Reducing performance variation of piglets in a multi-suckling system

Tianyue Tang

Propositions

1. Positive and negative behaviours do not affect feed intake of piglets in multi-suckling systems.
2. Assessment of individual feed intake of piglets from multiple sources is key to evaluate causes of variation in body weight gain (this thesis).
3. The more complex the data, the more important causal reasoning becomes.
4. Artificial intelligence is essential for progress in animal ethology.
5. Being insensitive to time helps to improve time-management.
6. Problem solving is hampered by excessively talking about the problem.

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Tianyue Tang

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Tianyue Tang

Thesis committee

Promotor
Prof. Dr W.J.J. Gerrits
Professor of Animal Nutrition Group
Wageningen University & Research

Co-promotors
Dr N.M. Soede
Associate professor, Adaptation Physiology Group
Wageningen University & Research

Dr I. Reimert
Senior researcher, Adaptation Physiology Group
Wageningen University & Research

Other members
Prof. Dr S.J. Oosting, Wageningen University & Research
Dr E. Baxter, Scotland's Rural College, Edinburgh, United Kingdom
Dr R.E. Nordquist, Utrecht University
Dr P. Langendijk, Trouw Nutrition Research & Development

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Tianyue Tang

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Contents

Chapter 1: General Introduction

1.1 Introduction

This thesis will focus on unravelling the role of piglet (including birthweight, nutrient intake, nutrient intake associated behaviours and other behaviours) and management related factors in explaining variation in growth rate of piglets raised in a multi-suckling (**MS**) system and on intervention strategies to reduce this variation. This thesis is part of a larger project of which the main goal is to design an integral group housing system for sows and piglets, in which the transitions between gestation and lactation (sows), and between suckling and weaning (piglets) are adapted to accommodate natural behaviour.

In this chapter, a general introduction of the multi-suckling system, the natural living habit of pigs, as well as the knowledge gap within MS systems are reviewed. Subsequently, potential factors affecting variation in growth rate of piglets are discussed. Next, the relevance and possibilities to measure individual feed intake of group housed piglets are introduced. Lastly, the aim and outline of this thesis are presented.

1.2 Multi-suckling systems

In recent years, group housing systems, or MS systems for lactating sows and their litters have been developed (Dybkjær et al., 2003; van Nieuwamerongen, 2017; Nicolaisen et al., 2019) as a solution to welfare problems of conventional farrowing pens by mimicking natural living conditions of pigs. In MS systems, the welfare of sows and piglets is improved by more space allowance, enhanced possibilities to show natural behaviour and improved social development of piglets (Šilerová et al., 2010; Van Nieuwamerongen et al., 2014; Nielsen et al., 2022). In the MS system developed by van Nieuwamerongen (2017) which was located at the Swine Innovation Center Sterksel, the Netherlands (Figure 1a), five sows are introduced into the system one week before farrowing. Loose farrowing takes place in individual farrowing pens, but the sows can move freely in the system at all times. Piglets are only detained in the farrowing pen for the first week of life and can join the other sows and litters at around days 8-9 postpartum (**p.p.**). The room has a relatively large communal area with enrichment materials (e.g. ropes, jute bags) and sows are floor fed in open feeding crates where piglets have the opportunity to eat with the sows. Piglets are provided with various nutrient sources including milk, sow feed, piglet feed and roughage so that they can largely make their own choices. The system also has a separate sow area to which the piglets have no access. This allows the application of intermittent suckling (**IS**), which not only increases piglet feed intake, but also results in a more gradual weaning process during an extended lactation period. Additionally, the application of IS combined with boar contact, induces lactational oestrus in the sows which allows lactational inseminations. In the study of van Nieuwamerongen (2017), each MS unit contains five farrowing pens with piglet nests (A), a communal MS area which includes a lying area (B), a feeding area (C) and a dunging area (D). Connected to the MS unit is the IS area (E), which includes feeding stalls for sows (F) and a boar pen (G). Differently from van Nieuwamerongen (2017), no farrowing crates or farrowing pens is included in another type of MS system (Health et al., 2022)(Figure 1b). Sow and her offspring are initially housed in individual farrowing crates or pens until 1-2 weeks p.p. and are transferred to the MS system (Health et al., 2022) (Figure 1b).

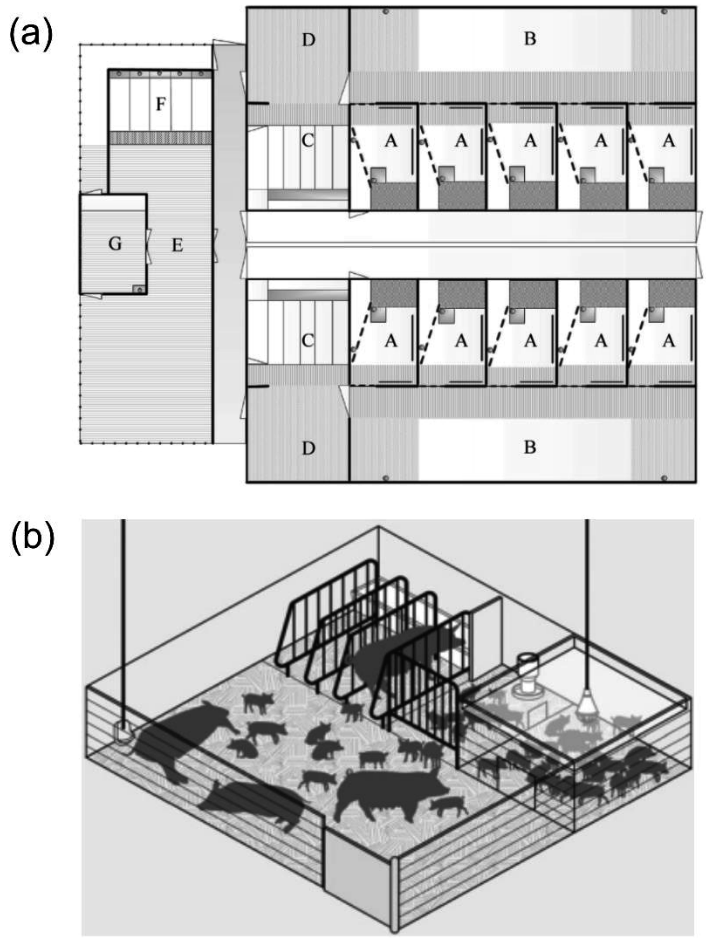

Figure 1. Layout of two types of multi-suckling systems for sows and piglets.(a) van Nieuwamerongen (2017); (b) Health et al. (2022).

In natural conditions, pigs usually live in family groups. Each family group consists of several adult females, their collective progenies, and occasionally several subadult males (Graves, 1984). A few days before farrowing, a pregnant sow leaves her group and travels for kilometers to seek a suitable place to build a farrowing nest. For the first 9 days p.p., sow stays close to the nest to nurse her offspring (Jensen, 1986). During this period, nose to nose contact between the sow and her litter occurs frequently to establish a strong bond and recognition. At around 10 days p.p., the piglets start to join the sow on trips out of the nest to search for food (Jensen and Redbo, 1987). At around 2 weeks p.p., they re-join the family group, after which piglets can socialize with other litters and sows (Petersen et al., 1989). Under these conditions, weaning is a gradual process. Lactation may last for 9-20 weeks, during which suckling activity gradually decreases and feeding activity gradually increases (Widowski et al., 2008).

Studies in the MS system at Swine Innovation Center Sterksel showed that rearing in the MS system had clear advantages for piglets. Compared to piglets raised in a conventional single litter system, piglets kept in the MS system during week 0-4 p.p. showed more play behaviour, less damaging oral manipulation such as tail biting, and a better growth performance after weaning at 4 weeks of age over the entire 5-week postweaning phase (Van Nieuwamerongen et al., 2015). Also, when comparing abrupt weaning at 4 weeks of age to gradual weaning up to 9 weeks of age in the MS system, the gradually weaned piglets showed less negative behaviours including damaging oral manipulation and belly nosing between 4-18 weeks of age (Van Nieuwamerongen et al., 2017).

However, an aspect that needs to be further investigated is the variation in growth rate found in the MS piglets, which is also the knowledge gap of the MS system. Body weight (**BW**) uniformity of piglets during lactation is essential for production efficiency of growing and finishing pigs in current all-in-all-out pig production systems (Douglas et al., 2014) and sizeable variation in BW gain was observed in MS systems at weaning after 6 weeks (Thomsson et al., 2016) and 9 weeks of lactation (BW of piglets in week 9: 12-34 kg) (Van Nieuwamerongen et al., 2017). To find the causes of this variation and to reduce this variation, it is important to understand the role of the piglet, sow and management factors that influence variation in growth rate of piglets in MS systems.

1.3 Potential factors affecting variation in growth rate of piglets in MS systems

Several studies have investigated factors explaining variation in BW and BW gain in conventional housing systems, which includes piglet related factors, sow related factors and management related factors. These factors are discussed below as potential factors affecting variation in growth rate of piglets in MS systems, as the investigation of these factors was rarely done in MS systems.

1.3.1 Piglet related factors

Piglet related factors affecting growth rate include e.g. birthweight (**BiW**), nutrient intake, nutrient intake associated behaviours and other behaviours. Among these piglet related factors, birthweight has been considered as a critical indicator of postnatal performance. For example, Fraser et al. (1986) reported that birthweight explained 30%-40% of within-litter variation in BW on day 14 p.p. In other studies, BiW was positively related with BW gain up to 4 weeks of age during lactation (r=0.39, P<0.001) (Muns et al., 2013) and was positively related with BW up to 10 weeks of age during the nursery period (P<0.001) (Paredes et al., 2012).

Besides BiW, obviously the intake of milk and feed determine BW, and they affect BW differently among different growth phases. During early lactation, milk intake plays an important role for growth rate of piglets (Hojgaard et al., 2020) and feed intake is usually negligible until around day 20 p.p. (Pajor et al., 1991; Widowski et al., 2008). At 3-4 weeks p.p., milk output of sows reaches its peak and declines afterwards (Quesnel et al., 2015), while feed intake plays a more important role for growth rate of piglets after 4 weeks of age (Paredes et al., 2014; Van Nieuwamerongen et al., 2017). For instance, Lodge and McDonald (1959) found that at 8 weeks of age, feed intake explained much more between-litter variation in BW of piglets compared to milk intake (77% vs. 10%). Also, feed intake varies greatly both within and between litters (Pajor et al., 1991; Fraser et al., 1994). Individual variation of total feed intake was, for example, found to range from 13 to 1911 g/piglet on days 10-28 p.p. (Pajor et al., 1991). In conventional housing, it was found that litters which were separated from the sow for 12 h/day from day 14 p.p. until weaning on day 25 p.p. had more visits towards feeders before weaning, compared to litters which were not separated from sows before weaning (Kuller et al., 2010). In addition, in conventional housing, it was found that IS beginning on day 14 p.p. combined with an extended lactation until weaning on day 43 p.p. helped piglets to be gradually independent from milk (Berkeveld et al., 2007). Therefore, in MS systems that combine the application of IS with extended lactations, piglets may also have different feeding patterns compared to piglets reared in commercial single housing systems where no IS or extended lactations are applied. It is also unclear how such MS settings that combine the application of IS with extended lactation influence variation in milk and feed intake and thus variation in growth rate of piglets in different periods.

Furthermore, feed and milk associated behaviours may also influence BW. For feed related behaviours, it was found that piglets with a higher frequency of eating at the feeder before weaning were often slow-growing piglets (Appleby et al., 1992), possibly these piglets have a lower ability to compete for teats and therefore visit the feeder more often, which is called the ‘compensatory feeding hypothesis’ (Middelkoop et al., 2019). However, due to their immature digestive system for ingesting solid feed (Michiels et al., 2013), these piglets grow less than their counterparts that mainly drink milk. In addition, the synchronization of feeding behaviour among pigs (Nielsen et al., 1996) could affect their feed intake and thereby affect BW gain. For milk associated behaviours, it was reported that suckling position (anterior to posterior) and teat fidelity (consistency of suckling position) were the two behavioural factors which were most closely associated with BW on day 14 p.p. of piglets (Fraser and Thompson, 1986), probably through their influence on milk intake. For example, it was reported that piglets consumed a significantly higher quantity of milk from the anterior and middle teats than posterior teats (Skok et al., 2007). In another study, BW gain was found to be highest in piglets suckling the front teats, intermediate in those suckling the middle teats and lowest in those suckling the rear teats (Nielsen et al., 2001). Piglets establish a relatively stable teat order after the first week p.p. (Skok and Škorjanc, 2013) and this teat fidelity is related with variation in BW gain, as it was observed that during days 2-10 p.p., piglets that gained more weight were more consistent in the use of their preferred teats, had fewer teat disputes and missed nursing bouts less often (De Passillé et al., 1988). Cross-suckling, i.e. suckling at alien sows instead of the own sow, was observed in MS systems (Van Nieuwamerongen et al., 2014), which could cause increased competition at the teats, making it more difficult for vulnerable piglets to get access to the udder, and thereby possibly increase the variation in BW gain among piglets in MS systems.

Besides milk and feed intake associated behaviours, also other behaviours have been found to be related to growth rate of piglets. For example, it was found that play behaviour was positively related with BW gain of piglets in early life (Šilerová et al., 2010; Brown et al., 2015). Play activity involves vigorous physical exercise which is regarded as a positive behaviour and could stimulate muscle and bone development (Fagen, 1976) and thus could be able to facilitate growth of piglets. Additionally, it was found that aggressive behaviour was performed mainly by the piglets which were relatively heavy (Mason et al., 2003); it is plausible that heavier pigs fight more to compete for food. Different litters were reported to perform the same behaviour but at different levels, for example play behaviour (Rauw, 2013; Brown et al., 2018). Therefore, litter variation and possibly individual variation in behaviours could have an influence on the variation in growth rate of piglets.

