and to discriminate eaters from non-eaters at each observation day. Piglets were also classified into different eater classes according to Middelkoop et al. (2018). Briefly, piglets that were observed eating on three observation days were classified as good eaters and piglets eating on two observation days as moderate eaters. Piglets eating on one observation day were classed as bad eaters and piglets that were never seen eating as non-eaters. If a piglet was scored as an eater based on behavioural observations, it was also investigated which feed items it consumed over the three observation days. Behaviours after weaning were recorded live on 35 and 42 days of age (d7 and 14 post-weaning) by 2-min instantaneous scan sampling for six one-hour sessions per day using a Psion hand-held computer with the Pocket Observer 3.1 software package (Noldus Information Technology, Wageningen, the Netherlands). The ethogram with behaviours of interest in the weaner rooms is listed in **Supplementary Table S4**. The observation sessions in the farrowing rooms started at 8:00, 9:15, 10:30, 14:00, 15:30 and 16:45 h and in the weaner rooms at 8:00, 9:15, 10:30, 14:00, 15:15 and 16:30 h.

Table 1. Feeding behaviours during the suckling period of piglets that had access to two feeders with four feeding spaces/bowls each. Litters were either provided with creep feed as a monotonous diet or four feed items simultaneously (one feed item per bowl) as a diverse diet. The feed was presented without or with substrate in one of two feeders. When given the choice between substrate or not, it was noted at/from which feeder (i.e. with sand, without sand or unknown) the piglets were exploring/eating to study feed presentation preferences. When given the choice between different feed items, it was scored to which feed item (creep feed, celery, cereal honey loops, peanuts or unknown) the behaviour was directed to test feed item preferences.

Behaviour	Description
Exploring feeder	Sniffing, touching with snout, rooting or chewing on the feeders
Exploring feed	Sniffing, touching with snout or rooting the content (feed or feed + sand) of the feeders (snout is in the feeder) or sniffing or touching feed on the floor (snout is outside the feeders)
Playing with feed	Rolling feed over the floor, walking around the pen with feed item in mouth or (energetically) shaking head while having feed item in mouth
Eating feed	Eating or chewing feed from the feeders or the floor
Suckling	Drinking milk from teat of sow (soft suckling noises)

Identification of creep feed eaters (based on rectal swabs)

Rectal swabs were taken at d12, 19, 23, 26 and 28 to determine the intake of creep feed including feed colourant Indigo carmine qualitatively (yes or no) on piglet level at each measurement day. Piglets were also classified into different eater classes according to Collins et al. (2013). In short, piglets of which blue colour was present on the swab on four or five measurement days were classified as 'good/early eaters'. 'Moderate eaters' had blue colour on the swab on two or three measurement days and 'bad eaters' on only one measurement day. This may not necessarily concern the last measurement day(s) and therefore these piglets cannot be called 'late eaters'. Swabs of 'non-eaters' did not include blue colour at any of the measurement days.

Feed intake

Pre-weaning feed intake was determined in fresh weight between d4-12, d12-19, d19-23 and d23-28, thereby resulting in four feeding phases. It was measured on litter level per feeder (with or without substrate within SUB) and per feed item (within DD) by weighing feed remains from the feeders and floor (including peanut shells). Feed remains derived from SUB-pens were sieved before weighing them to minimize the attachment of sand, but sieving did not fully remove the sand attached to the feed items. To assess the effects of sand on the feed intake measures (attachment of sand to the items and moisture from the sand taken up by the items) we weighed the feed remains in two pens with no animals in it. In these 'test pens' the feed was presented and handled as DD-SUB. In addition to pre-weaning feed intake, the number of refills (i.e. number of times extra feed was added to the bowls in the pen) were recorded on litter level per feeder (with or without substrate within SUB) and per feed item (within DD) as an estimate of the extra feed provided per feeding phase. The volume of the different feed items within DD were equal per refill and was 1:12 (feed:sand) up to d19 and 1:6 thereafter. Standard refreshment procedures (as mentioned for creep feed, celery and cereal honey loops in sand) were not included in the number of refills. Post-weaning feed intake was determined on pen level between 0-4 h, 4-24 h, d1-2, d2-5 and d5-15 post-weaning. Feed wastage was kept to a minimum by placing the feeders on the solid floor in the farrowing and weaner pens.

Sow and piglet body weight development

Sow body weight and back fat thickness at left and right P2 positions were measured using an ultrasonic Renco Lean Meater (MS Schippers, Bladel, the Netherlands) at one week before farrowing and at weaning. Piglets were individually weighed at d0 (within 24 h after birth), d4 (before commencing feeding), 19, 26, 28 (at weaning) before weaning and d1, 2, 5 and 15 after weaning (at the end of the experiment).

Body lesions and damage on piglets

The number of body lesions on the piglets were monitored as a measure of aggression according to Turner et al. (2006) at 4 hours and d1, d2 and d15 post-weaning. Bite injuries on ears and tails of the piglets were classified into no damage, bite marks, small wound or medium wound as a measure of oral manipulation according to van Nieuwamerongen et al. (2015) at d15 post-weaning.

Faecal consistency scores of piglets

Faeces in the weaner pens were scored daily for consistency according to Pedersen and Toft (2011). Score 1 (firm and shaped) and 2 (soft and shaped) represent normal faeces. Score 3 (loose) and 4 (watery) represent diarrhoea. The highest faecal consistency score that was observed in a pen was selected on each measurement day and averaged over two weeks post-weaning to calculate the mean faecal consistency score per pen. Faeces were removed on a daily basis after scoring to guarantee consistency scoring of fresh faeces.

Statistical analyses

Data processing

Results regarding pre-weaning feed intake from the 'test pens' indicated that in the feeders with sand, feed intake calculations based on weighing back the feed may have overestimated the intake of celery and underestimated the intake of creep feed and cereal honey loops. In the end, we did not use these results to correct the feed intake measurements, as the 'test pens' did not resemble closely enough the situation in pens with animals. Firstly the feed, and thus also celery, disappeared at a faster rate in pens with animals due to consumption. Secondly, sand was regularly rooted by the piglets and freshly added as required, and thirdly feed could get in contact with saliva due to chewing efforts of the piglets. Feed intake data of SUB-pens were therefore excluded from analyses.

Piglet behaviours in the home pen were averaged per piglet per day and expressed as proportions of time. Behavioural element 'chewing faeces' was excluded from analyses because it was seen very rarely (0.01% of observation time). The ear with the highest damage score (either the left or right ear of the piglet) was used in the analyses of ear damage. Only five piglets had small wounds on their ears and only one piglet had a medium ear wound, therefore ear damage was analysed as 0-1 variable, i.e. no damage (0) vs. damage (1: bite marks + small wound + medium wound). Only four piglets had medium tail wounds, therefore small and medium tail wounds were combined into one score prior to data analyses. Faecal consistency score 1 and 2 were combined into one score prior to data analyses, as they both represent normal faeces. The presence of outliers was tested by a Grubb's test and two outliers were excluded from further analyses, i.e. one piglet was excluded from the calculation of average daily gain between d19-26 and one piglet from the calculation of average daily gain between d26-28.

Data analyses

Data were analysed with generalised linear (GLIMMIX) and linear (MIXED) mixed models in the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Proportions of time spent on the different behaviours and the proportion of eaters per litter (based on home pen observations and blue coloured rectal swabs) were analysed in a GLIMMIX with a binomial distribution, logit link function and an additional multiplicative overdispersion parameter. The proportion of piglets playing with feed, the proportion of weaner piglets with tail damage and individual creep feed classification of piglets based on behavioural observations were analysed in a Fisher's exact test, because there were empty classification categories scoring 0 only (i.e. 0 MO-piglets were playing with feed, 0 DD-CON piglets had bite marks on their tails and 0 DD-litters had non-eaters). Individual creep feed classification of piglets based on blue coloured rectal swabs was analysed in a GLIMMIX with a multinomial distribution and a cumulative logit link function. Subsequently, data on individual creep feed classification were expressed as binary data (good + moderate eaters vs. bad eaters + non-eaters) and analysed in a GLIMMIX with a binary distribution and a logit link. The number of feed refills, the number of body lesions and the number days with post-weaning diarrhoea were analysed in a GLIMMIX with a Poisson distribution, log link function and an additional multiplicative overdispersion parameter. The occurrence of watery diarrhoea and ear damage were expressed as binary data and analysed in a GLIMMIX with a logit link and binary distribution. Feed intake before weaning, average daily feed intake (ADFI), average daily gain (ADG), body weight (BW), uniformity in BW expressed as coefficient of variation, feed conversion ratio, mean faecal consistency score as well as sow BW and back fat loss were analysed in a MIXED procedure. For feed intake and ADG, totals over the pre- (d4-28) and post-weaning period (d0-15 post-weaning) were analysed, as well as effects on separate periods. Model residuals of the MIXED procedure were checked for normality. Creep feed intake between d4-12 and d4-28, and feed intake in the first four hours after weaning were log transformed before analyses.

The models included the fixed effects of dietary diversity (DD vs. MO), feed presentation (SUB vs. CON), their interactions, as well as batch (batch 1, 2 or 3 before weaning and batch 1 or 2 after weaning). Treatment DD-SUB had 100% eaters at d27 based on home pen observations, therefore the interaction between DV x FP was excluded from the model at d27. In addition, for behaviour, BW, ADG and the number of body lesions, the model included a random pen effect, nested within treatments and batch (farrowing pen for observations pre-weaning and weaner pen for observations post-weaning). Back fat thickness at one week before farrowing was used as covariate in the analyses of back fat loss during lactation and CV at d4 was used as covariate in the analyses of CV at d28. Moreover, BW at weaning was used as covariate in the analyses of BW and ADG after weaning, but excluded from analyses when not significant.

To study the effects of sand (with or without sand in the feeder) within FP, fixed effects of sand, DV and their interaction, as well as batch, were used. Furthermore, to test feed item preferences within DD, fixed effects of feed item and batch were used. Pen (nested within dietary treatment (DV or FP respectively) and batch) was also used as random effect in the analyses within treatments.

Significant fixed effects were further analysed using post-hoc pairwise comparisons of least squares means. Data are presented as (untransformed) means ± SEM based on pen averages. Differences at $P < 0.05$ were considered statistically significant and differences at $0.05 \leq P < 0.10$ were considered a trend.

Results

Feed-related behaviour before weaning

DD-piglets spent at least two and a half times more time on feed exploration (DD vs. MO, d11: 0.60 ± 0.09 vs 0.22 ± 0.05%; d18: 1.60 ± 0.25 vs. 0.15 ± 0.03%; d27: 1.87 ± 0.33 vs. 0.10 ± 0.02%) and eating (DD vs. MO, d11: 1.83 ± 0.29 vs. 0.55 ± 0.14%; d18: 5.66 ± 0.88 vs. 1.67 ± 0.31%; d27: 12.32 ± 1.02 vs. 4.59 ± 0.51%) than MO-piglets at all observation days in lactation (**Figure 3**). When looking at creep feed only, however, MO-piglets were seen eating this feed item two times more than DD-piglets at d18 (MO: 1.67 ± 0.31 vs. DD: 0.83 ± 0.17%) and d27 (MO: 4.59 ± 0.51 vs. DD: 1.63 ± 0.24%). Playing with feed was only shown by DD-piglets and not performed by MO-piglets (DD: 0.15 ± 0.03 vs MO: 0% of the time). The percentage of piglets playing with feed was therefore 0% at all observation days in MO and differed from DD that had 12.6 ± 4.4%, 19.9 ± 6.1%, and 24.0 ± 4.6% of the piglets playing with feed at d11, 18 and 27 respectively. SUB-piglets tended to spend more time on feed exploration than CON-piglets at d11 (SUB: 0.50 ± 0.08 vs. CON: 0.32 ± 0.08% of time), but a lower proportion of SUB- (2.6 ± 1.5%) than CON-piglets (10.1 ± 4.5%) were seen playing with feed at this age. Interactions between DV x FP were found on exploration of the feeder at d18, indicating that DD-SUB piglets spent more time exploring the feeder than DD-CON piglets, whereas in MO piglets no significant differences were found between SUB and CON. Suckling was not affected by the treatments. Time spent exploring the feed positively correlated with time spent eating at all observation days (Piglet level: r = 0.42, 0.62, 0.65 at d11, 18 and 27 respectively; Litter level: r = 0.74, 0.76, 0.77; *P* < 0.0001 for all correlations).

Percentage of eaters identified by behavioural observations

DD enhanced the percentage of piglets within the litter observed to be eating at d11 and d18 and tended to at d27 compared to MO (DD vs. MO, d11: 78.7 ± 5.1 vs. 40.3 ± 6.7%; d18: 95.6 ± 2.3 vs. 69.3 ± 5.8%; d27: 99.6 ± 0.4 vs. 89.5 ± 4.1%, **Figure 4A**). In line with this, when we classified the piglets in each eater category, we found that DD resulted in a higher number of piglets in better eater classes (**Figure 4B**), as it enhanced the number of good eaters by three times compared to MO (DD: 75.8 vs. MO: 23.5%, *P* < 0.0001). Notably, all piglets within DD were observed to be eating on at least one observation day (DD: 100 vs. MO: 96% of the piglets). SUB also increased the number of piglets in better eater classes, but only within MO (SUB effect within MO: *P* = 0.02), showing more good eaters (MO-SUB: 29.8 vs. MO-CON: 17.2%, *P* = 0.02), and less moderate eaters (MO-SUB: 48.9 vs. MO-CON: 63.2%, *P* = 0.02).

Percentage of creep feed eaters identified by blue coloured rectal swabs

In accordance with behavioural observations of eating the creep feed (**Figure 3**), which included feed colourant Indigo carmine, the percentage of creep feed eaters per litter identified by blue coloured rectal swabs was higher or tended to be higher in MO-litters compared to DD-litters from d19 onwards (MO vs. DD, d19: 29.5 ± 6.3 vs. 13.4 ± 3.2%; d23: 32.2 ± 5.2 vs. 19.3 ± 5.6%; d26: 47.3 ± 5.8 vs. 30.8 ± 6.1%; d28: 69.8 ± 6.1 vs. 45.3 ± 7.1%; **Figure 4C**). In addition, MO-piglets were more likely to be better creep feed eaters compared to DD-piglets (*P* < 0.01, OR = 4.27, 95% CI = 1.56-11.67, **Figure 4D**), particularly to be moderate and good creep feed eaters (moderate + good creep feed eaters, MO: 59.6 vs. DD: 35.7%, *P* < 0.01).

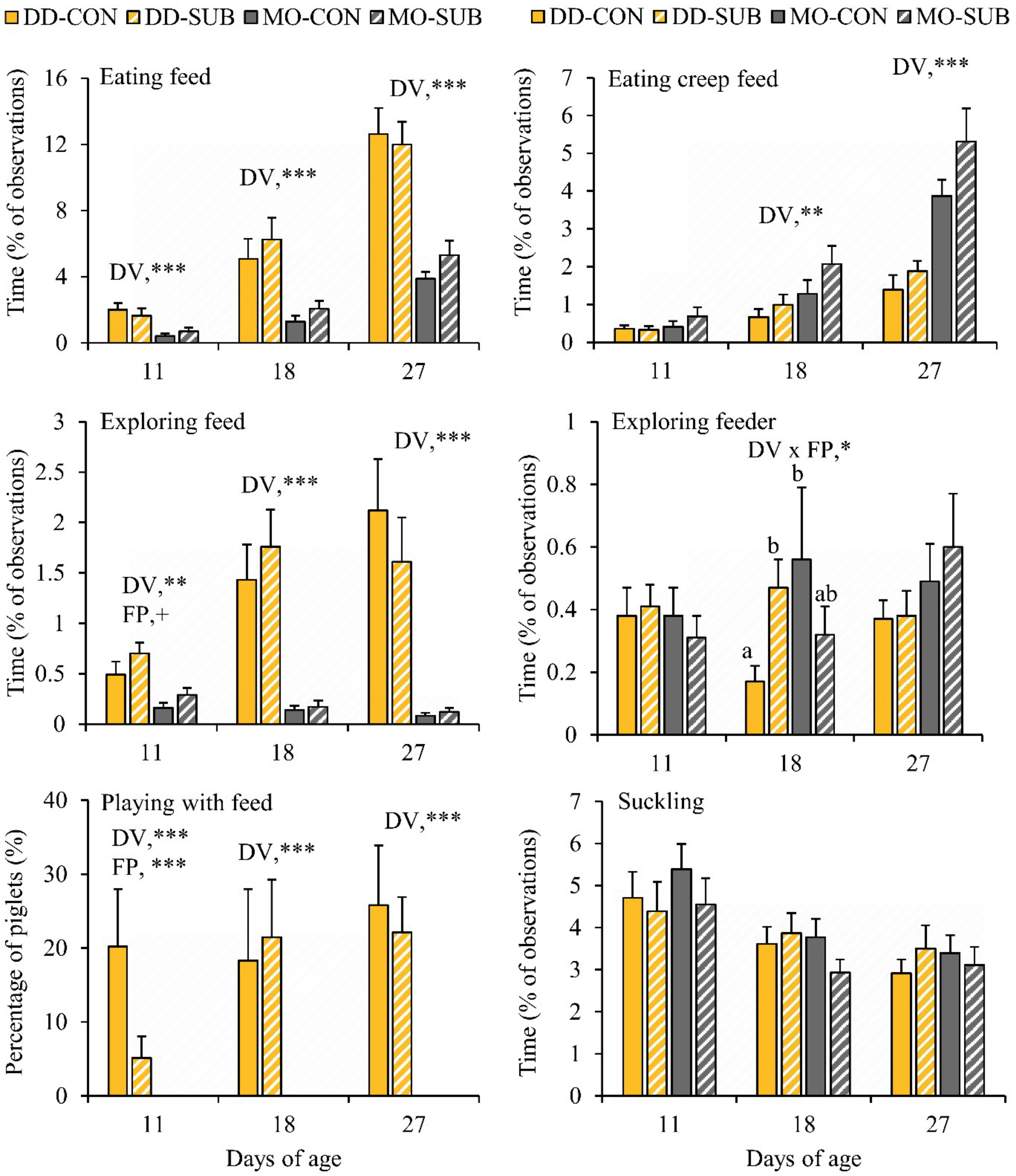


Figure 3. Feed-related behavioural activities (% of total observations) of litters provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD). The feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages. +, *, **, ***Significant effects at $P < 0.10$, < 0.05, < 0.01, < 0.001 respectively. Within a day superscripts without a common letter differ at $P < 0.05$.

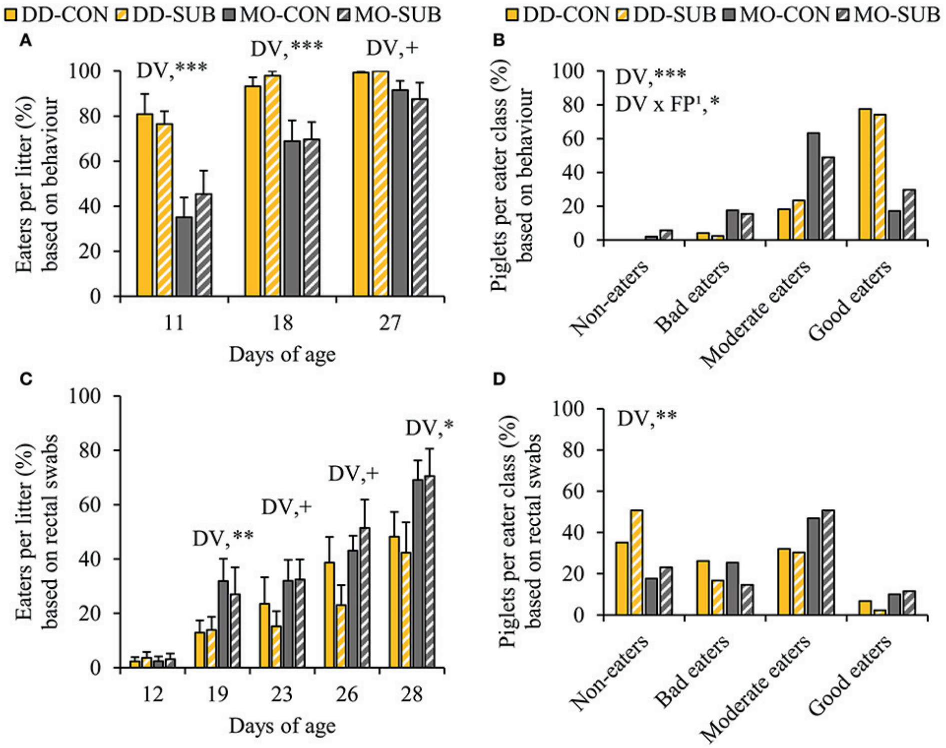


Figure 4. The percentage of eaters per litter over time and individual eater classification of piglets based on live home pen observations (**A, B**) and blue coloured rectal swabs (**C, D**). Litters were either provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD). The feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages. ^{1}SUB effect within MO. +, *, **, ***Significant effects at *P* < 0.10, < 0.05, < 0.01, < 0.001 respectively.

Feed intake before weaning

Feed in DD-pens (sum of all feed items) needed to be refilled more often compared to feed in MO-pens (12.7 ± 1.1 vs. 3.9 ± 0.4 refills/pen/d between d4-28) in all four feeding phases (double as much in phase 1, three times as much in phase 2 and four times as much in phase 3 and 4; **Table 2**). When looking at creep feed only, however, this feed item was refilled half the number of times in DD-pens than in MO-pens (2.0 ± 0.2 vs. 3.9 ± 0.4 refills/pen/d between d4-28). Based on weighing fresh weight of the feed remains, DD-piglets consumed 1267 ± 169 g of feed during lactation (d4-28), of which 178 ± 34 g creep feed (2839 kJ ME), while MO-piglets consumed 260 ± 38 g creep feed (4147 kJ ME) during lactation. Besides, DD-piglets consumed 566 ± 84 g celery (340 kJ ME), 252 ± 47 g cereal honey loops (4024 kJ ME) and 270 ± 54 g peanuts (7058 kJ ME). DD-piglets thereby consumed 1 kg more in total during lactation than MO-piglets, but MO-piglets tended to consume more creep feed than DD-piglets before weaning. Although SUB-pens did not differ from CON-pens in the total number of refills, SUB-pens tended to be refilled more often with creep feed than CON-pens between d12-19 (2.8 ± 0.5 vs. 2.0 ± 0.3 refills/pen/d) and MO-SUB was more often refilled with creep feed than the other 3 treatments between d19-23.

Table 2. Feed intake during the suckling period, based on the number of refills and weighing feed remains. Piglets were provided with creep feed as monotonous diet (MO) or four solid feed items (creep feed, celery, cereal honey loops and peanuts) simultaneously as diverse diet (DD) before weaning and their pre-weaning diet was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are means ± SEM. Within a row superscripts without a common letter differ at $P < 0.05$.

	DD		**MO**		**Significance**		
	CON	**SUB**	**CON**	**SUB**	**DV**	**FP**	**DV x FP**
Total number of refills (sum of all feed items), per pen/d							
d 4 - 12	3.7 ± 0.9	2.8 ± 0.7	1.8 ± 0.7	1.6 ± 0.5	**0.002**	0.50	0.72
d 12 - 19	9.9 ± 1.5^{a}	9.4 ± 1.6^{a}	2.7 ± 0.4^{b}	4.0 ± 0.9^{b}	**< 0.0001**	0.20	**< 0.05**
d 19 - 23	15.7 ± 2.8^{a}	12.6 ± 1.9^{a}	2.5 ± 0.4^{b}	4.2 ± 0.8^{b}	**< 0.0001**	0.54	**0.09**
d 23 - 28	35.1 ± 4.4^{a}	27.0 ± 3.4^{a}	7.1 ± 1.1^{b}	10.0 ± 1.5^{b}	**< 0.0001**	0.86	**0.08**
Total, d 4 - 28	14.1 ± 1.8^{a}	11.4 ± 1.3^{a}	3.3 ± 0.5^{b}	4.5 ± 0.6^{b}	**< 0.0001**	0.72	**0.07**
Number of creep feed refills, per pen/d							
d 4 - 12	0.8 ± 0.2	0.6 ± 0.2	1.8 ± 0.7	1.6 ± 0.5	**0.002**	0.77	0.96
d 12 - 19	1.4 ± 0.3	1.7 ± 0.2	2.7 ± 0.4	4.0 ± 0.9	**< 0.001**	**0.09**	0.46
d 19 - 23	2.2 ± 0.4^{a}	1.8 ± 0.3^{a}	2.5 ± 0.4^{a}	4.2 ± 0.8^{b}	**0.005**	0.42	**0.02**
d 23 - 28	5.0 ± 1.1	4.4 ± 0.8	7.1 ± 1.1	10.0 ± 1.5	**0.002**	0.62	0.21
Total, d 4 - 28	2.1 ± 0.3	1.9 ± 0.2	3.3 ± 0.5	4.5 ± 0.6	**0.0001**	0.41	0.18
Feed intake, g/piglet							
d 4 - 12	72 ± 17	-	9 ± 6	-	**< 0.0001**	-	-
d 12 - 19	206 ± 38	-	64 ± 17	-	**< 0.001**	-	-
d 19 - 23	291 ± 53	-	58 ± 14	-	**< 0.001**	-	-
d 23 - 28	696 ± 96	-	129 ± 18	-	**< 0.001**	-	-
Total, d 4 - 28	1267 ± 169	-	260 ± 38	-	**< 0.0001**	-	-
Creep feed intake, g/piglet							
d 4 - 12	6 ± 2	-	9 ± 6	-	0.82	-	-
d 12 - 19	37 ± 10	-	64 ± 17	-	**0.07**	-	-
d 19 - 23	44 ± 12	-	58 ± 14	-	0.49	-	-
d 23 - 28	92 ± 21	-	129 ± 18	-	0.17	-	-
Total, d 4 - 28	178 ± 34	-	260 ± 38	-	**0.08**	-	-

Effects of sand (with or without sand in the feeder) within feed presentation strategy (SUB)

When given the choice between sand in the feeder or not, piglets spent more time exploring the feed (plus sand) in the feeder with sand (S) compared to exploring the feed in the feeder without sand (NS) at all observation days (S vs. NS, d11: 0.25 ± 0.05 vs. 0.14 ± 0.03%; d18: 0.30 ± 0.08 vs. 0.11 ± 0.03%; d27: 0.22 ± 0.05 vs. 0.06 ± 0.02%; **Figure 5**). At d18, sand x DV tended to interact on eating feed, showing that SUB-piglets spent more time eating from the feeder with sand than from the feeder without sand when fed a monotonous diet. The interaction was significant at d27, and showed that the effect of sand was more pronounced within MO ($P < 0.0001$) than within DD ($P = 0.02$). Moreover, SUB-piglets spent more time exploring the feeder with sand than the feeder without sand at d18, and also at d27 when fed a monotonous diet. Vice versa was observed at d11, when SUB-piglets spent less time exploring the feeder with sand and eating from the feeder with sand than the feeder without sand (S vs. NS, exploring feeder at d11: 0.14 ± 0.03 vs. 0.22 ± 0.03%; eating feed at d11: 0.42 ± 0.10 vs. 0.65 ± 0.19%). In agreement, between d4-12, the feeder with sand was refilled less often compared to the feeder without sand (0.8 ± 0.2 vs. 1.4 ± 0.3 refills/pen/d, $P = 0.003$), but no differences in the number of refills were observed between the feeder with and without sand from d12 onwards (data not shown) and in total between d4-28 (S vs. NS: 3.9 ± 0.5 vs. 4.1 ± 0.6 refills/pen/d, $P = 0.95$).

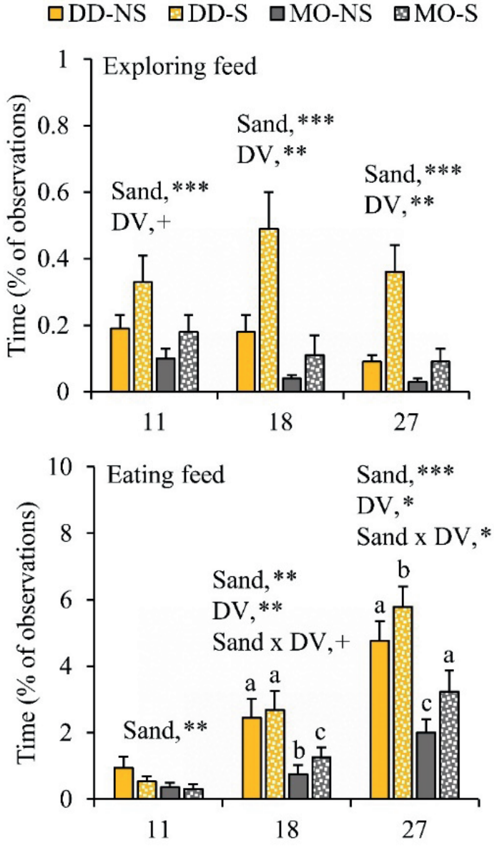
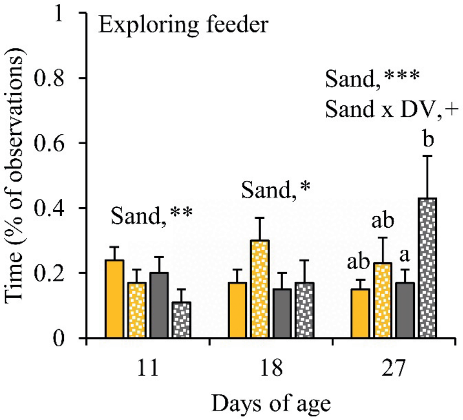


Figure 5. Feed-related behavioural activities (% of total observations) of litters that had their feed presented in one feeder without substrate (NS) and in a second feeder with substrate (S). The litters were either provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD). F, feeder; DV, dietary variety. Data are expressed as means ± SEM based on pen averages. +, *, **, ***Significant effects at $P < 0.10$, < 0.05, < 0.01, < 0.001 respectively. Within a day superscripts without a common letter differ at $P < 0.05$.