1.3.2 Sow related factors

Sow related factors obviously include milk production, but also maternal behaviour and nursing behaviours of the sow. Milk production of sows was reported to be positively related with growth rate of litters (Ramanau et al., 2004), therefore the individual variation in milk production of sows has potential to influence the between-litter variation in growth rate of piglets. In addition, the maternal behaviour of sows (Wallenbeck et al., 2008) such as attentiveness and lack of aggression towards piglets, could influence the availability of milk for piglets and thus the growth rate of piglets. For example, in MS systems where sows have the opportunity to get away from piglets and control their nursing frequency (Van Nieuwamerongen et al., 2014), and in conventional housing where a sow could roll over on her belly and hide her udders, variation in milk availability of sows for piglets exists which subsequently influences growth rate of piglets. Additionally, in MS systems, cross-suckling could occur (Van Nieuwamerongen et al., 2014) and piglets may have access to sow feed. It is yet unknown how such MS systems could influence the extent to which variation in milk production, maternal behaviour and nursing behaviours of sows that may explain the variation in growth rate of piglets.

1.3.3 Management related factors

Management related factors include the feeding management which allows maternal and social learning of feed intake between sows and piglets, the feeding management which allows social learning of feed intake among piglets, the time that piglet access to the communal area, and the group size in MS systems.

The way in which nutrients are presented can affect intake and thus growth rate of piglets. When providing piglets with the opportunity to access sow feed and interact with the sow when she eats, piglets will eat more feed before weaning and consequently have better growth after weaning (Oostindjer et al., 2011; Oostindjer et al., 2014). Thus, providing the opportunity of maternal learning in an MS system might increase growth rate of piglets. In addition, it was reported that group housed growing pigs highly synchronize their feeding bouts (Nielsen et al., 1996). Piglets can also learn from other piglets which is called ‘social learning’ or ‘social facilitation’, as seeing experienced piglets eat may increase their motivation to also start eating (Keeling and Hurnik, 1996), which could probably benefit growth of piglets in MS systems as well.

Co-mingling non-littermates in group lactation systems, is to allow different litters which are reared in separate farrowing pens in the first few days p.p. access the communal area and other farrowing pens, and to allow these non-littermates to interact with other sows and litters (Van Nieuwamerongen et al., 2014). This strategy aims to help piglets to develop social skills in early age and reduce aggression towards unfamiliar pigs when they are transferred to growing facilities after weaning (Bohnenkamp et al., 2013). Several studies compared the effect of different starting times of co-mingling non-littermates on the growth performance of piglets in MS systems (Wattanakul et al., 1997; Thomsson et al., 2016; Verdon et al., 2020). For example, Verdon et al. (2020) found that in a group lactation system, in which piglets were mixed at day 14 p.p. had less variation in BW at weaning on day 26 p.p. and higher BW gain during days 0-26 p.p. compared with piglets mixed at day 7 p.p. Since different studies have different experimental settings for example different group sizes, it is unknown how different grouping dates can influence the variation in growth rate of piglets in the MS system of our current project.

For group size, it was reported that weanling pigs reared in a group size of 100 pigs/pen had a greater within-pen CV in BW at week 9 p.p. (weaned on day 15 p.p.) than pigs reared in a group size of 20 pigs/pen (Wolter et al., 2000). Group size may therefore also influence the within-group variation in growth rate of piglets in MS systems. The split-weaning strategy, which is to remove the heaviest piglet(s) from a sow before the planned weaning date, can reduce the competition at the udder for the lighter piglets and increase their BW gain (Vesseur et al., 1997). It can be speculated that split-weaning in MS systems could increase BW gain of specifically the smaller piglets, thereby reducing variation in piglet BW within the MS system.

1.4 The determination of individual feed intake of piglets

As previously discussed, the variation in nutrient intake contributes to the variation in growth rate of piglets. Nonetheless, the determination of feed intake in individual piglets is technically challenging, both in conventional single litter housing and in group lactation systems. Attempts to measure individual feed intake have been made, however, observations are often restricted to the classification of piglets into eaters and non-eaters. For example, by adding green colour chromic oxide to piglet feed together with computerized feeding stations, piglets with green faeces were considered as eaters (Sulabo et al., 2010). The individual nutrient intake from various sources is even more difficult to estimate, not only in single litter housing, but also in MS systems. In addition, some of the markers for example chromic oxide are restricted in use because of its negative influence on the environment (Coetzee et al., 2020).

In recent years, the dual marker method, which is widely used in ruminant studies (Ferreira et al., 2007; Chavez et al., 2011; Andriarimalala et al., 2020), has received increased attention for its application in pigs as well (Sehested et al., 1999; Ferre et al., 2000; Mendes et al., 2007; Staals et al., 2007; Kanga et al., 2012). Alkanes have been often applied as markers, as they naturally occur in cuticular waxes of herbages, are non-toxic to animals, and have a low digestibility by animals (Wright, 2017). Therefore, they are regarded as a potential substitution for metal markers to estimate individual feed intake of animals. By supplementing a known concentration of in-feed alkane marker in feed and providing animals with a certain amount of reference alkane marker for some days, individual-level feed intake of animals could be calculated by analysing the concentration of two alkane markers in faeces (Chavez et al., 2011). However, the majority of the studies on pigs concentrated on the estimating the intake of pasture and herbage of sows and growing pigs. The feasibility of the dual alkane method in measuring individual nutrient intake from various sources of suckling piglets in MS systems still needs to be studied. Also, how different frequency of administration of reference markers and faecal sampling strategy in MS systems influences the accuracy of estimation of feed intake still need to be studied.

1.5 Conclusions

In conclusion, multi-suckling systems for lactating sows and their litters mimic natural living conditions of pigs, which is regarded as a solution to welfare problems of conventional farrowing pens. However, the large variation in body weight of piglets at weaning is a problem in multi-suckling systems. As body weight uniformity of piglets during lactation is essential for production efficiency of growing and finishing pigs in current all-in-all-out pig production systems, for the successful application of multi-suckling systems, it is important to reduce the variation in body weight at weaning, or the variation in growth rate of piglets during lactation. Several factors that may influence variation in growth rate of piglets have been discussed, which can be grouped as piglet (including birthweight, nutrient intake, nutrient intake associated behaviours and other behaviours), sow and management related factors. Therein, for piglet nutrient intake related factors, in order to better understand their role in explaining the variation in growth rate of piglets, a novel method, the 'dual alkane method' was proposed to apply in multi-suckling systems to estimate individual feed and milk intake of piglets.

1.6 Aim and outline of this thesis

The main aim of this thesis is to improve the feasibility of an MS system which can be used in the current all-in-all-out pig production systems, by reducing the variation in growth rates of individual piglets in the MS system during lactation. For effectively designing strategies to reduce this variation, it is crucial to understand the factors that cause this variation, which involves piglet (including birthweight, nutrient intake, nutrient intake associated behaviours and other behaviours), sow and management related factors. A pilot study (**Chapter 2)** explored the use of the dual marker technique for the estimation of individual nutrient intake of piglets, to be used to estimate intake of multiple feed sources in MS systems. The 1st study conducted in the MS system (**Chapter 3)** are used to clarify the factors that explain variation in BW gain and feed intake of piglets in three different periods during a 9-week lactation. The 2nd study conducted in the MS system (**Chapter 4**) applied two intervention strategies, i.e. the timing of grouping of litters and split-weaning, during a 7-week lactation in order to reduce variation in growth rate of piglets. **Chapter 5** describes the consequences of birthweight on piglet performance, behaviours and catchup growth, from the data of both the 1st and 2nd study. In **Chapter 6** (General discussion), the results of the previous chapters are summarized, integrated and discussed. The scientific and societal relevance of this thesis are discussed throughout Chapter 6.

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Chapter 2: A dual marker technique to estimate individual feed intake in young pigs

T. Tang [a, b], C. M. C. van der Peet-Schwering [c], N. M. Soede [b], B. F. A. Laurenssen [b], Bruininx. E. M. A. M. [1,a], E. J. Bos [1], W. J. J. Gerrits [a]

[a] Animal Nutrition Group, Department of Animal Sciences, Wageningen University & Research, The Netherlands
[b] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University & Research, The Netherlands
[c] Wageningen Livestock Research, Wageningen University & Research, The Netherlands
[1] Agrifirm Innovation Center, P.O. Box 20000 7302 HA Apeldoorn, The Netherlands
Corresponding author: Walter Gerrits. E-mail: walter.gerrits@wur.nl

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Abstract

Accurate estimation of individual feed intake (**FI**) of pigs could help better understand the variation in performance between individual animals. We studied dual marker methods to estimate individual FI in pigs. This method is based on the measurement of the ratio between two indigestible markers in faeces. Twelve 6.5-week-old individually housed male pigs were assigned to one of three oral dosing treatments supplying 180 mg of ytterbium chloride (**$YbCl_3$**)/day and 111 mg of dotriacontane (**C32**)/day as reference markers, either once (**R1**), three times (**R3**) or five times (**R5**) daily. Pigs were offered a diet containing 0.46 g/kg of chromium chloride (**$CrCl_3$**) and 0.15 g/kg of hexatriacontane (**C36**) as in-feed markers. The experiment lasted for 10 days: days -5-0: adaptation; days 1-3: dosing of reference marker; days 2-4: total faecal collection (**TFC**). Spot faecal samples were taken on day 3 at 1200 h, 1700 h and on day 4 at 0700 h. Pigs were fed restrictedly three times daily, at 133.6 g/kg $BW^{0.60}$. Individual measured FI was recorded daily, and was compared to predicted FI using the ratio of the dual marker pairs (Yb:Cr and C32:C36), both in TFC and spot samples. Due to unequal variance, R1 pigs were omitted from the statistical treatment comparison. When using TFC samples, the absolute prediction error (**APE**) (predicted FI minus measured FI) in R3 and R5 pigs were numerically lower than in R1 pigs, regardless of the marker pair used. The APE measured by C32:C36 was numerically lower than measured by Yb:Cr at all frequencies, and significantly (P =0.039) in R3 pigs (C32:C36: 0.15 ± 0.02 kg/day; Yb:Cr: 0.29 ± 0.04 kg/day). This was related to a larger difference in faecal recovery between Yb and Cr compared with C32 and C36. Daily TFC revealed that for R3 pigs, starting faecal collections 2 days after the onset of provision of the reference marker improved the APE when compare with starting after 1 day. When using C32:C36 to predict feed intake, pooled, but not single spot samples gave similar APEs compared with total faecal collections. Therefore, we recommend dosing the reference marker for three times per day for 2 days on days 1 and 2, combined with pooled spot faecal sampling collected on days 3 and 4. In this way, absolute prediction errors of 10%-15% of simultaneously measured intakes of multiple nutrient resources in a complex housing system are feasible using the dual marker technique.

Keywords

N-alkanes, Faecal recovery, Ytterbium, Chromium, Faecal sampling

Implications

In this study, we investigated the dual marker method for the estimation of individual feed intake in young pigs, for use in complex housing systems. A protocol is suggested with recommendations for frequency of administration of reference markers and faecal sampling strategy.

2.1 Introduction

Accurate estimation of individual feed intake of pigs could help us to better understand the variation in performance between individual animals. Although technically demanding, the introduction of individual feeding stations has yielded valuable information in this respect (Kim et al., 2010). In the farrowing pen, however, measurement of individual feed intake of pigs is often considered impossible, and observations have been restricted to the classification of piglets into eaters and non-eaters (Van Nieuwamerongen et al., 2015), or time spent eating (Middelkoop, 2020). Moreover, in more spacious rearing systems which catering for the increasing demand for animal welfare, individual nutrient intake from various sources is even more difficult to estimate. These systems include for example outdoor organic pig farming (Von Borell and Sørensen, 2004) and multi-suckling systems (Van Nieuwamerongen et al., 2017).

Trivalent metal markers such as ytterbium chloride (**$YbCl_3$**) (Mavromichalis et al., 2001) and chromium oxide (**Cr_2O_3**) (Clawson et al., 1955) have been successfully used for the estimation of nutrient digestibility in pigs. Kim et al. (2010) estimated individual feed intake in post-weaned pigs by using two indigestible metal markers, one of which was Lanthanum oxide (**La_2O_3**) which was orally administered to pigs, the other one was Yttrium oxide (**Y_2O_3**) supplemented in diets. The individual feed intake was then calculated based on measurement of the ratio between the two metal markers in faeces. However, there are some constraints about the use of markers. The daily manipulation of the animals can cause stress and thus biases data. In addition, the excretion of markers might not be stable since their passage rate through the digestive tract is not uniform. In addition, spot sampling of the faecal output has to be representative. Moreover, the measurement of markers requires complex and costly chemical analyses. For the use of double marker technique, the constraint would be that external markers (e.g. Cr_2O_3) may exhibit a different passage rate behaviour compared with intrinsic or mordanted markers (de Vries and Gerrits, 2018). Additionally for metal markers, some of them are limited in use since the resulting manure can cause pollution of the environment (e.g. chromium) (Coetzee et al., 2020).