Effects of feed item within dietary diversity (DD)

The majority of DD-piglets, i.e. 86%, was seen eating all four feed items before weaning, whereas 10% was seen eating three feed items, 3% two feed items and 1% only one out of four feed items. Some piglets developed strong feed preferences for either one feed item (e.g. piglet G43: 0.6, 10.0, 1.7 and 1.1% of the observation time eating creep feed, celery, cereal honey loops and peanuts respectively at d27) or multiple (e.g. piglet F43: 4.4, 10.6, 1,1, 13.3% respectively), while others divided their feeding time equally over the feed items (e.g. piglet B18: 2.8, 2.2, 2.2 and 3.3% respectively). Strong feed preferences were also observed between pens, with pens that mainly consumed one feed item (e.g. pen 3.14.2: 18, 25, 115 and 36 refills of creep feed, celery, cereal honey loops and peanuts respectively between d23-28), two or three feed items (e.g. pen 1.15.1: 34, 62, 64, 32 refills respectively) versus pens that divided their feeding time equally (e.g. pen 1.15.7: 47, 57, 51 and 48 refills respectively). Overall, DD-piglets spent more time eating peanuts and exploring plus playing with peanuts than with the other three feed items (**Figure 6**). Only at d11, DD-piglets spent less time eating peanuts compared to the other three feed items. Next to peanuts, piglets spent less time eating creep feed than celery and spent less time exploring creep feed than celery and cereal honey loops at d18. Piglets spent as well less time eating creep feed than celery and cereal honey loops at d27. In addition, all feed items differed in the amount of time that was spent on exploring plus playing towards them at d11, with the lowest amount of time exploring plus playing towards creep feed, followed by cereal honey loops, celery and the largest amount of time towards peanuts. Comparing the different feed items in DD-pens in terms of the number of refills, no differences were found in how often the different feed items were refilled in the first feeding phase (data not shown, $P = 0.22$). From the second feeding phase onwards, pellets were refilled half the number of times than peanuts, celery and cereal honey loops in DD-pens (data not shown, $P < 0.01$ for all phases). In total before weaning, pellets were refilled less often than the other three items (2.0 ± 0.2, 3.8 ± 0.3, 3.7 ± 0.5 and 3.3 ± 0.4 refills/pen/d of creep feed, celery, cereal honey loops and peanuts respectively between d4-28, $P \leq 0.001$).

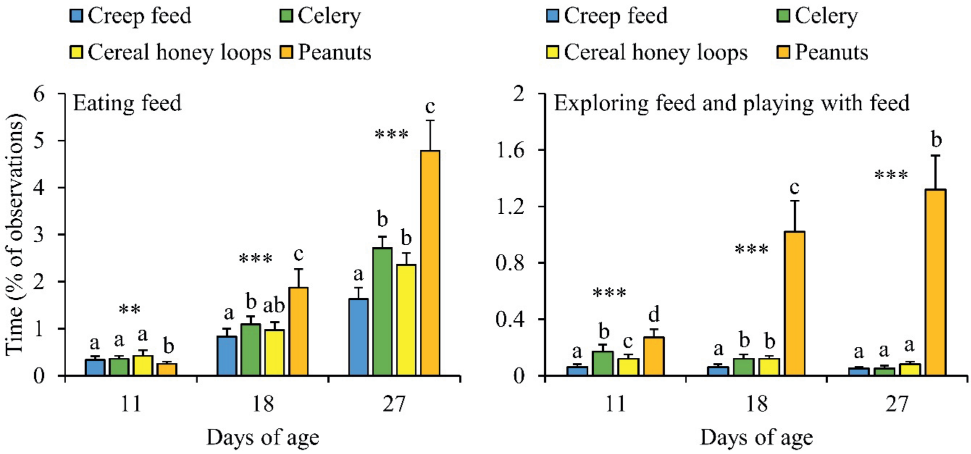


Figure 6. Feed-related behavioural activities (% of total observations) towards four different feed items of litters provided with those four feed items simultaneously as a diverse diet. Data are expressed as means ± SEM based on pen averages. **, ***Significant effects at $P < 0.01$ and < 0.001 respectively. Within a day superscripts without a common letter differ at $P < 0.05$.

Sow and piglet body weight development

The number of weaned piglets and sow body weight and back fat loss during lactation were not affected by dietary variety, feed presentation or their interaction (**Table 3**). Dietary variety and feed presentation tended to interact on ADG between d4-19 ($P = 0.06$), in which DD-SUB piglets grew faster than MO-SUB piglets ($P = 0.01$). DD-piglets grew faster in the last two days prior to weaning compared to MO-piglets (DD: 280 ± 10 vs. MO: 251 ± 11 g/d), but the treatment groups did not differ in weaning weight at d28 and homogeneity in weaning weight within litters (CV). Time spent eating at d27 positively correlated with ADG between d26-28 in MO-piglets (r = 0.40; $P < 0.0001$ on piglet level and r = 0.74; $P = 0.0002$ on litter level), but not in DD-piglets (r = 0.05; $P = 0.44$ on piglet level and r = 0.31; $P = 0.18$ on litter level).

Table 3. Performance of sows and piglets during lactation. Litters were either provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD). The feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are means ± SEM based on pen averages. Within a row superscripts without a common letter differ at $P < 0.05$.

	DD		**MO**		**Significance**		
	CON	**SUB**	**CON**	**SUB**	**DV**	**FP**	**DV x FP**
Sow back fat loss, mm	4.3 ± 0.5	6.3 ± 0.9	5.1 ± 0.8	4.4 ± 0.7	0.59	0.25	0.57
Sow BW loss, kg	35 ± 3	39 ± 3	43 ± 5	37 ± 3	0.42	0.85	0.29
ADG, g/piglet/d							
d 4 - 19	210 ± 8^{ab}	225 ± 6^{a}	209 ± 8^{ab}	198 ± 11^{b}	**0.06**	0.86	**0.06**
d 19 - 26	223 ± 14	210 ± 14	205 ± 10	217 ± 11	0.66	0.96	0.39
d 26 - 28	292 ± 13	269 ± 15	244 ± 15	257 ± 16	**0.04**	0.77	0.33
Total, d 4 - 28	220 ± 8	224 ± 8	211 ± 9	208 ± 9	0.13	0.99	0.53
Body weight, kg							
d 0	1.38 ± 0.05	1.38 ± 0.07	1.40 ± 0.07	1.44 ± 0.09	0.53	0.84	0.79
d 4	1.86 ± 0.09	1.85 ± 0.10	1.86 ± 0.09	1.86 ± 0.10	0.93	0.99	0.89
d 28	7.17 ± 0.25	7.22 ± 0.24	6.95 ± 0.25	6.86 ± 0.27	0.21	0.96	0.65
Litter CV in BW, %							
d 4	20.0 ± 1.5	19.1 ± 0.8	18.2 ± 1.4	16.7 ± 1.4	**< 0.10**	0.41	0.88
d 28	19.0 ± 1.3	18.3 ± 1.4	18.1 ± 1.2	19.2 ± 2.5	0.48	0.55	0.32

Piglet behaviour after weaning

Ingestive behaviour

Time spent eating tended to be higher in SUB-piglets compared to CON-piglets at week 1 post-weaning (SUB: 12.6 ± 0.8 vs. CON: 11.3 ± 0.6%; **Table 4**). The treatments tended to interact in their effect on time spent drinking at week 2 post-weaning (**Table 5**), but no significant differences were observed using post-hoc pairwise comparisons of least squares means.

Exploratory behaviour

DD-piglets spent less time exploring the feed(er) and drinker than MO-piglets at week 1 post-weaning (DD: 2.4 ± 0.2 vs. MO: 3.2 ± 0.3%). DD-piglets also explored their environment less than MO-piglets in this period (DD: 19.8 ± 1.0 vs. MO: 24.7 ± 1.3%), which was reflected by lower levels of chewing the environment (DD: 6.3 ± 0.8 vs. MO: 10.5 ± 1.2%) and chewing air (DD: 1.7 ± 0.2 vs. MO: 2.6 ± 0.2%). At week 2 post-weaning, interactions between DV x FP were found on exploring the environment ($P < 0.01$) and chewing the environment ($P < 0.01$), showing that DD-CON piglets spent less time exploring their environment than the other three treatment groups ($P \leq 0.01$ for all) and DD-CON piglets had lower levels of chewing their environment than DD-SUB and MO-CON piglets ($P < 0.01$ for both). DD-piglets showed less rooting of their environment compared to MO-piglets (DD: 1.5 ± 0.3 vs. MO: 2.0 ± 0.3%) and SUB-piglets showed more rooting of their environment compared to CON-piglets (SUB: 2.1 ± 0.3 vs. CON: 1.4 ± 0.2%) at week 2 post-weaning. No effects were found on nosing the environment.

Postures and locomotion

DD-piglets tended to be inactive for a larger amount of time than MO-piglets at week 1 post-weaning (DD: 46.7 ± 2.0 vs. MO: 41.9 ± 2.0%). CON-piglets were inactive for a larger amount of time than SUB-piglets at week 1 post-weaning (CON: 46.3 ± 2.0 vs. SUB: 42.3 ± 2.1%). Also in week 2 post-weaning, but only in DD (DV x FP, $P = 0.08$), as DD-CON piglets spent more time inactive than the other three treatment groups ($P \leq 0.03$ for all). CON-piglets also showed less standing and walking at week 2 post-weaning than SUB-piglets (CON: 2.9 ± 0.2 vs. SUB: 3.7 ± 0.2%).

Pig-directed behaviour

Nosing pen mates was higher for DD-piglets than MO-piglets at week 1 post-weaning (DD: 4.2 ± 0.3 vs. MO: 3.1 ± 0.2%). SUB-piglets had higher levels of manipulating pen mates (SUB: 0.8 ± 0.1 vs. CON: 0.5 ± 0.1%) and aggression (SUB: 0.2 ± 0.04 vs. CON: 0.1 ± 0.03%) than CON-piglets at week 1 after weaning. At week 2, DD-piglets also had higher levels of nosing pen mates than MO-piglets, but only within CON (DV x FP, $P = 0.03$). A trend for a DV x FP interaction was found on manipulating pen mates at week 2 after weaning ($P = 0.09$), but no significant differences were observed using post-hoc pairwise comparisons of least squares means. Ear biting, tail biting, belly nosing and mounting pen mates were not affected by treatments.

Table 4. Behavioural activities (% of total observations) at week 1 after weaning (35 days of age). Piglets were provided with one solid feed item as monotonous diet (MO) or four solid feed items as diverse diet (DD) before weaning and their pre-weaning diet was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are means ± SEM based on pen averages. Within a row superscripts without a common letter differ at $P < 0.05$.

Behaviour at week 1 after weaning	**DD**		**MO**		**Significance**		
	CON	**SUB**	**CON**	**SUB**	**DV**	**FP**	**DV x FP**
‘Ingestive behaviour’							
Eating feed	11.7 ± 1.0	11.9 ± 0.7	10.8 ± 0.7	13.4 ± 1.4	0.91	**0.06**	0.11
Drinking	1.0 ± 0.2	0.7 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	0.23	0.16	0.47
‘Exploratory behaviour’							
Exploring feed(er) and drinker	2.4 ± 0.3	2.4 ± 0.2	2.6 ± 0.3	3.8 ± 0.4	**0.03**	0.11	0.13
Exploring environment	19.4 ± 1.6	20.2 ± 1.2	24.9 ± 1.5	24.5 ± 2.2	**< 0.01**	0.84	0.76
Nosing environment	9.8 ± 0.7	10.8 ± 0.7	9.8 ± 1.0	10.0 ± 0.8	0.43	0.39	0.68
Rooting environment	1.2 ± 0.2	1.6 ± 0.4	1.8 ± 0.4	1.6 ± 0.3	0.23	0.79	0.21
Chewing environment	6.9 ± 1.2	5.8 ± 0.9	10.8 ± 1.2	10.2 ± 2.2	**< 0.01**	0.41	0.85
Chewing air	1.6 ± 0.3	1.9 ± 0.3	2.5 ± 0.3	2.7 ± 0.3	**0.01**	0.39	0.60
‘Postures and locomotion’							
Inactive behaviour	48.5 ± 2.9	44.9 ± 2.7	44.0 ± 2.6	39.8 ± 3.1	**0.05**	**0.04**	0.63
Standing and walking	5.5 ± 0.7	7.5 ± 0.8	6.5 ± 1.0	6.7 ± 1.0	0.98	0.14	0.33
‘Pig-directed behaviour’							
Nosing pen mates	4.1 ± 0.4	4.4 ± 0.4	3.2 ± 0.3	3.0 ± 0.4	**< 0.001**	0.76	0.62
Ear biting	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.63	0.59	0.55
Tail biting	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.98	0.17	0.59
Belly nosing	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.61	0.43	0.61
Manipulating pen mates	0.4 ± 0.1	1.0 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.87	**0.02**	0.28
Mounting pen mates	0.8 ± 0.1	0.9 ± 0.4	0.6 ± 0.2	0.8 ± 0.2	0.50	0.28	0.60
Aggression	0.1 ± 0.03	0.2 ± 0.04	0.1 ± 0.1	0.2 ± 0.1	0.83	**0.03**	0.94
‘Other behaviour’							
Playing	3.4 ± 0.5	3.0 ± 0.3	2.8 ± 0.4	3.6 ± 0.5	0.99	0.66	0.24
Comfort behaviour	0.6 ± 0.1^{ab}	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}	0.3 ± 0.1^{b}	0.10	0.24	**0.02**
Eliminating	0.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	0.95	0.21	0.29

Table 5. Behavioural activities (% of total observations) at week 2 after weaning (42 days of age). Piglets were provided with one solid feed item as monotonous diet (MO) or four solid feed items as diverse diet (DD) before weaning and their pre-weaning diet was presented without substrate (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are means ± SEM based on pen averages. Within a row superscripts without a common letter differ at $P < 0.05$.

Behaviour at week 2 after weaning	**DD**		**MO**		**Significance**		
	CON	**SUB**	**CON**	**SUB**	**DV**	**FP**	**DV x FP**
‘Ingestive behaviour’							
Eating feed	11.1 ± 0.6	11.3 ± 0.5	10.5 ± 0.6	11.7 ± 0.5	0.88	0.23	0.41
Drinking	2.0 ± 0.3	1.5 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	0.49	0.40	**0.07**^{1}
‘Exploratory behaviour’							
Exploring feed(er) and drinker	3.1 ± 0.3	3.3 ± 0.5	3.4 ± 0.5	3.8 ± 0.4	0.43	0.39	0.68
Exploring environment	18.8 ± 1.2^{a}	29.4 ± 2.6^{b}	28.3 ± 3.2^{b}	26.5 ± 1.6^{b}	**0.09**	0.04	**< 0.01**
Nosing environment	9.5 ± 1.2	12.0 ± 1.1	11.7 ± 1.1	11.3 ± 0.7	0.47	0.22	0.18
Rooting environment	1.1 ± 0.2	2.0 ± 0.5	1.7 ± 0.5	2.2 ± 0.4	**0.04**	**0.03**	0.18
Chewing environment	6.6 ± 1.3^{a}	13.1 ± 2.1^{b}	12.8 ± 2.0^{b}	10.5 ± 1.4^{ab}	0.15	0.14	**<0.01**
Chewing air	1.7 ± 0.2	2.3 ± 0.4	2.0 ± 0.5	2.5 ± 0.5	0.64	0.23	0.75
‘Postures and locomotion’							
Inactive behaviour	50.5 ± 2.2^{a}	40.5 ± 2.5^{b}	43.6 ± 2.5^{b}	41.1 ± 2.0^{b}	0.17	**< 0.01**	0.08
Standing and walking	2.8 ± 0.2	3.8 ± 0.2	3.1 ± 0.3	3.6 ± 0.2	0.73	**< 0.01**	0.27
‘Pig-directed behaviour’							
Nosing pen mates	4.1 ± 0.3^{a}	3.3 ± 0.2^{ab}	2.9 ± 0.3^{b}	3.6 ± 0.3^{ab}	**< 0.10**	0.98	**0.03**
Ear biting	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.32	0.88	0.19
Tail biting	0.6 ± 0.1	0.8 ± 0.3	0.5 ± 0.1	0.6 ± 0.1	0.32	0.48	0.81
Belly nosing	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.4	0.93	0.73	0.89
Manipulating pen mates	1.1 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	0.90	0.64	**0.09**^{1}
Mounting pen mates	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.99	0.28	0.94
Aggression	0.1 ± 0.05	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.70	0.63	0.48
‘Other behaviour’							
Playing	3.2 ± 0.4	2.5 ± 0.4	2.3 ± 0.4	3.3 ± 0.3	0.80	0.61	**0.03**^{1}
Comfort behaviour	0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.11	0.54	0.29
Eliminating	1.0 ± 0.1	1.2 ± 0.2	0.8 ± 0.1	0.9 ± 0.2	**0.05**	0.37	0.76

[1] No significant differences were observed using post-hoc pairwise comparisons of least squares means

Other behaviour

DV x FP interacted on comfort behaviour at week 1 post-weaning (*P* = 0.02), showing lower levels of comfort behaviour for MO-SUB piglets in comparison with MO-CON (*P* = 0.03) and DD-SUB piglets (*P* = 0.01). In addition, DV x FP interacted on playing at week 2 post-weaning (*P* = 0.03), but no significant differences were observed using post-hoc pairwise comparisons of least squares means. Lastly, DD-piglets tended to eliminate more than MO-piglets at week 2 post-weaning (DD: 1.1 ± 0.1 vs. MO: 0.8 ± 0.1%).

Body lesions in the first two days after weaning

Dietary diversity before weaning reduced the number of body lesions at 4 hours after weaning (DD: 15.8 ± 1.9 vs. MO: 24.1 ± 3.3 lesions), while feed presentation in substrate during lactation, in contrast, increased the number of body lesions (SUB: 23.7 ± 3.0 vs. CON: 16.1 ± 2.4 lesions; **Table 6**). A DV x FP effect was found on the number of body lesions at 24 hours and 48 hours after weaning, showing that MO-SUB piglets had more lesions on their body than the other three treatment groups at 24 hours after weaning, but no significant pairwise differences were observed at 48 hours after weaning.

Body lesions and damage at two weeks post-weaning

Dietary diversity and feed presentation in substrate before weaning increased the number of body lesions at d15 after weaning (DD: 5.6 ± 0.7 vs. MO: 4.1 ± 0.6 lesions; SUB: 5.6 ± 0.7 vs. CON: 4.0 ± 0.6 lesions; **Table 6**). Dietary variety, feed presentation and their interaction did not affect the percentage of piglets with ear damage at two weeks post-weaning (**Figure 7A**). The percentage of piglets with tail damage, however, was affected by the interaction between DV x FP, as DD-CON piglets had less often higher tail damage scores than DD-SUB piglets (**Figure 7B**).

Table 6. Body lesions at 4, 24, 48 hours and 15 days post-weaning of piglets provided with one solid feed item as monotonous diet (MO) or four solid feed items as diverse diet (DD) before weaning and their pre-weaning diet presented without substrate (CON) or with substrate (SUB). DV = dietary variety. FP = feed presentation. Data are means ± SEM based on pen averages. Within a row superscripts without a common letter differ at $P < 0.05$.

Body lesions	**DD**		**MO**		**Significance**		
	CON	**SUB**	**CON**	**SUB**	**DV**	**FP**	**DV x FP**
4 h	14.5 ± 2.5	17.2 ± 2.8	17.8 ± 4.1	30.3 ± 4.5	**0.06**	**0.04**	0.29
24 h	3.2 ± 1.1^a	1.8 ± 0.7^a	2.4 ± 0.6^a	6.5 ± 1.1^b	**0.06**	0.37	**< 0.01**
48 h	0.8 ± 0.3	1.8 ± 0.5	1.7 ± 0.5	1.0 ± 0.2	0.97	0.63	**0.04**[1]
d 15	4.8 ± 1.0	6.3 ± 0.9	3.2 ± 0.6	5.0 ± 1.1	**0.03**	**0.02**	0.47

[1] No significant differences were observed using post-hoc pairwise comparisons of least squares means

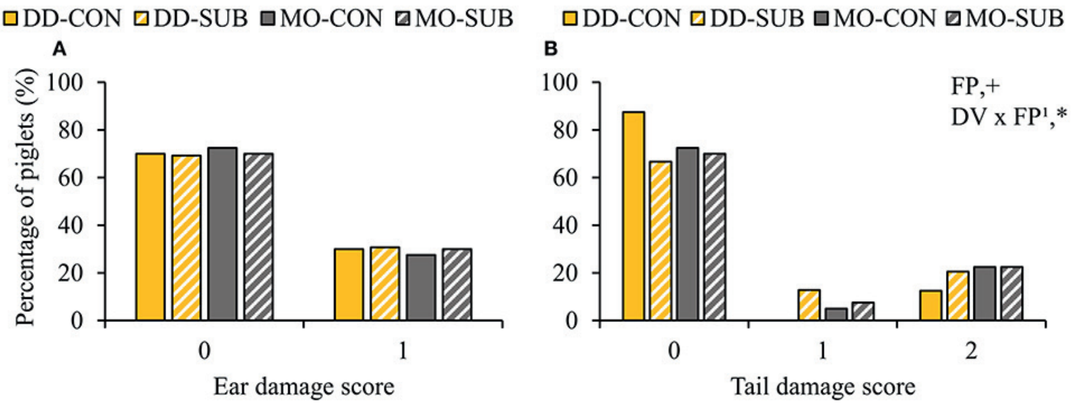


Figure 7. Occurrence of ear (**A**) and tail damage (**B**) (% of piglets with each score) at two weeks post-weaning on piglets provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) before weaning and their pre-weaning diet was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Ear damage was classified as 0: no damage or 1: bite marks or small wound. Tail damage was classified as 0: no damage, 1: bite marks or 2: small or medium wound. Data are expressed as means. ^{1}FP effect within DD. +, *Significant effects at $P < 0.10$ and < 0.05 respectively.

Feed intake after weaning

Dietary variety and feed presentation tended to interact in their effect on feed intake in the first four hours after weaning (**Table 7**), but no significant differences were found using post-hoc pairwise comparisons of least squares means. SUB-piglets had a lower feed intake on the second day post-weaning (SUB: 243 ± 18 vs. CON: 318 ± 13 g/d) and between d0-2 post-weaning (SUB: 186 ± 15 vs. CON: 241 ± 17 g/d, $P = 0.02$) versus CON-piglets. They also had a lower ADFI between d0-5 (SUB: 286 ± 9 vs. CON: 324 ± 13 g/d, $P = 0.03$) and in total from d0-15 post-weaning (SUB: 500 ± 10 vs. CON: 537 ± 19 g/d). Dietary variety and feed presentation interacted in their effect on ADFI between d5-15 ($P = 0.04$). Post-hoc pairwise comparisons showed that DD-CON piglets had a higher ADFI in this period compared to the other three treatment groups ($P \leq 0.02$ for all comparisons).

Piglet growth and faecal consistency after weaning

SUB-piglets lost weight on the first day after weaning compared to CON-piglets (SUB: -100 ± 47 vs. CON: 40 ± 56 g/d) and gained less on the second day after weaning (SUB: 385 ± 23 vs. CON: 470 ± 33 g/d; **Table 7**). They also had a lower ADG between d0-2 post-weaning (SUB: 142 ± 24 vs. CON: 255 ± 24 g/d, $P < 0.01$), but tended to have a higher ADG between d2-5 post-weaning (SUB: 309 ± 17 vs. CON: 283 ± 17 g/d). Taken together, no differences were found in ADG between d0-5 post-weaning (data not shown). Dietary variety and feed presentation interacted in their effect on ADG between d5-15 ($P = 0.04$) and d0-15 ($P = 0.09$) and BW at d15 post-weaning ($P = 0.09$). Post-hoc pairwise comparisons showed that SUB-piglets had a lower ADG compared to CON-piglets in these two periods and a lower BW at d15 post-weaning, but only when fed a diverse diet before weaning ($P = 0.02$ for all). DD-piglets tended to have a higher feed conversion ratio than MO-piglets after weaning (1.31 ± 0.01 vs. 1.28 ± 0.01). Dietary variety, feed presentation and their interaction did not affect the prevalence, duration and severity of (watery) diarrhoea (**Table 7**).

Table 7. Weaner performance in the first two weeks after weaning (d0-15 post-weaning). Piglets were provided with one solid feed item as monotonous diet (MO) or four solid feed items as diverse diet (DD) before weaning and their pre-weaning diet was presented without substrate (CON) or with substrate (SUB). DV, dietary variety; FP, feed presentation. Data are means ± SEM based on pen averages. Within a row superscripts without a common letter differ at $P < 0.05$.

	DD		**MO**		**Significance**		
	CON	**SUB**	**CON**	**SUB**	**DV**	**FP**	**DV x FP**
FI, g/piglet							
0 - 4 h	27 ± 2	11 ± 6	6 ± 2	13 ± 4	0.27	0.92	**<0.10**[1]
4 - 24 h	146 ± 35	121 ± 19	150 ± 25	112 ± 32	0.99	0.23	0.73
ADFI, g/piglet/d							
d 0 - 1	172 ± 42	133 ± 23	156 ± 26	124 ± 34	0.76	0.21	0.99
d 1 - 2	326 ± 23	264 ± 19	310 ± 15	222 ± 29	0.20	**<0.01**	0.58
d 2 - 5	387 ± 20	357 ± 11	371 ± 21	347 ± 19	0.27	0.11	0.66
d 5 - 15	668 ± 26^{a}	605 ± 14^{b}	618 ± 36^{b}	610 ± 26^{b}	**0.09**	**0.08**	**0.04**
Total, d 0 - 15	556 ± 23	500 ± 11	517 ± 29	499 ± 18	0.10	**0.03**	0.11
ADG, g/piglet/d							
d 0 - 1	-2 ± 95	-62 ± 54	83 ± 63	-139 ± 78	0.91	**0.04**	0.22
d 1 - 2	474 ± 48	358 ± 29	467 ± 47	412 ± 36	0.62	**0.04**	0.34
d 2 - 5	292 ± 29	301 ± 21	275 ± 19	318 ± 28	0.76	**0.07**	0.19
d 5 - 15	508 ± 23^{a}	453 ± 12^{b}	474 ± 33^{ab}	469 ± 27^{ab}	0.98	0.19	**0.04**
Total, d 0 - 15	429 ± 23^{a}	382 ± 9^{b}	407 ± 28^{ab}	395 ± 20^{ab}	0.91	**<0.10**	**0.09**
BW at d 15, kg	13.79 ± 0.33^{a}	13.09 ± 0.15^{b}	13.19 ± 0.53^{ab}	12.87 ± 0.33^{ab}	0.91	**<0.10**	**0.09**
Feed conversion ratio, d 0 - 15	1.31 ± 0.02	1.31 ± 0.02	1.28 ± 0.02	1.27 ± 0.02	**0.08**	0.94	0.43
Faecal consistency and diarrhoea							
Faecal consistency score	0.41 ± 0.10	0.34 ± 0.06	0.35 ± 0.11	0.37 ± 0.10	0.94	0.44	0.70
# days with diarrhoea	4.50 ± 0.89	3.90 ± 0.59	4.10 ± 1.22	4.10 ± 0.90	0.89	0.51	0.89
# days with watery diarrhoea	1.70 ± 0.67	1.20 ± 0.47	1.20 ± 0.47	1.40 ± 0.65	0.96	0.56	0.59
% pens with watery diarrhoea	50	70	50	50	0.62	0.62	0.39

[1] No significant differences were observed using post-hoc pairwise comparisons of least squares means

Discussion

We investigated the effects of dietary variety (versus monotony, DD vs. MO) and feed presentation (hidden in sand as substrate or not, SUB vs. CON) before weaning on the feeding behaviour and performance of piglets up to two weeks after weaning. Dietary diversity highly stimulated the feeding behaviour of suckling piglets and, in contrast with piglets on a monotonous diet, all piglets were observed to eat feed prior to weaning. Presenting a part of the feed in substrate hardly increased foraging behaviour before weaning, although piglets spent more time exploring and eating from the feeder with sand than from the feeder without sand. Dietary diversity (that was only given pre-weaning) only affected piglet development after weaning to a limited extent, but piglets that were given a diverse diet presented in feeders without substrate seemed to perform the best in the post-weaning period of the four groups in terms of feed intake, body weight gain and tail damage. Presenting the pre-weaning diet in substrate negatively affected weaner piglet performance, as post-weaning feed intake and growth were reduced and weaning-stress-induced behaviours were increased.

Effects of dietary variety

Dietary diversity stimulated time spent exploring the feed and eating it by at least 2.5 times at all observation days. In total DD-piglets spent 14% of their time interacting with the feed of which they spent 12% of the time eating it at four weeks of age, and consumed 1267 g feed during lactation (determined in fresh weight), which seems exceptionally high compared to MO-piglets (5% time spent eating, 260 g feed intake) and previous studies (2% and 50-90 g/piglet in week 4 in Appleby et al. (1992); 3.5-4.5% at week 3 and 601-693 g/piglet during lactation in Van den Brand et al. (2014); 4.4% at week 4 and 397 g/piglet during lactation in Middelkoop et al., under revision). Moreover, dietary diversity enhanced the percentage of piglets observed to be eating by 38% early in lactation and by 10% shortly before weaning and all DD-piglets were seen eating feed during lactation as compared with a monotonous diet. Dietary diversity particularly stimulated piglets to start eating early, as more than 75% of the piglets were observed eating from 11 days of age, whereas in MO it was 23%. The success of dietary variety on the feeding behaviour of suckling piglets may be the result of a reduction in sensory-specific satiety that is induced by exposure to a monotonous diet. This is supported by our observation that 99% of the DD-piglets consumed more than one of the four solid feed items before weaning, of which 86% consumed all four feed items, thereby indicating that piglets prefer to eat diverse feed items when provided the choice. We observed a similar effect when piglets were given the choice between two feeds, in which 88% of the eaters consumed both (Middelkoop et al., 2018). As a result, DD-piglets experienced variety in all senses, including sight (e.g. colour, size and shape), smell, taste (e.g. sweet, bitter), touch (e.g. texture) and hearing (e.g. crunch). The variety in feed items may have reduced sensory-specific satiety, resulting in an increase in feed intake. It should be noted that there were large individual differences in preferences for the different feed items, and in the strength of the preferences, as some piglets distributed their eating time more or less equally over the items, whereas others fed on one, two or three feed items mainly. The stimulation of feed intake could therefore also partly be the result of more choice allowing piglets to select their preferred item. The feeding behaviour of DD-piglets may also have been enhanced by intrinsic exploration towards the feed (Van den Brand et al., 2014; Middelkoop et al., 2018), elicited by differential sensorial experiences of the feeds. Our previous study in piglets suggests that feeds with multiple sensory differences can enhance feed exploration and intake more than feeds that vary in flavour only (Middelkoop et al., 2018), whereas specific properties of the feed may also stimulate feed intake, such as a larger size (Van den Brand et al., 2014). This mechanism is further supported by the positive correlation between time spent exploring the feed and eating it. Alternatively, post-ingestive signals may have mediated the increased feed intake by DD-piglets, as differences in nutrient profiles existed between the diets of DD- and MO-piglets. Changes in post-ingestive signals may lead to physiological changes in the animal, such as modification of appetite-controlling hormones and, as such, affected feed intake. Alternatively, the increase in feed intake caused by dietary diversity may have exerted changes in appetite-controlling hormonal profiles as result of changes in the feeding pattern of the animals, as discussed by Villalba et al. (2011).

DD-piglets grew faster than MO-piglets in the last two days before weaning and thereby seems in agreement with lambs in a diversity treatment that tended to grow faster than lambs in the other monotonous treatments (Villalba et al., 2011). Although DD-piglets spent four times more time on feeding on solids than mothers' milk, they continued to suckle milk and no differences were found in time spent suckling between DD- and MO-piglets. It is, therefore, suggested that the higher weight gain of DD-piglets compared to MO-piglets in the last two days prior to weaning might be the result of an earlier uptake of feed (thereby stimulating the development of the gastrointestinal tract and gut microbiota) or a greater uptake of feed (resulting in a higher energy intake and/or heavier digesta in the gut). Both may play a role as bowls of DD-pens were refilled more often already from the first feeding phase onwards, and the metabolizable energy intake from solid feed by DD-piglets was roughly 3.4 times higher than the metabolizable energy intake from solid feed by MO-piglets. This difference in ME intake is sufficiently high to account for the higher growth in DD-piglets as compared with MO-piglets towards weaning. Using the method described by Pluske et al. (1995), the contribution of solid feed to the total energy intake before weaning was estimated to be 11.9% for DD-piglets and 3.7% for MO-piglets. The energy intake from solid feed by DD-piglets could be explained for approximately 50, 28, 20 and 2% by the intake of peanuts, cereal honey loops, creep feed and celery respectively. Time spent eating did not correlate with average daily gain between d26-28 in DD-piglets, while it did in MO-piglets. An underlying reason may be that the consumption of feed items, and thus nutrient and energy intake, was more variable between DD-piglets than between MO-piglets, of which the latter could only consume creep feed.

When given the choice between creep feed, celery, cereal honey loops and peanuts, piglets were seen more often eating peanuts at d18 and d27 than the other items, although peanuts were not refilled more often than celery and cereal honey loops. We therefore assume that the time piglets spent eating peanuts was longer, but not resulting in more actual intake than the other items, because piglets may have spent more time chewing on the peanuts to break the peanut into smaller pieces before ingestion could occur or to crack the shell before they could ingest the nuts. This may also have affected the correlation between time spent eating and average daily gain between d26-28. In addition, creep feed was less preferred to the other three feed items from d18 (based on behavioural observations and refills), which were larger in size and more complex in texture than the pellets. Larger feed items have been found previously to stimulate feed intake, as they are easier to handle for piglets (Van den Brand et al., 2014), which may at least partly explain why creep feed was least preferred. It should be noted, though, that the four feed items differed in various sensory properties which may, apart from size differences, have affected preferences. Pen differences were also observed in these patterns, which may indicate social transmission of feed preferences within a pen, as piglets have been shown to acquire information concerning feed from their siblings (Nicol and Pope, 1994), although a genetic influence on feed preferences cannot be excluded.

On top of a positive effect of dietary diversity on piglet performance before weaning, dietary diversity may also improve animal welfare before weaning, by providing individuals with food choices (as suggested in laying hens by Edgar et al. 2013) and stimulating playing with the feed. Play has been proposed to both induce and reflect positive welfare (reviewed by Held and Špinka 2011), although this does not seem straightforward in all cases (Ahloy-Dallaire et al., 2018). The main potential advantage of a high feed intake before weaning is, however, that it may facilitate coping with weaning due to its expected benefits for post-weaning feed intake, body weight gain (Kuller et al., 2004; Berkeveld et al., 2007b), net absorption in the small intestine (Kuller et al., 2007b), gut physiology and gut microbiota development. This is of importance because several studies have shown that eaters outperform non-eaters (Bruininx et al., 2002, 2004; Kuller et al., 2007a; Sulabo et al., 2010a) and good/early eaters outperform bad eaters (Carstensen et al., 2005; Pluske et al., 2007) in terms of feed intake and weight gain. Based on these studies, one would expect large beneficial effects of dietary variety on post-weaning feed intake and gain, as this treatment successfully stimulated solid feed intake and piglets to become eaters before weaning. Contrary to expectations, however, the beneficial effects were minor, as piglets that were given the diverse diet in two feeders without substrate during lactation only had the highest feed intake and body weight gain in the period from day 5 to 15 post-weaning. In terms of behaviour, it could not be concluded whether the effects of dietary diversity on the behavioural development of piglets after weaning were beneficial for piglet welfare or not. Firstly, DD-piglets had a lower number of body lesions at 4 hours after weaning compared to MO-piglets, which could point to a lower level of aggression. Aggression between unfamiliar piglets immediately after weaning is mainly aimed at establishing a new social hierarchy (Meese and Ewbank, 1973), but mixing of piglets was performed equally over treatments in this study. As frustration can also induce aggression in pigs (Arnone and Dantzer, 1980), the lower number of body lesions at 4 hours after weaning in DD piglets may potentially also reflect less frustration-related aggression as compared with MO-piglets. However, DD-piglets had a higher number body lesions than MO-piglets at 15 days after weaning. Secondly, DD-piglets spent less time exploring the feed(er) and drinker, chewing the environment (i.e. chain and parts of the pen) and chewing air (i.e. sham or vacuum chewing), but showed more nosing pen mates and tended to be inactive for a longer period of time at week 1 post-weaning compared to MO-piglets. Chewing on a chain (Dybkjær, 1992), pen fixtures and/or air (Haskell et al. 1996; Bolhuis et al. 2005), nosing pen mates (Beattie et al., 1995; Oostindjer et al., 2011d) and inactive behaviour (e.g. Haskell et al., 1996; Bolhuis et al., 2005; Oostindjer et al., 2011d) have been found to increase in barren as compared with enriched housing, which is commonly thought to result from unfulfilled needs for exploration in the absence of suitable rooting substrates and may reflect stress. At week 2 post-weaning, effects seemed more pronounced in piglets that were fed a diverse diet in feeders without substrate, as they had the lowest level of chewing environment, highest level of nosing pen mates and being inactive and lowest number of piglets with higher tail damage scores.

One of the reasons that the beneficial effects of dietary diversity on post-weaning performance might have been minor, in spite of its impact on pre-weaning feed intake and the number of eaters, is that DD-piglets seemed to appreciate the creep feed the least compared to the other three feed items. They spent less time eating creep feed than MO-piglets at d18 and d27 and a lower number of DD-piglets were classed as creep feed eaters from d19. DD-piglets thereby mainly ingested the other feed items, which connected less well to the commercial weaner diet in terms of structure and ingredient composition. The latter may also explain the trend for a higher feed conversion ratio in DD-piglets that was found after weaning. The interaction between the composition of the pre- and post-weaning diet is one of the determinants of post-weaning performance (Torrallardona et al., 2012) and may therefore play an important role in the success of a high feed intake before weaning on post-weaning performance. Secondly, it has been well-documented in children that they begin to show 'picky eating' behaviour, such as strong food preferences, when they are exposed to an increasingly diverse diet during weaning, as reviewed by Samuel et al. (2018). Indeed, some DD-piglets were observed to develop strong feed preferences during exposure to the diverse pre-weaning diet, which could make them less willing to try the monotonous post-weaning diet than MO-piglets, resulting in a lower post-weaning feed intake than expected based on the high pre-weaning feed intake level that we observed. A third explanation for the limited post-weaning effects of DV may be the loss of diversity, as the diverse feed items were only provided before weaning, particularly because DD-piglets not only explored and ate the feed items, but also used them to play with. The loss of environmental enrichment has shown detrimental effects on pig welfare and production, also in early life (Munsterhjelm et al., 2009; Oostindjer et al., 2010, 2011d; Brajon et al., 2017). Therefore it is recommended to investigate the effects of dietary diversity by continuing the provision of the diverse diet after weaning or even strengthening the diversity in the diet after weaning, thereby creating a more gradual dietary change. The potential negative effects of loss of diversity may be less when a pre-weaning diet is provided that is less diverse, such as the two pellet types that were given as diverse diet in our previous study, but piglets were only followed up to weaning (Middelkoop et al., 2018). We suggest that dietary diversity provided from the post-weaning period onwards may also be beneficial for piglet performance, but this warrants further investigation, as it has only been studied in 42-day-old nursery piglets by performing flavour variety trials of 90 minutes (Figueroa et al., 2018).

Effects of feed presentation

The provision of substrate, such as earth, wood bark and the combination of wood shavings, straw, peat and branches has been found to highly stimulate exploratory behaviour (Wood-Gush and Beilharz, 1983; Oostindjer et al., 2010; Yang et al., 2018). We therefore expected that supplementing (a part of) the pre-weaning diet with substrate may encourage litters to spend more time at the feeder to explore. SUB-piglets, however, only tended to spend more time on feed exploration than CON-piglets at d11, which may be because other studies used a larger amount of substrate and different substrate sources, which were edible, which is not the case for sand that was used as substrate in our experiment. We also expected that SUB would increase pre-weaning feed intake, but our data on the number of refills, time spent eating and eater classification do not support this, except that SUB-litters consisted of more good/early eaters than CON-litters when fed with creep feed, when classified based on home pen observations. This is a mild indicator that SUB has the potential to improve the early intake of piglets. These results correspond to the findings of Wood-Gush and Beilharz (1983), in which early weaned piglets that had access to a trough with earth did not seem to have a higher intake of the feed provided, but the number of piglets in this group that was seen eating during observations was larger.

Presenting the feed in substrate before weaning did not positively, but even negatively affected piglet performance and behaviour after weaning. This is shown by a reduced feed intake in the two weeks after weaning, a reduced body weight gain particularly in the first two days after weaning and a trend for a lower body weight at two weeks post-weaning. In terms of post-weaning behaviour, SUB resulted in an increase in manipulation and aggression between pen mates at week 1 after weaning and a higher number of body lesions at 4 hours and week 2 post-weaning. These results indicate that SUB-piglets had a higher level of frustration and a poorer adaptation to the post-weaning environment than CON-piglets, which also here likely resulted from losing enrichment as substrate was not provided any longer. Similar detrimental effects of losing enrichment were found previously (straw: Day et al. 2002; wood shavings plus straw: Munsterhjelm et al. 2009), although one study reported that the provision of straw as pre-weaning enrichment tended to increase feed intake in the first two days after weaning and reduced body weight loss in this period compared to pre-weaning barren housing, although no straw was provided any longer after weaning. However, in terms of behaviour, losing the straw at weaning reduced play and increased belly nosing (Brajon et al., 2017). Moreover, no detrimental effects of losing a box with wood bark as enrichment from the pre- to post-weaning period were seen on body weight gain and skin lesions and even positive effects on salivary cortisol the first day after weaning were described (Yang et al., 2018). The detrimental effects of removing enrichment might reflect altered behavioural needs of pigs due to their prior rearing experience, as suggested by Day et al. (2002). Continuing the feed presentation strategy in a foraging-stimulating context after weaning therefore deserves further attention.

Despite that SUB-piglets did not significantly spend more time on feed(er) exploration than CON-piglets, SUB-piglets did spent more time on exploration at the feeder with sand compared to the feeder without sand at all observation days. This may derive from intrinsic foraging needs and indicates that piglets are motivated to forage from early in lactation onwards. In addition, piglets spent more time eating at the feeder with sand than at the feeder without sand (except for the first observation day), which was more pronounced within MO. This may indicate that piglets are willing to ‘work’ for feed by rooting through the substrate, while the same feed was freely available nearby at the same time. This is often referred to as ‘contrafreeloading’, and has been previously observed in older pigs (De Jonge et al., 2008; Holm et al., 2008). However, to prove this phenomenon in suckling piglets, a feeder with substrate only would be needed as a control (next to the feeder with feed and feeder with feed plus sand). Moreover, Wood-Gush and Beilharz (1983) reported that piglets mostly went to eat and drink after using an earth trough to forage, suggesting foraging is indeed an appetitive component for eating. After rooting the sand in our study, piglets may therefore have stayed at the feeder to eat, which may partly explain why piglets spent more time eating at the feeder with sand at d18 and d27 compared to the feeder without sand.

Conclusion

In conclusion, a diverse feeding regime for suckling piglets highly stimulated feed exploration, eating, feed intake and the percentage of eaters from an early age onwards, and enhanced their growth towards weaning. Dietary diversity thus has the potential to get all suckling piglets to eat and to improve piglet performance and, potentially, their welfare before weaning. Feed presentation in a foraging-stimulating context, i.e. in substrate, only subtly stimulated exploratory behaviour and the percentage of good eaters before weaning, but piglets seemed motivated to forage as they spent more time at the feeder with sand than the feeder without sand to explore and, to a lesser extent, eat. Against expectations, post-weaning benefits as result of pre-weaning dietary diversity were minor and detrimental effects of feed presentation in a foraging-stimulating context were found on post-weaning adaptation of piglets. This could be due to the loss of diversity and substrate piglets experienced at weaning, therefore the reinforcement of dietary diversity and feed presentation in substrate after weaning deserves further attention. Piglets that were provided dietary diversity in feeders without sand before weaning seemed to perform the best in the post-weaning period of the four groups. Piglets in this treatment did not experience loss of sand, but were positively affected by dietary diversity before weaning, despite the loss of diversity at weaning that may have partly suppressed the positive effects of dietary diversity.

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Supplementary data

Supplementary Table S1. Nutrient profile of celery, cereal honey loops and peanuts in shell.

Nutritional value per 100 g[1]	Celery	Cereal honey loops	Peanuts	Unit
Metabolisable energy	60	1597	2614	kJ
Water	92	6	2.3	g
Protein	1	9	25.2	g
Carbohydrates	2	74	12.9	g
of which mono/disaccharides	1	29	4.8	g
Polysaccharides	1	45	8.1	g
Fat	0	3.5	51.7	g
Fatty acids	0	3.3	49.1	g
of which saturated	0	0.7	7.5	g
Monounsaturated cis	0	1.3	32.1	g
Polyunsaturated	0	1.4	9.5	g
Fibre	1.1	7	6.8	g
Sodium	60	550	0	mg
Potassium	320	270	757	mg
Calcium	80	456	56	mg
Phosphorus	40	320	456	mg
Magnesium	12	70	216	mg
Iron	0.5	8	1.9	mg
Copper	0.01		0.61	mg
Selenium	0		16	µg
Zinc	0.1	2	3.3	mg
Iodine	1.1	2.4	2.5	µg
Retinol activity equivalents	241	0	0	µg
Retinol equivalents	484	0	1	µg
Beta-carotene	2900		4	µg
Lutein	7200		5	µg
β-cryptoxanthin			1	µg
Vitamin B1	0.08	0.91	0.16	mg
Vitamin B2	0.15	1.2	0.08	mg
Vitamin B6	0.1	1.2	0.158	mg
Vitamin B12	0	2.1	0	µg
Nicotinic acid	0.8	14.9	17.5	mg
Folate equivalents	16	268.2	56.6	µg
Folic acid	0	146	0	µg
Vitamin C	25	0	0	mg
Vitamin D	0	4.2	0	µg
Vitamin E	0.2		6.4	mg
Vitamin K	29.3			µg

[1] As-fed, data from the Dutch Food Composition Database of the Dutch National Institute for Public Health and the Environment (2016)

Supplementary Table S2. Nutrient profile of the creep feed.

Calculated nutrient composition[1]	Creep feed
Net energy	1180
Dry matter	891
Starch	290
Sugars	41
Non-starch polysaccharides[2]	261
Crude protein	195
Crude fat	61
Crude fibre	44
Crude ash	57
Calcium	9.1
Phosphorus	6.1
Sodium	2.2
Standardized ileal digestible lysine	11.9
Standardized ileal digestible methionine	4.8
Standardized ileal digestible threonine	7.1
Standardized ileal digestible tryptophan	2.4

[1] According to CVB (2007). Nutrients are presented in g/kg dry matter, except for dry matter (g/kg) and net energy (kJ/100 g). Metabolisable energy is estimated to be 1595 kJ/100 g (Noblet et al., 1994)

[2] Calculated as the difference between dry matter and the sum of starch, sugars, crude protein, crude fat and crude ash

Supplementary Table S3. Ingredient composition of the creep feed.

Ingredient component[1]	**%**
Wheat	21.9
Barley	15
Maize	15
Soy protein concentrate	7
Soybeans (heat treated)	5
Galacto-oligosaccharides	5
Potato protein	4
Sugarbeet pulp (dehydrated)	4
Oat hulls	4
Inulin	4
High-amylose starch (± 75% amylose)	4
Soybean oil	3
Blood meal (spray dried)	2
Dicalcium phosphate	1.7
Sucrose	1.5
Calcium carbonate	1.0
Sodium chloride	0.5
Premix[2]	0.5
Potassium bicarbonate	0.3
L-lysine hydrochloride	0.3
DL-methionine	0.2
L-threonine	0.04
L-tryptophan	0.04
Total	**100**

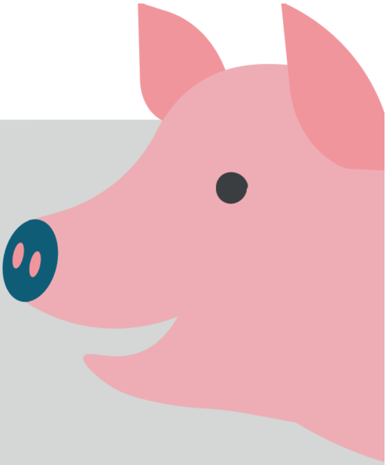
[1] Feed colourant Indigo carmine was included in the feed (5 g/kg feed)

[2] Vitamin and mineral premix (per kg of feed): vitamin A: 10000 IU, vitamin D3: 2000 IU, vitamin E: 40 mg, vitamin K: 1.5 mg, vitamin B1: 1 mg, vitamin B2: 4 mg, vitamin B6: 1.5 mg, vitamin B12: 0.02 mg, niacin: 30 mg, D-pantothenic acid: 15 mg, choline chloride: 150 mg, folate: 0.4 mg, biotin: 0.05 mg, iron: 100 mg, copper: 20 mg, manganese: 30 mg, zinc: 70 mg, iodine: 0.7 mg, selenium: 0.25 mg, anti-oxidant: 125 mg

Supplementary Table S4. Behaviours of piglets after weaning.

Behaviour	**Description**
'Ingestive behaviour'	
Eating feed	Eating or chewing feed (at the feeder)
Drinking	Drinking water from drinking trough
'Exploring feed(er) and drinker'	
Exploring feeder	Sniffing, touching (with snout), rooting or chewing on feeder
Exploring feed	Sniffing, touching (with snout) or rooting the feed in the feeder
Exploring drinking trough	Sniffing, touching (with snout) or chewing on drinking trough
'Exploring environment'	
Nosing environment	Sniffing, touching (with snout) part of the pen (e.g. floor, wall)
Rooting environment	Rooting part of the pen, scraping floor with one the front legs
Chewing environment	Chewing or nibbling part of the pen, including chew object
Chewing air	Sham chewing (not chewing on part of the pen, feed or chew object)
Chewing faeces	Chewing and/or swallowing faeces
'Inactive behaviour'	
Lying eyes closed	Lying on side or belly with eyes closed without performing any other described behaviour
Lying eyes open	Lying on side or belly with eyes open, sitting or kneeling without performing any other described behaviour
'Standing and walking'	
Standing	Piglet is upright, standing, without performing any other described behaviour
Walking	Piglet is walking, without performing any other described behaviour
'Play behaviour'	
Playing individually	Play activities that involve one player; running across pen, rolling, pivoting, tossing head, flopping, sliding, scampering, nudging
Playing socially	Play activities that involve more players; e.g. running, pivoting, scampering, sliding across pen together, play fighting.
Playing with chew object	(Energetically) shaking head with chew object in mouth
'Nosing pen mates'	
Nosing body	Sniffing, touching (with snout) part of the body of a pen mate excluding snout
Snout contact	Mutual snout contact with pen mate
'Pig-directed behaviour'	
Ear biting	Chewing, nibbling or sucking the ear of a pen mate (except ear tag)
Tail biting	Chewing, nibbling or sucking the tail of a pen mate
Belly nosing	Rubbing belly of a pen mate with ≥ 3 up and down movements of the snout or sucking the navel or skin of the abdominal area of a pen mate
Manipulating pen mates	Chewing, nibbling or sucking part of the body of a pen mate excluding ear, tail and abdominal area
Mounting pen mates	Standing on hind legs while having front legs on pen mate
Aggression	Aggressively ramming, pushing, head-knocking, lifting or biting a pen mate, including mutual fighting
'Other behaviour'	
Comfort	Rubbing body against objects or pen mates, scratching body with hind legs or stretching (part of) body
Eliminating	Defaecating or urinating

CHAPTER 8



Early feeding experiences of piglets and their impact on open-field behaviour and food neophobia

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Abstract

This study determined whether early feeding experiences of piglets during lactation influence open-field behaviour before weaning and the acceptance of novel feed before and after weaning. The early feeding experiences were hypothesized to result in a reduced food neophobia. Forty litters were assigned to four treatments in a 2 × 2 factorial arrangement (n = 40 litters in total) at 4 days of age and either received creep feed as a monotonous diet (MO) or four feed items simultaneously (creep feed, celery, cereal honey loops and peanuts) as a diverse diet (DD) and the feed was either provided without substrate (CON) or partly hidden in substrate (SUB), which was sand. Per litter, two groups of four piglets were exposed to an open field test at d21, a familiar food test at d22 and a food neophobia test (FNT) at d24, which was repeated with the same animals at d25 to study acceptance of novel food over time (cheese and chocolate candy on both days). At weaning (d28), four piglets from the same treatment were grouped (n = 40 pens in total) and all fed a monotonous weaner diet presented without substrate. At d41 a post-weaning food neophobia test (dried apple and crisps) was performed on pen level. No differences were observed in open-field behaviour. DD-piglets had a longer latency to eat creep feed and less DD-piglets were seen eating creep feed than MO-piglets in the familiar food test. More feed items were consumed, and a higher feed intake and less vocalisations were reported on the second compared to the first day of the pre-weaning FNT. DD-piglets performed more vocalisations during the pre-weaning FNTs and had a shorter latency to the first vocalisation on day 2 of the FNT. The latency to sample and explore the feed decreased from the first to the second day of the FNT, but this was not or less the case for DD-CON respectively. DD-piglets spent less time on exploring (particularly chocolate) and sampling feed (particularly cheese) than MO-piglets. There tended to be more SUB-piglets sampling the feed on the second day compared to the first day of the FNT. In the post-weaning FNT, DD-CON had a longer latency to explore the feed than MO-CON and DD-piglets tended to sample the feed sooner after the start of the test than MO-piglets. DD-piglets seemed more attracted to crisps, while MO-piglets seemed more interested in dried apple. No differences between treatments were found in the number and weight of the feed items consumed in the pre- and post-weaning FNT. To summarize, early feeding experiences did not influence open-field behaviour before weaning and feed intake in the FNTs, but affected the short- (pre-weaning FNT) and long-term (post-weaning FNT) exploratory response to novel feed. Unexpectedly, piglets provided with a diverse diet before weaning did not show signs of a reduced food neophobia.

Introduction

In commercial pig farming, pigs are frequently exposed to new feeds to provide them with rations appropriate to their stage of life. However, providing a new diet to pigs may trigger a decrease in feed intake due to food neophobia (Clouard et al., 2012). Food neophobia is defined as 'the behavioural response of humans and animals to prevent overconsumption of toxins or nutrients from foods with unknown post-ingestive effects' (Catanese et al., 2012). Animals therefore commonly sample novel feed items with caution, as indicated by a long feeding latency, slow rate of eating and a low intake (Costa et al., 2014; Modlinska and Stryjek, 2016; Callon et al., 2017). To obtain nutritional information about the novel feed before ingesting it, animals likely use nutritional information from previous feed items they consumed (Burritt and Provenza, 1989, 1997). This process is called 'stimulus generalization' and implies that animals recognize sensory cues (such as flavour, colour and texture) from the novel feed that they associate with specific post-ingestive consequences of feeds that they consumed in the past (Launchbaugh and Provenza, 1993, 1994).

Strategies that reduce food neophobia may be of particular interest for newly-weaned pigs. Firstly, most piglets face a sudden change from sow's milk to solid feed at weaning, as a significant proportion of piglets do not consume solid feed prior to weaning or only limited amounts (Pluske et al., 2007; Collins et al., 2013), even though it is generally provided during lactation. Consequently, weaned piglets have little nutritional information from previous feed items. Secondly, weaning involves, next to the change in diet, also social and environmental stressors, as piglets are separated from the sow, and are often transported to a new pen and housed in a large group of non-littermates. Stress negatively affects feed intake and increases food neophobia (Bolhuis et al., 2009), since animals are more reluctant to eat novel food in an unfamiliar compared to a familiar environment (lambs: Launchbaugh et al., 1997; piglets: Hötzel et al., 2011). Thirdly, timely intake of feed at weaning is important for gastrointestinal development and functioning. The latency to eat after weaning can vary between piglets from several minutes up to 97 hours (Bruininx et al., 2001b, 2002). The period of anorexia in late eaters results in low post-weaning growth rates or even body weight loss (Le Dividich and Sève, 2000, 2001; Dunshea, 2003) and perturbs gut health, which may lead to a high occurrence of post-weaning diarrhoea (Pluske et al., 2018). Reducing food neophobia in piglets at weaning may therefore be particularly helpful to stimulate timely intake of solid feed after weaning, and may reduce the health and welfare problems that are associated with a low post-weaning feed intake.

Several human and animal studies show that a diverse feeding experience in early life mitigates food neophobia. Breast-fed infants displayed lower neophobia towards new food than formula-fed infants (Sullivan and Birch, 1994; Maier et al., 2008) and breast feeding is a diverse feeding experience since the maternal diet affects the odour and flavour of the milk (Hausner et al., 2010). Moreover, prior exposure to unfamiliar foods reduced not only neophobia towards those food items, but also towards other unfamiliar foods in infants (Pliner et al., 1993; Loewen and Pliner, 1999; Gerrish and Mennella, 2001; Maier et al., 2008) and lambs (Launchbach et al., 1997). It was also shown in lambs that a varied diet after weaning (4 of 6 diverse feeds were given simultaneously from 2 weeks post-weaning and altered every 5 days until all combinations were offered) enhanced the acceptance rate of novel flavours and feeds that were given later in life compared to a monotonous diet (mixture of the 6 feeds; Catenese et al., 2012; Villalba et al., 2012). How long the effects of early feeding experiences persist when dietary variety is not continued remain unknown, but these results suggest that dietary variety may be crucial in the development of cognitive abilities related to feeding behaviour (Catanese et al., 2012). In turkeys, exposure to successive variety in feed colours (two feed colours that alternated weekly from 0 to 28 days of age) reduced neophobia towards novel feed colours as compared with previous exposure to one feed colour (Lecuelle et al., 2011). In piglets, enriched housing during lactation by provision of edible substrates (straw, wood shavings, peat and branches) and extra space reduced neophobia towards novel feed items just before weaning as compared with barren housing without substrates (Oostindjer et al., 2011c), which may have been the result of the diverse substrate experience. Dietary variety may not only be effective in reducing food neophobia, but may also reduce neophobia in general. For example, a varied versus monotonous diet given to lambs, as previously described, tended to result in a lower increase in rectal temperature after exposure to an open field test that was done later in life than the diets were given (Villalba et al., 2012).

Thus, there are strong indications that dietary variety may reduce food neophobia, and some studies even suggest a decline in general fearfulness in animals fed a diverse diet. Therefore, in this study we determined whether provision of a diverse diet before weaning as opposed to a monotonous diet influenced general fearfulness as measured by an open field test (OFT), and the readiness to sample novel feed items in food neophobia tests (FNT) that were performed during and after exposure to the feeding treatments. For half of the piglets, in a 2 × 2 arrangement, feed was hidden in substrate (sand) to stimulate foraging behaviour and thereby to increase the number of suckling piglets that would eat. These early feeding experiences, i.e. dietary variety and feed presentation in substrate, were hypothesized to result in a reduced food neophobia.

Methods

The study was conducted at research facility Carus (Wageningen University & Research, the Netherlands) according to the protocol of the experiment (AVD104002016515) approved by the Animal Care and Use committee of Wageningen University & Research (Wageningen, the Netherlands) and in accordance with the Dutch law on animal experimentation, which complies with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The use of Indigo carmine as colourant in the creep feed was approved by the Medicines Evaluation Board (Utrecht, the Netherlands).