From the 1980s, the double n-alkane technique has been used as a reliable method for estimating individual intake in herbivores, due to their low digestibility, non-toxicity to animals, and natural presence in a wide range of herbage species (Wright, 2017). In herbivores, various dual marker techniques have been used for the determination of individual intake of forages, grass, clover and browses (Andriarimalala et al., 2020). These methods are based on the measurement of the ratio between two indigestible markers in faeces. The external marker (hereafter referred to as reference marker) is dosed frequently and independent from the forage of study which is used to determine the faecal output. The internal marker (hereafter referred to as in-feed marker) is associated with the forage of interest (Chavez et al., 2011). The ratio measured quantitatively reflects forage intake, and can be estimated from the analysis of spot faecal samples, obtained in a steady state (Chavez et al., 2011).

Around 90% of the naturally present n-alkanes are odd-chained. Therefore, odd-chained alkanes are often used as in-feed markers, and synthetic even-chained alkanes are often used as reference markers (Chavez et al., 2011). It is important to note that n-alkanes are not completely indigestible and that digestibility varies between and within animal species (e.g. the physiological status of animals) as well as diet type. Faecal recovery is an important indicator of the reliability of a marker, which is the quantity recovered from the total collection of faeces expressed as a proportion of that consumed in diet (Morais et al., 2011). In goats, for example, faecal recoveries of the alkane C25 ranged from 43.0% to 72.4%, while C35 recoveries ranged from 87.9% to 99.9% (Hilburger, 2017). The complete faecal recoveries of markers are important to attain unbiased estimates of intake in digestibility studies. However, it is not a requirement for dual marker technique, as the main assumption of the technique is that the faecal recoveries between the reference and in-feed marker used are similar (de Vries and Gerrits, 2018).

Dual marker methods might be well-suited to estimate individual feed intake of group housed pigs, particularly in a setting where multiple ration components are offered, e.g. in outdoor farming or in multi-suckling systems. Prior to application of such a dual marker method for the estimation of individual feed intake in pigs, assumptions regarding the steady state conditions, involving dosing frequency of the reference marker, collection procedure of spot faecal samples, and the faecal recoveries of the selected markers need to be verified. In choosing marker pairs, it is important that these markers can be analysed using a single procedure, e.g. gas chromatography for n-alkanes of different chain length (Smit et al., 2005), or inductively coupled plasma optical emission spectrometry for metal oxides or chlorides (Williams et al., 1962). For estimation of each additional source of nutrients, an extra marker can be added.

There have been very few attempts of the application of the dual alkane method to estimate individual feed intake in pigs: Mendez et al. (2007) and Ferraz de Oliveira et al. (2006) investigated dual alkane technique to estimate intake of roughages in growing pigs. They also investigated the minimum dosing duration to get a steady concentration of alkanes in faeces. Kanga et al. (2012) applied the dual alkane technique to determine individual voluntary forage intake in 8-week-old male pigs which were fed a mixed forage and concentrate diet. So far, alkane faecal recovery data are mostly available in sows (Wilson et al., 1999) and growing pigs (Ferraz de Oliveira et al., 2006; Ribeiro et al., 2007), but few cases in post-weaned pigs (Staals et al., 2007). Observations from other species indicate that faecal recoveries of n-alkanes increase curvilinearly with increasing carbon chain length (Wright et al., 2020). However, in pigs, an increase in faecal recovery with increased chain length of long-chain alkanes is not apparent from the limited data available (Wilson et al., 1999; Ferraz de Oliveira et al., 2006).

The aim of the current study was to assess the dual marker method to estimate individual feed intake of young pigs. To achieve this, we explored the effects of the frequency and duration of oral administration of the reference marker on the accuracy of the feed intake estimation using two n-alkanes (dotriacontane (**C32**) and hexatriacontane (**C36**)) and two metal chlorides ($YbCl_3$ and Chromium chloride (**$CrCl_3$**)) as marker pairs. As spot-sampling is key to the success of the dual marker method, we compared variation among single and pooled spot samples. We also compared spot faecal sampling methods with quantitative faecal collections to estimate feed intake. Marker pair comparison and duration of faecal sampling were also investigated.

2.2 Material and methods

2.2.1 Experimental design

The experiment was conducted at the research farm 'Laverdonk' of Agrifirm Innovation Center (Heeswijk-Dinther, the Netherlands). Pigs were housed individually in pens of 0.76 x 2.28 m, but they could smell, see, hear and touch (e.g. nose to nose contact) the animals in neighbouring pens. The pens consisted of a solid (rubber) floor and a slatted (approximately 1/6 of total area) floor. The pens were enriched with toys that were changed every 2 days. Room temperature was kept at 24-26 °C. Lights were turned on from 0700 h to 1900 h, and during feeding events or bolus administration outside this period.

Twelve individually housed male pigs (Tempo × Topigs 20, Topigs Norsvin, 18.8 ± 0.6 kg) of 6.5 weeks of age were used in this experiment for a period of 10 days, including an adaptation period of 6 days (days -5-0) and an experimental period of 4 days (days 1-4). Pigs were grouped into 4 blocks of 3 pigs in each block based on initial BW. Within block, pigs were assigned to one of three treatments that differed in the frequency of administration of the reference marker: once (**R1**), three (**R3**) or five (**R5**) times a day. Two pairs of dual markers were tested: $YbCl_3$ with $CrCl_3$ and the n-alkanes C32 with C36. $YbCl_3$ (180 mg/d) and C32 (111 mg/d) were considered as reference markers and were provided with a small amount of feed (~ 2 g/feeding bolus). Chromium chloride ($CrCl_3$, 460 mg/kg of feed) and C36 (150 mg/kg of feed) were considered as in-feed markers and were mixed into a commercial mash feed (net energy: 9.6 MJ/kg, crude protein: 160 g/kg)(Big Rendement 1, Agrifirm, NL), providing 150 mg of Cr and 150 mg of C36/kg of feed. The marker dosage was calculated based on the expected concentration in faeces of 1000 mg/kg, assuming feed intake of 750 g/d and a DM digestibility of 85%. The sensitivity of metal-chlorides and alkane analytical methods is then well-suited to pick up differences in this concentration range. Feeding boluses were prepared fresh by mixing the pre-weighed amount of reference markers and a small amount of feed (~ 2 g/bolus) and kneaded to a firm bolus using a few drops of sucrose-based lemonade syrup (Karvan Cevitam, Amsterdam, NL).

From day -5 onwards, pig diet was supplied restrictedly, at 133.6 g/kg $BW^{0.60}$, in three equal meals per day, at 0700 h, 1200 h and 1700 h. The restricted feeding was used for controlling the variation of feed intake between individual pigs in order to reduce the variation of the excretion of markers in pigs at different administration frequencies. The restricted feeding levels were set to around 80% of the estimated *ad libitum* feed intake of the pigs, assuming that the estimated *ad libitum* feed intake is 3.5 times the maintenance energy requirement, according to National Research Council (1998). Each meal was mixed with 400 mL of water to stimulate feed intake. During the adaptation period pigs were allowed access to the feed for a maximum of 45 minutes per meal to make pigs get used to being fed restrictedly. During the experimental period, pigs were allowed access to the feed for a maximum of 30 minutes per meal, after which feed refusals were removed and weighed.

Water was freely available during 24 h/day. Boluses with reference marker were given to the pigs by hand for three consecutive days (days 1, 2, 3 of the experimental period), at 0900 h (R1); at 0900 h, 1500 h and 2100 h (R3); at 0600 h, 0900 h, 1500 h, 1800 h and 2100 h (R5). For R1 and R3 pigs, placebo boluses without reference markers were provided at the timepoints that they did not receive boluses containing reference markers, as indicated in Fig. 1.

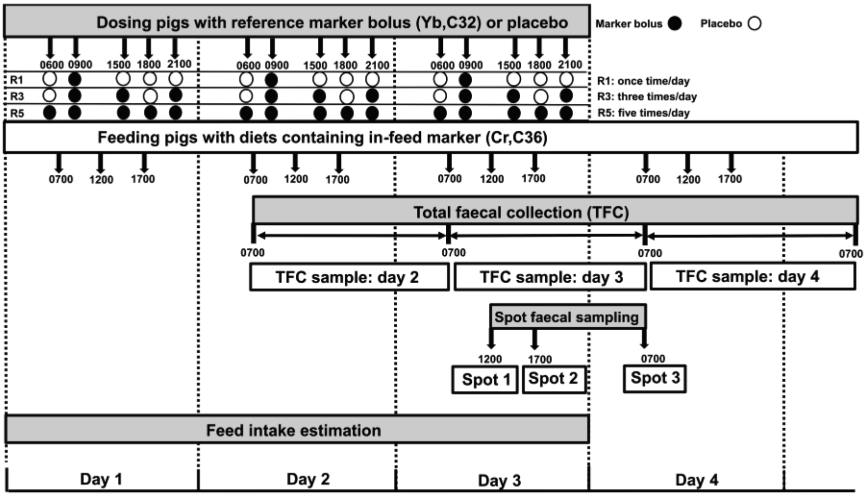

Figure 1. The time schedule (time use 24-h clock) of the oral administration of reference marker, feeding pigs with diets containing in-feed marker, and faecal sampling procedure during the experimental period on days 1-4.

2.2.2 Faecal sampling

During adaptation period, faeces was collected quantitatively using faecal collection bags attached to a patch with ring, glued around the anus using adhesive spray (Hollister, Libertyville, USA) on day -3. The faeces collected during adaptation period was discarded. During the experimental period, faeces was collected quantitatively for 72 h on days 2, 3 and 4, as indicated in Fig. 1: the faecal bags were replaced twice per 24 h period at 0700 h and 1700h and three times on day 3 for an extra timepoint at 1200 h. The faecal bags were pooled and stored per day per pig. In addition, for all pigs, three spot samples of about 50 g were taken from the faecal collection bags at three points in time, immediately after removing the bags: spot sample 1: day 3 at 1200 h; spot sample 2: day 3 at 1700 h; spot sample 3: day 4 at 0700 h. The three spot samples per pig were stored separately. All faecal samples were stored at -20 ˚C for further lab analysis.

2.2.3 Chemical analyses and calculation

The faecal samples were oven-dried at 65 °C and ground to pass a 1 mm screen. The concentrations of Yb and Cr in faeces and feed were determined using inductively coupled plasma optical emission spectrometry as described by (Williams et al., 1962). N-alkanes in faeces and feed were extracted by heptane and analysed by gas chromatography according to the method of Smit et al. (2005) using tetratriacontane (**C34**) as an internal standard. The feed intake was estimated using total faecal collection samples and spot faecal samples separately. For the feed intake estimation using total faecal collection samples, the removal of markers by removing spot sampling from the faecal collection bags was corrected for.

The estimated feed intake (kg/d), percent difference between measured and estimated feed intake (%), faecal recovery of markers (%) and within marker pair faecal recovery difference (%) were calculated for the averaged value from day 1 till day 3. The absolute prediction error (**APE**, kg/d) was calculated both for the averaged value from day 1 till day 3 and for each day separately. For days 1 to 3, the APE was calculated from the faecal samples collected on days 2 to 4.

The calculations were as follows:

Estimated feed intake (kg/day)

$$= \frac{\left(\frac{\text{concentration of in-feed marker in faeces (mg/kg)}}{\text{concentration of reference marker in faeces (mg/kg)}} \times \text{daily intake of reference marker (mg/day)}\right)}{\text{concentration of in-feed marker in diet (mg/kg)}} \quad (1)$$

Absolute prediction error (kg/day) = | predicted feed intake (kg/day) – measured feed intake (kg/day) | (2)

Percent difference between measured and estimated feed intake (%)=

$$= \frac{\text{absolute prediction error (kg/day)}}{\text{measured feed intake (kg/day)}} \times 100 \quad (3)$$

$$\text{Faecal recovery of marker (i) (\%)} = \frac{\text{excretion of marker (i) in faeces on days 2-4 (g)}}{\text{intake of marker (i) on days 1-3 (g)}} \times 100$$

with marker (i) referring to Yb, Cr, C32 or C36. (4)

Within marker pair faecal recovery difference (%) =

| in-feed marker recovery (%) – reference marker recovery (%) | (5)

Estimated feed intake (kg/day) correcting for the differences in faecal recoveries of in-feed and reference marker:

$$= \frac{\text{concentration of in-feed marker in faeces (mg/kg) / faecal recovery of in-feed marker (\%)}}{\text{concentration of reference marker in faeces (mg/kg) / faecal recovery of reference marker (\%)}} \times \frac{\text{daily intake of reference marker (mg/day)}}{\text{concentration of in-feed marker in diet (mg/kg)}} \quad (6)$$

2.2.4 Statistics

SAS 9.4 was used for all statistical analyses. The data were analysed as a randomised complete block design, with the treatment as the fixed effect and the pig as the experimental unit. The effect of the frequency of administration of the reference markers on the APE of feed intake was analysed by ANOVA, using a model that included BW block and frequency of reference marker administration and all two-way interactions as fixed effects. R1 pigs were excluded from statistical comparison with R3 and R5, as the R1 pigs showed much larger variation in APEs of feed intake than R3 and R5 pigs. Due to the single replication of all treatments within block, Tukey 1df test was used to check non-additivity of treatment x block interaction to determine whether the interaction is present prior to ANOVA (Montgomery, 2017; Marasinghe and Koehler, 2018). As the interaction was not significant in Tukey 1df test in either total faecal collection samples (Yb:Cr: P =0.765; C32:C36: P =0.806) or pooled spot faecal samples (Yb:Cr: P =0.654; C32:C36: P =0.540), it was omitted from the model. Also as BW block was not significant in ANOVA in either total faecal collection samples (Yb:Cr: P =0.144; C32:C36: P =0.087) or pooled spot faecal samples (Yb:Cr: P =0.246; C32:C36: P =0.326), it was omitted from the model. After removing R1 pigs and block effect, the comparison of APEs of feed intake between R3 and R5 pigs was analysed by two-sample t-test using PROC TTEST. The normality of the model was checked using PROC UNIVARIATE with Skewness-Kurtosis test and Shapiro-Wilk test. The assumption of homogeneity of variance was checked using PROC TTEST with folded F statistic, according to SAS/STAT® 15.1 user’s guide (SAS®, 2018). The results showed that the assumption of normality and homogeneity of variance was met in total faecal collection samples and pooled spot faecal samples.