Animals, housing and treatments

The experimental design of the study is described in Middelkoop et al. (2019b). Briefly, forty litters were housed in farrowing pens (2.85 x 1.80 m), divided over two farrowing rooms, in three consecutive batches. During the study, litters were reared by multiparous sows, which were Topigs-20 and Norwegian Landrace x Topigs-20 sows inseminated by Tempo boar semen. From four days of age, piglets had *ad libitum* access to a concrete piglet feeding area (1.37 x 1.80 m), in front of the sow, including two feeders with four bowls/feeding spaces each (17.5 x 13.5 cm per bowl). Litters were assigned to one of four treatment combinations (10 litters/treatment) in a 2 × 2 arrangement, with dietary variety (DV) and feed presentation (FP) as experimental factors. In short, piglets received either one solid feed item, creep feed, as a monotonous diet (**MO**) or received four solid feed items simultaneously (creep feed, celery, cereal honey loops and peanuts in shell) as a diverse diet (**DD**), and the feed was either presented without substrate (**CON**) or hidden in substrate (**SUB**), which was sand, in one of two feeders to stimulate natural foraging behaviour. Treatments were continued until weaning at 28.5 ± 0.2 days of age. Litter size was on average

12.9 ± 0.2 piglets/litter at weaning. At weaning, a subset of 160 piglets (n = 10 weaner pens per treatment, with 4 piglets per pen) was relocated in two weaner rooms (in two consecutive batches) until two weeks post-weaning. Piglets were mixed with conspecifics from the same pre-weaning treatment, and housed with two males and two females, which derived from three litters. Piglets were housed in pens of 2.76 × 1.20 m. All weaner pens were identical and all piglets were fed a novel commercially available nursery diet *ad libitum*.

To the aim of this study, eight piglets from each litter were selected on 21 days of age to study their behaviour in behavioural tests. Piglets were selected based on sex (equivalent male to female ratio) and body weight (close to the average weight of the litter and close to the average weight of the treatment group). Piglets with a history of medication and leg/claw problems were excluded from selection. The eight selected piglets per litter were tested in two fixed groups of four piglets, resulting in 20 groups per treatment in the test before weaning. At weaning, piglets were selected based on sex, body weight and health (see Middelkoop et al. (2019b) for details), and only piglets that were exposed to the pre-weaning behavioural tests were selected. The selected piglets originated from eight litters in MO-SUB and DD-CON and from seven litters in MO-CON and DD-SUB. Each weaner pen was tested as group of four piglets, resulting in 10 pens per treatment in the test after weaning.

Behavioural tests

Piglets were individually marked using dark permanent hair dye to enable behavioural observations. Piglets were observed through direct observation using Psion hand-held computers and tablets with the Pocket Observer 3.1 and 3.3 software package respectively (Noldus Information Technology, Wageningen, the Netherlands). Behavioural tests were all conducted in a 5.3 × 5.3 m arena, located in a different room, with wooden walls of 1.2 m and a concrete floor. A 2 × 2 m square, painted on the floor in the centre of the arena, served as the feeding place when feed was provided in the tests. Transfer to the test arena was done in a transport cart. At the test arena piglets were put in the start box, in front of the entrance of the test arena and calmly guided into the arena. The tests started when all four piglets entered the arena and the total test time was 5 minutes per test. After the tests, piglets were returned to their home pen and faeces and urine were removed from the test arena. The order of testing was balanced for treatment and room. Temperature in the test room was kept the same as the temperature in the home pens.

Open field test

An open field test (OFT) was conducted in the test arena on 21 days of age. Piglets encountered the test arena for the first time and their frequencies of vocalising, defaecating, urinating and escape attempts (**Table 1**) were scored live on group level. The observer was blind to the treatment.

Familiar feed test

On 22 days of age, creep feed (same as provided in the home pen of the piglets) was distributed in piles at the feeding place of the test arena. The latency to sample creep feed was recorded for each piglet. Sampling was defined as ‘taking a feed item in the mouth and chewing or eating it’. The intake of creep feed was also measured by weighing the feed before and after testing, but the amount eaten was negligible and therefore excluded from analyses.

Table 1. Vocalisations and other behavioural events of suckling piglets during behavioural tests.

Behaviour	Description
'Low-pitched vocalisations'	
Short grunt	A low tone of less than half a second (one note)
Long grunt	A low tone of more than half a second (one note)
Bark	A low tone that sounds like 'wuff'
'High-pitched vocalisations'	
Squeal	A high tone (different notes)
Grunt-squeal	A low tone that transforms into a high tone
Scream	A high, long and loud tone, often as long as an expiration
'Other behavioural events'	
Defaecating	Defaecating
Urinating	Urinating
Escape attempt	Jumping in air or against wall of the test arena trying to escape

Food neophobia tests

A food neophobia test (FNT) was conducted on 24, 25 (pre-weaning) and 41 days of age (post-weaning) in the test arena. Two novel feeds were evenly distributed over the feeding place of the arena. In the pre-weaning FNT at 24 days of age, five cheese cubes (2.4 gram per cheese cube sized 1.5 x 1.5 x 1.5 cm) and five differently coloured chocolate candies, i.e. button-shaped chocolates surrounded by a colourful candy shell (2.8 gram per chocolate sized 1.3 x 1.3 x 0.7), were provided per piglet as novel feed items in the test. The test was repeated with the same animals at 25 days of age to study the acceptance rate of novel feed (cheese and chocolate candy) across time. In the post-weaning FNT at 41 days of age, five pieces of dried apple (12.2 gram per apple piece sized 2.5 x 2 x 0.3 cm) and five curled paprika crisps (1.4 gram per crisp piece sized 5 x 2.5 x 2.5) were used per piglet.

Feed-related behaviours were observed live for each piglet by continuous sampling for 5 minutes. Sniffing or touching the feed with snout were defined as 'exploring feed'. Taking a feed item in the mouth, chewing or eating feed were defined as 'sampling feed', as previously described. Moreover, for each group of piglets, the latencies of vocalising, defaecating, urinating and escape attempts during the test were recorded (**Table 1**), as well as the consumption of the feed items by weighing them before and after the test. From this data, the time spent exploring and sampling feed (per piglet), the latency to explore and sample feed (per piglet), the percentage of piglets that explored and sampled feed (per group) and the number and weight of feed items consumed (per group) were calculated.

Statistical analyses

Data processing

One MO-SUB piglet was ill during the pre-weaning FNTs and laid down during the test and was therefore excluded from analyses of time spent exploring and sampling. Data of one DD-CON piglet in the post-weaning FNT were excluded from analyses, because of a technical error with the hand-held computer. DD-SUB had two groups of three instead of four piglets tested in the pre- and post-weaning FNTs, as two piglets died in the period after the familiar food test. The number of vocalisations and the number and weight of the feed items consumed were therefore divided by the number of piglets in the test before data analyses. Urinating and escape attempts were seen very rarely in all tests and defaecating was seen very rarely in the FNT, and these response variables were therefore excluded from analyses. The percentage of piglets exploring the feed items was not analysed in the post-weaning FNT since all piglets explored the feed.

Data analyses

Latencies (to vocalise, explore and sample), counts (the number of vocalisations and feed items) and feed intake were analysed in a linear mixed model (MIXED procedure). Model residuals were checked for normality and data were transformed before analyses if model residuals were not normally distributed. No pieces of apple were consumed by DD-CON in the post-weaning FNT and data on the number of apple pieces eaten were therefore expressed as binary data (percentage of groups that consumed apple pieces) and analysed in a Fisher's exact test.

Time spent exploring and sampling feed were analysed in a generalised linear mixed model (GLIMMIX procedure) with a binomial distribution, logit link function and an additional multiplicative overdispersion parameter. In addition, data on the occurrence of exploring and sampling feed were expressed as binary data per piglet (0-1 variable) to analyse the percentage of piglets exploring and sampling the feed in a GLIMMIX procedure with a logit link and binary distribution. Data on the occurrence of defaecating (per group) were also analysed in this model. The percentage of piglets exploring or sampling the feed was analysed in a Fisher's exact test if a sub-classification category for the interaction effects was scoring 1 only.

The (generalised) linear mixed models included the fixed effects of dietary variety (DD vs. MO), feed presentation (SUB vs. CON), their interactions, as well as batch (batch 1, 2 or 3 for the pre-weaning tests and batch 1 and 2 for the post-weaning test). Variables that were measured on piglet level were analysed with a random group effect (nested within pen, treatments and batch) and variables that were measured on group level were analysed with a random pen effect (nested within treatments and batch). Day was used as fixed effect in the analyses of the pre-weaning FNT, which consisted of two days, and used as repeated measure in the MIXED procedure.

Significant fixed effects were further analysed using post-hoc pairwise comparisons of least squares means using Tukey's adjustment for three-way interactions. Data are presented as (untransformed) means ± SEM based on pen averages. Differences at $P < 0.05$ were considered statistically significant and differences at $0.05 \leq P < 0.10$ were considered a trend.

Results

Open field test

The total number of vocalisations per piglet during the OFT was not affected by treatments (**Table 2**). SUB-piglets tended to perform more low-pitched vocalisations than CON-piglets during the OFT however (102.8 ± 5.8 vs. 89.9 ± 3.8, $P = 0.08$), due to a trend for a higher number of short grunts (99.1 ± 5.7 vs. 85.9 ± 4.4, $P = 0.08$). Treatments did not affect the latency of groups to vocalise and the percentage of groups that defaecated during the OFT (**Table 2**).

Familiar food test

MO-piglets had a shorter latency to sample the creep feed in the familiar food test than DD-piglets (154.2 ± 13.9 vs. 194.7 ± 13.5 sec, $P < 0.05$) and also more MO- than DD-piglets were sampling it (70.6 vs. 50.9%, $P = 0.02$). Feed presentation (SUB vs. CON) did not affect behaviour towards the creep feed (**Table 3**).

Food neophobia tests

Pre-weaning food neophobia test

Vocalisations. The latency to vocalise was affected by dietary variety $(P = 0.01)$ and dietary variety x day ($P = 0.04$), showing that the latency to vocalise increased from day 1 to day 2 in MO-piglets, but not in DD-piglets, resulting in a higher latency to vocalise of MO-piglets on the second day of the FNT versus the other groups on both days (**Figure 1A**). Irrespective of treatments, the total number of vocalisations (**Figure 1B**) was lower on the second compared to the first day of the FNT (60.5 ± 4.3 vs. 68.2 ± 4.3, $P = 0.02$), which held both for low-pitched (57.4 ± 4.0 vs. 64.4 ± 3.8, $P = 0.03$) and high-pitched vocalisations (3.0 ± 0.8 vs. 3.7 ± 0.8, $P = 0.03$). DD-piglets vocalised more than MO-piglets during the FNT (73.7 ± 3.7 vs. 55.0 ± 4.5, $P = 0.03$), due to a higher number of low-pitched vocalisations (69.3 ± 3.2 ± vs. 52.5 ± 4.1, $P = 0.03$), of which the short grunts tended to be higher (62.6 ± 3.2 vs. 48.7 ± 4.1, $P = 0.07$) and the number of long grunts was higher (6.7 ± 0.7 vs. 3.8 ± 0.4, $P < 0.01$) for DD- compared to MO-piglets.

Table 2. Vocalising and defaecating behaviour of a group of four 21-day-old suckling piglets in the open field test. In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages. Trends are presented in bold.

Behaviour	**DD**		**MO**		**Significance**		
	CON	**SUB**	**CON**	**SUB**	**DV**	**FP**	**DV x FP**
No. of vocalisations / piglet	99.0 ± 8.8	111.4 ± 3.9	95.4 ± 5.0	108.9 ± 12.7	0.82	0.14	0.99
No. of low-pitched vocalisations / piglet	89.3 ± 6.5	106.1 ± 3.7	90.4 ± 4.5	99.5 ± 11.2	0.81	**0.08**	0.55
No. of high-pitched vocalisations / piglet	9.7 ± 3.1	5.3 ± 1.1	5.0 ± 1.3	9.4 ± 2.7	0.86	0.91	0.21
Latency to vocalise (sec)	5.9 ± 0.8	6.0 ± 0.6	8.4 ± 1.5	6.5 ± 0.9	0.13	0.39	0.57
Percentage of groups defaecating (%)	35	10	10	10	0.96	0.96	0.47

Table 3. Behaviour of 22-day-old suckling piglets towards familiar creep feed in the familiar food test. In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages. Significant *P*-values are presented in bold.

Behaviour towards creep feed	**DD**		**MO**		**Significance**		
	CON	**SUB**	**CON**	**SUB**	**DV**	**FP**	**DV x FP**
Latency to sample (sec)	187.4 ± 22.1	202.8 ± 15.5	149.8 ± 19.7	158.1 ± 20.5	< **0.05**	0.57	0.66
Percentage of piglets sampling (%)	50	51.9	67.5	73.8	**0.02**	0.63	0.71

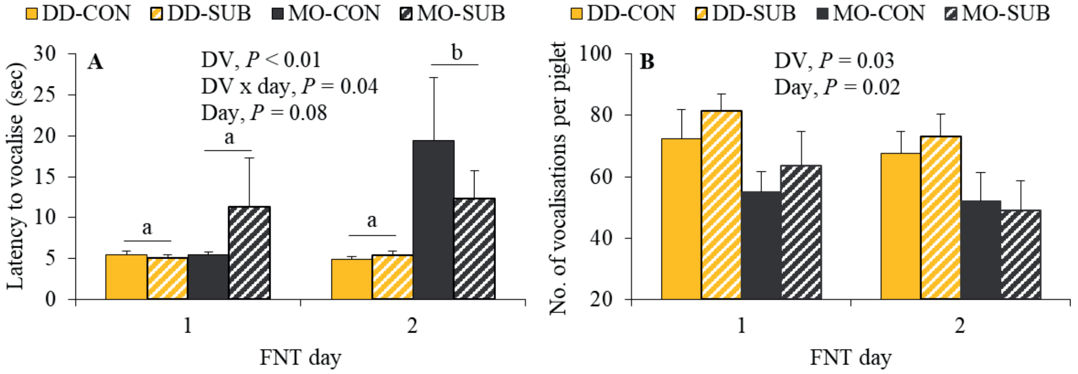

Figure 1. The latency to vocalise (**A**) and the total number of vocalisations (**B**) of piglets in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages. Superscripts without a common letter differ at $P < 0.05$.

Feed-related behaviour and feed intake. The latency to explore the feed (cheese plus chocolate) was affected by day ($P < 0.0001$) and the dietary variety x feed presentation x day interaction ($P = 0.02$). The latency to explore the feed decreased in all groups from the first to the second day of the pre-weaning FNT, but DD-CON decreased the least (**Figure 2A**). The latency to sample the feed was affected by day ($P < 0.0001$), dietary variety x day ($P < 0.01$) and the dietary variety x feed presentation x day interaction ($P < 0.05$). The latter interaction showed that the latency to sample the feed decreased from the first to the second day for DD-SUB, MO-CON and MO-SUB, but not for DD-CON (**Figure 2B**).

Time spent exploring the feed was higher on the first than on the second day of the FNT (day 1: 11.1 ± 0.7 vs. day 2: 9.7 ± 0.5%, $P < 0.01$, **Figure 2C**), while time spent sampling the feed was higher on the second than on the first day of the FNT (day 1: 25.7 ± 0.3 vs. day 2: 32.0 ± 3.1%, $P < 0.0001$, **Figure 2D**). Time spent exploring (DD: 9.2 ± 0.6 vs. MO: 11.5 ± 0.6%, $P = 0.03$) and sampling the feed (DD: 23.1 ± 2.7 vs. MO: 34.6 ± 2.8%, $P < 0.01$) were both lower in DD- than in MO-piglets. The percentage of piglets exploring the feed did not differ on the first day of the FNT. On the second day of the FNT, however, there was a trend for a higher percentage of MO-SUB piglets exploring the feed compared to MO-CON and DD-SUB with an intermediate percentage for DD-CON (**Figure 2E**). The percentage of piglets sampling the feed tended to be affected by the interaction between feed presentation and day ($P = 0.07$), showing that there were more SUB-piglets sampling the feed on the second than on first day of the FNT (**Figure 2F**).

The number of feed items eaten during the pre-weaning FNT was higher on the second (1.70 ± 0.23 feed items) compared to the first day (1.05 ± 0.17 feed items, $P < 0.001$, **Supplementary Figure S1A**). Also the intake of feed in grams was higher on the second (2.12 ± 0.27) than on the first day (1.14 ± 0.15, $P < 0.0001$, **Supplementary Figure S1B**).

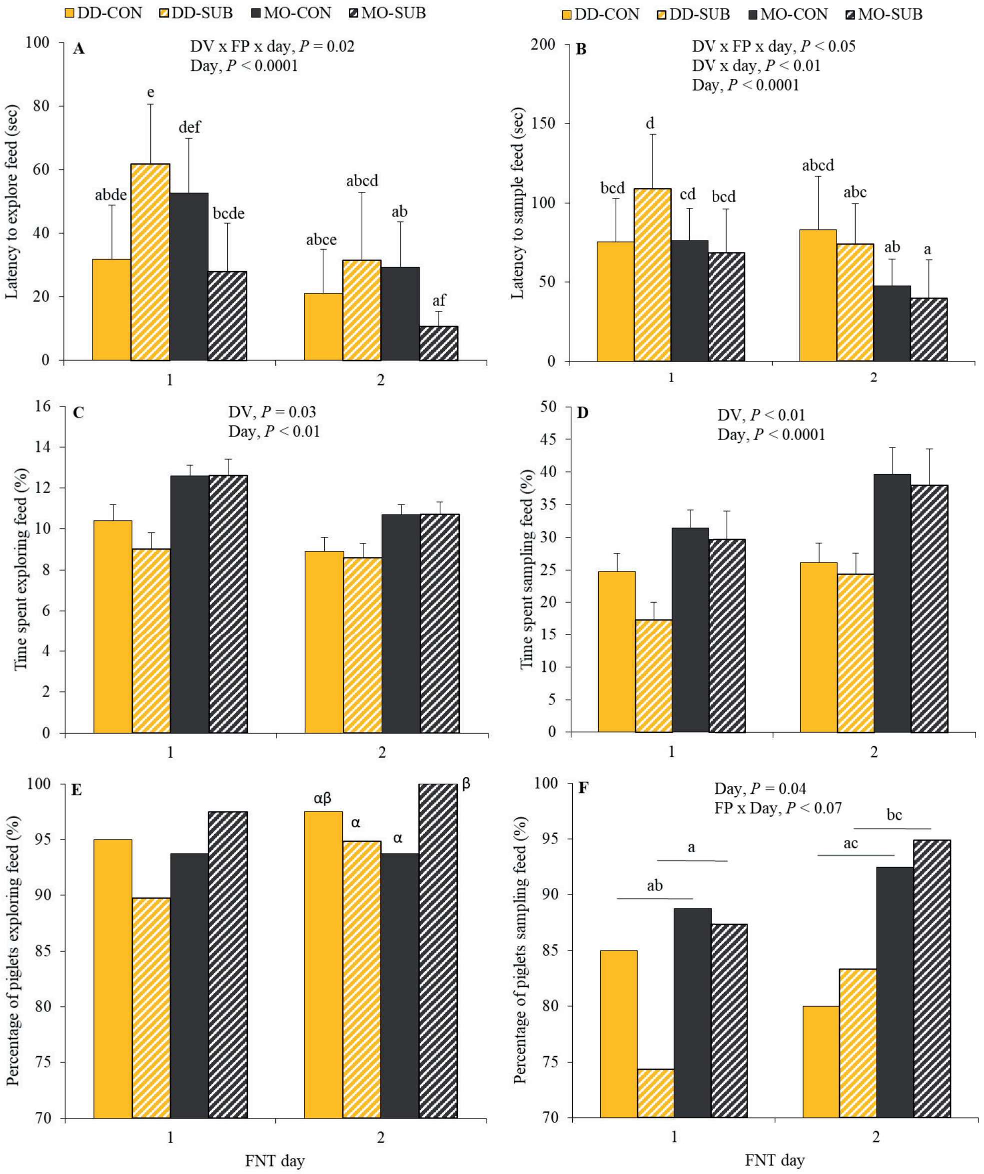

Figure 2. Feed-related behaviour of piglets towards novel feed, i.e. cheese and chocolate, in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). The latency to explore (**A**), the latency to sample (**B**), time spent exploring (**C**), time spent sampling (**D**), the percentage of piglets exploring (**E**) and the percentage of piglet sampling (**F**) were studied. In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages. Superscripts without a common letter differ at $P < 0.05$. Greek superscripts without a common letter differ at $P < 0.10$.

Feed-related behaviour towards cheese and intake of cheese. The latency to explore cheese was affected by day ($P < 0.001$) and the dietary variety x feed presentation x day interaction ($P = 0.03$). MO-CON, MO-SUB and DD-SUB had a shorter latency to explore cheese on the second compared to the first day of the FNT, which was not the case for DD-CON (**Figure 3A**). The latency to sample cheese was affected by dietary variety ($P < 0.05$), day ($P < 0.01$) and tended to be affected by their interaction ($P = 0.05$). The interaction showed that the latency to sample cheese decreased from the first to the second day of the FNT for MO-piglets, but not for DD-piglets, resulting in a shorter latency to sample cheese of MO-piglets on the second day of the FNT compared to the other groups on both days (**Figure 3B**).

A trend for a dietary variety x feed presentation x day interaction was found on time spent exploring cheese ($P = 0.05$), showing that DD-CON piglets spent less time exploring cheese on the second than the first day of the FNT, whereas the other treatment groups did not differ over time (**Figure 3C**). Time spent sampling cheese was higher on the second compared to the first day of the FNT (day 1: 12.2 ± 1.7 vs. day 2: 15.4 ± 2.2%, $P < 0.01$) and lower in DD- versus MO-piglets (DD: 9.2 ± 1.4 vs. MO: 18.4 ± 2.1%, $P < 0.001$, **Figure 3D**).

The percentage of piglets exploring cheese did not differ between treatments on the first day of the FNT. On the second day of the FNT, however, MO-SUB had a higher percentage of piglets exploring cheese than DD-SUB, and tended to have a higher percentage of piglets exploring cheese than MO-CON, with an intermediate percentage for DD-CON (**Figure 3E**). The percentage of piglets consuming cheese increased over time ($P = 0.02$), with more piglets eating cheese on the second (71.6%) than on the first day of the pre-weaning FNT (64.7%, **Figure 3F**). Dietary variety tended to reduce the percentage of piglets sampling cheese (60.5 vs. 76%, $P = 0.07$).

The number of cheese cubes eaten was not affected by treatments, but affected by day ($P = 0.02$) and increased from the first (0.18 ± 0.05 cheese cubes) to the second day (0.32 ± 0.07, **Supplementary Figure S2A**). The intake of cheese in grams was also influenced by day (day 1: 0.41 ± 0.07 vs. day 2: 0.74 ± 0.13, $P < 0.01$, **Supplementary Figure S2B**).

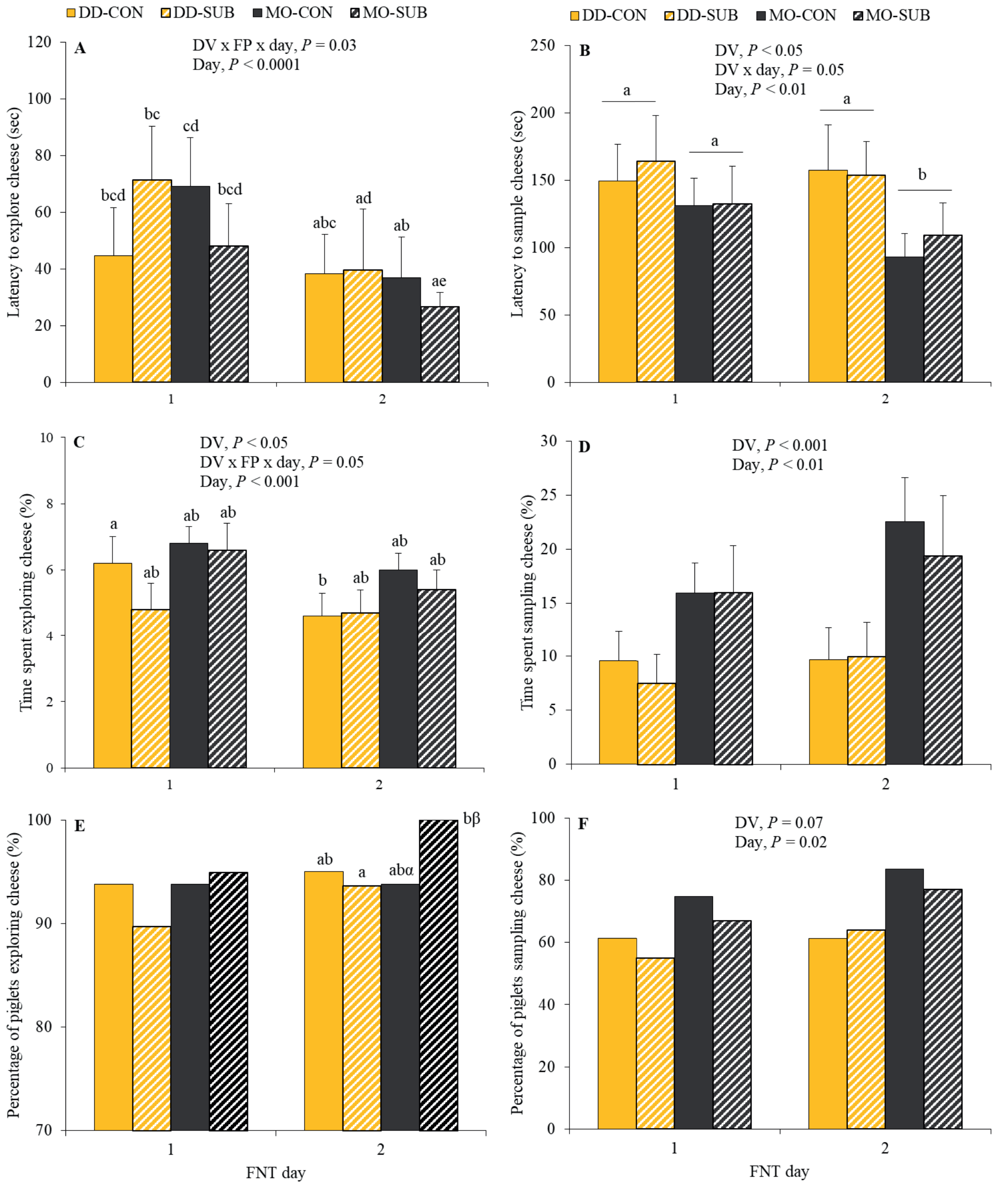

Figure 3. Feed-related behaviour of piglets towards cheese in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). The latency to explore (**A**), the latency to sample (**B**), time spent exploring (**C**), time spent sampling (**D**), the percentage of piglets exploring (**E**) and the percentage of piglets sampling (**F**) were studied. In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages. Superscripts without a common letter differ at $P < 0.05$. Greek superscripts without a common letter differ at $P < 0.10$.

Feed-related behaviour towards chocolate and intake of chocolate. The latency to explore chocolate was affected by day (day 1: 69.2 ± 8.1 vs. day 2: 30.6 ± 6.4 sec, $P < 0.0001$) and tended to be affected by dietary variety x day ($P = 0.098$), but no post-hoc pairwise differences were found on top of the day effect (**Figure 4A**). The latency to sample chocolate was not affected by day, treatments or their interactions (**Figure 4B**).

DD-piglets spent less time exploring chocolate than MO-piglets (DD: 4.1 ± 0.3 vs. MO: 5.3 ± 0.3%, $P = 0.03$, **Figure 4C**). Time spent sampling chocolate was affected by day ($P < 0.01$) and tended to be affected by feed presentation x day ($P = 0.08$), showing that time spent sampling chocolate was higher for SUB-piglets on the second compared to the first day of the FNT, but did not differ from the other groups on both days (**Figure 4D**).

The percentage of piglets exploring chocolate did not differ between treatments on the first day of the FNT. On the second day of the FNT, however, there was a higher percentage of MO-SUB versus MO-CON and DD-SUB piglets that was exploring chocolate, with an intermediate percentage of DD-CON piglets (**Figure 4E**). The percentage of piglets sampling chocolate tended to be affected by the interaction between feed presentation and day ($P < 0.10$) without significant post-hoc differences, but the percentage of piglets sampling chocolate seemed to show a numerical increase from the first to the second day of the FNT within SUB, but not within CON (**Figure 4F**).

Treatments did not affect the number of chocolates eaten (**Supplementary Figure S3A**) and the intake of chocolate in grams (**Supplementary Figure S3B**). Both the number of chocolates eaten (day 1: 0.87 ± 0.15 vs. day 2: 1.38 ± 0.21, $P < 0.01$) and the intake of chocolate in grams (day 1: 0.73 ± 0.13 vs. day 2: 1.38 ± 0.21 $P < 0.0001$) were higher on the second compared to the first day of the FNT.

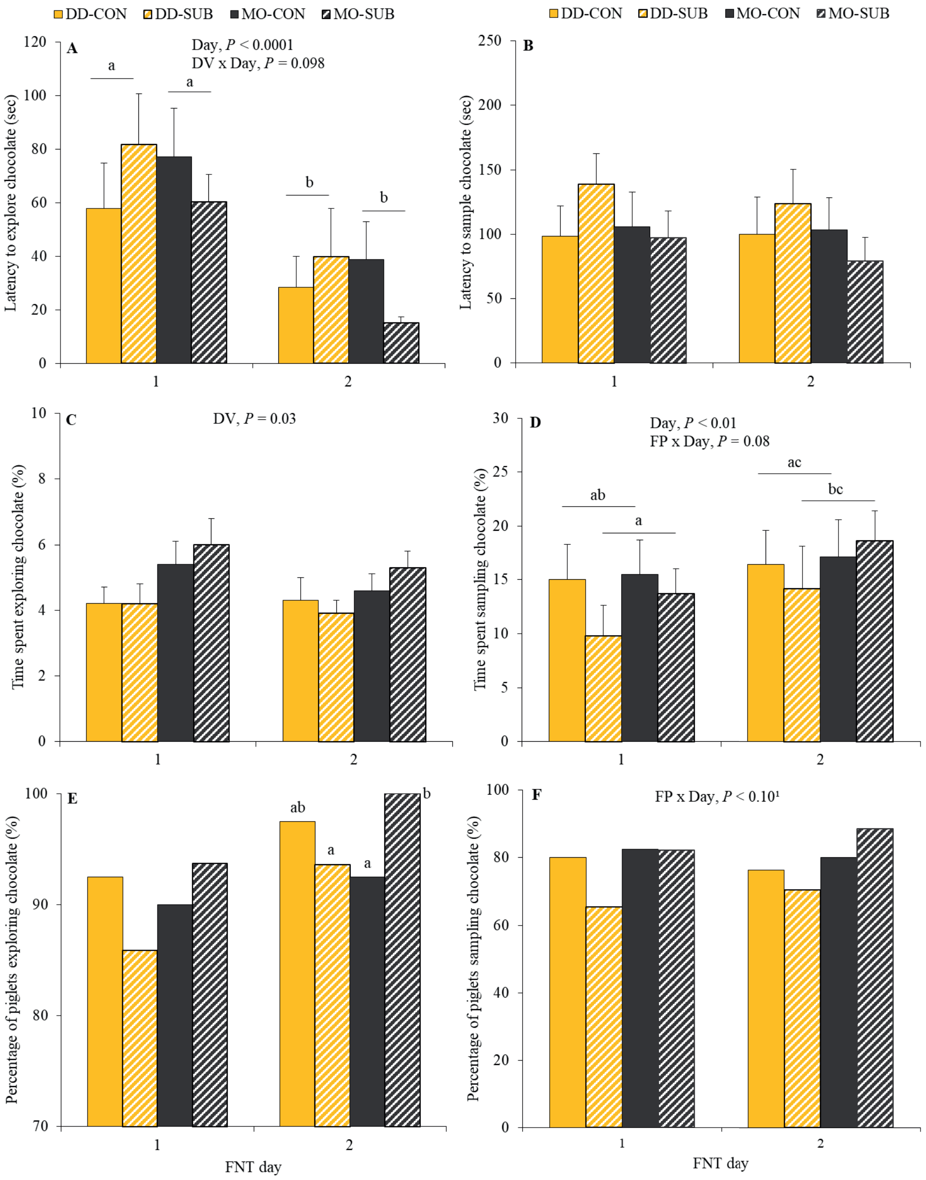

Figure 4. Feed-related behaviour of piglets towards chocolate in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). The latency to explore (**A**), the latency to sample (**B**), time spent exploring (**C**), time spent sampling (**D**), the percentage of piglets exploring (**E**) and the percentage of piglets sampling (**F**) were studied. In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages. Superscripts without a common letter differ at $P < 0.05$.

Post-weaning food neophobia test

The latency to explore the feed (apple plus crisps) tended to be affected by the interaction between dietary variety and feed presentation (**Table 4**), showing that DD-CON had a longer latency to explore the feed than MO-CON (post-hoc, $P < 0.01$), with the SUB-groups in between. The latency to sample the feed tended to be shorter for DD- compared to MO-piglets (33.0 ± 5.1 vs. ± 54.0 ± 11.2 sec, $P = 0.08$). Time spent on exploring and sampling the feed, the percentage of piglets sampling the feed and the number of feed items eaten were not affected by treatments (**Table 4**).

The response of weaner piglets towards apple was affected by dietary variety or the dietary variety x feed presentation interaction (**Table 4**). DD-CON had a longer latency to explore apple than the other three treatments (post-hoc, $P \leq 0.01$) and DD-piglets had a longer latency to sample apple (147.1 ± 10.1 vs. 98.2 ± 11.6 sec). DD-piglets also spent less time exploring (4.0 ± 0.4 vs. 5.4 ± 0.4%, $P < 0.01$) and sampling apple (5.8 ± 0.6 vs. 9.2 ± 0.9%, $P = 0.01$) compared to MO-piglets. There was a lower percentage of DD-SUB than MO-SUB piglets that were seen sampling apple (post-hoc, $P < 0.01$), but there tended to be a higher percentage of DD-SUB groups that consumed apple pieces compared to groups of DD-CON, with an intermediate percentage of groups for MO-CON and MO-SUB (**Table 4**).

The latency to explore crisps tended to be affected by the interaction between dietary variety and feed presentation ($P = 0.06$) and the latency to sample crisps and the percentage of piglets sampling crisps were influenced by dietary variety (**Table 4**). DD-CON had a longer latency to explore crisps versus MO-CON (post-hoc, $P = 0.04$). In addition, DD-piglets had a shorter latency to eat crisps versus MO-piglets (57.2 ± 9.5 vs. 91.8 ± 12.2 sec, $P = 0.01$) and a larger number of DD-piglets were observed to eat crisps than MO-piglets (96.1 vs. 85%, $P = 0.02$). No differences between treatment groups were observed in the time spent exploring and eating crisps and the number of crisps eaten per piglet in the test (**Table 4**).

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Table 4. Behaviour of 41-day-old weaner piglets towards novel feed, i.e. dried apple and crisps, in the post-weaning food neophobia test. Piglets were exposed to the test in groups of pen mates. During lactation, piglets were fed creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV = dietary variety (DD vs. MO). FP = feed presentation (SUB vs. CON). Data are expressed as means ± SEM based on pen averages. Significant *P*-values and trends are presented in bold. Superscripts without a common letter differ at $P < 0.05$.

Behaviour	**DD**		**MO**		**Significance**		
	CON	**SUB**	**CON**	**SUB**	**DV**	**FP**	**DV x FP**
Apple + crisps							
Latency to explore	14.5 ± 2.1^{a}	10.9 ± 1.5^{ab}	8.4 ± 1.0^{b}	10.8 ± 1.5^{ab}	**0.04**	0.74	**0.08**
Latency to sample	30.2 ± 7.0	35.9 ± 7.7	68.5 ± 20.9	39.4 ± 6.8	**0.08**	0.32	0.22
Time spent exploring	14.8 ± 1.1	14.1 ± 1.0	15.9 ± 0.8	14.7 ± 1.1	0.34	0.30	0.71
Time spent sampling	19.6 ± 3.0	21.0 ± 1.9	23.3 ± 4.2	25.5 ± 3.1	0.25	0.40	0.76
Percentage of piglets sampling	100	97.4	90	100	0.37	0.37	0.12^{1}
No. eaten per piglet	0.39 ± 0.08	0.68 ± 0.18	0.58 ± 0.17	0.83 ± 0.31	0.68	0.21	0.80
Apple							
Latency to explore	26.3 ± 2.6^{a}	17.9 ± 1.7^{b}	15.7 ± 1.7^{b}	18.8 ± 2.3^{b}	**0.03**	0.15	**< 0.01**
Latency to sample	140.9 ± 16.9	153.3 ± 11.8	111.3 ± 19.4	85.2 ± 12.4	**0.02**	0.47	0.70
Time spent exploring	4.0 ± 0.5	4.0 ± 0.5	5.5 ± 0.6	5.4 ± 0.5	**< 0.01**	0.92	0.90
Time spent sampling	6.2 ± 1.0	5.5 ± 0.9	8.3 ± 1.4	10.1 ± 1.0	**0.01**	0.81	0.33
Percentage of piglets sampling	79.5^{ab}	63.2^{a}	82.5^{ab}	92.5^{b}	**0.02**	0.15	**0.05**
Percentage of groups that consumed items^{2}	0	40	20	30	1.00	0.13	**0.09**
Crisps							
Latency to explore	18.6 ± 3.0^{a}	16.2 ± 2.9^{ab}	11.1 ± 1.6^{b}	17.5 ± 2.1^{ab}	0.32	0.45	**0.06**
Latency to sample	48.8 ± 11.1	65.5 ± 15.6	100.6 ± 18.8	82.9 ± 15.8	**0.01**	0.73	0.22
Time spent exploring	10.7 ± 0.8	10.1 ± 0.7	10.5 ± 0.6	9.2 ± 0.8	0.47	0.20	0.66
Time spent sampling	13.4 ± 2.4	15.5 ± 2.1	15.0 ± 3.0	15.3 ± 3.2	0.99	0.52	0.83
Percentage of piglets sampling	97.4	94.7	85	85	**0.02**	0.69	0.48
No. eaten per piglet	0.39 ± 0.08	0.52 ± 0.12	0.41 ± 0.09	0.50 ± 0.15	0.93	0.27	0.92

[1] Analysed in a Fisher's exact test. $P \geq 0.12$ for all comparisons

[2] The no. of apple pieces eaten by DD-CON was 0. Data were therefore expressed as binary data (group consumed items yes or no) and analysed in a Fisher's exact test. DD-SUB tended to have a higher percentage of groups that consumed apple pieces than DD-CON

Discussion

This study determined whether early feeding experiences by piglets during lactation would influence the behavioural response of piglets in an open field test, familiar food test and two-day food neophobia test before weaning and in a one-day food neophobia test after weaning. Early feeding experiences did not significantly affect behaviour in the open field test, but affected behaviour in all feed-related tests. The effects of early feeding experiences in the pre-weaning tests (open field test, familiar food test and two-day food neophobia test) and in the post-weaning food neophobia test will be discussed separately below.

Effects of early feeding experiences in the pre-weaning tests

We hypothesized that piglets exposed to dietary variety would have an attenuated fear response towards a novel environment, displayed by a lower frequency of vocalising, defaecating and escape attempts. The latter behaviour was seen rarely and therefore not analysed. The latency to vocalise, the overall number of vocalisations, as well as low- and high-pitched vocalisations produced in the open field test, and the percentage of groups that defaecated in the OFT did not differ between the treatments however. We therefore conclude that we found no behavioural indications of differences in fearfulness in the open field test as result of the treatments. Villalba et al. (2012) also reported no differences in vocalisations and escape attempts during an OFT between lambs exposed to a monotonous and diverse diet. However, they reported that lambs exposed to a diverse diet tended to have a lower stress-induced-hyperthermia after an OFT than lambs exposed to a monotonous diet.

In the familiar food test, DD-piglets had a longer latency to eat creep feed and less DD- than MO-piglets were observed to eat it. Negative emotions of fear and anxiety did likely not cause these findings, as no differences were found in behaviour in the open field test as previously described. In contrast, differences in food neophobia and feeding preferences towards the creep feed prior to the familiar food test may have played a role. Creep feed was provided to both DD- and MO-litters, but DD-litters tended to consume less creep feed during lactation and consisted of a lower percentage of piglets that consumed creep feed from 19 days of age compared to MO-litters (Middelkoop et al., 2019b). DD-litters were, however, more familiar with solid feed in general, since DD-litters ate more solid feed than MO-litters and consisted of more piglets that consumed solid feed, but apparently seemed to prefer creep feed the least of the four items that were provided (Middelkoop et al., 2019b).

Irrespective of treatments, piglets tended to vocalise later after the start of the FNT on the second compared to the first day and produced a lower number of vocalisations, including both low- and high-pitched vocalisations. Other studies have shown that piglets exposed to negative conditions produced a higher overall number of vocalisations and a higher number of low-pitched vocalisations compared to piglets exposed to positive conditions (Leliveld et al., 2016), and that high-pitched vocalisations in piglets were strongly associated with negative conditions (Tallet et al., 2013), suggesting that piglets were less fearful and less emotionally stressed on the second compared to the first day of the FNT. In correspondence, piglets became less neophobic towards the feed items presented in the test over time, as the time spent sampling the feed, the number of feed items eaten and the intake of feed in grams increased from the first to the second day of the test. Also the number of 'neophobic' piglets towards cheese decreased over time, as more piglets were sampling cheese over time, but this was not the case for chocolate. However, the percentage of piglets eating chocolate was observed to be higher than the percentage of piglets eating cheese on the first day of the FNT, suggesting the initial neophobic response towards cheese seemed to be stronger than towards chocolate. We expected the decline in fear-related behaviour and increase in eating behaviour to be stronger for dietary variety and feed hidden in substrate. However, the findings of the current study do not support this hypothesis, and, for some parameters the opposite seemed to be true for piglets with a diverse versus monotonous diet.

Feed presentation in substrate did not affect the feeding behaviour of piglets in the pre- and post-weaning FNT, which is in line with the absence of a feed presentation effect on feed intake and the percentage of eaters in the home pen (Middelkoop et al., 2019b).

Piglets with a diverse diet did not differ in intake of the novel feed items from piglets with a monotonous diet. When we exposed the piglets to a novel feed at weaning that was provided *ad libitum* as described in Middelkoop et al. (2019b), DD- and MO- piglets did also not differ in their feed intake in the first four hours after weaning, neither between d0-1, d1-2 and d2-5 post-weaning. Together with these previous reported findings, the current results suggest that DD- and MO- piglets did not differ in food neophobia. Nevertheless, the test duration may also have been too short to detect differences in the intake of novel feed. The amount of feed consumed during the test was negligible and longer test durations were used previously in pigs (7 min: Oostindjer et al., 2011c) and sheep (10-20 min: Villalba et al., 2009, 2011, 2012; Catanese et al., 2012), of which all studies reported treatment differences in feed intake. Since the ingestion of feed items was limited, sampling mainly involved exploratory chewing/biting.

Opposite to our expectations, DD-piglets spent less time on exploring (particularly chocolate) and sampling feed (particularly cheese) than MO-piglets. Moreover, the latency to sample and explore the feed decreased from the first to the second day of the FNT, but this was not or less the case for DD-CON respectively. Potential explanations why the results of this study do not match with, or for some parameters even seem contradictory to, our hypothesis will be discussed below.

DD-piglets vocalised more in the pre-weaning FNT, which has been associated with negative emotions (Leliveld et al., 2016). Due to the lack of exploratory stimulation in the home pen, MO-piglets may have been more motivated to explore the novel feed items during testing than DD-piglets, as reflected by a larger time spent on exploring and sampling the feed, which mainly involved exploratory chewing/biting, of MO- versus DD-piglets. The increased motivation to perform a behaviour when it has been prevented by certain housing conditions has been called a 'rebound effect' (Edwards-Callaway, 2015). Others have suggested previously that barren housing may result in such a rebound effect during testing in a novel object and human interaction test, and that barren-housed piglets were therefore more explorative and less anxious towards novelty compared to enriched-housed piglets in such tests (Olsson, 1999; Backus et al., 2017). Others found, however, no signs of such a rebound effect in piglets in a FNT (Oostindjer et al., 2011c). In addition, based on the rebound hypothesis one would expect CON-piglets to be more involved in feed-related behaviour than SUB-piglets, since their intrinsic motivation to explore may be less satisfied due to the absence of substrate in the home pen. In contrast, more SUB- than CON-piglets tended to explore and sample the feed on the second day of the FNT. Although the piglets ingested the novel feed with caution, exposure to the novel feed items may not necessarily elicit fear, as some groups of piglets displayed object play with the feed items and locomotory play during the test, which are indications of positive emotions. This seemed to occur more often in the post- than in the pre-weaning FNT (personal observation by the observers).

Feed items have a characteristic set of sensory properties (appearance, flavour, taste and texture) which enable the animal to identify it. The novel feed items in the FNT may have differed too largely in sensory properties from the diverse feed items in the home pen of DD-piglets to enable stimulus generalization and thereby to increase the intake of the novel feed items (Mennella et al., 2008). Besides, DD-piglets developed strong feeding preferences in the home pen (Middelkoop et al., 2019b) and may therefore be more ‘picky’ in their eating behaviour (Dovey et al., 2008). Although not tested, DD-piglets may have preferred the feed items in their home pen over the feed items in the FNT, resulting in a negative contrast between the test situation and the home pen. Conversely, the monotonous diet in the home pen might have resulted in a positive contrast between the test situation with the choice of two feeds compared to the home pen with only one feed, which appeared to be least preferred by DD-piglets (Middelkoop et al., 2019b). To conclude, the food items experienced in the home pen and how they relate to the novel food items likely play an important role in the acceptance of novel food in pigs.

Effects of early feeding experiences in the post-weaning food neophobia test

Although piglets from all treatments were housed under the same conditions and received the same feed post-weaning, DD-piglets still differed in their behaviour towards novel feed from MO-piglets when tested two weeks after weaning. DD-piglets seemed more attracted to crisps, while MO-piglets seemed more interested in dried apple. Consequently, no major differences were reported in the overall response towards the novel feed items. DD-piglets may have been more attracted to crisps as result of their saltier diet in the home pen (Middelkoop et al., 2019b), as the acceptance pattern of novel food appeared to be specific to the previous food profile experienced by infants (Mennella et al., 2008). This may also be the case in piglets, as the diet composition of the pre-weaning and post-weaning diet were found to interact on the amount of weaner feed ingested after weaning (Torrallardona et al., 2012; Heo et al., 2018). The findings of the current study suggest that early feeding experiences can have long-lasting effects on feed preferences, at least up to two weeks after the treatments were not reinforced anymore. Our findings are consistent with previous studies, who also reported differences in short- (during dietary treatment) and long-term feeding preferences (after end of dietary treatments) in piglets exposed to different diets early in life (Figueroa et al., 2013; Blavi et al., 2016; Clouard et al., 2016).

Conclusions

Early feeding experiences did not influence open-field behaviour before weaning and feed intake in the food neophobia tests. Against expectations, piglets provided with a diverse diet showed lower levels of feed exploration and sampling of novel feed items than piglets provided with a monotonous diet before weaning. Whether these changes in exploratory behaviour towards the feed were accompanied by differences in food neophobia or motivation to explore remained inconclusive. Moreover, feed preferences were observed in the food neophobia test after weaning. Yet, this study showed that early feeding experiences can have short-term and long-lasting effects on the responsiveness to novel feed in young piglets.

Acknowledgements

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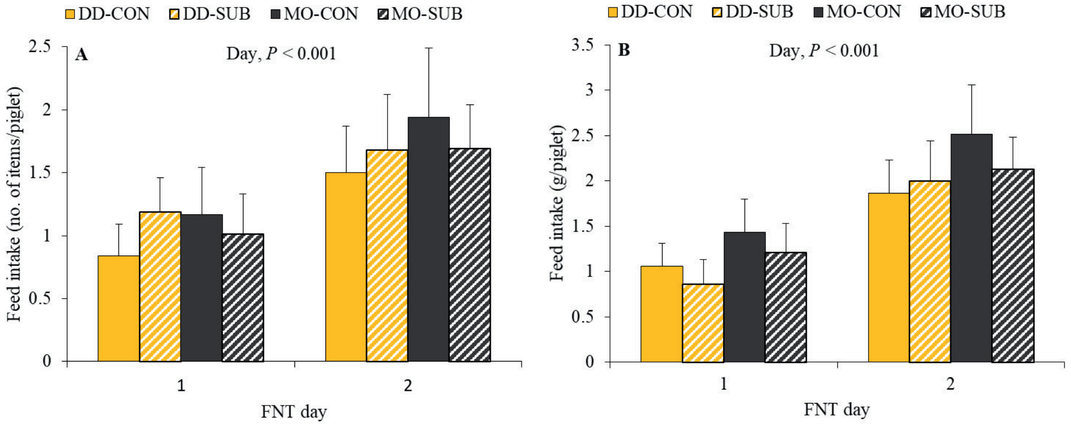
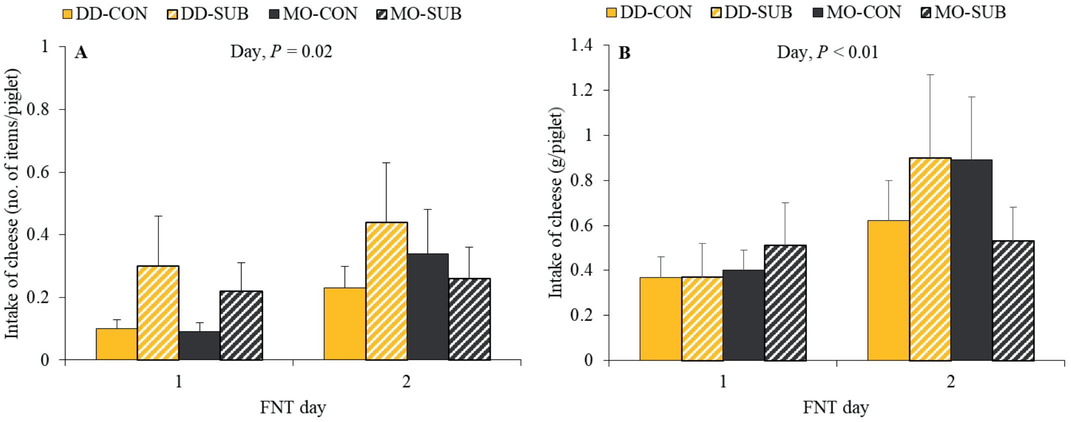
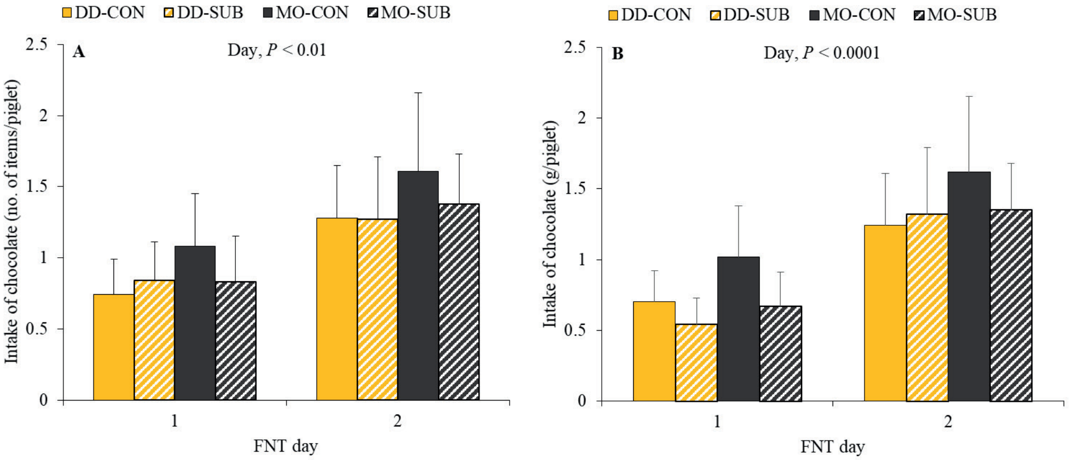
Supplementary data



Supplementary Figure S1. Feed intake of novel feed (cheese and chocolate) in number of items eaten (**A**) and in grams eaten (**B**) by piglets in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. Data are expressed as means ± SEM based on pen averages.

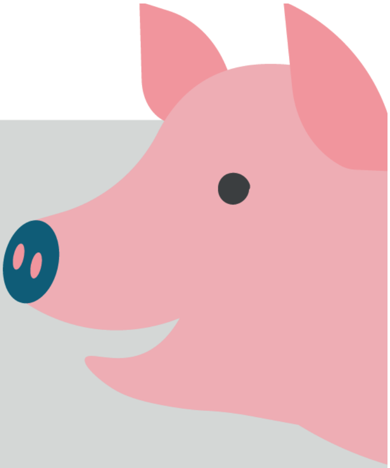


Supplementary Figure S2. Feed intake of cheese in number of cubes eaten (**A**) and in grams eaten (**B**) by piglets in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. Data are expressed as means ± SEM based on pen averages.



Supplementary Figure S3. Feed intake of chocolate in number of pieces eaten (**A**) and in grams eaten (**B**) by piglets in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. Data are expressed as means ± SEM based on pen averages.

CHAPTER 9



General discussion

Chapter 9

General discussion

On commercial farms, weaning is commonly seen as the most stressful event of a pig's life. It happens abruptly and earlier than in the wild (3-4 weeks versus 3-4 months) and is associated with social, environmental and dietary changes. These stressors together often lead to a low feed intake, gastrointestinal problems, gut microbiota dysbiosis, reduced growth and behavioural disturbances after weaning, thereby compromising the health, welfare and performance of newly-weaned piglets (**Chapter 1**). This thesis aimed to reduce weaning-induced problems in pigs by preparing piglets for the dietary transition that they will face at weaning. This was done by applying nature-inspired features that stimulate foraging behaviour of piglets before weaning. The first aim of this thesis was to investigate the effects of creep feed provision on piglet development, with a focus on the transition around weaning (**Chapter 2** and **3**). The second aim of this thesis was to develop foraging and feeding-stimulating strategies for suckling piglets and to study their effect on piglets' ability to deal with the weaning transition (**Chapter 4** to **8**). For the aims of this thesis it was essential to measure individual feed intake of suckling piglets, which is challenging. The different methods used to measure individual feed intake will be discussed first in this chapter. Secondly, potential mechanisms how creep feed provision may reduce weaning-induced problems will be discussed. Thereafter the effects of the feeding strategies that were tested in this thesis on pre-weaning feed intake and post-weaning adaptation will be discussed. To conclude, the relevance for and impact of this thesis on society and industry are discussed and recommendations are given regarding the practical implementation of the feeding strategies and future research.

Methods to determine individual creep feed intake

Determination of individual creep feed intake in this thesis was aimed at quantifying individual creep feed intake over time and describing individual variation in creep feed intake within the litter (**Chapter 2** and **3**). To this aim live behavioural instantaneous scan sampling and continuous video recording and behaviour sampling were conducted to report the (observation) time each piglet spent eating. Secondly, we aimed to compare the creep feeding behaviour between treatments over time to test the success of foraging strategies on feed intake of the litter and individual piglets (**Chapter 4** to **7**). Creep feed intake on litter level was determined by consistently placing the creep feeders on solid flooring, to consider the creep feed eaten and not wasted through the slats. Creep feed intake on piglet level was determined by time spent eating using live behavioural scan sampling observations, and by calculating the percentage of eaters within the litter from live behavioural scan sampling observations as well as from faecal colour by the use of a colour marker in the creep feed. Three techniques were thus explored in this thesis to determine individual creep feed intake: method 1) live behavioural observations using instantaneous scan sampling, 2) behavioural observations from video using continuous recording and behaviour sampling and 3) visual inspection of faecal colour by the use of a colour marker in the creep feed. The measures were repeatedly taken during lactation to study the development of creep feeding behaviour over time.

Measuring creep feed intake of individual piglets is challenging, because 1) piglets are reared in litters, 2) piglets only consume a small quantity of creep feed, particularly early in lactation, 3) piglets tend to eat together, 4) there is substantial variation in feed intake between littermates and 5) because of difficulties to take spillage of feed on the floor and wastage through the slats into account. These factors make it difficult to find a reliable method to measure individual creep feed intake. This paragraph will discuss the measurement techniques to determine individual creep feed intake and their pros and cons.

Behavioural observations

Behavioural observations in this thesis were conducted to quantify the eating behaviour of piglets in their home pen by reporting the (observation) time each piglet spent eating. This was either done by instantaneous scan sampling (**Chapter 2, 4** to **7**) or by continuous recording and behavioural sampling (**Chapter 2** and **3**). Scan sampling was performed during day-time only, but results from continuous observations revealed that piglets eat mostly during day-time compared to night-time (**Figure 1**), which is in agreement with previous studies (Fraser et al., 1994; Baumann et al., 2012). On average 14% of the feeding activity occurred during the night. Moreover, continuous observations showed that the feeding activity during the day was mostly concentrated after 13:30 h with some smaller peaks in the morning (**Figure 2**). This confirms that pigs show a diurnal 'alternans' feeding pattern: a small peak of feed intake at the beginning of the active period and a larger peak at the end of the active period (Boumans et al., 2017). The eating pattern of piglets is distinct from their suckling pattern, since piglets suckle the sow approximately once per hour, decreasing from 1.3 times per hour at 1 week of age to 0.95 times per hour at 4 weeks of age (Jensen, 1988; Puppe and Tuchscherer, 2000). In order to score the majority of the feeding behaviour it is important for future studies to include the main creep feed activity peaks in the scan sampling observations. In addition, it is required to scan the eating behaviour of piglets as often as possible within the observation time to minimize short eating bouts of piglets from being missed. In my thesis work it was feasible for one person to observe the feed-related behavioural repertoire of seven litters with on average 12 to 13 piglets with 2-min scan sampling.

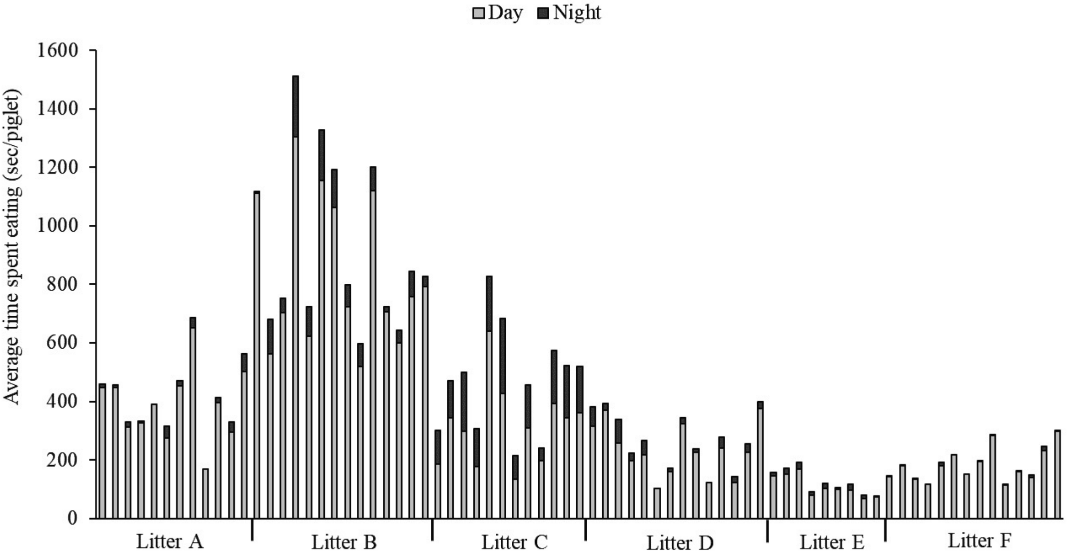


Figure 1. Average daily eating activity of individual piglets of six litters (batch 1 of Chapter 2) when lights are on (day: 7:00-19:00h) and lights are dimmed (night: 19:00-7:00h), determined between 22 and 26 days of age.