The effects of faecal sampling method (quantitative collection samples vs. spot samples) on APEs of feed intake were analysed by one-way repeated measures ANOVA including pig as repeated subject. The Dunnett test was used for the model testing differences between faecal sampling methods to compare each of spot faecal samples with total faecal collection samples as a control. For the comparison of APEs among days 1, 2, 3 within treatment, one-way repeated measures ANOVA including pig as repeated subject and the Tukey-Kramer test were used. The effects of marker pair types (metals vs. alkanes) and the effects of day of faecal collection (days 2-4 vs. days 3-4) on APEs of feed intake were analysed by paired t-test. The comparison of faecal recovery between Yb and Cr, between C32 and C36 within treatment was analysed by paired t-test as well. R1 pigs were excluded from all analyses; however, the descriptive results of R1 pigs are reported in tables and figures.

In all analyses, the normality of model residuals was checked using PROC UNIVARIATE with Skewness-Kurtosis test and Shapiro-Wilk test. For models using one-way repeated measures ANOVA, when the assumption that normality of residuals was met, PROC MIXED with REPEATED statement was used, with type = cs option to specify a compound symmetry covariance structure; when the assumption that normality was not met, the lognormal distribution in PROC GLIMMIX with RANDOM statement was used, with RESIDUAL and type = cs option, according to SAS/STAT® 15.1 user's guide (SAS®, 2018), (Westfall et al., 2011). The specific analysis used in each model is given in Supplementary Table S1.

Feed intake was kept constant relative to $BW^{0.6}$, hence some variation appeared in the absolute rate of feed intake between pigs resulting from differences in BW. Prediction of this variation was evaluated by linear regression.

2.3 Results

2.3.1 The effects of dosing frequency and faecal sampling method on absolute prediction error

The predicted feed intake overestimated the measured feed intake in all treatment groups (Table 1) and in each pig (Fig. 2). For Yb:Cr, feed intake from total faecal collection samples was overestimated by 43.2 ± 15.5%, 37.1 ± 4.5%, 21.4 ± 4.0% in R1, R3 and R5 pigs respectively, while feed intake from pooled spot samples (spot123) was overestimated by 59.2 ± 20.6%, 17.1 ± 3.1%, 5.1 ± 2.2% in R1, R3 and R5 pigs respectively. For C32:C36, feed intake from total faecal collection samples was overestimated by 40.8 ± 22.3%, 19.5 ± 1.9%, 17.9 ± 2.5% in R1, R3 and R5 pigs respectively, while feed intake from pooled spot samples was overestimated by 59.0 ± 26.2%, 23.9 ± 5.2%, 9.6 ± 3.2% in R1, R3 and R5 pigs respectively. There was no significant difference (P >0.05) in APE between R3 and R5 pigs in total faecal collection samples for either marker pair (Table 1). Comparing pooled spot samples with total faecal collection samples for R3 and R5 pigs yielded some significant differences in both directions: the APEs in pooled spot samples were lower (Yb:Cr in R3 pigs, P =0.004) or not significantly different (Yb:Cr in R5 pigs, P =0.086; C32:C36 in R3 pigs, P =0.966; C32:C36 in R5 pigs, P =0.283), compared with total faecal collection samples. When correcting for the differences in faecal recoveries of in-feed and reference marker, for C32:C36, APEs were reduced to zero in R3 and R5 pigs (Table 1).

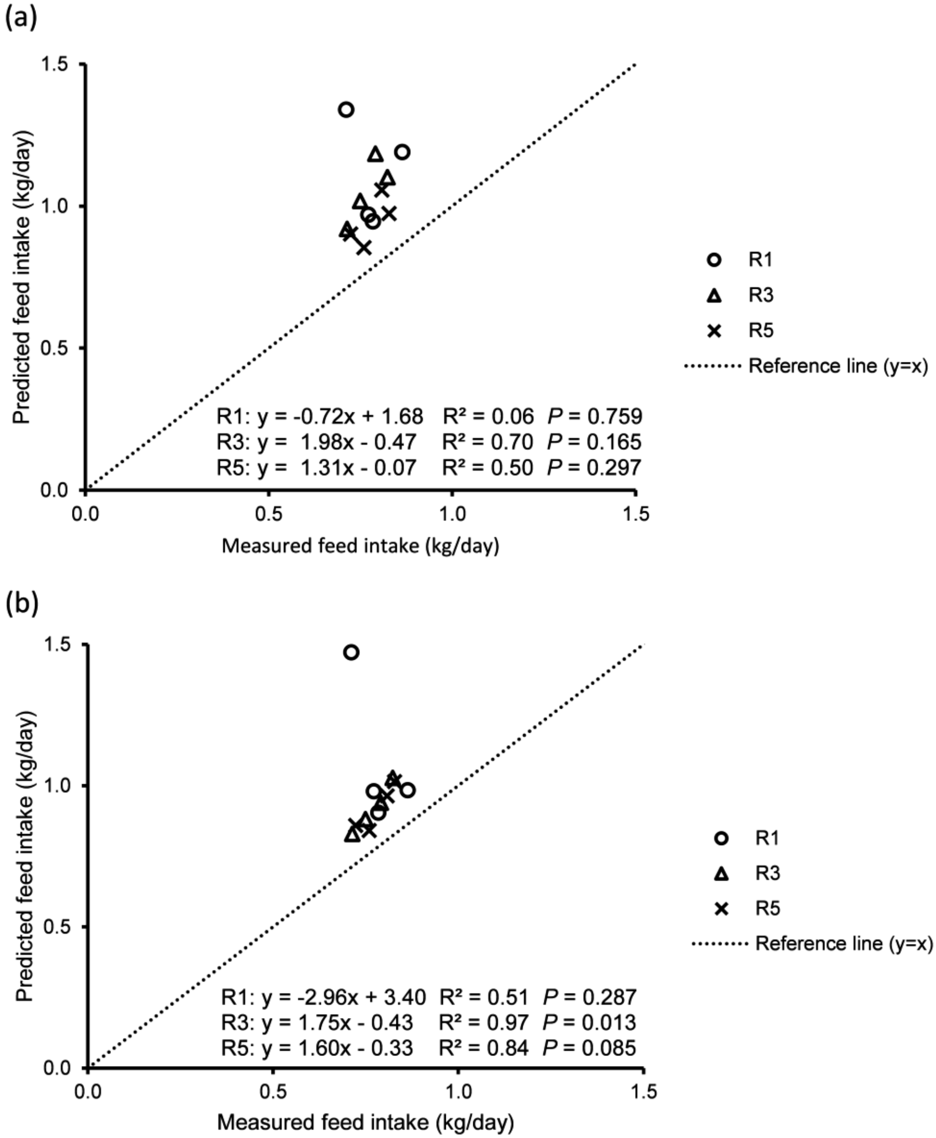

Figure 2. Linear relationship between measured individual DM feed intake (kg/day per pig) and predicted individual DM feed intake (kg/day per pig) averaged on day 1 till day 3 predicted by two types of marker pair in total faecal collection samples for 6.5-week-old pigs in three administration frequency of reference marker bolus (R1: once/day, R3: three times/day, R5: five times/day). (a) predicted by Yb:Cr; (b) predicted by C32:C36.

Table 1. Measured DM feed intake (DFI; kg/day per pig) and absolute prediction error of DM feed intake (APE; kg/day per pig) averaged over days 1-3, predicted by two types of marker pairs (Yb:Cr and C32:C36) using different faecal sampling methods[1] for 6.5-week-old pigs in three administration frequency of reference marker bolus (R1: once/day, R3: three times/day, R5: five times/day) [2].

	Frequency administration of reference marker				
	R1	R3	R5	SEM of R3+R5	Comparison R3-R5 *P*-value[3]
Measured DFI (kg/day per pig)	0.78±0.03	0.77±0.02	0.78±0.02		---
APE for Yb:Cr[4]					
Faecal sampling method[2]					
TFC	0.33±0.11	0.29^{x}±0.04	0.17^{X}±0.03	0.04	0.056
Spot1	0.40±0.13	0.11^{y}±0.07	0.17±0.06	---	---
Spot2	0.88±0.22	0.14^{y}±0.02	0.08±0.03	---	---
Spot3	0.77±0.27	0.13^{y}±0.04	0.09±0.03	---	---
Spot123	0.46±0.15	0.13^{y}±0.03	0.04^{Y}±0.02	0.02	0.027
SEM	---	0.03	0.04	---	---
P-value[5]	---	0.004	0.083	---	---
APE for C32:C36[4]					
Faecal sampling method[2]					
TFC	0.30±0.15	0.15±0.02	0.14^{x}±0.02	0.02	0.732
Spot1	0.28±0.21	0.12±0.02	0.27^{y}±0.03	---	---
Spot2	0.75±0.36	0.19±0.05	0.12±0.02	---	---
Spot3	1.03±0.47	0.21±0.09	0.06±0.02	---	---
Spot123	0.44±0.18	0.18±0.04	0.08±0.03	0.03	0.068
SEM	---	0.05	0.02	---	---
P-value[5]	---	0.846	<0.001	---	---
APE in TFC assuming reference markers have equal faecal recovery with in-feed markers					
C32:C36 in TFC	0.00±0.00	0.00±0.00	0.00±0.00	---	---

[1] TFC=total faecal collection on days 2, 3 and 4; Spot1, 2 and 3: spot faeces samples on day 3 at 1200 h and 1700 h and day 4 at 0700 h, respectively; Spot123: pooled spot samples.

[2] Data are presented as mean ± SEM, n=4 for each treatment.

[3] Comparison between R3 and R5 pigs using two-sample t-test.

[4] APE: absolute prediction error of DM feed intake (kg/day). Absolute prediction error (kg/day) = | predicted DM feed intake (kg/day) – measured DM feed intake (kg/day) |.

[5] Comparison between TFC samples and other types of faeces samples using Dunnett's test within treatment R3 and R5 within the same marker pair. ^{x,y} values with different letters differ significantly (P <0.05); ^{X,Y} values with different letters tend to be different (0.05 < P <0.10).

2.3.2 Comparison between metal pair and alkane pair in total faecal collection samples

In the total faecal collection samples, the APE was significantly lower in C32:C36 than in Yb:Cr in R3 pigs (P =0.039), while it did not differ significantly in R5 pigs (P =0.414) (Fig. 3).

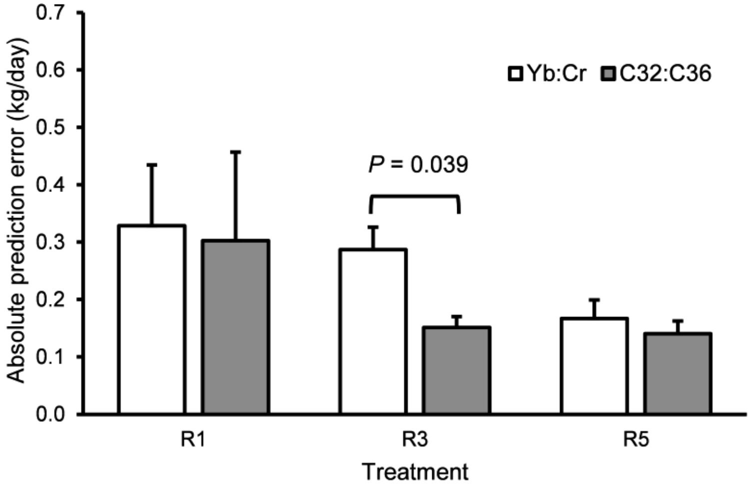

Figure 3. Comparison of absolute prediction error of DM feed intake (kg/day per pig) averaged on day 1 till day 3 between the prediction from metal pair (Yb:Cr) and from alkane pair (C32:C36) using total faecal collection samples for 6.5-week-old pigs within two administration frequency of reference marker bolus (R3: three times/day, R5: five times/day). R1 pigs (once/day) were excluded from the statistical analysis. Absolute prediction error (kg/day) = | predicted DM feed intake (kg/day) – measured DM feed intake (kg/day) |. Error bar: SEM.

2.3.3 Marker recovery and within marker pair recovery difference

The faecal recovery of Yb and Cr averaged over days 1-3 was 54.0 ± 5.2% and 74.3 ± 2.2% (R1), 55.6 ± 2.2% and 76.2 ± 2.9% (R3) and 62.8 ± 2.0% and 76.5 ± 3.0% (R5)(Fig. 4a), being significantly lower in Yb compared to Cr within treatment (R3: P =0.004, R5: P =0.013). The faecal recovery of C32 and C36 averaged over days 1-3 was 57.2 ± 8.6% and 74.9 ± 4.1% (R1), 60.8 ± 1.5% and 72.6 ± 0.8% (R3), 63.5 ± 1.2% and 75.1% ± 2.3% (R5), being significantly lower in C32 compared to C36 (R3: P =0.002, R5: P =0.006). The faecal recovery difference between Cr and Yb was 20.2 ± 4.4% (R1), 20.6 ± 2.4% (R3), 13.7 ± 2.6% (R5); the faecal recovery difference between C36 and C32 was 17.7 ± 6.1% (R1), 11.8 ± 1.2% (R3), 11.7 ± 1.6% (R5) (Fig. 4b). There was a strong relationship between APE and the difference in faecal recovery rates of the marker pairs used (Supplementary Figure S1), with R^2=0.84 (R3) and 0.89 (R5) for Yb:Cr and R^2=0.78 (R3) and 0.73 (R5) for C32:C36.