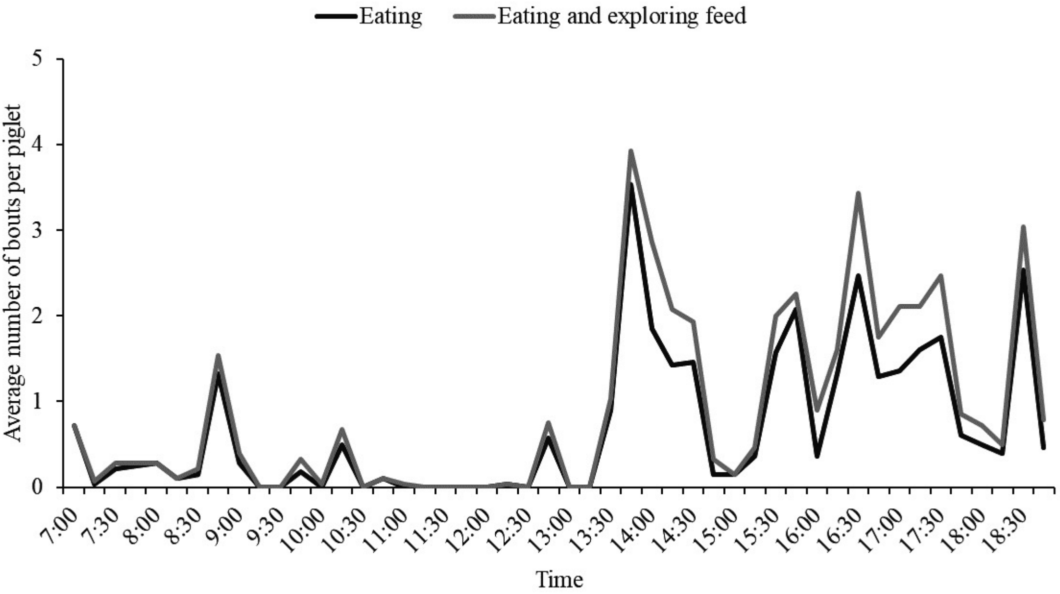


Figure 2. Eating activity pattern of piglets during day-time when lights are on (7:00-19:00h), determined for one litter of 14 piglets (batch 2 of **Chapter 2**) between two and three days before weaning. Sows were fed at 7:30 h and 16:00 h.

There are some limitations of using behavioural observations to score individual feed intake in piglets compared to other measures. Behavioural observations are laborious as a significant amount of observation hours is needed for reliable results and because piglets need to be marked to allow individual recognition. Marking requires piglet handling, which imposes stress to piglets and their sows. Frequent marking and thus handling may moreover affect piglets' activity and thereby their feeding behaviour. Experimenters should also be trained to establish a high degree of inter-observer reliability.

It is hard to distinguish between eating and chewing by behavioural observations. During live scan sampling observations eating was defined as 'eating or chewing feed from the feeder or the floor' (**Chapter 2, 4** to **7**). This definition may underestimate the eating behaviour on one hand as it assumes that piglets that show chewing movements away from the feeder without indications of feed in their mouths are not chewing on feed. On the other hand using this definition may overestimate the eating behaviour as it assumes that piglets will swallow the feed after chewing on it. During continuous video observations eating was defined as 'placing the snout into the feeder for a minimum of 5 seconds' (**Chapter 2** and **3**), based on Pajor et al. (1991) and Adeleye et al. (2014). With this method you may score piglets eating, while they actually just visited the feeder to sniff and root through the feed. To distinguish between eating or chewing feed and other foraging behaviours it is therefore important to observe if piglets are making the jaw movements associated with eating. Particularly for piglets early in lactation, it was hard to observe from video whether piglets were chewing, but this can possibly be solved in future research by a better position of the camera relative to the feeder. These two definitions of eating used in this thesis assume an equal eating rate (g/min) over time and between littermates. However, during the first days that a piglet starts eating it tends to eat slower (Appleby et al., 1991), resulting in a variable eating rate of littermates due to variation in their start of feed consumption.

Using behavioural observations it remains unknown how much piglets actually take in, but significant correlations were found between creep feeding behaviour and creep feed intake at litter level. Creep feed intake on litter level strongly correlated with time spent eating creep feed determined by 2-min (r = 0.83, **Chapter 5**) and 4-min instantaneous scan sampling (r = 0.91, **Chapter 6**) and moderately correlated with time spent eating creep feed determined by 4.5-min instantaneous scan sampling (r = 0.58, $P < 0.001$, unpublished data from **Chapter 2**). This corresponds to the findings of Delumeau and Meunier-Salaun (1995) that reported a significant correlation between the frequency of eating and litter creep feed intake determined by 5-min scan sampling (r = 0.80). Creep feed intake and time spent eating based on scan sampling also strongly correlated with time spent eating based on continuous sampling of the litter during day-time in **Chapter 2** of the present thesis (r = 0.92, $P = 0.0001$ and r = 0.86, $P < 0.0001$ respectively, unpublished data from **Chapter 2**). This is in accordance with Huting et al. (2017) who also found a significant correlation between creep feed intake and time spent eating based on continuous sampling (r = 0.69). Time spent eating based on continuous sampling was related to several individual microbial groups in the colon in this thesis (**Chapter 3**), supporting the relationship between time spent eating based on continuous sampling and creep feed intake. Time spent eating based on scan and continuous sampling were also correlated on piglet level in this dissertation (r = 0.75, $P < 0.0001$, measured on the same days, unpublished data from **Chapter 2**). Taken together, these data suggest that both scan and continuous sampling can be good estimates of creep feed intake and can provide quantitative information about the time spent eating by each piglet in the litter. However, no correlation between time spent exploring feed based on scan sampling and feed intake was found before weaning (**Chapter 6**). Nevertheless, time spent exploring feed did correlate with time spent eating on piglet (r = 0.65) and litter level (r = 0.77, **Chapter 7**). Time spent exploring the feeder was weakly associated with time spent eating at 16 (r = 0.29, $P < 0.0001$) and 23 days of age (r = 0.18, $P = 0.01$), but not at 9 days of age (r = 0.06, $P = 0.35$, unpublished data from **Chapter 5**, based on piglets provided with a conventional feeder only). These findings suggest that it is important to observe the time piglets spent eating the feed rather than the time spent exploring the feed(er) or time spent at the feeder. To estimate how much individual piglets consume, the creep feed intake of the litter may be allocated to individual piglets based on the observation time each piglet within the litter spent eating.

After scoring the eating behaviour of piglets by observations, piglets were subsequently classified into ‘eaters’ and ‘non-eaters’ in this thesis. Piglets eating at least once were designated as eaters, while piglets that were never seen eating on that observation day were designated as non-eaters (**Chapter 2, 4** to **7**). This classification method was adapted from previous studies (Delumeau and Meunier-Salaün, 1995; Devillers and Farmer, 2009) by increasing the number of scans per observation day, by improving the distribution of scans over the day and by excluding feed exploration and waiting at the feeder from the definition of eating behaviour. These modifications may reduce false-negative and false-positive results respectively. However, since eating during one scan is not substantial, the number of eaters may still be overestimated, while potential eating behaviour between the scans may be missed and thereby underestimate the number of eaters. In this thesis, eaters were also grouped into different eater classes (**Chapter 3, 5, 6** and **7**) after

Bruininx et al. (2004) and Pluske et al. (2007), being either a 'bad', 'moderate' or 'good/early' eater of creep feed depending on the number of observation days the piglets were seen eating. Litters that start eating early consume more creep feed than litters that can only start eating late (Sulabo et al., 2010d; Yan et al., 2011), indicating that if piglets are observed eating on more days than others, they likely also have a more regular feed intake pattern and an overall higher feed intake. Once scored as an eater the majority of the piglets remained eaters at subsequent observation days (73% in **Chapter 2**, 91% in **Chapter 5**, 96% in **Chapter 6** and 90% in **Chapter 7**).

Faecal colour and markers

The most commonly used method to determine creep feed intake on individual piglet level is the use of colour markers in the creep feed that distinguish whether a piglet is an eater or not. When piglets consume the creep feed, the colour marker, which is assumed to be inert, is transported through the gastrointestinal tract and ends up colouring the faeces. Subsequent inspection for colouration of faeces determines the intake of creep feed in a qualitative way (yes or no subjectively by eye). Recently, Huting et al. (2017) determined that faecal colour can also be assessed objectively and in a quantitative manner by a colour reader. The numerical colour data did correlate well with creep feed intake assessed on pen level, indicating that litters consuming more feed had indeed more intensely coloured faeces. Similar treatment effects were observed when individual creep feed intake was determined qualitatively by subjective inspection of the faecal colour by the experimenter or quantitatively by objective inspection of the colour by the colour reader (Huting et al., 2017, 2019), suggesting the use of one of the methods is sufficient to study treatment differences.

The use of faecal colour to estimate individual creep feed intake has some disadvantages that are similar to those of behavioural observations. Firstly, obtaining faecal material from each piglet takes time and imposes discomfort. Faecal material can be obtained by inserting a swab in the rectum (e.g. De Ruyter et al., 2017; Muns and Magowan, 2018) or by stimulating defaecation by temporarily placing piglets individually (Collins et al., 2013; Huting et al., 2019). Another drawback are factors that affect the accuracy of the method. Ninety-nine percent of the piglets can be classified accurately by the use of a colour marker when faecal samples are inspected visually once daily for four consecutive days (Kuller et al., 2007c). However, the accuracy of a colour marker in the creep feed is dependent on the assessment of the number of faecal samples taken per piglet per day and over consecutive days (Kuller et al., 2007c). Using a single faecal sample, 40% of the piglets were wrongly classified as non-eaters, which lowered to 15% of the piglets when multiple faecal samples were taken per piglet per day (Kuller et al., 2007c). As a result, the number of eaters may be under-estimated. This may particularly be the case for piglets with a low creep feed intake (Barnett et al., 1989; Kuller et al., 2007c) and for piglets with an irregular feed intake and thus irregular excretion pattern (Kuller et al., 2007c). Piglets may especially have an irregular feed intake pattern in early lactation when they eat creep feed for the first time, since they did not always continue eating during the following days (unpublished data from **Chapter 2** and **3**). Indeed, when the percentage of creep feed eaters based on rectal swabs is compared with the percentage of creep feed eaters based on behavioural observations in the same study, it was found that behavioural observations classified more piglets as eaters than faecal colour of rectal swabs taken the day after, particularly early in lactation when creep feed intake is low (**Chapter 5**: behaviour: d9: 2%, d16: 22%, d23: 60% vs. faecal colour: d10: 0%, d17: 5%, d24: 37%;

Chapter 7: behaviour: d11: 40%, d18: 69%, d27: 90% vs. faecal colour: d12: 3%, d19: 30%, d28: 70%). Once scored as a creep feed eater the majority of the piglets remained creep feed eaters at subsequent faecal sampling days (92% in **Chapter 5** and 69% in **Chapter 7**). The number of eaters per litter based on behavioural observations (seen eating at least once per observation day) correlated with the number of eaters per litter determined by rectal swabs (blue colour on the swab) that were taken the day after ($r = 0.71$, **Chapter 5**).

The average transit time of a colour marker from ingestion to excretion is to date unknown in suckling piglets, but excretion of chromic oxide (a green colour marker) can start within 24 hours after ingestion and last up to four days after ingestion in suckling piglets (most frequent from 48 to 58 hours after ingestion: Kuller et al., 2007c) and excretion of Indigo carmine (a blue colour marker) occurred between 10 and 40 hours after ingestion in weaner piglets (most frequent from 13 to 24 hours after ingestion: Tan et al., 2016). This variation in oral-rectal transit time may be due to individual variation in sensitivity to stress, gut capacity and/or intestinal peristalsis (Kim et al., 2007). The occurrence of diarrhoea may mask a low creep feed intake and watery faecal samples were therefore excluded in previous research by others (Callesen et al., 2007b; Tucker et al., 2010; Huting et al., 2017, 2019), although visually blue diarrhoeic samples have been observed in studies presented in this thesis (unpublished data).

Indigo carmine was used as a colour marker in this thesis to visually inspect blue colouring of rectal swabs (**Figure 3A**). Blue colour markers have been used more recently in research to determine eaters than green colour markers like chromic oxide, as blue appears to stain the faeces more clearly than green. All piglets could be classed into eating categories when Indigo carmine was used (**Chapter 5** and **7**; Pluske et al., 2007), while 27 to 33% of the piglets could not be designated into any eating category due to indistinguishable colours of the faeces when chromic oxide was used (Bruininx et al., 2002, 2004). Colour markers are not recommended to be used in studies on host physiology and gut microbiota. Although such markers are assumed to be inert, clear effects of feed colourant titanium dioxide were reported on gut homeostasis in mice, such as changes in the colonic gut microbial composition, colonic crypt depth and the release of bacterial metabolites (Pinget et al., 2019). Whether the use of Indigo carmine or other dietary colour markers in piglets interfere with their gut homeostasis has not been studied yet. The creep feed in **Chapter 3** did therefore not include a colour marker since measures on gut morphology and gut microbiota were taken.

Next to colour markers, the 'normal' colour of the faeces, i.e. without colour marker, may indicate whether a piglet is an eater or non-eater. According to Van Nieuwamerongen et al. (2015) eaters have brown faeces with a coarse structure and non-eaters have yellow faeces with a smooth structure, which was determined with a sensitivity of 96.2% and a specificity of 73.5%. The advantage of using normal faecal colour over using colour markers is that the feed does not need to be prepared with a colour marker (during feed production or thereafter by adding it manually to the feed), which is time-consuming. On the other hand, using normal faecal colour as method may be affected by diarrhoea and diet composition to a larger extent than using colour markers, as faecal consistency is taken into account with this method, but this has not been investigated yet.

Figure 3. Blue colouration of a rectal swab (**A**) and faeces (**B**) by the use of colour marker Indigo carmine in the creep feed. Piglets that did not consume creep feed had faecal samples ranging from yellow to dark brown.

Electronic feeders

The most reliable and time-efficient way to determine creep feed intake on piglet level may be the use of electronic feeders that collect individual data on feed intake (disappearance of feed) and feeding behaviour, such as the number and duration of eating bouts and the distribution of feeding behaviour over the day. Pajor et al. (1991) developed an electronic feeder for suckling piglets by implementation of two load cells in the feeder. These load cells monitored the feed removed from the feeder by continuous weighing of the feeder. Although a lot of information can be collected, commercially available electronic feeders do not allow pigs to feed from the feeder at the same time and the experimental feeder of Pajor et al. (1991) only allowed two piglets simultaneously, which may limit local enhancement, synchronization and social facilitation of feeding behaviour between littermates (Keeling and Hurnik, 1996; Wattanakul et al., 2005; Sulabo et al., 2010b). In addition, during validation of an electronic feeder for weaned piglets, Bruininx et al. (2001a) reported that the feed intake in the first two weeks post-weaning of piglets fed in an electronic feeder was 10% lower than the feed intake of piglets fed in a commercial feeder, while both feeders were single-spaced. Next to social facilitation, the more ‘enclosed’ design of electronic feeders may therefore limit the feeding behaviour of piglets and may explain why piglets need more time to get familiar with an electronic compared to a commercial feeder. The disadvantage of currently available electronic feeders is thus that they may impact the feeding behaviour of piglets negatively, but this may be solved in the future by developing one with more feeding spaces allowing more piglets to eat at the same time.

Select the best method by your aim

Although all methods mentioned above have their own pros and cons, to date there are, to my knowledge, no other means to determine individual creep feed intake of piglets within a litter. I would like to argue that the best method to determine individual creep feed intake in piglets depends on the research aim(s). Electronic feeders are preferred to quantify the exact amount of feed individual piglets ingested, but (semi-)quantitative data can also be collected using behavioural observations and faecal samples in combination with a colour reader. The following decision tree can be used to select the method or combination of methods that suit the aim(s) of the research regarding the measurement of individual feed intake by piglets (**Figure 4**), until more sophisticated strategies have been developed.

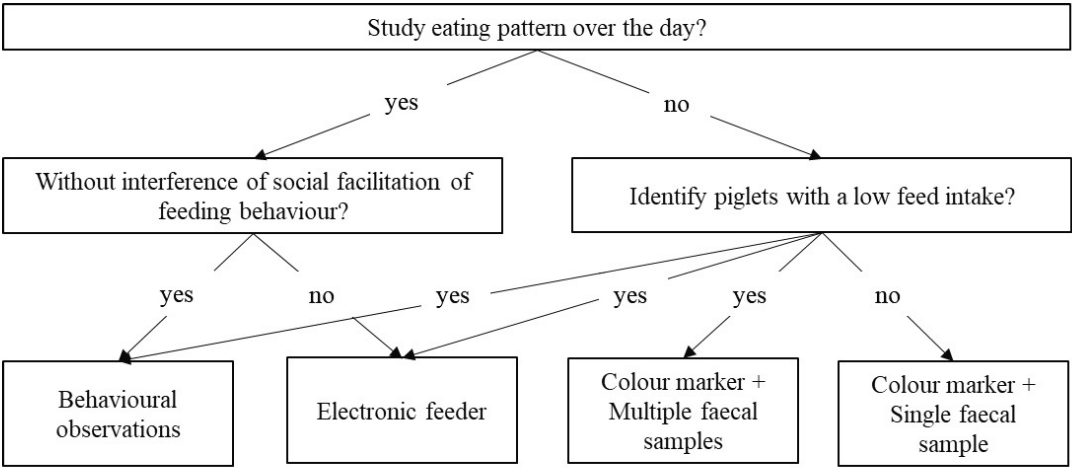


Figure 4. Decision tree to select method(s) to measure individual creep feed intake of piglets within a litter.

Methods of the future?

More research is needed to assess the intake of feed from faeces in a quantitative manner. There are n-alkane markers available that can be provided to pigs to estimate the intake of concentrates (but also roughage) via the n-alkane content of faeces by gas chromatography. Nevertheless, this has only been limitedly tested in pigs up to now (Mendes et al., 2007; Ribeiro et al., 2007; Kanga et al., 2012) and not validated in suckling piglets yet albeit currently studied (Tang et al., ongoing project at Wageningen University & Research). Besides, it would be very attractive to design techniques that do not require the provision of markers to pigs. In the experiments presented in **Chapter 2** and **3** of this thesis it was attempted to estimate creep feed intake in piglets by detection of specific diet-derived mono-saccharides in acid hydrolysates of faecal samples. However, this approach failed to discriminate eaters from non-eaters, indicating that undigested diet-derived (poly-)saccharides and their monosaccharide hydrolysates are not suitable for this purpose (Choudhury, Middelkoop et al., unpublished data). The gut microbiome may also be employed to estimate the amount of creep feed intake, as a positive correlation was found between creep feed intake and the abundance of several gut microbiota strains (**Chapter 3**). Nevertheless, the effect of creep feed intake on the gut microbiota will largely depend on the composition of the creep feed and should therefore be carefully taken into account.

Observations to score individual feeding behaviour of piglets are very laborious. Digitisation of the agricultural sector may shed new light on this in the near future. Researchers are working on the individual recognition of group-housed animals and their behaviours by the use of cameras and farm technology, such as the detection of a lowered tail posture by 3D cameras to predict tail biting (D'Eath et al., 2018). Presence at the feeder is not a good indication of the eating behaviour of piglets, but when linked to removal of feed from the feeder it allows to estimate individual feed intake. This requires techniques that employ advanced image analysis technology that automatically distinguish multiple animals at the feeder.

Creep feed provision

Piglets that consume (sufficient amounts of) solid feed before weaning consume more feed and grow faster after weaning than piglets that consume no (or little amounts of) solid feed (**Chapter 1**). Nevertheless, the increased feed intake of these piglets after weaning does not seem to fully explain the difference in growth rate observed. In the experiment of **Chapter 2** and **3** the mode of action of creep feed provision (and intake) on piglet development was therefore investigated. Before weaning, creep feed provision was hypothesized to improve the growth rate of piglets while suckling the sow (Williams, 2003; Pluske et al., 2018) and to stimulate development of the gut microbiota and digestive system (Yao et al., 2011; Pluske et al., 2018). After weaning, it was expected to reduce food neophobia (Bolhuis et al., 2009; Muns and Magowan, 2018; Figueroa et al., 2019) and stress (Van der Meulen et al., 2010) as result of a more gradual dietary transition. These potential mechanisms are discussed separately below.

Growth before weaning

The provision of creep feed improved the growth performance of piglets before weaning, particularly in the last week, and resulted in a trend for a higher weaning weight (**Chapter 2**). Moreover, some of the intervention strategies of this thesis that led to a higher creep feed intake also resulted in a higher growth performance while suckling the sow (**Chapter 4** in combination with results on growth reported in Luo, Reimert, Middelkoop et al. (under revision), **Chapter 7**), while another did not improve growth before weaning (**Chapter 6**). It should be noted that other potential explanatory variables, such as gut functioning and stress, cannot be excluded, but time spent on eating creep feed positively correlated with body weight gain, at least in the last two days before weaning (**Chapter 7**). In literature, benefits on growth before weaning were mainly reported in piglets with a high creep feed intake (**Chapter 1**). The effects on pre-weaning growth may therefore be dependent on the amount of feed ingested as well as on its composition.

Development of the gastrointestinal tract and gut microbiota

Creep feed intake (eaters from litters given creep feed versus piglets from litters withheld from creep feed) resulted in a longer and heavier gastrointestinal tract, and in a lower pH, higher concentrations of short-chain fatty acids and a different microbiota composition in the large intestine before weaning (**Chapter 3**). Moreover, creep feed intake tended to increase the weight of the pancreas and to reduce the villus:crypt ratio in the jejunum (**Chapter 3**). Such effects were not seen before (Bruininx et al., 2004; Hedemann et al., 2007; Van der Meulen et al., 2010; Muns and Magowan, 2018). Fermentable fibres in the creep feed of this thesis may therefore have contributed to most of the observed effects, since the gut microbiota of creep-fed piglets was mostly characterized by ‘fibrolytic’ taxa such as *Prevotella*, *Megasphaera*, *Roseburia* and *Faecalibacterium*, while that of piglets withheld from creep fed was characterised by *Bacteroides*, *Butyicimonas* and *Cloacibacillus*. Yet to prove this, comparing piglets on a low fermentable fibre creep feed, with piglets on a high fermentable fibre creep feed, and piglets withheld from creep feed in one study is needed to dissect the effects of creep feed intake and fermentable fibre on gut (microbiota) development per se. Van Hees et al. (2019) reported that a creep feed with high levels of long-chain arabinoxylan, which is a fermentable fibre, resulted in a larger fill of the large intestine and a heavier large intestine weight compared to a low-fibre creep feed, supporting the potential role of fermentable fibre on gut microbiota and digestive system development that is suggested in this thesis.

Food neophobia

It was hypothesized that piglets provided with creep feed would consume more of a novel weaner feed in the first days after weaning than piglets that are not provided with creep feed. However, even when the same feed is provided before and after weaning, food neophobia may occur, as a significant part of the piglets do not consume feed or only little amounts before weaning (**Table 5** in **Chapter 1**). It is therefore also suggested that piglets provided with solid feed before weaning would consume more solid feed in the first days after weaning, when the same diet is provided. First, I will discuss the potential effects of creep feed provision (and intake) on food neophobia when a familiar diet is given, and thereafter I will discuss the effects on food neophobia when a novel diet is given.

In **Chapter 5**, maternal feed restriction increased feed intake the last two days prior to weaning (a mix of creep feed and the nursery diet was given these days), doubled the number of eaters at weaning, and resulted in a higher feed intake in the first four hours, on the first and second day after weaning, as well as in improved body weight gain on the first and second day after weaning. Although the difference in pre-weaning feed intake between the groups was relatively small (<120 g/piglet during lactation), the amount of feed ingested before weaning thus seemed to reduce food neophobia after weaning when the piglets were familiarised to the weaner feed before weaning. This corresponds to the positive relation found in literature between the amount of feed eaten before and after weaning (Kuller et al., 2004; Berkeveld et al., 2007b; Langendijk et al., 2007) and between the amount of feed eaten before weaning and the amount of body weight gain during the initial days after weaning (Berkeveld et al., 2007b; Langendijk et al., 2007). The same feed was provided before and after weaning in these studies.

Concerning the hypothesized effects of a reduced neophobia towards novel feed in piglets offered creep feed, equivocal results were obtained in this thesis. In **Chapter 7**, dietary diversity increased pre-weaning feed intake and the number of eaters from early in lactation, but initial post-weaning intake of the novel weaner diet was unaffected. Although stress levels in these piglets may have been higher as dietary diversity was not continued at weaning, and therefore have increased food neophobia at weaning (Bolhuis et al., 2009), results from the food neophobia tests do not provide indications either of reduced food neophobia in piglets provided with a diverse diet compared to a monotonous diet (**Chapter 8**). When comparing piglets provided with creep feed with piglets withheld from creep feed, piglets withheld from creep feed ate more of the novel weaner diet in the first 4 hours after weaning (20 ± 5 vs. 8 ± 3 g/piglet, *P* = 0.04), while creep-fed piglets consumed more feed on the second day after weaning (308 ± 26 vs. 239 ± 21 g/piglet, *P* = 0.03, Choudhury, Middelkoop et al., unpublished data) and between 2 and 5 days after weaning (**Chapter 2,** 582 ± 13 vs. 458 ± 14, *P* < 0.0001). Over the first two days there was no difference in feed intake between piglets provided with creep feed or not in Choudhury, Middelkoop et al. (unpublished data**,** withheld from creep feed: 167 ± 15 vs. provided with creep feed: 186 ± 13 g/piglet/day, *P* = 0.34), while piglets that were not given creep feed tended to consume more feed in the first two days after weaning than piglets given creep feed in **Chapter 2**. It is unknown, however, how long it takes before neophobia towards the weaner diet disappears in piglets, and which time period after weaning thus provides most information regarding food neophobia. Bruininx et al. (2002, 2004) reported no differences in initial intake of a novel weaner diet comparing eaters with non-eaters and good eaters with moderate and non-eaters, but the feeding behaviour of the piglets did differ. Of the piglets that were withheld from creep feed, 27% and 10% of the piglets did not consume the novel weaner feed on the first and second day after weaning respectively, while of the creep-fed piglets all eaters of creep feed consumed the novel weaner feed within 20 hours after weaning, and 15% and 5% of the non-eaters did not consume feed on the first and second day after weaning respectively (Bruininx et al., 2002). On top of a shorter latency to eat, eaters had more feeder visits with intake in the first week after weaning than non-eaters and piglets withheld from creep feed (Bruininx et al., 2002). In a follow-up study, good eaters also showed more feeder visits with intake than non-eaters measured in the first four days after weaning, but did not affect latency to eat (Bruininx et al., 2004) as reported for eaters versus non-eaters (Bruininx et al., 2002). Although literature suggests some positive effects of creep feed intake on the feeding behaviour towards a novel weaner diet (Bruininx et al., 2002, 2004), results on the actual intake of the novel diet remain inconsistent. The relation between the intake of creep feed and the intake of a novel weaner diet therefore warrants further investigation, as relations between pre-and post-weaning feed intake have only been studied when the same feed was provided before and after the weaning transition.

Supplementation of a novel weaner diet with a limited amount of familiar creep feed was hypothesized to reduce food neophobia after weaning, but feed intake in the first days after weaning remained unaffected in this thesis (**Chapter 2**). Nevertheless, Heo et al. (2018) suggested that familiarity with the feed may be more important than the amount of feed ingested before weaning, since piglets that were given the weaner diet already before weaning consumed more feed in the first two weeks after weaning than piglets that were given creep feed before weaning, although the latter piglets consumed more feed in the pre-weaning period (Heo et al., 2018). Diet composition seems important for such an interaction between pre- and post-weaning feed intake (Torrallardona et al., 2012), but maybe also sensory properties can contribute (**Chapter 7**).

Stress

Given the benefits of creep feed intake on initial post-weaning performance (**Table 2** to **4** in **Chapter 1; Chapter 2, 4** and **5**), it may be hypothesized that creep feed intake may reduce stress at weaning (Van der Meulen et al., 2010) and, consequently, reduce weaning-stress-induced behaviours. However, the provision of creep feed did not significantly affect piglets' response to novelty before weaning, haptoglobin concentrations before and after weaning and weaning-stress-induced behaviours in **Chapter 2**. When comparing treatment groups within the same study that differ in pre-weaning feed intake, some subtle indications appear of an association between creep feed intake and reduced levels of stress. Maternal feed restriction increased the number of eaters and the amount of creep feed eaten in the last days before weaning and lowered the number of body lesions in the first two days post-weaning and the occurrence of ear biting at two weeks post-weaning (**Chapter 5**). A lower number of injuries from aggression and a lower time spent on biting pen mates may be related to a reduced stress level (Schrøder-Petersen and Simonsen, 2001; McGlone and Anderson, 2002; Munsterhjelm et al., 2013).

This thesis provided evidence of the impact of creep feed intake on growth (**Chapter 2**), digestive system and gut microbiota development before weaning (**Chapter 3**), while after weaning benefits were reflected by improved feed intake and growth, a stimulating effect on exploratory behaviour and an inhibitory effect on weaning-stress-induced behaviour (**Chapter 5**). The effects of creep feed intake on performance, behaviour as well as on gastrointestinal health are summarized in **Figure 5**. It is important to note that the effects may depend on the intake of creep feed and its composition.