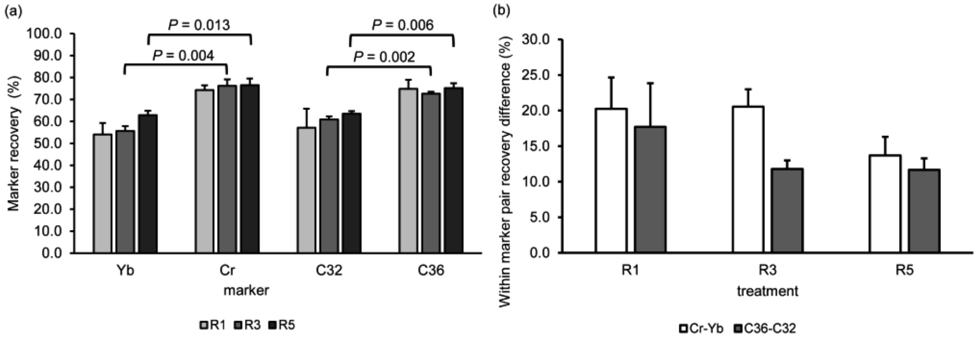

Figure 4. (a) Comparison of marker recoveries (%) between Yb and Cr, between C32 and C36 in total faecal collection samples for 6.5-week-old pigs within two administration frequency of reference marker bolus (R3: three times/day, R5: five times/day). Faecal recovery of marker (%)= excretion of marker on days 2-4 (g)/ intake of marker on days 1-3(g) × 100. Error bar: SEM. R1 pigs (once/day) were excluded from the statistical analysis. (b) Within marker pair difference of recoveries in total faecal collection samples. Error bar: SEM. Within marker pair faecal recovery difference = | in-feed marker recovery (%) – reference marker recovery (%) |.

2.3.4 Comparison between faecal collection days

Using total faecal collection samples, the APE in Yb:Cr was significantly lower on day 3 (faeces collected on day 4) than on day 1 (faeces collected on day 2) in R3 pigs (P =0.008), while there was no significant difference of APE between day 2 and day 3 in R3 pigs (Fig. 5a). In C32:C36 (Fig. 5b), the APE on day 3 tended to be lower than that on day 1 in R3 pigs (P =0.061), while there was no significant difference of APE between day 2 and day 3 in R3 pigs. There were no significant differences in APE among the three days in R5 pigs, predicted either by Yb:Cr or by C32:C36.

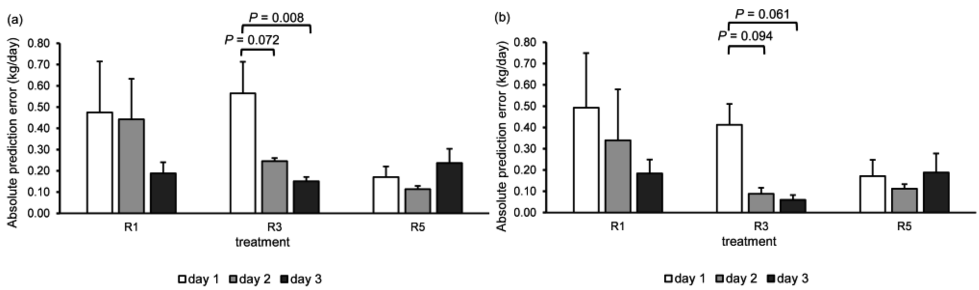

Figure 5. Comparison of absolute prediction error of DM feed intake (kg/day per pig) among days 1, 2, 3 (faeces samples were collected on days 2, 3, 4) using total faecal collection samples for 6.5-week-old pigs within two administration frequency of reference marker bolus (R3: three times/day, R5: five times/day). R1 pigs (once/day) were excluded from the statistical analysis. Absolute prediction error (kg/day) = | predicted DM feed intake (kg/day) – measured DM feed intake (kg/day) |. The administration of reference marker was conducted on days 1, 2, 3. (a) predicted by Yb:Cr; (b) predicted by C32:C36. Error bar: SEM.

Using total faecal collection samples, the APE averaged over days 2+3 (faeces collected on days 3+4) tended to be lower than that when averaged on days 1 to 3 (faeces collected on days 2+3+4) in R3 pigs, predicted either by Yb:Cr (*P* =0.064) or by C32:C36 (*P* =0.069) (Fig. 6). Using total faecal collection samples, there was no significant difference of APE between days 2+3 and days 1+2+3 either predicted by Yb:Cr or by C32:C36 in R5 pigs.

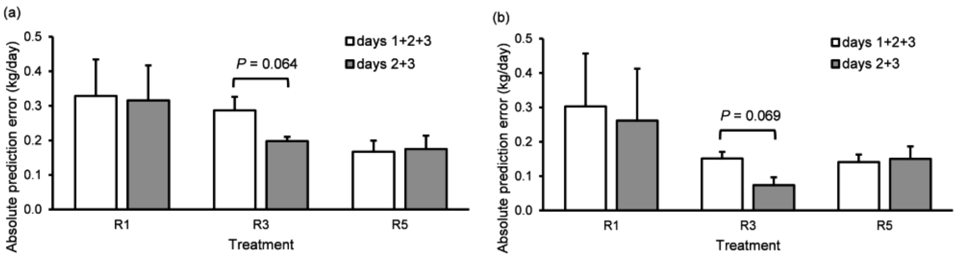

Figure 6. Comparison of absolute prediction error of DM feed intake (kg/day per pig) between days 2+3 (faeces collected on days 3+4) and days 1+2+3 (faeces collected on days 2+3+4) using total faecal collection samples for 6.5-week-old pigs within two administration frequency of marker bolus (R3: three times/day, R5: five times/day) using two types of marker pair. R1 pigs (once/day) were excluded from the statistical analysis. Absolute prediction error (kg/day) = | predicted DM feed intake (kg/day) – measured DM feed intake (kg/day) |. (a) predicted by Yb:Cr. (b) predicted by C32:C36.

2.4 Discussion

2.4.1 The effect of dosing frequency on absolute prediction error and estimation accuracy

In our study, we tested the extent to which a fixed amount of reference marker in a single daily dose (R1) or split in three (R3) or five (R5) daily dose allows accurate estimates of feed intake of pigs. Increasing the frequency of administration of the reference marker by definition leads to more stable excretion patterns of the reference marker, which is beneficial for the feed intake estimation. This is of particular importance when applying this technique under complex housing conditions, in which feed intake patterns are unknown. Increasing the frequency, on the other hand, results in increased labour requirements and may disrupt the animals.

We excluded R1 pigs from statistical comparison with R3 and R5, as the R1 pigs showed much larger variation in APEs of feed intake than R3 and R5 pigs: i.e. the provision of the reference marker once daily apparently leads to erratic excretion patterns. Hence, even in the total faecal collection samples, APEs of feed intake in R3 and R5 pigs were numerically lower than in R1 pigs. It corroborates the findings of Kim et al. (2010), who concluded that dosing a reference marker La_2O_3 three times per day to post-weaned pigs showed reasonable accuracy (R^2= 0.85) of individual feed intake estimation, while once per day showed weak accuracy (R^2= 0.47). Ferraz de Oliveira et al. (2006) found that dosing a reference marker (C32) twice daily to growing pigs reduced variation in faecal C32 concentrations compared to dosing once daily. We conclude that dosing the reference marker three or five times per day improves estimation of the feed intake compared with dosing once per day. No improvements in APE were observed when increasing the frequency from three to five times per day.

2.4.2 The effect of dosing duration on absolute prediction error

Administering the reference marker long enough prior to the start of faecal sampling is important for obtaining steady faecal excretion rates and thereby obtaining accurate estimates of feed intake. With regard to feasibility, particularly when using the dual marker approach in a complex housing system, a shorter duration of administration is preferred. When using the total faecal collection samples, for R3 pigs, feed intake prediction errors were larger at day 1 compared with days 2 and 3 for both marker pairs. For R5 pigs this effect was not observed. Therefore, two dosing days would likely be the minimum requirement for a reference marker to reach a steady excretion pattern when dosing it to pigs three times daily.

Other studies have reported stabilized alkane excretion on the third day of dosing for once or twice daily in growing pigs (Ferraz de Oliveira et al., 2006), on the fifth day of dosing alkanes for once or twice daily in growing pigs (Ribeiro et al., 2007), after 7 days of dosing alkanes with unknown frequency in adult sows as observed by (Sehested et al., 1999) as cited by (Ribeiro et al., 2007). The required number of dosing days naturally depends on the mean retention time of digesta inside the gastrointestinal tract. This varies with age (Wilson and Leibholz, 1981) and feeding level (Schop et al., 2019). We conclude that, the different minimum dosing days reported in these studies is related to the different dosing frequency and different mean retention time of digesta in gastro-intestinal tract in pigs. When applying the dual marker technique, this should be carefully considered, or tested prior to the measurement, e.g. via a pulse-dose of a coloured marker and monitoring the time of first appearance in faeces.

A potential effect of a difference in diurnal variation in faecal marker excretion between reference and in-feed markers on feed intake estimates should be considered. The reference markers were administrated once, three and five times per day at different intervals between each dosing, which could create fluctuations in excretion pattern that would differ from the in-feed markers. The limited number of daily defaecations, however, complicates the measurement of such effect.

2.4.3 Comparison between metal pair and alkane pair

In our study, the APE using C32:C36 was numerically lower than using Yb:Cr at all frequencies, and significantly in R3 pigs, indicating that using C32:C36 leads to improved estimates of feed intake compared with Yb:Cr. This is caused by the higher similarity in faecal recovery of C32 and C36 compared with Yb and Cr. Similarly, a strong relationship was found between the prediction error of feed intake and the difference in faecal recovery of the alkane pair in cattle (R^2=0.75, P <0.001 for both C31:C32 and C32:C33) (Bezabih et al., 2012). In addition, a meta-analysis in ruminants showed a strong relationship between the difference in faecal recovery of n-alkane pairs (=1-natural/dosed) and feed intake prediction error (1-predicted/measured), with the adjusted R^2 of 0.83 (P <0.001) for C31:C32 and adjusted R^2 of 0.93 (P <0.001) for C32:C33 (Andriarimalala et al., 2020). In our study C32:C36 seems to have better estimation accuracy of feed intake than Yb:Cr. However, the final choice of marker pairs for estimation of feed intake also depends on the availability of equipment and the precision of analytical procedures.

2.4.4 The effect of faecal sampling method on absolute prediction error

For successful application of the dual marker technique in a complex housing environment, quantitative faecal collections are impossible to perform, and the technique has to be applied using faecal spot sampling. In our study, pooled faecal spot samples had similar or lower SEM of APE than single spot samples in most cases. It was found that in growing pigs, feed intake estimates from morning and evening spot faecal samples underestimated the measured feed intake by 16.8% (± 2.79%) and 20.4% (± 6.30%), while from the average concentrations of the two faecal samples underestimated by 6.1% (± 2.66%)(Méndez Cante, 2013). Circadian variations of marker faecal concentrations was suggested to explain the discrepancy of feed intake estimation between pooled and single spot samples (Méndez Cante, 2013). In addition, single spot samples may also introduce a lot of random variation, particularly so at lower frequencies of administration of the reference marker.

In our study, the APEs were similar or lower in pooled samples compared with total faecal collection samples, reaching statistical significance only for Yb:Cr in R3 pigs. This may be related to the later timing of the first spot sample, compared with the onset of total faecal collection.

2.4.5 Marker faecal recovery and estimation accuracy of feed intake

Differences in faecal recovery between reference and in-feed markers lead to feed intake prediction errors, but recoveries deviating from 100% are not necessarily a problem, if faecal recoveries of the reference and in-feed marker are similar or if the difference can be corrected for (de Vries and Gerrits, 2018). Marker recoveries have been reported variable in metals (Köhler et al., 1990) and alkanes (Bachmann et al., 2018). In our study, the faecal recovery of Yb in R3 and R5 pigs averaged 59.2% while it was higher (83%) in post-weaned piglets along the gastrointestinal tract (Low et al., 2020); the recovery of Cr in R3 and R5 pigs averaged 76.4%, while it was higher (averaged 87.3%) in gastrointestinal compartment in milk-fed veal calves (Pluschke et al., 2016) and similar (73%) along the gastrointestinal tract in post-weaned piglets (Low et al., 2020). In our study, the faecal recovery of the reference marker C32 in R3 and R5 pigs averaged 62.1%, which was rather low compared to the 72.6% (Ferraz de Oliveira et al., 2006) and to the value even exceeding 100% (Ribeiro et al., 2007) found in growing pigs; the faecal recovery of in-feed C36 in R3 and R5 pigs averaged 73.9% in our study, which was close to the recovery of pulse dosed C36 of 69.1% (Ferraz de Oliveira et al., 2006) but was rather low compared to the value even exceeding 100% (Ribeiro et al., 2007) found in growing pigs.

In our study, the faecal recoveries of both in-feed markers were higher than that of both reference markers. A plausible explanation for this could be the longer adaptation period to get steady faecal excretion rates of the in-feed markers. In addition, the in-feed markers may have been better mixed in digesta than the reference markers. The longer chain length of C36 than C32 could also explain the higher recovery of C36 (Wright et al., 2020). It is remarkable and consistent with Méndez Cante (2013) that the errors in the prediction are merely related to erroneous faecal recoveries of the reference rather than that of the in-feed markers.

2.4.6 Correcting feed intake estimations for differences in faecal recoveries of reference and in-feed markers

If the inherent assumption of equal faecal recoveries of the reference and in-feed markers is violated, correcting estimations for differences in recoveries can be done provided that there is reason to assume these differences are consistent across studies. For the metal markers, as discussed above, this is not the case, and hence, corrections are not considered. For n-alkanes, observations from other species indicate that faecal recoveries of n-alkanes increase curvilinearly with increasing carbon chain length (Wright et al., 2020). For pigs, information from literature is more ambiguous: in pregnant sows, faecal recoveries of alkanes increased only numerically with increasing chain length from 71% (C29) to 79% (C35) (Wilson et al., 1999). Ferraz-de Oliveira et al. (2006) found in growing pigs, that mean faecal recoveries of n-alkanes did increase curvilinearly with increasing carbon-chain length (C25 to C36) from 38% to 69%, with little differences among C29, C32, C33 and C36.

In our study, the difference in faecal recovery between C32 and C36 were 11.8 ± 1.2% (R3), 11.7 ± 1.6% (R5). In our study, in C32:C36, the APEs from total faecal collection samples were 0.15 kg/day and 0.14 kg/day in R3 and R5 pigs respectively. When assuming equal faecal recoveries, for C32 and C36, APEs were reduced to zero in R3 and R5 pigs. This is further supported by the strong linear relationship between the APEs and the difference in faecal recovery rates of C32:C36 (Supplementary Figure S1).

2.4.7 Comparison between faecal collection days

In total faecal collection samples of R3 pigs, the APE using total faecal collection samples collected on days 3-4 (the third and fourth day after the first dosing day) tended to be lower (Yb:Cr, *P* =0.064; C32:C36, *P* =0.069) than using total faecal collection samples collected on days 2-4 (the second till fourth day after the first dosing day). Apparently, the start of the collection period on day 2 caused additional variation, and appeared to be too soon after the onset of pulse-dosing the reference marker. Not surprisingly, this effect was absent in R5 pigs. It is concluded that properly timed faecal spot sampling is key to reducing the bias of estimation. It makes especially sense for the estimation of individual feed intake in pigs reared in free-grazing conditions, which could help to minimize the labour for oral administration duration and faeces sampling duration.

2.5 Conclusion

In our study, the absolute prediction error measured by C32:C36 was numerically lower than measured by Yb:Cr at all frequencies, and significantly in R3 pigs (0.15±0.02 kg/day vs. 0.29±0.04 kg/day). This was related to a larger difference in faecal recovery between Yb and Cr compared with C32 and C36. For successful application of the dual marker technique in a complex housing environment, marker administration and the timing of spot sampling relative to the onset of pulse dosing the reference marker is crucial. Our study indicates that dosing the reference marker for three times per day for 2 days on days 1 and 2, combined with pooled spot faecal sampling, collected on days 3 and 4 appeared the minimum requirement for obtaining acceptable estimates of feed intake. In this way, absolute prediction errors of 10%-15% are feasible using the dual marker technique. Hence, this technique is promising to simultaneously provide semi-quantitative estimates of the intake of nutrients from various sources for individual pigs in a complex housing environment.

2.6 Reference

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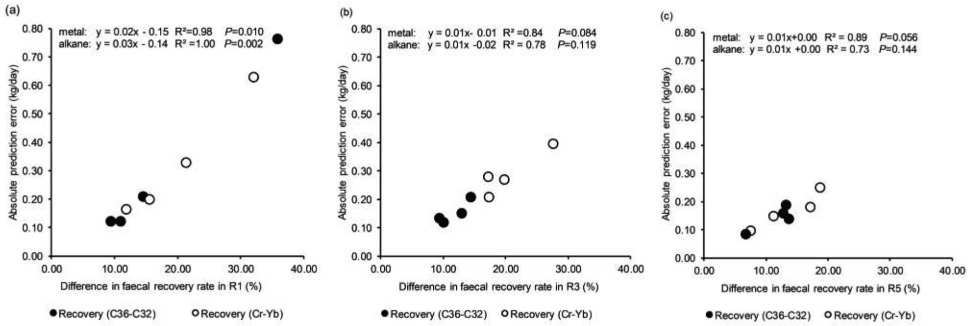
2.7 Supplementary material



Supplementary Figure S1. Linear relationship between the difference in faecal recovery rate of (C36-C32, Cr-Yb) and absolute prediction error (kg/day per pig) averaged on day 1 till day 3 predicted by two types of marker pair (C32:C36, Yb:Cr) in total faecal collection samples for 6.5-week-old pigs in three administration frequency of reference marker bolus (R1: once/day, R3: three times/day, R5: five times/day). (a) R1; (b) R3; (c) R5.

Supplementary Table S1:

Statistical analyses used in the models, which include testing the effect of the frequency of administration of the reference markers (three times/day vs. five times/day), faecal sampling method (quantitative collection samples vs. spot samples), marker pair types (metals vs. alkanes) on the absolute prediction error of DM feed intake (APE; kg/day per pig) for 6.5-week-old pigs, as well as the comparison of APEs among different days (days 1, 2, 3), the comparison of APEs between days 2+3 and days 1+2+3, and the comparison of faecal recoveries (%) between Yb and Cr, between C32 and C36.

Statistics	Normality of residuals	Homogeneity of variance	SAS Procedure	Test	Name	Comparison
Two-sample t-test	Met	Met	PROC TTEST	---	Table1	Comparison of APEs between R3[2] pigs and R5[3] pigs using Yb:Cr and C32:C36
Paired t-test	Met	---	PROC TTEST, paired statement	---	Fig.3	Comparison of APEs between Yb:Cr and C32:C36 within R3 and R5 pigs
					Fig.4	Comparison of marker recoveries between Yb and Cr, between C32 and C36 within R3 and R5 pigs
					Fig.6	Comparison of APEs between days 2+3 and days 1+2+3 within R3 and R5 pigs using Yb:Cr and C32:C36

[1] Absolute prediction error (APE; kg/day per pig) = | predicted DM feed intake (kg/day per pig) – measured

One-way repeated ANOVA	Met	---	PROC MIXED; REPEATED statement with type=cs option;	Dunnett test;	Table1	Comparison of APEs between spot faecal samples with TFC[4] samples as a control within R3 and R5 pigs
				Tukey-Kramer test	Fig.5	Comparison of APEs among days1,2,3 within R5 pigs using Yb:Cr and C32:C36
	Not met	---	PROC GLIMMIX, the lognormal distribution; RANDOM statement with RESIDUAL and type=cs option ;	Tukey-Kramer test	Fig.5	Comparison of APEs among days1,2,3 within R3 pigs using Yb:Cr and C32:C36

DM feed intake (kg/day per pig) |.

[2] R3: administration frequency of reference marker bolus for three times/day for pigs.

[3] R5: administration frequency of reference marker bolus for five times/day for pigs.

[4] TFC: total faecal collection.

Chapter 3: Variation in piglet body weight gain and feed intake during a 9-week lactation in a multi-suckling system

T. Tang [a, b], W. J. J. Gerrits [a], I. Reimert [b], C. M. C. van der Peet-Schwering [c], N. M. Soede [b]

[a] Animal Nutrition Group, Department of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[b] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[c] Wageningen Livestock Research, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
Corresponding author: Nicoline Soede. E-mail: nicoline.soede@wur.nl

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Abstract

A multi-suckling (**MS**) system for sows and piglets has been developed aiming to improve animal welfare. In this system, large variation in BW gain exists between piglets up to weaning at 9 weeks of age. We aimed to study causes of variation in BW gain and DM intake of solid feed (**DFI**) (piglet + sow feed) of piglets during lactation in the MS system. A total of 15 sows and 60 focal piglets across three batches were studied. Individual intake of piglet and sow feed was measured by the dual marker method, and multiple variables were recorded. Multiple linear regression analysis with forward selection was conducted on BW gain and DFI after correcting for piglet sex and batch, using multiple explanatory variables including genetic background, birthweight (**BiW**), DM feed intake, behaviours and number of skin lesions. These factors jointly explained less than 45% and 21% of the variation in BW gain and DFI, respectively. In week 2−4, variation in BW gain was mainly explained by BiW (12.0%) and play and nosing behaviours (7.6%). In week 4−6 and 6−8, it was largely explained by DM intake of piglet feed with 15.1% and 25.9%, respectively. Individual variation in DFI in week 2−4 was explained by the presence at front and middle teats during suckling bouts (2.9%), in week 4−6 by BiW (9.6%), and in week 6−8 by the number of skin lesions (5.1%). The unexplained variation in BW gain and DFI warrants further investigation.

Keywords

Individual growth variation, Individual feed intake, N-alkanes, Group lactation, Behaviour

Implications

In this study, we investigated causes of variation in BW gain and feed intake of piglets during lactation in multi-suckling systems. Our findings revealed that variation in BW gain of piglets was mainly explained by piglet’s birthweight and positive behaviour in week 2−4, and by its solid feed intake in week 4−8; variation in feed intake was explained by piglet’s teat presence in week 2−4, by its birthweight in week 4−6, and by its number of skin lesions in week 6−8. These findings give direction to future investigations to reduce variation in performance of piglets in multi-suckling systems.

3.1 Introduction

Multi-suckling (**MS**) systems for sows and piglets have been developed to improve animal welfare (Thomsson et al., 2016; Van Nieuwamerongen et al., 2017; Verdon et al., 2020). Such systems accommodate groups of lactating sows and their litters, allowing pigs more freedom of movement and social interactions (Weary et al., 2002; Van Nieuwamerongen et al., 2014; Grimberg-Henrici et al., 2018), as well as expression of play, explorative and other natural behaviours compared to conventional housing systems (Van Nieuwamerongen et al., 2015; van Nieuwamerongen, 2017; Schrey et al., 2019). In the study of Van Nieuwamerongen et al. (2017), the gradual weaning during a lactation period of 9 weeks helps to ease the weaning transition and improved the performance of piglets. In this system, piglets can access multiple food sources. They can access sow milk and piglet feed and can join sows to eat sow feed (Van Nieuwamerongen et al., 2015). Such settings resemble the semi-natural conditions, in which piglets are gradually weaned at 17 weeks of age (Jensen and Recén, 1989). During the long lactation period, sows spend an increasing amount of time away from piglets and piglets make a gradual transition from drinking milk to solid feed sources, and ultimately achieve nutritional independency from the sow (Newberry and Wood-Gush, 1986; Petersen et al., 1989).

Body weight homogeneity of piglets during lactation is important for successfully incorporating MS systems in all-in-all-out pig production systems. However, substantial variation in BW gain was observed in MS systems at weaning during week 6 (Thomsson et al., 2016) and week 9 (Van Nieuwamerongen et al., 2017), and a better understanding of the factors affecting piglet BW gain variation is needed. As dry matter feed intake (**DFI**) also varies in piglets (Van Nieuwamerongen et al., 2017), it is of interest to study factors affecting piglet DFI in the MS system as well.

Therefore, the objective of the current study was to investigate how different variables including genetic background, birthweight (**BiW**), feed intake, piglet behaviours and skin lesions explain variation in BW gain and DFI of piglets in three periods (week 2−4, week 4−6, week 6−8) during a 9-week lactation in an MS system.

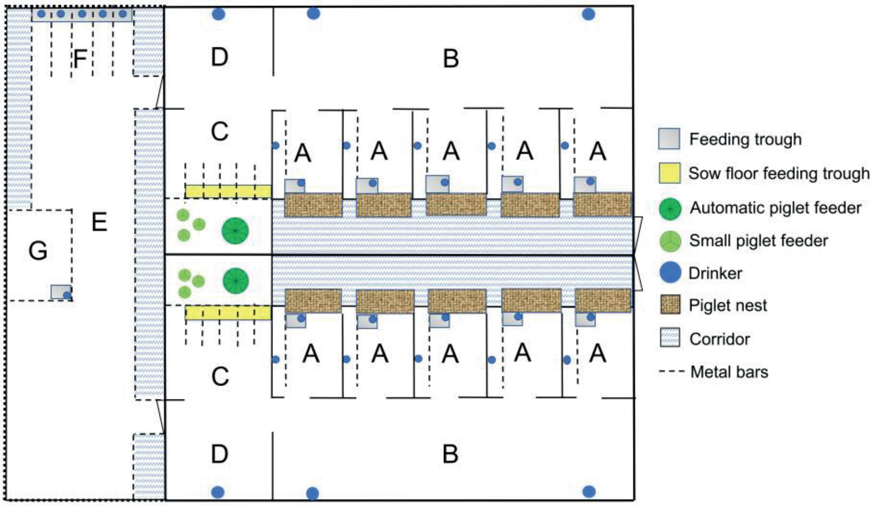
3.2 Material and methods

3.2.1 Animals, housing and experimental design

Fifteen multiparous sows (parity: 3.9 ± 0.4) (Topigs 20) (Topigs Norsvin, Beuningen, the Netherlands) and their litters (Tempo × Topigs 20) across three consecutive batches were studied at the animal facilities of Swine Innovation Centre Sterksel, the Netherlands. On day 14 postpartum (**p.p.**), in each litter, the surviving second lowest and highest BiW piglets from both sexes were selected as focal piglets (total: n=60), and were intensively followed up to weaning at 64 days of age. Focal piglets were selected from original litters and were never cross-fostered piglets. Sows and piglets received a mark with stock marker spray to distinguish sows and piglets at individual level.