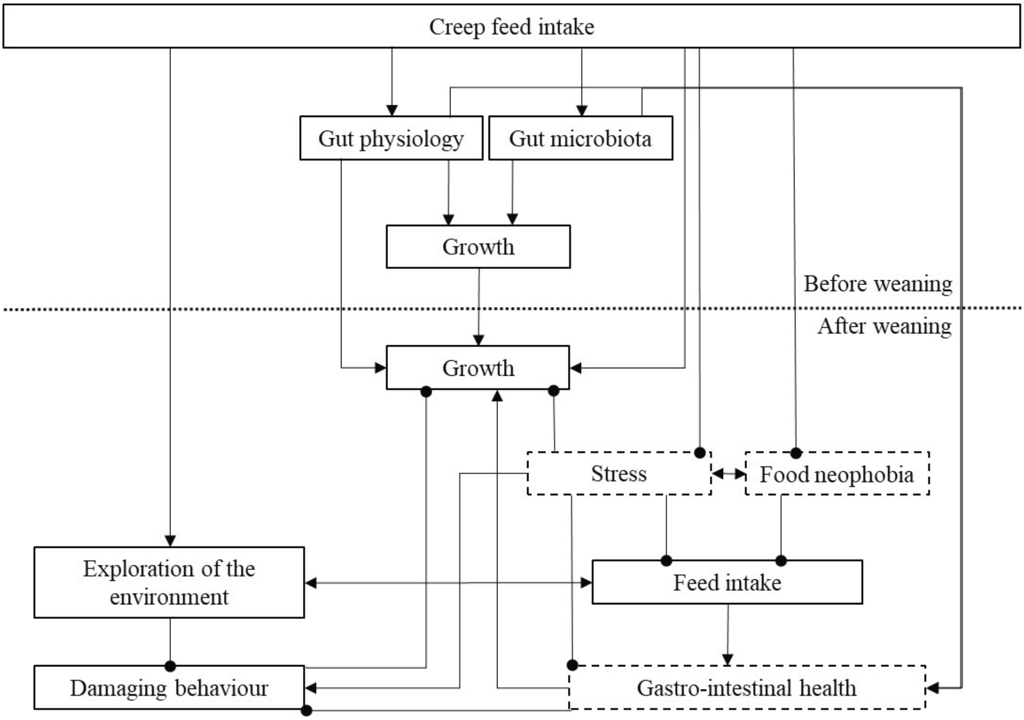


Figure 5. Effects of creep feed intake on piglet development before and after weaning. A normal arrow indicates a stimulatory effect, while an oval arrow indicates an inhibitory effect. Boxes with dashed lines are hypothesized effects of creep feed intake. Boxes with solid lines are effects of creep feed intake supported by the results of this thesis.

Success of the strategies

The second aim of this thesis was to stimulate early foraging and feeding activities in suckling piglets to ease the weaning transition. Four strategies were investigated that aimed at stimulating foraging and feeding: enriching the pen with foraging materials on the floor (straw, saw dust and peat) and extra space, and alternating chew objects (**Chapter 4)**, a foraging-stimulating play-feeder (**Chapter 5**), dietary diversity (**Chapter 6** and **7**) and feed presentation in sand (**Chapter 7**). The main effects of the strategies on piglet development up to two weeks post-weaning are summarized in **Table 1**.

Firstly, all four strategies succeeded in stimulating the exploratory / foraging behaviour of piglets before weaning (**Table 1**). Although the effect of enriching the pen on exploration in pigs was not reported in **Chapter 4** of this thesis, exploration was recorded in Luo, Reimert, Middelkoop et al. (under revision), showing that enriched-housed piglets spent more time on exploration and less time on manipulating pen mates before and after weaning. Additionally, enriched-housed piglets showed less aggression and tended to perform more play behaviour before weaning (Luo, Reimert, Middelkoop et al., under revision).

Secondly, the feeding behaviour of piglets before weaning (determined by feed intake, percentage of eaters and individual feeding behaviour) was also improved by the four strategies (**Table 1**), implying early foraging is important for the development of early feeding. Two of the four strategies stimulated the feeding behaviour on their own (enriching the pen and dietary diversity) with the other two strategies interacting with another intervention on the percentage of eaters (play-feeder in combination with feed restriction of the sow, and feed hidden in sand in combination with a monotonous diet). We showed that the energy intake of piglets from milk is one of the fundamental factors that influences their creep feed intake, but we do not recommend maternal feed restriction as a feeding strategy to improve creep feed intake and to facilitate the weaning transition of piglets, as feed restriction is detrimental for piglet welfare (Pastorelli et al., 2012), sow welfare (Appleby and Lawrence, 1987) and sow future reproductive performance (Sulabo et al., 2010a; De Bettio et al., 2016; Costermans et al., 2019).

The best strategy to improve the performance of piglets during the weaning transition is hard to choose from this thesis, as three of the four strategies were not continued after weaning, resulting in loss of resources: loss of the play-feeder (**Chapter 5**), loss of dietary diversity (**Chapter 7**) and loss of sand given in a feeder (**Chapter 7**), which likely affected post-weaning adaptation in a negative manner. The pigs described in **Chapter 4** were followed until the growing-finishing phase for other study purposes and were either exposed to a shift in environment (from enriched to barren or from barren to enriched housing conditions) at 3 weeks after weaning or were continuously reared in their current housing conditions (enriched or barren, Luo, Reimert, Middelkoop et al., under revision). Piglets that ‘downgraded’ from an enriched to a barren environment had the lowest levels of play, highest inactivity levels and showed most oral manipulation of pen mates compared to the other three groups (Luo, Reimert, Middelkoop et al., under revision). This is supported by literature, in which most studies found evidence that loss of enrichment could be more frustrating to pigs than barren housing throughout their life (Day et al. 2002; Munsterhjelm et al. 2009; Oostindjer et al. 2010, 2011d; Douglas et al., 2012). Nevertheless, no indications of detrimental effects were found in Yang et al. (2018), while Brajon et al. (2017) found behavioural indicators of frustration, but, in contrast, the productivity of the piglets was improved. Different enrichment strategies may thus lead to variation in the impact on the adaptive capacity of piglets. The different effects on the coping ability of the piglets after weaning among the foraging strategies tested in this thesis imply that the strategies may stimulate foraging and play differently, and that the loss of some resources may be more frustrating than others. The play-feeder improved post-weaning feed intake, growth, diarrhoea and body lesions and damage, despite no main effect of the play-feeder was seen on the feeding behaviour of piglets before weaning. The play-feeder therefore stands out from the other two strategies, dietary diversity and feed hidden in sand, that showed no major post-weaning effects or negative post-weaning effects, respectively. The play-feeder was most effective in stimulating object play compared to the other two strategies, which may have played a role in the stimulating effects on coping, since object play is suggested to be self-rewarding (Nahallage et al., 2016) and may have resulted in a positive association with feed. The positive impact of the play-feeder on post-weaning adaptation suggests that not only early feeding may exert positive effects for the weaning transition (as suggested in this thesis and in studies by others), but that foraging, the appetitive phase of feeding, in early life can exert large beneficial effects on piglet adaptability. In summary, the discontinuation of strategies at weaning may have resulted in frustration, which could have overshadowed the positive effects that were expected. To select the strategy that improves piglet performance during the weaning transition the most from the strategies studied in this thesis, future research is needed with continuation of the strategies after weaning. Until those results are obtained, foraging materials on the floor in combination with extra space and alternating chew objects, and the play-feeder are two promising strategies that showed large and broad beneficial effects on both welfare and performance of the pigs (**Table 1** and Luo, Reimert, Middelkoop et al., under revision). If one wants to improve post-weaning adaptation of piglets, a 'mismatch' in environment should be avoided.

Table 1. Effects of feeding strategies (**Chapter 4** to **7**) and other interventions (**Chapter 2, 3** and **5**) on piglet behaviour and performance up to two weeks post-weaning. All strategies and interventions were applied until weaning, except for creep feed supplementation on top of the weaner diet that was applied after weaning and except for the provision of foraging materials on the floor, extra space and alternating chew objects that was continued after weaning.

	Chapter 2 + 3		**Chapter 4**	**Chapter 5**		**Chapter 6**	**Chapter 7**	
	Creep feed provision	Creep feed supplement on top of weaner diet	Foraging materials on the floor, extra space and alternating objects	Maternal feed restriction	Play-feeder	Dietary diversity vs. flavour novelty	Dietary diversity vs. monotony	Feed hidden in sand
Before weaning (d0 = birth)								
Feed intake	not applicable		↑ 1.8x d28-30 ↗ 1.4x total intake d7-30	↑ 3x d22-24 ↗ 2x total intake d10-24	= d4-24	↑ 1.5x total intake d2-22	↑ 5x total intake d4-28	= d4-28
Eater %	not applicable		= at d22	↑ 2x at d24 ↑ piglets fed in a play-feeder in better eater classes ↗ piglets fed in a conventional feeder in better eater classes	↑ piglets reared by restrictedly-fed sows in better eater classes	↑ good and moderate eaters	↑ 2x at d11 ↑ 1.4x at d18 ↗ 1.1x at d28 ↑ piglets in better eater classes	↑ piglets fed a monotonous diet in better eater classes
Exploration towards feed(er)	↘ 1.6x exploring feeder			=	↑ 3.5-6x exploring feeder ↑ 1.3-2.5x piglets visiting feeder	↑ 2.6x exploring feed at d14	↑ 2.7-18.7x exploring feed	↗ 1.6x exploring feed at d11
Eating feed	not applicable		= at d22	↑ 2x at d23	=	=	↑ 3x	=
Growth	↑ 12% d21-29 ↑ 5% total growth d2-29			↓ 20% total growth d10-24	=	=	=	=
Weaning weight	↗ 5%			↓ 12%	=	=	=	=
Uniformity in weaning weight	=			=	=		=	=

	Chapter 2 + 3		Chapter 4	Chapter 5		Chapter 6	Chapter 7	
	Creep feed provision	Creep feed supplement on top of weaner diet	Foraging materials on the floor, extra space and alternating objects	Maternal feed restriction	Play-feeder	Dietary diversity vs. flavour novelty	Dietary diversity vs. monotony	Feed hidden in sand
After weaning (d0 = weaning)								
Feed intake	↓ 9% d9-14	↑ 11% d9-14	↑ 22% d0-5 ↑ 9% d5-19 ↑ 10% d0-19					
d0-2	↘ 22%	=		↑ 2x (113%)	↑ 1.4x (36%)		=	↓ 23%
d2-5	↑ 24%	=		↑ 19%	↑ 23%		=	=
d0-15	=	=		=	↑ 15%		=	↓ 7%
Growth	↑ 11% d9-14	=						
d0-2	=	=		↑ (128 vs -32 g/d)	↑ 1416% (91 vs. 6 g/d)		=	↓ 44%
d2-5	↑ 26%	=		=	↑ 18%		=	↗ 9%
d0-15	=	=		=	↑ 16%		=	↓ 11% when fed a diverse diet
Final BW	↑ 6%	=		=	↑ 5%		=	↓ 5% when fed a diverse diet
Uniformity in final BW	↓ 10%	=						
Feed efficiency	=	=		=	=		↗ 2%	=
Diarrhoea	=	=		=	↓ 73% prevalence ↓ 50% consistency ↓ 43% duration		=	=
Exploratory behaviour	↑ 1.8x rooting environment ↓ 1.8x chewing faeces or sham chewing	↑ 3x exploring feed		↑ 1.3x exploring environment			↓ 1.7x chewing environment ↓ 1.5x chewing air ↓ 1.3x rooting environment	↑ 1.5x rooting environment

Continue

Table 1. Effects of feeding strategies (**Chapter 4** to **7**) and other interventions (**Chapter 2, 3** and **5**) on piglet behaviour and performance up to two weeks post-weaning. All strategies and interventions were applied until weaning, except for creep feed supplementation on top of the weaner diet that was applied after weaning and except for the provision of foraging materials on the floor, extra space and alternating chew objects that was continued after weaning.

	Chapter 2 + 3		**Chapter 4**	**Chapter 5**		**Chapter 6**	**Chapter 7**	
	Creep feed provision	Creep feed supplement on top of weaner diet	Foraging materials on the floor, extra space and alternating objects	Maternal feed restriction	Play-feeder	Dietary diversity vs. flavour novelty	Dietary diversity vs. monotony	Feed hidden in sand
Damaging behaviour	=	=		↓ 1.75x ear biting ↓ 2.2x body lesions first 2 days when fed in a conventional feeder ↘ piglets fed in a play-feeder with tail damage	↓ 1.5x body lesions at d15 ↓ piglets reared by full-fed sows with ear damage ↓ piglets reared by restrictedly-fed sows with tail damage		↑ 1.35x nosing pen mates ↓ 1.5x body lesions at 4h ↑ 1.4x body lesions at d15	↑ 1.6x manipulating pen mates ↑ 2x aggression ↑ 1.5x body lesions at 4h ↑ 1.4x body lesions at d15
Inactive behaviour	=	=						↓ 1.1x

↑ Significant increase, ↗ tendency to increase
↓ Significant decrease, ↘ tendency to decrease
Cell left empty means not studied in this thesis
= No difference

Relevance, impact and recommendations

To feed the growing world population it is expected that pork production will need to increase, while concurrently there is increasing pressure from the general public, legislators and other stakeholders to improve sustainability of the pig industry and animal welfare. One of the major issues in pig production are weaning-induced problems, because they impair piglet health, welfare and performance. Reducing weaning-induced problems in pigs will therefore contribute to the societal and economic perspectives of the pig industry. The aim of this thesis was to facilitate the dietary transition of pigs at weaning and thereby to reduce weaning-induced problems within current indoor pig production systems. This PhD project led to successful strategies that stimulated feed intake (**Chapter 4, 6** and **7**) and the number of (good) eaters before weaning (**Chapter 5, 6** and **7**) and that improved piglet adaptation after weaning in a broad manner (**Chapter 4, 5,** Luo, Reimert, Middelkoop et al., under revision) by stimulating post-weaning feed intake, growth and body weight, reducing the prevalence, duration and severity of post-weaning diarrhoea and decreasing the number of body lesions, and the proportion of piglets with ear and tail damage. Implementing the innovative strategies of this thesis into current pig production systems will simultaneously improve welfare and productivity of pigs and contribute to reduce problems such as antibiotic use (and thus antibiotic resistance) and tail biting. The latter is of increasing importance due to the urgent societal pressure to enforce legislation on the prohibition of routine tail docking in the EU. Strategies of this dissertation were tested at research facilities. To assess the impact of the strategies on the European pig sector it is recommended to test the strategies on a larger scale at multiple commercial farms. Weaning-induced problems were reduced using an ethological approach: by stimulating the natural foraging behaviour of pigs. Creep feed intake is partly driven by a low energy intake from milk, but the success of the strategies suggest that foraging and play are also important aspects for the development of feeding behaviour in pigs. This implicates that feeding means more to pigs than just taking in feed to obtain energy, but also concerns foraging and 'fun'. Given the broad positive effects that were found, facilitating the natural behaviour of pigs is a promising perspective for the pig sector to improve piglet health, welfare and performance.

Based on the results of this thesis the following changes in management, feed and feeder design that can be easily implemented in existing systems are recommendable:

- Provide *ad libitum* solid feed to piglets before weaning to stimulate their behavioural, physiological and gut microbial development. Creep feed intake is at least partially driven by a low milk intake and supplementation of sow's milk with solid feed is thus even more important for litters of sows with a low milk production/quality (due to e.g. heat stress, illness, first parity, large litter size). These litters will grow less and consume more creep feed than litters of sows with proper milk production/quality. The extra consumption of creep feed during lactation will help them, by making the dietary transition more gradual, to catch up in body weight within two weeks after weaning.
- Stimulate early foraging to accelerate the development of feeding behaviour:
    - Provide foraging and alternating chewing materials to piglets, as well as extra space when possible.
    - Provide pigs with a diverse diet. For example, by providing by-products on top of the creep feed or edible foraging materials. Another example is simultaneous provision of creep feed, weaner feed and sow feed before weaning. The creep feed and sow feed will likely stimulate

pre-weaning feed intake, because the first is tasty and the latter because of sow-facilitated learning, and the weaner diet prepares the piglet for the future diet. Ideally, the items provided are as diverse as possible in sensory properties to prevent sensory-specific satiety.

  - Provide feed items and feeders that stimulate object play, such as large feed items and chewable/destructible objects at/in the feeder, to stimulate positive emotions and to ease weaning. This will also help litters with a low milk (quality) intake to start eating.

- Match the pre-weaning environment with the post-weaning environment. Continue dietary and non-dietary strategies implemented before weaning throughout a pig's life.

Current opportunities of piglets to forage for feed are limited, while the European Commission Directive 2001/93/EC states that pigs in the EU must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities. Moreover, the EU law prohibits routine tail docking and requires farmers to try to prevent tail biting, which is known to be more frequent when no proper foraging materials are provided. To be effective in enabling pigs to engage in proper investigation and manipulation, effective enrichment materials should be chewable, deformable, destructible, edible and odorous (reviewed by Van de Weerd and Ison, 2019).

The effectiveness of the feeding strategies tested in this thesis as enrichment is shown in **Table 2**. To stimulate the food-seeking behaviour of pigs to a larger extent, hiding the feed in sand can be optimized by hiding the feed in a larger amount of sand on the floor instead of in a feeder like in this thesis. Sand may also be replaced by a more edible foraging material, such as peat. The play-feeder can be optimized by attaching destructible and edible items. Although the objects attached to the feeder need to be replaced after a batch of pigs, the feeder itself can be used repeatedly. Implementation of the strategies have their cost, but one option may be to re-use materials such as attaching parts of the jute sack, that was used by the sow as nesting material, to the play-feeder. This may even trigger more interest to the piglets by the smell of the mother.

Table 2. Feeding strategies tested in this thesis and how effective they are as enrichment materials for pigs.

		Rootable	**Chewable**	**Deformable**	**Destructible**	**Edible**	**Playable**	**Odorous**	**Heterogenous**	**Effects on feeding**
Strategy	Foraging materials on the floor, extra space and alternating chew objects	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Play-feeder	±	✓	✓	×	×	✓	±	✓	± for slow-growing litters
	Dietary diversity	±	✓	✓	✓	✓	✓	✓	✓	✓
	Feed hidden in sand	✓	×	✓	×	×	±	×	×	±

The recommendations of this thesis are easily applicable to various existing farming systems. However, to feed the growing world population, drastic changes in the food production system will likely take place. In the past year, there has been mass unrest concerning the current animal production system in the Netherlands. Farmers protested because of upcoming policy plans regarding nitrogen and animal activists occupied a pig farm to draw attention to pig welfare. This has led to tension between stakeholders, including citizens, and implies that changes are needed in the current food production system. Also in a changing pig industry, stimulating foraging, feeding and play activities in pigs remain important, while a 'mismatch' in feed and environment between early and later life should be prevented. Future systems are therefore recommended to be designed with a view to stimulate the natural behaviour of pigs, such as suitable flooring to provide foraging materials. A part of the pigs may be used in landscape management in which they can forage for natural resources in a large area, for example on pastures with (invasive) weeds or harvest remains. Dietary diversity may be provided by using residues of the (local) food industry to improve circularity.

The following recommendations for future research can be formulated based on this thesis:

- Integrating multiple successful intervention strategies. This approach has the potential to test if interventions have an additive or synergetic effect maximizing solid feed intake before and after weaning. Moreover, it would be interesting to integrate strategies with a different mode of action. For example, feeding strategies that target the gut (microbiome) by modifications of the diet only make sense if the creep feed is consumed by the piglets, suggesting the integration of strategies that increase creep feed intake with strategies that stimulate gut (microbiota) development. Another interesting combination would be the provision of dietary diversity, that exerted large beneficial effects before weaning, and the play-feeder, that exerted large beneficial effects after weaning. Successful intervention strategies from this thesis as well as from previous research (e.g. social facilitation) should be considered.
- This thesis showed that the development of the gastrointestinal tract and gut microbiota of piglets can be modulated by the provision of creep feed before weaning. This provides opportunities to develop feeding strategies that result in a gut microbiome that is better able to withstand challenges and to maintain host health. For this it is first needed, however, to define what a robust gastrointestinal tract and gut microbiome for piglets around weaning is. This is important to enable the identification of alternatives for the use of antibiotics and zinc in the pig industry. An example of such a feeding strategy may be the inclusion of pre-, pro- and synbiotics in the creep feed. In this thesis, fermentable fibres, including prebiotics, were used in the creep feed.
- The gut microbiota can impact behaviour through the microbiota-gut-brain axis. There may also be a relation between gut microbiota and tail biting, which could be identified by selecting piglets with a high tail biting activity and piglets with no or low tail biting activity and comparing their gut microbiota composition.
- Gain a more comprehensive understanding of the interplay between post-weaning feed intake and gut health. Effects of weaning on post-weaning gut development are widely studied, however the exact role of a low post-weaning intake, which is associated with weaning, is not completely clear.

- Determine the correlation between pre-weaning feed intake and post-weaning feed intake and body weight gain when a novel diet is given at weaning, to better understand the role of food neophobia at weaning. Moreover, correlations between pre-weaning feed intake and behavioural development, such as exploration of the environment, belly-nosing and play, and mechanistic understanding of the potential correlations require further attention.
- Dietary diversity, the play-feeder and hiding the feed in sand were not continued after weaning, resulting in loss of foraging opportunities. Implications of these strategies when also given post-weaning warrant further investigation, including the underlying mechanisms behind the implications. Particularly relations between pre-weaning play and foraging behaviour and post-weaning behaviour and performance remain to be investigated.
- It is recommended to quantify individual creep feed intake by using an electronic feeder with multiple feeding spaces for suckling piglets. For this purpose it is required that multiple animals at the feeder can be distinguished automatically by either using electronic ear tags or video recordings, and that this information is automatically linked to the weight information of the electronic feeder. This will allow more accurate and direct determination of individual creep feed intake, while limiting labour and preventing discomfort for the animals, as compared to rectal swabs and behavioural observations.
- Stimulating foraging behaviour was only studied in young piglets in this thesis. However, foraging is a behavioural need of pigs throughout their life. Strategies should therefore also be tested in older pigs.
- The long-term effects of creep feed provision (**Chapter 2)**, creep feed intake **(Chapter 3)** and the strategies tested in **Chapter 4** to **8** cannot be identified on the basis of this thesis. This thesis focused on the transition phase around weaning and pigs were therefore only followed until two weeks post-weaning. Effects up to slaughter remain to be studied.
- Strategies in this thesis stimulated feeding behaviour in piglets by using an ethological approach of applying features and processes from a (semi-)natural environment to the commercial environment. Not all of these features and processes have been tested in indoor housing conditions yet. For example, pigs spend a significant amount of time grazing in (semi-)natural conditions and they will encounter novel feed items over time (not only simultaneous but also successive dietary diversity) and in different places (multiple feeders to eat from). It is unknown whether providing diverse feed items in a mixture, such as in a muesli, is just as successful as providing the diverse feed items in separate places. It is also unknown whether simultaneous or successive dietary variety or their combination is most successful in stimulating feed intake in pigs. Lastly, flavour novelty using aromas that are found in the natural environment of the animal may be more successful than flavour novelty using synthetic aromas.
- Scientists in other fields may benefit from the knowledge gained in this thesis, as weaning is generally also an abrupt process in commercially-housed calves, lambs, goat kids and rabbits. Although dietary diversity is known to play an important role in how human infants learn about food, the role of play behaviour (e.g. ‘here comes the airplane’, playing with handheld foods and cutlery) on their food acceptance is not yet known to my knowledge.

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Summary

In common pig production systems, piglets are suddenly separated from their mother at 3 to 4 weeks of age, resulting in an abrupt transition from drinking sow's milk to independent feeding on solid feed. This process is called weaning, and is often accompanied by transport of piglets (to another pen or another company) and mixing with piglets reared by other sows. These abrupt changes in diet, environment and social group, can lead to a lot of stress, which results in poor eating and growing, diarrhoea and the occurrence of stress-related behaviours, such as tail biting. Weaning is therefore often seen as the most stressful event in a pig's life. Piglets that have already learned to eat solid feed before weaning, eat and grow better after weaning, especially if they have eaten a large amount of this feed before weaning. However, a significant proportion of piglets do not eat before they are weaned or only eat small amounts, despite being offered the feed from a few days after birth onwards. It is therefore important to investigate how piglets can be stimulated to consume solid feed before weaning. Furthermore, the influence of providing solid feed on behaviour and the influence of eating solid feed on gut physiology and gut microbiota are still largely unknown.

This dissertation is divided into two parts. In the first part we investigated the effects of providing solid feed from a young age onwards on the behaviour and the (intestinal) physiology of piglets. In the second part of this dissertation, several strategies were investigated to get more piglets to eat and also to increase the amount they eat before weaning, in order to facilitate adaptation to solid feed after weaning. This was done by giving piglets more opportunities to display foraging behaviour, such as rooting and chewing. Under more natural conditions, piglets already forage from a few days after birth and try out various, partly fibre-rich food resources. In conventional pig farming, however, the possibility to forage is limited, because usually no suitable rooting materials are offered and piglets only receive one type of feed. In this dissertation, foraging in piglets was stimulated by 1) enriching the pen with rooting materials on the floor and extra space, and by alternating chew objects 2) attaching rooting, chewing and playing materials to the feeder to create a 'play-feeder', 3) providing diverse feeds in the feeder and 4) hiding feed in the feeder in sand to stimulate food-seeking behaviour.

Providing solid feed from a young age

In **Chapter 2** and **3** it was investigated whether early provision of solid feed before weaning (i.e. creep feed, including fermentable fibres) can contribute positively to the behavioural and physiological development of piglets and thus reduce problems after weaning. Piglets given creep feed before weaning grew 12% better in the last week before weaning than piglets who did not receive it, resulting in a trend for a 5% higher weaning weight (**Chapter 2**). Over the two weeks after weaning, the groups did not differ in feed intake, growth, feed efficiency and diarrhoea, but at the end of these two weeks the piglets that received creep feed before weaning were 6% heavier and 10% more uniform in their body weight. The groups did not differ in a stress marker in the blood and in damaging behaviours associated with stress. We therefore found no indications that providing creep feed before weaning makes the weaning process less stressful. Providing creep feed before weaning had little influence on piglet behaviour, as only the types of exploratory behaviour differed between groups, with piglets given creep feed showing more rooting of the environment but less chewing on faeces or sham chewing than piglets without creep feed. Just before weaning, a subset of piglets was sacrificed to study the digestive system and gut microbiota (**Chapter 3**).

Piglets that ate the creep feed that they were offered before weaning had a longer (9.6 ± 0.2 vs. 9.0 ± 0.2 metre) and heavier gastrointestinal tract (484 ± 23 vs. 438 ± 14 gram without digesta) than piglets without creep feed. They also had a lower pH in the caecum (6.3 ± 0.05 vs. 6.7 ± 0.06) and colon (6.9 ± 0.08 vs. 7.2 ± 0.08) and a higher concentration of short-chain fatty acids in the colon (69.3 ± 12.4 vs. 41.2 ± 4.3 µmol/gram wet weight). In addition, their colonic microbiome differed before weaning, whereby the microbiota of eating piglets could be associated with fibre-rich feed and the amount of feed they consumed.

Some of the piglets in **Chapter 2** received a small amount of the creep feed they received before weaning as familiar feed on top of the feed they received after weaning, which was an unfamiliar feed. We expected that by providing a small amount of familiar creep feed on top of the unfamiliar weaner feed, we would improve feed intake just after weaning and reduce stress. Supplementing the unfamiliar weaner diet with the familiar creep feed stimulated feed exploration up to 3 times, but feed intake was only higher (by 11%) between 9 and 14 days after weaning. Growth and diarrhoea were not affected.

Foraging strategies

In **Chapter 4** to **8**, the effectiveness of different foraging strategies in the farrowing room was determined with a focus on the transition phase around weaning. This was measured by studying the eating behaviour of piglets before weaning and their adaptability after weaning, reflected in behaviour, skin lesions and damage, faecal consistency and production characteristics.

In **Chapter 4**, enrichment was added to the farrowing pen by providing rooting materials (straw, sawdust and peat) on the floor and extra space, and by alternating chew objects. Enrichment of the farrowing pen resulted in a higher feed intake before weaning, which was twice as high in the last two days before weaning, compared to barren farrowing pens. Enrichment was continued after weaning and increased feed intake by 10% in the post-weaning period.

In **Chapter 5**, piglets were given a conventional feeder or the same feeder with rooting, chewing and playing materials attached to it. This created a play-feeder, which allowed piglets to explore and play with it. The play-feeder was indeed visited more often and by more piglets to nose, root, chew and play compared to a conventional feeder. However, the play-feeder did not cause a higher feed intake or more creep feed eaters than the conventional feeder. More ‘good eaters’ (piglets that were scored as an eater on multiple days) were found, however, when the play-feeder was given instead of a conventional feeder to piglets from sows with a low milk production. Although all piglets were fed in a conventional feeder after weaning, the piglets that had a play-feeder before weaning did considerably better after weaning. Over the two weeks after weaning, they ate 15% more and grew 16% more, diarrhoea was 73% less prevalent, 50% less severe (better faecal consistency) and 43% shorter in duration, and piglets had 1.5 times fewer skin lesions. There were also fewer piglets with ear and tail damage in this group.

In **Chapter 6**, the effect of dietary diversity was tested on the eating behaviour of piglets before weaning. Half of the litters were offered two different feed items at the same time and the other half of the litters one of the feed items, to which a different flavour was added daily (four alternating flavours in total). Litters with two feed items showed more feed-oriented behaviour: they explored the feed 2.6 times as much, had more good eaters, and ate 1.5 times more feed than litters with one feed item with alternating flavours. The positive effect of dietary diversity on early feed intake before weaning was confirmed in **Chapter 7**, in which the piglets were also monitored after weaning.

In **Chapter 7**, the piglets were given one feed item or four different feed items simultaneously. Litters with a diverse diet explored the feed 3 to 19 times more, ate 5 times more and contained more piglets that were eating during the suckling period than litters with a monotonous diet consisting of only one feed item. Litters with a diverse diet consisted of twice as many eaters at 11 days of age and all piglets were seen eating before weaning. However, piglets that were given a diverse diet before weaning did not do better after weaning, possibly because dietary diversity was not continued after weaning and they thus suddenly received a monotonous diet. We expected that piglets on a diverse diet would eat more from unfamiliar feed due to the experience with various feed items, but found no indications for this. This was investigated by measuring the feed intake of the unfamiliar weaner diet in the first hours and days after weaning (**Chapter 7**) and using behavioural tests in which piglets were exposed to unfamiliar food, such as cheese and crisps (**Chapter 8**).

In **Chapter 7**, half of the litters were also given the opportunity to display food-seeking behaviour by hiding feed in sand in one feeder of the pen and offering feed without sand in the other feeder of the pen. Piglets explored and ate more often from the feeder with sand than from the feeder without sand. The other half of the litters got two feeders without sand in it. However, litters with the possibility of food-seeking behaviour did not explore and eat the feed more often than litters without the possibility of food-seeking behaviour, but did have more good eaters of creep feed. Piglets that had the possibility of food-seeking behaviour before weaning seemed to have more difficulty to cope with weaning than piglets that did not have these possibilities. This was reflected in 1.4 times more damaging behaviour and 2 times more aggressive behaviour, more lesions on the skin (5.6 ± 0.7 vs. 4.0 ± 0.6 lesions), and a 23% lower feed intake and 44% lower growth, particular in the first two days.

Finally, **Chapter 5** demonstrated that the intake of creep feed before weaning is not only stimulated by exploration (**Chapter 4** to **7**), but is also driven by a low energy intake from milk. In this study, half of the litters were reared by restrictedly-fed sows and the other half were reared by full-fed sows. Piglets of restrictedly-fed sows grew 20% less before weaning and therefore had a 12% lower weaning weight than piglets of full-fed sows, because they received less milk and milk with a lower fat percentage. This caused twice as many piglets to eat before weaning and tended to double feed intake before weaning, and therefore also doubled the feed intake in the first days after weaning. As a result, weaning in this group did not result in a growth dip (128 vs. -32 gram/day in the first two days after weaning) and this group spent less time on ear biting (0.4 ± 0.05 vs. 0.7 ± 0.10% of the time). Two weeks after weaning, piglets from restrictedly- or full-fed sows did no longer differ in their body weight (10.18 ± 0.17 vs. 10.43 ± 0.14 kg). This suggests that litters with a low milk consumption can catch up in growth in the period after weaning by eating more solid feed before and after weaning.

Conclusion

Providing solid feed from a young age in the farrowing room influences the exploratory behaviour of piglets and can improve the growth development of piglets before and after weaning. The adapted gut physiology and gut microbiota of eating piglets before weaning may have played a role in this. In summary, the results from this dissertation support that the provision and intake of solid feed before weaning can accelerate the (intestinal) development of piglets.

The results of this thesis have also led to new feeding strategies that can improve the welfare and productivity of piglets. This was achieved by stimulating the development of natural exploration, play and eating behaviour of piglets from a young age. The foraging strategies in this thesis all encouraged the eating behaviour of suckling piglets, with strategies 1 and 3 being more successful in promoting feed intake behaviour than strategies 2 and 4. However, not all strategies promoted piglet adaptability after weaning. This may be due to the discontinuation of strategies after weaning, except for strategy 1, resulting in loss of enrichment. Nevertheless, the play-feeder before weaning provided a greatly improved adaptability of piglets after weaning. To ensure a good performance of piglets after weaning, a gradual transition to solid feed is important and the housing conditions before and after weaning should be aligned. On the basis of this dissertation, it can be stated that piglets should be given the opportunity to forage and play from a young age onwards, and that this possibility should be retained in the growth phases that follow.

Samenvatting

Biggen worden in de gangbare varkenshouderij op 3 tot 4 weken na hun geboorte plotseling gescheiden van hun moeder, waardoor ze geen melk meer kunnen drinken en zelfstandig voer moeten gaan eten. Dit proces wordt spenen genoemd, en gaat vaak samen met het overplaatsen van biggen (naar een ander hok of een ander bedrijf) en het samenvoegen met biggen opgegroeid bij andere zeugen. Deze abrupte veranderingen in voer, omgeving en sociale groep leiden tot veel stress, wat zich uit in slecht eten en groeien, diarree en het vertonen van stress-gerelateerde gedragingen, zoals staartbijten. Spenen wordt daarom vaak gezien als de meest stressvolle periode in het leven van een varken. Biggen die al geleerd hebben vast voer op te nemen voordat ze gespeend worden, eten en groeien beter na spenen, met name als ze veel van dit voer vóór spenen gegeten hebben. Een aanzienlijk deel van de biggen eet echter niet voor ze gespeend worden of slechts kleine hoeveelheden, ondanks dat ze het voer vroeg aangeboden krijgen. Het is daarom belangrijk om te onderzoeken hoe biggen gestimuleerd kunnen worden om vast voer op te nemen vóór spenen. Verder is de invloed van het vroeg aanbieden van vast voer op gedrag, en het eten van vast voer op de darmfysiologie en darm microbiota nog grotendeels onbekend.

Dit proefschrift is opgedeeld in twee delen. In het eerste deel onderzochten we de effecten van het verstrekken van vast voer vanaf een jonge leeftijd op het gedrag en de (darm)fysiologie van de biggen. In het tweede deel van het proefschrift zijn verscheidene strategieën onderzocht om meer biggen aan het eten te krijgen en ook de hoeveelheid die ze eten vóór spenen te verhogen, om zo de aanpassing aan vast voer na spenen te vergemakkelijken. Dit werd gedaan door biggen meer mogelijkheden te geven om foerageergedrag te vertonen, zoals wroeten en kauwen. Onder meer natuurlijke omstandigheden foerageren biggen namelijk al vanaf een paar dagen na de geboorte en proberen ze hierbij verscheidene, deels vezelrijke voedselbronnen uit. In de gangbare varkenshouderij is de mogelijkheid tot foerageren echter beperkt, omdat er meestal geen geschikte wroetmaterialen aangeboden worden en de biggen slechts één type voer verstrekt krijgen. In dit proefschrift werd foerageren van biggen gestimuleerd door 1) het hok te verrijken met wroetmaterialen, afwisselende speeltjes en extra ruimte, 2) wroet-, kauw- en speel-materialen aan de voerbak te bevestigen zodat een 'speel-voerbak' ontstaat, 3) divers voer in de voerbak aan te bieden en 4) voer in de voerbak te verstoppen in zand om zo voedselzoekgedrag te bevorderen.

Verstrekken van vast voer vanaf jonge leeftijd

In hoofdstuk 2 en 3 werd onderzocht of het vroeg verstrekken (vanaf 2 dagen leeftijd) van vast voer met fermenteerbare vezels vóór spenen positief kan bijdragen aan de gedrags- en fysiologische ontwikkeling van biggen en zo problemen na spenen kan verminderen. Biggen die voer kregen vóór spenen groeiden 12% beter in de laatste week vóór spenen dan biggen die dit niet kregen, waardoor ze een 5% hoger speengewicht neigden te hebben (**Hoofdstuk 2**). In de twee weken na spenen verschilden de groepen niet in voeropname, groei, voerefficiëntie en diarree, maar aan het einde van deze twee weken waren de biggen die voer vóór spenen kregen wel 6% zwaarder en 10% uniformer in hun lichaamsgewicht. De groepen verschilden niet in een stress-marker in het bloed en in beschadigende gedragingen die geassocieerd worden met stress. We vonden dus geen aanwijzingen dat het verstrekken van vast voer vóór spenen het speenproces minder stressvol maakt. Het verstrekken van voer vóór spenen beïnvloedde het gedrag van de biggen in geringe mate, aangezien alleen de typen exploratiegedragingen verschilden tussen te groepen, waarbij biggen met voer vaker wroetgedrag in het hok lieten zien, maar minder vaak op mest of lucht kauwden dan biggen zonder voer. Net vóór spenen werd een deel van de biggen opgeofferd om het spijsverteringsstelsel en de darmmicrobiota te bestuderen (**Hoofdstuk 3**). Biggen die vóór spenen het voer aten dat ze aangeboden kregen, hadden een langere (9.6 ± 0.2 vs. 9.0 ± 0.2 meter) en zwaardere darm (484 ± 23 vs. 438 ± 14 gram zonder darminhoud) dan biggen zonder voer. Ook hadden ze een lagere pH in de blinde (6.3 ± 0.05 vs. 6.7 ± 0.06) en dikke darm (6.9 ± 0.08 vs. 7.2 ± 0.08) en een hogere concentratie van korte-keten vetzuren in de dikke darm (69.3 ± 12.4 vs. 41.2 ± 4.3 µmol/gram nat gewicht). Bovendien verschilden hun microbioom in de dikke darm vóór spenen, waarbij de microbiota van etende biggen geassocieerd kon worden met vezelrijk voer en de hoeveelheid voer die ze tot zich namen.

Een deel van de biggen in **Hoofdstuk 2** kreeg na spenen een kleine hoeveelheid van het voer dat ze vóór spenen kregen (bekend voer) bovenop het voer verstrekt dat ze na spenen kregen (onbekend voer). We verwachtten dat door een kleine hoeveelheid bekend voer te verstrekken naast het onbekende voer, we de voeropname net na spenen zouden verbeteren en stress zouden verminderen. Het aanvullen van onbekend speenvoer met een kleine hoeveelheid bekend voer stimuleerde het exploreren van voer tot 3 keer, maar de voeropname was alleen 11% hoger tussen 9 en 14 dagen na spenen. Groei en diarree werden niet beïnvloed.

Foerageerstrategieën

In **Hoofdstuk 4** tot en met **8** werd de effectiviteit van verschillende foerageerstrategieën in de kraamstal bepaald met een focus op de overgangsfase rondom spenen. Dit werd gemeten door het eetgedrag van biggen vóór spenen en hun aanpassingsvermogen na spenen, gereflecteerd in gedrag, huidkrassen en –schade, mestconsistentie en productiekenmerken te bestuderen.

In **Hoofdstuk 4** werd een deel van het kraamhok verrijkt door wroetmaterialen (stro, zaagsel en turf) te verstrekken op de vloer en de biggen afwisselende speeltjes en extra ruimte te geven. Verrijking van het kraamhok zorgde voor een hogere voeropname vergeleken met kale hokken, welke dubbel zo hoog was in de laatste 2 dagen vóór spenen. De verrijking werd voortgezet na spenen en verhoogde in de periode na spenen de voeropname met 10%.

In **Hoofdstuk 5** kregen biggen een reguliere voerbak of dezelfde voerbak met wroet-, kauw- en speelmaterialen eraan bevestigt. Hierdoor ontstond een speel-voerbak, waarmee biggen konden exploreren en spelen. De speel-voerbak werd inderdaad vaker en door meer biggen bezocht om te snuffelen, wroeten, kauwen en spelen dan een reguliere voerbak. De speel-voerbak zorgde echter niet voor een hogere voeropname dan de reguliere voerbak of voor meer etende biggen. Er werden wel meer ‘goede eters’ (biggen die op meerdere dagen als eter gescoord werden) gevonden als de speel-voerbak in plaats van een reguliere voerbak werd gegeven aan biggen van zeugen met een lage melkproductie. Ondanks dat alle biggen een gewone voerbak kregen na spenen, deden de biggen die een speel-voerbak hadden vóór spenen het aanzienlijk beter na spenen. In de twee weken na spenen aten ze 15% meer en groeiden ze 16% meer, was diarree 73% minder prevalent, 50% minder ernstig (vastere mestconsistentie) en 43% korter en hadden de biggen anderhalf keer minder huidkrassen. Ook waren er in deze groep minder biggen met oor- en staartschade.

In **Hoofdstuk 6** werd het effect van een divers voeraanbod getest op het eetgedrag van biggen vóór spenen. De helft van de tomen kreeg gelijktijdig twee verschillende voeritems aangeboden en de andere helft van de tomen één van de voeritems, waaraan dagelijks een andere geur werd toegevoegd (vier geuren in totaal). De tomen met twee voeritems lieten meer voergericht gedrag zien: ze exploreerden het voer 2.6 keer zoveel, hadden meer goede eters, en aten 1.5 keer meer voer dan de tomen met één voeritem met afwisselende geuren. Het positieve effect van het aanbieden van meerdere voeritems op de vroege voeropname vóór spenen werd bevestigd in **Hoofdstuk 7**, waarin de biggen ook na spenen gevolgd werden.

In **Hoofdstuk 7** werd aan de biggen één voeritem gegeven of gelijktijdig vier diverse voeritems. Tomen met een divers dieet exploreerden het voer 3 tot 19 keer meer, aten 5 keer meer en hadden aanzienlijk meer etende biggen tijdens de zoogperiode dan tomen met een monotoon dieet bestaande uit maar één voeritem. In tomen met een divers dieet aten namelijk dubbel zoveel biggen op 11 dagen leeftijd en aten alle biggen vóór spenen. Toch deden de biggen met het diverse dieet het niet beter na spenen, mogelijk omdat de behandeling na spenen niet werd voorgezet en ze dus ineens een monotoon dieet kregen. We verwachtten dat biggen op een divers dieet door de ervaring met diverse voeritems meer zouden eten van onbekend voer, maar vonden hier geen aanwijzingen voor. Dit werd onderzocht door de voeropname van het onbekende speenvoer te meten in de eerste uren en dagen na spenen (**Hoofdstuk 7**) en met behulp van gedragstesten waarin biggen werden blootgesteld aan onbekend voedsel, zoals kaas en chips (**Hoofdstuk 8**).

In **Hoofdstuk 7** kreeg de helft van de tomen ook de mogelijkheid om voedselzoekgedrag te vertonen door in de ene voerbak in het hok voer te verstoppen in zand en in de andere voerbak in het hok voer aan te bieden zonder zand. Biggen exploreerden en aten vaker uit de voerbak met zand dan uit de voerbak zonder zand. De andere helft van de tomen kreeg twee voerbakken zonder zand erin. Tomen met de mogelijkheid tot voedselzoekgedrag exploreerden en aten het voer echter niet vaker dan tomen zonder mogelijkheid tot voedselzoekgedrag, maar hadden wel meer goede eters van regulier vast voer. Biggen die voor spenen de mogelijkheid hadden om voedselzoekgedrag te vertonen leken meer moeite te hebben met de speenovergang dan biggen zonder deze mogelijkheid. Dit werd weerspiegeld in 1.4 keer zoveel beschadigend en 2 keer zoveel agressief gedrag, meer krassen op de huid (5.6 ± 0.7 vs. 4.0 ± 0.6 krassen), en met name in de eerste twee dagen een 23% lagere voeropname en 44% lagere groei.

Ten slotte werd in **Hoofdstuk 5** aangetoond dat de inname van vast voer vóór spenen niet alleen wordt gestimuleerd door exploratie (**Hoofdstuk 4** tot en met **7**), maar ook wordt gedreven door een lage energie inname van melk. In deze proef groeide de helft van de tomen op met een zeug op een gehalveerd voerniveau en de andere helft met een zeug op een normaal voerniveau. Biggen van zeugen op een gehalveerd voerniveau groeiden 20% minder hard vóór spenen en hadden daardoor een 12% lager speengewicht dan biggen van zeugen op een normaal voerniveau, doordat ze minder melk kregen en melk met een lager vetpercentage. Dit zorgde voor dubbel zoveel biggen dat at vóór spenen en een trend voor een dubbel zo hoge voeropname vóór spenen, en zorgde vervolgens ook voor een dubbel zo hoge voeropname in de eerste dagen na spenen. Hierdoor resulteerde spenen in deze groep niet in een groeidip (128 vs. -32 gram/dag in eerste twee dagen na spenen) en vertoonde deze groep minder oorbijten (0.4 ± 0.05 vs. 0.7 ± 0.10% van de tijd). Twee weken na spenen verschilden biggen van zeugen op een gehalveerd of normaal voerniveau niet meer in hun gewicht (10.18 ± 0.17 vs. 10.43 ± 0.14 kg). Dit suggereert dat tomen met een lage melkconsumptie de groeiachterstand die ze ontwikkelen tijdens de zoogperiode kunnen inhalen in de periode na spenen door meer vast voer vóór en na spenen te eten.

Conclusie

Het verstrekken van vast voer vanaf een jonge leeftijd in de kraamstal beïnvloedt het exploreergedrag van biggen en kan de groei-ontwikkeling van biggen voor en na spenen verbeteren. De aangepaste darmfysiologie en darmmicrobiota van etende biggen vóór spenen kan hierin een rol gespeeld hebben. Samenvattend ondersteunen de resultaten uit dit proefschrift dat het verstrekken en eten van vast voer vóór spenen de (darm)ontwikkeling van biggen kan versnellen.

De resultaten van dit proefschrift hebben bovendien geleid tot nieuwe voerstrategieën die het welzijn en de productiviteit van biggen kunnen verbeteren. Dit werd behaald door de ontwikkeling van natuurlijk exploratie-, spel- en eetgedrag van biggen te stimuleren vanaf een jonge leeftijd. De foerageerstrategieën in dit proefschrift stimuleerden allen het eetgedrag van zogende biggen, waarbij strategie 1 en 3 succesvoller waren in het bevorderen van voeropnamegedrag dan strategie 2 en 4. Niet alle strategieën bevorderden het aanpassingsvermogen van de biggen na spenen echter. Dit komt mogelijk omdat, behalve strategie 1, de strategieën niet voortgezet werden na spenen en er daardoor verlies van verrijking optrad. Desondanks zorgde de speel-voerbak vóór spenen voor een sterk verbeterd aanpassingsvermogen van biggen na spenen. Voor een goede prestatie van biggen na spenen is een geleidelijke overgang naar een dieet van vast voer belangrijk en lijken de huisvestingscondities vóór en na spenen op elkaar aan te moeten sluiten. Op basis van dit proefschrift kan worden gesteld dat biggen de mogelijkheid moeten krijgen om vanaf jonge leeftijd te foerageren en spelen, en deze mogelijkheid moeten behouden in de opgroeifases die volgen.

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Anouschka

Curriculum Vitae

Anouschka Middelkoop was born on the first of May 1992 in Noordeloos, the Netherlands. She started her BSc in Animal Sciences at Wageningen University in 2010. After she completed her BSc thesis concerning the relation between inflammation and reproductive success in pigs, she continued with the MSc Animal Sciences and obtained her Research Master degree in 2015. During her MSc, she specialised in two research areas, the first being Animal Health and Behaviour at the Adaptation Physiology Group. Her minor MSc thesis investigated the effects of pre- and/or postnatal exposure to a Western diet on behaviour, particularly fearfulness, in juvenile pigs. At the end of 2014, she moved to Linköping, Sweden, to study the intestinal physiology of feed restricted broiler breeder pullets at the AVIAN Behavioural Genomics and Physiology Group of Linköping University. Back in the Netherlands she also specialised in Applied Zoology at the Cell Biology and Immunology Group of Wageningen University in the topic ‘improving the diagnostics and treatment (allergen-specific immunotherapy) of insect-bite hypersensitivity in horses’. She graduated in December 2015 and was employed as PhD candidate at the Adaptation Physiology Group of Wageningen University from January 2016. During her PhD, she has been studying early nutrition and the weaning transition in piglets, specifically 1) understanding the effects of early feed intake on the behaviour and performance of piglets and 2) developing strategies based on the natural foraging behaviour of pigs to increase early feed intake and improve adaptability around weaning. The results of this PhD research are presented in the current thesis. While working on this PhD thesis she was awarded with two poster prizes and one presentation prize, and became Alltech Young Scientist of Europe in 2019. From January 2020 onwards, Anouschka started working at the R&D department of Schothorst Feed Research in Lelystad as Researcher Swine Nutrition. During her career she would like to continue to improve the health and welfare of animals by finding innovative strategies that promote positive behaviour and performance.

List of publications

Refereed journal publications

Middelkoop, A., Van Marwijk, M.A., Kemp, B., Bolhuis, J.E., 2019. Pigs like it varied; Feeding behavior and pre- and post-weaning performance of piglets exposed to dietary diversity and feed hidden in substrate during lactation. Front. Vet. Sci. 6, 408. doi:10.3389/fvets.2019.00408

Middelkoop, A., Costermans, N.G.J., Kemp, B., Bolhuis, J.E., 2019. Feed intake of the sow and playful creep feeding of piglets influence piglet behaviour and performance before and after weaning. Sci. Rep. 9, 16140. doi:10.1038/s41598-019-52530-w

Costermans, N.G.J, Teerds, K.J., Middelkoop, A., Roele, B.A., Schoevers, E.J., Van Tol, H.T.A., Laurenssen, B.F.A. Koopmanschap, R.E, Zhao, Y., Blokland, M., van Tricht, F., Zak, L.J., Keijer, J., Kemp, B., Soede, N.M., 2019. Consequences of negative energy balance on follicular development and oocyte quality in primiparous sows, Biol. Reprod. 102, 388-398. doi:10.1093/biolre/ioz175

Choudhury, R., Middelkoop, A., Bolhuis, J.E., Kleerebezem, M., 2019. Legitimate and reliable determination of the age-related intestinal microbiome in young piglets; Rectal swabs and fecal samples provide comparable insights. Front. Microbiol. 10, 1886. doi:10.3389/fmicb.2019.01886

Middelkoop, A., Choudhury, R., Gerrits, W.J.J., Kemp, B., Kleerebezem, M., Bolhuis, J.E., 2018. Dietary diversity affects feeding behaviour of suckling piglets. Appl. Anim. Behav. Sci. 205, 151-158. doi:10.1016/j.applanim.2018.05.006

Expected journal publications

Costermans, N.G.J., Soede, N.M., Middelkoop, A., Laurenssen, B.F.A., Koopmanschap, R.E, Zak, L.J., Knol, E.F., Keijer, J., Teerds, K.J., Kemp B. Influence of the metabolic state during lactation on milk production in modern sows. Manuscript under revision.

Middelkoop, A., Choudhury, R., Gerrits, W.J.J., Kemp, B., Kleerebezem, M., Bolhuis, J.E. Effects of creep feed provision on behaviour and performance of piglets around weaning. Manuscript under revision.

Luo, L., Reimert, I., Middelkoop, A., Kemp, B. and Bolhuis, J.E. Effects of early and current environmental enrichent on behaviour and growth in pigs. Manuscript under revision.

Choudhury, R., Middelkoop, A., Gerrits, W.J.J., Kemp, B., Bolhuis, J.E., Kleerebezem, M. Impact of early life feeding on local intestinal microbiota and digestive system development in piglets. Manuscript in preparation.

Middelkoop, A., Kemp, B., Bolhuis, J.E. Early feeding experiences of piglets and their impact on open-field behaviour and food neophobia. Manuscript in preparation.

Conference proceedings and abstracts

Middelkoop, A., Kemp, B., Bolhuis, J.E., 2019. Foraging in the farrowing room to stimulate feeding behaviour. In R. C. Newberry, & B. O. Braastad (Eds.), *Proceedings of the 53rd Congress of the International Society for Applied Ethology (ISAE): Animal Lives Worth Living* (pp. 119-119). Wageningen, the Netherlands: Wageningen Academic Publishers.

Middelkoop, A., Costermans, N.G.J., Kemp, B., Bolhuis, J.E., 2019. Piglet behaviour after weaning is affected by feed intake of the sow and creep feed presentation method. In *Trade-offs in science – Keeping the balance: Abstracts of the WIAS Science Day 2019* (pp. 28-28). Wageningen University & Research.

Middelkoop, A., Choudhury, R., Gerrits, W.J.J., Kemp, B., Kleerebezem, M., Bolhuis, J.E., 2018. Pre-weaning feeding stimulates gut development of piglets at weaning. In *Proceedings of the 14th International Symposium on Digestive Physiology of Pigs (DPP 2018), August 21-24, 2018, Brisbane, Australia* (pp. 112). Advances in Animal Biosciences 9 (S2).

Middelkoop, A., Costermans, N.G.J., Kemp, B., Bolhuis, J.E., 2018. Piglet nutritional needs during lactation and playfulness at the creep feeder ease weaning in piglets. In *Proceedings of the ISAE Benelux conference 2018* (pp. 16). International Society for Applied Ethology (ISAE).

Middelkoop, A., Choudhury, R., Gerrits, W.J.J., Kemp, B., Kleerebezem, M., Bolhuis, J.E., 2018. Pre-weaning feeding stimulates gut development of piglets at weaning. In *WIASS: Work on your Impact in Animal Sciences and Society: Abstract of the WIAS Science Day 2018* (pp. 13-14). Wageningen University & Research.

Middelkoop, A., Choudhury, R., Gerrits, W.J.J., Kemp, B., Kleerebezem, M., Bolhuis, J.E., 2017. Early feeding and the behavioural development of pigs around weaning. In *Proceedings of the ISAE Benelux conference 2017* (pp. 9). International Society for Applied Ethology (ISAE).

Middelkoop, A., Choudhury, R., Gerrits, W.J.J., Kemp, B., Kleerebezem, M., Bolhuis, J.E., 2017. If you give the pig a choice: suckling piglets eat more from a diverse diet. In M. Bak Jensen, M. S. Herskin, & J. Malmkvist (Eds.), *Proceedings of the 51st Congress of the International Society for Applied Ethology (ISAE), 7-10 August 2017, Aarhus, Denmark: Understanding animal behaviour* (pp. 101). Wageningen: Wageningen Academic Publishers.

Middelkoop, A., Choudhury, R., Van den Brand, H., Gerrits, W.J.J., Kemp, B., Kleerebezem, M., Bolhuis, J.E., 2017. Stimulating early foraging in piglets to facilitate their development and improve their performance around weaning. In *Beyond Sustainability: Abstracts of the WIAS Science Day 2017* (pp. 28-28). Wageningen University & Research.

Other publications

Ter Beek, V., Middelkoop, A. et al., 2019. Celery, cereals or peanuts? Piglets like variation. *Pig Progress*, November 2019, https://www.pigprogress.net/Piglets/Articles/2019/11/Celery-cereals-or-peanuts-Piglets-like-variation-496308E/.

Zimmerman, C., 2019. Alltech One19 Interview with Anouschka Middelkoop. Agwired, May 2018, https://agwired.com/2019/05/22/alltech-young-scientists-in-one19-spotlight/.

Koeleman, E., Middelkoop, A. et al., 2018. How to get small piglets to eat more? *Pig Progress*, July 2018, https://www.pigprogress.net/Piglets/Articles/2018/7/How-to-get-small-piglets-to-eat-more-308635E/.

Koeleman, E., Middelkoop, A. et al., 2018. How to influence higher feed intake in small piglets. *All About Feed*, July 2018, https://www.allaboutfeed.net/Compound-Feed/Articles/2018/7/How-to-influence-higher-feed-intake-in-small-piglets-306963E/.

Burgin, R. and Ter Beek, V., 2017. Pig Progress Interview with Anouschka Middelkoop. How to get every piglet eating. *Pig Progress*, November 2017, 32-34.

Education & Training certificate

Completed in fulfilment of the requirements of the Wageningen Institute of Animal Sciences

Description	**Year**
The Basic Package (3 ECTS)	
WIAS Introduction Day	2016
WGS course Ethics and Philosophy in Life Sciences	2016
WIAS course Essential Skills	2016
Disciplinary competences (19.6 ECTS)	
Writing a WIAS literature review	2016
WIAS/PE&RC advanced statistics course Design of Experiments	2016
Training school Gut Biology and Health, PiGutNet grant, Aarhus, Denmark	2017
Training school Prenatal and early life influences on the development of damaging behaviour in pigs and laying hens, GroupHouseNet, Bilbao, Spain	2017
Workshop from Beak to Tail - mechanisms underlying damaging behaviour in laying hens and pigs, FareWellDock and GroupHouseNet, Aarhus, Denmark	2017
WIAS course on The Fundamentals of Animal Emotion	2019
Workshop Future trends in the prevention of damaging behaviour, GroupHouseNet, Bergen, Norway	2019
Organisation of WIAS discussion group Animal Health and Immunology	2017-2019
Member of the WAPS council	2017-2019
Professional competences (10.6 ECTS)	
WGS course Data Management Planning	2016
WGS course Systemic approaches to reviewing literature	2016
WGS/Wageningen in'to Languages course Presenting with Impact	2016
WGS PhD Workshop Carousel	2016
WGS/Valley Consult course Project and Time Management	2016
WGS course PhD Peer Consultation Group	2016
Convener of 3rd Wageningen PhD Symposium	2016
WGS course Teaching and Supervising Thesis Students	2017
WGS course Communication with the Media and the General Public	2017
Participant Famelab Wageningen	2018
WGS course Reviewing a Scientific Paper	2019
WGS workshop Last Stretch of the PhD Programme	2019
WGS workshop Writing propositions for your PhD	2019

Presentation skills (4 ECTS)

WIAS Science Day Poster presentation (awarded Best Poster Presentation)	2017
NWO Co-creation? Naturally! Symposium Poster presentation, Amersfoort, the Netherlands	2017
Regional conference of the ISAE Benelux Oral presentation, Hoogeloon, the Netherlands	2017
51st International congress of ISAE Oral presentation, Aarhus, Denmark	2017
Workshop from Beak to Tail Oral presentation, FareWellDock and GroupHouseNet, Aarhus, Denmark	2017
WIAS Science Day Oral presentation	2018
AWA meeting Oral presentation	2018
Regional conference of the ISAE Benelux Oral presentation (awarded Best Presentation), Geel, Belgium	2018
14th International Symposium on Digestive Physiology in Pigs Poster presentation (awarded Third Best Poster Prize), Brisbane, Australia	2018
WIAS Science Day Oral presentation	2019
53st International conference of ISAE Poster presentation, Bergen, Norway	2019
One: The Alltech Ideas Conference, Lexington, Kentucky, USA (awarded Alltech Young Scientist Graduate Award Europe)	2019

Teaching competences (6 ECTS)

Supervising project group Introduction to the Animal Sciences	2017
Lecture in course Immunology and Thermoregulation	2017
Supervising practical Reproduction and Fertility	2019
Supervising 4 BSc and 6 MSc thesis students	2016-2019

Total 43.2 ECTS (One ECTS credit equals a study load of 28 hours)

Colophon
The research described in this thesis was financially supported by the Netherlands Organisation for Scientific Research (NWO), Cargill Animal Nutrition and Coppens Diervoeding and was part of research programme 'Genetics, nutrition and health of agricultural animals' with project number 868.15.010.

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