Per batch, five sows and their litters were housed in a MS housing system, which contained two MS units and an intermittent suckling (IS) area (Fig. 1a). Each MS unit contained five adjacent farrowing pens (2.2 x 3.2m) (A), a communal MS area including a partially slatted lying area (11.1 x 2.8m) (B), a solid concrete feeding area (3.2 x 3.3m) (C) and a fully slatted dunging area (2.8 x 3.3m) (D). Each farrowing pen consisted of a solid concrete floor (2.2 x 2.2m) and cast iron slatted floor (2.2 x 1.0 m) contained a heated nest for the piglets (0.65 x 1.6m), a feeding trough with a water nipple for the sows, and a drinking nipple for the piglets. There were two extra drinking bowls for the sows in the lying area and in the dunging area which were also accessible to the piglets. The feeding area contained five feeding places for sows with a stainless steel feeding trough on the floor separated by horizontal metal bars, which was accessible to both sows and piglets (Fig. 1b). The feeding area also contained a surrounding area, which was accessible only to the piglets, with three small round feeders (diameter: 28 cm) (remained in use until day 35 p.p.) and a sensor-controlled automatic piglet feeder containing ten feeding places (Rondomat, Fancom B.V., the Netherlands) (used from day 28 p.p.). The IS area was located beside both MS units. It was semi-outdoors and roofed, with three sides of 1.5 m high walls and it consisted of a communal area, five feeding stalls for sows and a boar pen.

(a)



(b)

Figure. 1. (a) Layout of the multi-suckling (MS) system for sows and piglets consisting of two MS units and an intermittent suckling (IS) area. Each MS unit contained 5 farrowing pens with piglet nests (A), a communal MS area which included a lying area (B), a feeding area (C) and a dunging area (D). Connected to the MS unit was the IS area (E), feeding stalls for sows (F) and a boar pen (G). (b) Communal feeding area.

One week before expected farrowing, five sows from the same gestation pen were moved to one MS unit, balanced for expected farrowing date. Sows could access all areas in the MS unit, but were restrained between bars in a temporary crate constructed within the farrowing pens from one night before the expected farrowing date until day 3 p.p. to prevent piglet crushing. The piglets were ear tagged and litter sizes were standardized (13.8 ± 0.3 piglets/litter) between 24−48 h p.p. based on the number of functional teats per sow. Within day 4 p.p., piglets received an iron injection, but were not tail docked, teeth resected or castrated. From day 4 onwards, the bars were opened to create loose housing and the sows were given access to the communal area. A 31 cm high piglet barrier was put in place to prevent piglets from leaving the farrowing pen until grouping (9.1 ± 0.1 days of age). On day 19, piglets were vaccinated (circovirus-mycoplasma /PRRS vaccine, Boehringer Ingelheim). From day 28 till 34, forced IS was applied by bringing the sows to the IS area for 10 h/day (from 0700 h to 1700 h), during which a sexually mature boar was kept in a boar pen in the IS area to stimulate oestrus. From day 35 until weaning at 64 days of age, sows could voluntarily access the IS and MS area by stepping over a flexible metal partition (height: 30−40cm) between the two areas but the piglets could not. After the week of forced IS until weaning, no boar was present in the IS area. This IS procedure results in a gradual weaning process for the piglets (Van Nieuwamerongen et al., 2017). The time schedule of the experiment setup per batch is shown in Fig. 2.

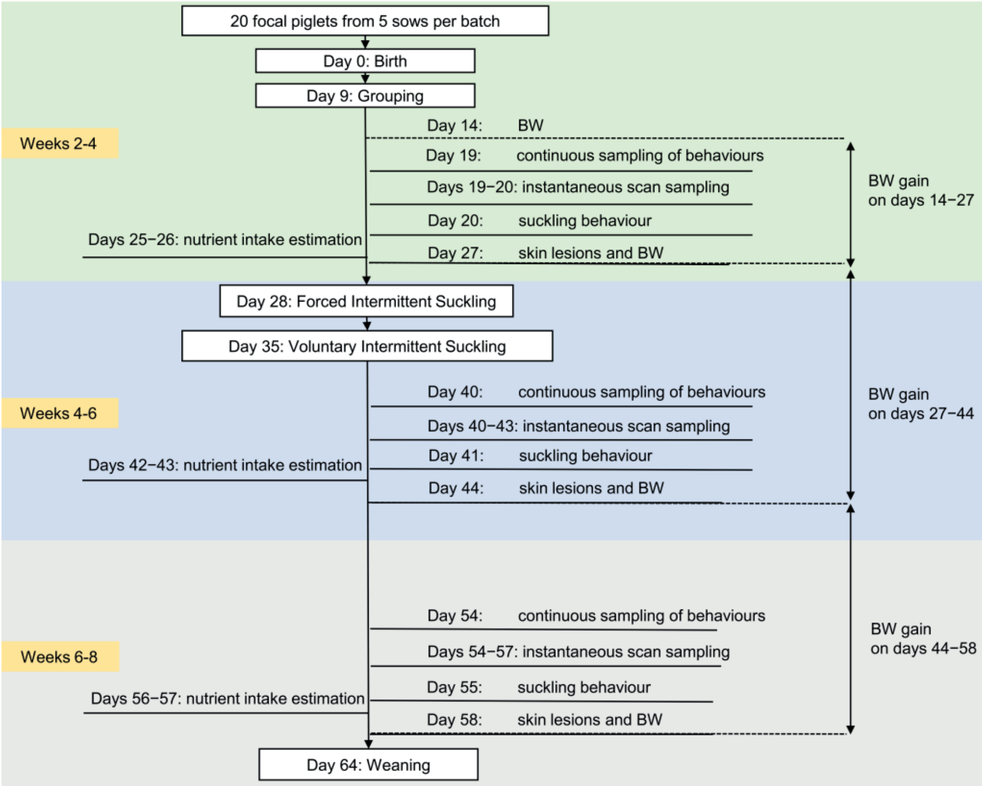


Figure 2. Time schedule of experiment setup of sows and piglets in each batch during a 9-week lactation in the multi-suckling system.

Enrichment materials were provided throughout lactation. During farrowing, two hessian sacks (110 cm x 60 cm) were provided on the solid concrete floor (2.2 x 2.2 m) for sows as nesting materials. From day 2 p.p., about 200 g of long straw was provided on the solid floor daily in each farrowing pen. During the entire lactation period, five sisal ropes (length: 1.2 m, 10 mm diameter) and five hessian sacks were hung on the wall in the MS lying area; in the IS area, five metal chains (length: 1.2 m, 11 mm diameter), one rope and two hessian sacks were available to the sows, and one metal chain and one rope were available to the boar.

The sows and their piglets were exposed to natural daylight and supplementary artificial lighting from 0700 until 1800 h. Ambient temperature in the MS unit was maintained around 18 °C with warmwater pipe heating. In the piglet nests the temperature was set at 33−35 °C (day 1 p.p.), 29−31 °C (day 7 p.p.) and 23−26 °C (from day 25 p.p.) with warmwater floor heating. The IS area had natural light and temperature. Manure and dirty straw were removed daily from the farrowing pens. The MS and IS area were also cleaned daily when necessary. Sawdust and dry powder were scattered when it was wet in the system. The MS system was disinfected and dried after the end of each batch.

3.2.2 Feeding regime

Sows were fed twice daily at around 0800 h and 1600 h, with 2.8 kg/day of sow diet before farrowing and gradually increased up to 8.5 kg/day p.p. (net energy: 9.4 MJ/kg, CP: 146.3 g/kg) (Aveve Biochem BV, Belgium). Before farrowing, sows were fed in floor troughs in the MS feeding area in the morning and in the farrowing pen in the afternoon. On days 0−3, sows were fed in the farrowing pens. On days 4−27, sows were floor fed in the MS feeding area. During forced IS on days 29−34, sows were fed in the IS area at 0700 h and in the MS feeding area at 1730 h. During voluntary IS on days 35−64, sows were fed in the IS area at 0800 h and were floor fed in the MS feeding area at 1600 h. Sows had *ad libitum* access to water via drinking bowls and nipples.

From day 2 p.p. until grouping at day 9, piglets were provided with a commercial diet of large creep feed pellets (8 mm diameter) (Research Diet Services BV, the Netherlands) which was spread over the floor of the farrowing pen which sows could not access. On days 9−11, the large creep feed pellets were provided in the three small round feeders in the MS feeding area together with a commercial weaner diet (Research Diet Services BV, the Netherlands), after which only the weaner diet was provided in small round feeders. Only piglets could access the small round feeders while sows could not. On days 20−21 the weaner diet was mixed with a pre-starter diet (net energy: 10.38 MJ/kg, CP: 164.4 g/kg), after which only the pre-starter diet was provided. On days 37−38 the pre-starter diet was mixed with a starter diet (net energy: 10.09 MJ/kg, CP: 173.5 g/kg), after which only starter diet was provided until weaning at day 64. The pellet size of all diets was 4 mm.

On days 9−28, feed was provided in the small round feeders in the feeding area, which were re-filled twice a day. On days 28−31 feed was also given in the trough of the automatic piglet feeder to make piglets get familiar with the trough. On days 32−64, the storage container of the automatic piglet feeder was filled with feed so that piglets could eat feed *ad libitum*. The piglets had *ad libitum* access to water via the drinking nipples and bowls in the MS system. The ingredient and calculated nutrient composition of sow, piglet pre-starter and starter diets are provided in Supplementary Tables S1, S2 and S3.

3.2.3 Measurements

BW

Piglets were weighed on days 0, 14, 27, 44, 58 and 64.

Estimated breeding value of BW gain

The estimated breeding value (**EBV**) of BW gain from birth to slaughter (g/day) of a piglet was calculated as the relative difference between the expected individual BW gain and the population average, based on the performance of the relatives and the genomic relationship matrix. Therefore, an EBV is an estimate of the genetic potential of the piglet. An ear tissue sample was collected from each piglet at the moment of ear tagging within 24 hours after birth. DNA extraction and genotyping was performed by Topigs Norsvin (Beuningen, the Netherlands), thus providing best linear unbiased predictions of the genetic potential of the piglets for the life time daily gain.

Feeding behaviour during sow feeding times and suckling behaviour

Feeding behaviour of the piglets was scored using 2-min instantaneous scan sampling during sow feeding times on days 19−20 (week 3) at 0800−0830 h and 1600−1630 h, and on days 40−43 (week 6) and days 54−57 at 1600−1630 h (week 8) (Fig. 2). During the forced and voluntary IS period, the sows were fed in the IS area in the morning, and therefore feeding behaviour of piglets in week 6 and week 8 was only observed in the afternoon. During these sow feeding times, every 2 min for each focal piglet it was noted whether it was in the feeding area, contacting (i.e. sniffing or eating) sow feed or contacting (i.e. sniffing or eating) piglet feed. From these observations, the percentage of time spent on contacting sow feed and piglet feed was calculated per week.

On days 20 (week 3), 41 (week 6) and 55 (week 8), suckling behaviour was observed at 0900−1600 h (Fig. 2). A nursing bout was scored as 'unsuccessful' and excluded from analysis when the nursing began within 20 min after a previous nursing (Weary et al., 2002), and no milk let-down was noted. Unsuccessful nursings comprised less than 3% of total nursings. The frequency of presence at teats of each focal piglet in all suckling bouts (at biological mother and other sows) was scored, on either the front (the first two pairs of teats), the rear (the last two pairs of teats) or the middle teats (the remaining teats). The frequency of presence at front and middle teats (**FM teats**) were summed into one variable for further analysis.

Other behavioural observations and lesions

On days 19 (week 3), 40 (week 6) and 54 (week 8) at 0900−1600 h (Fig. 2), continuous behaviour sampling was performed to determine the frequency of play, nosing, manipulation, aggression, exploration, and ingestion using the ethogram in Table 1. Continuous behavioural sampling was done during five observational blocks. The four focal piglets of each litter were observed for 10 min in each block, resulting in a total observation period per piglet of 50 min per day. Play is regarded as an indicator for good welfare (Brown et al., 2015) and nosing is regarded as a positive social interaction (Camerlink and Turner, 2013), therefore they were summed as positive behaviour. Manipulative and aggressive behaviour towards pigs (Schouten, 1985) are regarded as negative social interactions therefore they were summed as negative behaviour. Explorative behaviour belongs to inherent behavioural needs which is a biologically important behaviour to gain information about the surrounding environment and available food resources (Docking et al., 2008). Ingestion behaviour is related with the pigs' requirement of energy. Explorative and ingestion behaviour were analysed separately.

Table 1. Ethogram used for behavioural observations of play, nosing, exploration, manipulation, aggression, and ingestion of piglets in the multi-suckling system.

Category	Behaviour	Description
Play	Individual play	Scampering (forward hops in rapid succession), turning (rapid turn on the spot), head tossing, flopping (rapid drop from an upright position to lying), rolling on back, sliding, or running individually
	Social play (2 pigs or more)	Nudging (play invitation: gentle pushing of another piglet), gambolling (running together), play fighting, or scampering together
	Substrate play	Shaking of head while holding material (e.g. straw, rope) that protrudes from mouth (not scored when only chewing on material)
Nosing	Nosing head or body - piglets	Touching or sniffing any part of another piglet except nose contact, without manipulative behaviours
	Nose contact - piglets	Mutual nose contact between piglets, without manipulative behaviours
	Nosing head or body - sow	Touching or sniffing any part of a sow except nose contact, without manipulative behaviours or massaging the udder
	Nose contact - sow	Mutual nose contact between piglet and sow, without manipulative behaviours
Manipulation	Manipulating piglet	Nibbling, sucking, or chewing part of the body (ears, tail, or other part) of a piglet, not scored when a piglet clearly only bites an ear tag)
	Manipulating sow	Nibbling, sucking, or chewing part of the body (ears, tail, or other part) of a sow, not scored when a piglet clearly only bites an ear tag)
	Belly nosing	Rubbing belly of another pig with up and down snout movements (≥3 up and down movements)
Aggression	Individual or mutual fighting	Horizontal or vertical knocking with the head or forward thrusting with the snout toward another piglet (single event or short series of events) / Biting another piglet (single event or short series of events) / Intense mutual ramming or pushing (parallel or antiparallel), with or without biting, in rapid succession
	Aggression at feeder	Feed-related aggression: pushes, head knocks or bites given at feeder (not scored when e.g. pig gives a head knock at the feeder resulting from manipulative behaviour
Exploration	Exploring system	Nibbling, sucking or chewing on parts of the MS system such as iron bars, walls, door, floor, ropes, hessian sacks and straw
Ingestion	Sniffing/nosing sow feed	Sniffing/nosing sow feed
	Eating sow feed	Chewing on sow feed (jaw moves up and down)
	Sniffing/nosing piglet feed	Sniffing/nosing piglet feed
	Eating piglet feed	Chewing on piglet feed (jaw moves up and down)
	Drinking	Drinking from water nipple

On days 27 (week 4), 44 (week 6) and 58 (week 8) (Fig. 2), the number of skin lesions was counted per piglet by visual assessment as the number of fresh superficial and deep lesions on the whole body, except for ears and tails. Scoring was performed according to the procedure of Turner et al. (2006). The length or diameter per lesion was not taken into account. Lesions were deemed to be deep when haemorrhage was present, otherwise lesions were noted as being superficial. Skin lesions can be regarded as a proxy for aggressive behaviour given and received (Turner et al., 2006).

Estimation of individual feed and milk intake

Administration of alkanes

Individual daily DM intake of sow feed and piglet feed was measured for each focal piglet on days 25−26 (week 4), days 42−43 (week 6) and days 56−57 (week 8) using the dual alkane marker technique, according to Tang et al. (2022). Two pairs of dual markers were used: C32 and C31 for the estimation of DM intake of sow feed, C32 and C36 for the estimation of DM intake of piglet feed. C32 (42 mg/d, 60 mg/d and 78 mg/d in week 4, 6 and 8, respectively) was considered as a reference marker and was melted on a small amount of feed in a forced air oven (melting temperature: 69°C). Reference marker C32 boluses were prepared freshly before oral administration in an amount of ~ 1.4 g/bolus in week 4, ~ 2.0 g/bolus in week 6, ~ 2.6 g/bolus in week 8, kneaded to a firm bolus using a few drops of sucrose-based lemonade syrup (Karvan Cévitam, the Netherlands). C31 and C36 were considered as in-feed markers, therein C31 was provided via the inclusion of 15% alfalfa in the sow feed, and C36 was melted on soybean meal in a forced air oven (melting temperature: 72 °C) followed with the mixing into the pre-starter and starter diet, providing around 50 mg/kg of C31 in sow feed and 170 mg/kg of C36 in piglet feed respectively.

On days 22−23, focal piglets were habituated with placebo boluses without reference marker by hand twice per day. On days 25−26 in week 4, days 42−43 in week 6 and days 56−57 in week 8, focal piglets were orally administered one C32 bolus by hand for three times/day at 0830, 1430 and 2030 h, with a total dosing frequency of six times /week per piglet. The number of marker bolus consumed by the piglets was recorded. On days 27, 44 and 58, spot faecal samples of around 20~70 g were taken from the rectum with a cotton swab from each piglet at 0830 and 1230 h. Sow feed and piglet feed samples were collected for week 4, 6 and 8 separately. All feed and faecal samples were stored at −20 ˚C for further lab analysis.

Laboratory analysis

Faecal samples were pooled per piglet per day, and oven-dried at 65 °C and ground to pass a 1 mm screen. The concentrations of n-alkanes in faeces and feed were analysed using gas chromatography as described by Smit et al. (2005).

Dry matter intake of the sow feed and piglet feed in each batch were calculated for days 25−26, 42−43 and 56−57 separately, using eq. [1]:

$$= \frac{\left(\frac{\text{concentration of in-feed marker in faeces (mg/kg)}}{\text{concentration of reference marker C32 in faeces (mg/kg)}} \times \text{daily intake of reference marker C32 (mg/day)}\right)}{\text{concentration of in-feed marker in diet (mg/kg)}} \times 1000$$

[1]

Milk intake was calculated from BW gain, assuming a fixed efficiency of converting fresh milk into BW gain of 4.2 g/g for week 4 (Everts et al., 1995). For week 6 and 8, milk intake was calculated using eq. [2], by subtracting DM feed intake from BW gain, assuming fixed feed conversion ratios (**FCR**) of converting DM feed intake into BW gain of 1.5 g/g (week 6) and 1.7 g/g (week 8), and assuming a fixed efficiency of converting fresh milk into BW gain of 4.89 g/g (Theil et al., 2002). Resulting negative estimates were replaced by 0, assuming these piglets did not consume sow milk anymore.

Estimated intake of milk (g/day)= (BW gain (g/day) – intake of total feed (g/day) / FCR (g/g)) × 4.89 [2]

Dry matter intake of milk was then calculated assuming a DM content of 19 % (Hurley, 2015). The complete procedures for the calculation of nutrients intake can be found in Supplementary Material S1.

3.2.4 Statistics

SAS (SAS 9.4, SAS Institute Inc.) was used for all statistical analyses. Observations of individual focal piglets were used for all analyses. P<0.05 was regarded as significant.

A multiple linear regression analysis per period (week 2−4, week 4−6, week 6−8) was performed to explain variation in BW gain and DFI by multiple variables. Before the regression analysis, the observed BW gains and DFIs were corrected for sex and batch as fixed effect. The model residuals of BW gain and DFI were subsequently used as response variables.

In the initial model in which BW gain was the response variable, 12 variables were selected as explanatory variables based on biological plausibility, or single relationships between the explanatory variables and the response variable (Supplementary Table S4). In the initial model in which DFI was the response variable, 10 explanatory variables which were the same variables as in the BW gain model were included, except for 'DM intake of sow feed' and 'DM intake of piglet feed'.

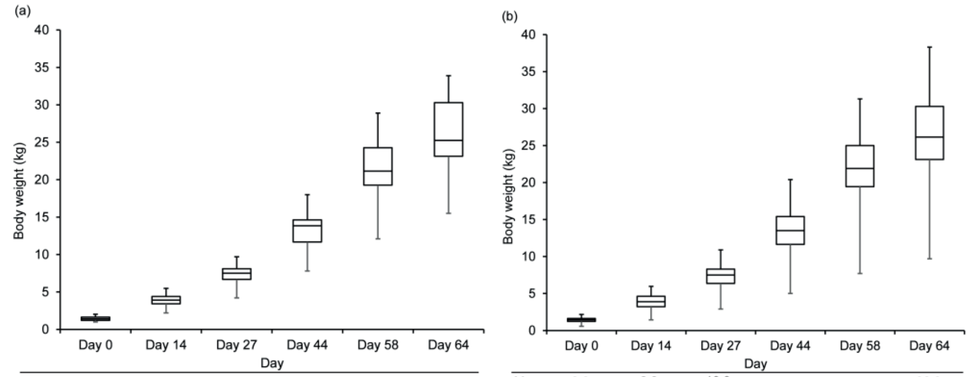
Regression functions were analysed using PROC REG. For the initial multiple regression models, forward selection with alpha=0.999999 were used to determine the significance of all 12 and 10 explanatory variables per period in the model of BW gain and DFI respectively. The initial models were also performed using backward selection with alpha=0.000001, and the results were similar with forward selection. For the final regression models, forward selection with alpha=0.25 was used to select the variables with $P<0.25$. The final models were also performed using stepwise selection with alpha =0.25, and using backward selection with alpha=0.25, of which the results were similar with using forward selection. Therefore the results of the forward selection are presented for both of the initial and the final models. The criteria for selection were based on the recommendations in the REG procedure in SAS user's guide ($P<0.10-0.25$) (SAS®, 2018), as well as considering the relevant biology of candidate variables.

Assumptions of the multiple regression analysis were checked. The normality of model residuals was checked with Skewness-Kurtosis test, Shapiro-Wilk test and quantile-quantile plot using PROC UNIVARIATE. Collinearity between the 12 explanatory variables was tested by checking the variable inflation factor and correlation analyses among the 12 variables (Supplementary Table S5). The variable inflation factors were less than 10 in all multiple linear regression models showing that there was no collinearity between the 12 explanatory variables, according to Kleinbaum et al. (2013).

3.3 Results

3.3.1 Descriptive statistics of performance and nutrient intake

During the 9 weeks of lactation, the BW of the focal piglets (Fig. 3a) showed a similar pattern as that of all piglets (Fig. 3b) thus being representative for all piglets in the system. As piglets grew older, DM intake of both sow feed and piglet feed increased over time, while DM intake of milk decreased over time (Fig. 4). The percentage of focal piglets with zero milk intake increased over time and was 0%, 26.3% and 43.1% in week 2−4, 4−6 and 6−8, respectively (data not shown). As piglets grew older, the variability in BW and BW gain, and the variability in DM intake of nutrients including sow feed, piglet feed, total feed and milk numerically increased (Table 2; Supplementary Fig. S1).



(a)

	Day 0	Day 14	Day 27	Day 44	Day 58	Day 64
Max	2.0	5.5	9.7	18.0	28.9	33.9
Min	1.0	2.2	4.2	7.8	12.1	15.5
Mean	1.4	3.9	7.3	13.4	21.7	26.1
Median	1.4	3.9	7.5	13.9	21.2	25.3
N	60	60	60	60	58	58

(b)

	Day 0	Day 14	Day 27	Day 44	Day 58	Day 64
Max	2.2	6.0	10.9	20.4	31.3	38.3
Min	0.6	1.4	2.9	5.0	7.7	9.7
Mean	1.4	3.9	7.3	13.4	21.8	26.1
Median	1.4	3.9	7.5	13.5	21.9	26.2
N	207	189	188	187	186	186

Figure 3. Boxplots showing the BW of (a) focal piglets (n = 58 on day 64) and (b) all piglets (n = 186 on day 64) during a 9-week lactation in the multi-suckling system at six weighing times, with indicating minimum, 25th percentile values of BW, median, 75% percentile values of BW, maximum of BW of piglets.

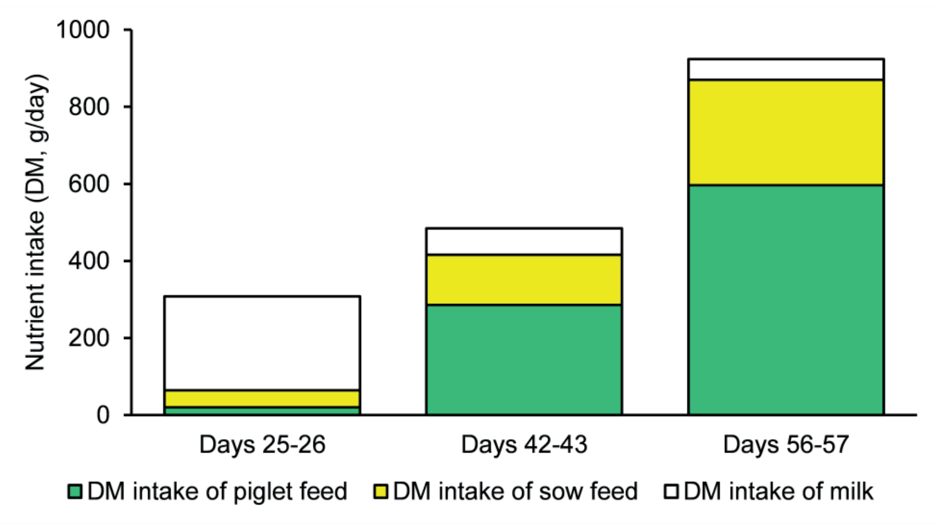


Figure 4. Stacked bar plot showing the average values of DM intake of piglet feed, sow feed and milk of focal piglets during a 9-week lactation in the multi-suckling system on day 25−26 (feed: n=52, milk: n=60), day 42−43 (n=57) and day 56−57 (n=58).

Table 2. Descriptive values for performance, genetics, nutrient intake, behaviours, and body lesions of focal piglets (n=60) in each of the three periods during a 9-week lactation in the multi-suckling system.

	Mean ± SE of individual means			Within-batch SD		
Variables[1]	Week 2-4	Week 4-6	Week 6-8	Week 2−4	Week 4−6	Week 6−8
Performance						
Birthweight (kg)	1.4±0.0	---	---	0.2	---	---
BW (kg)	7.3±0.2	13.4±0.3	21.7±0.5	1.1	2.0	3.4
BW gain (g/day)	262.5±6.4	359.5±10.5	578.6±17.4	40.7	72.0	132.9
Genetics						
EBV (g/day)	20.3±1.9	---	---	13.5	---	---
DM Intake (g/day)						
Sow feed	44.2±6.1	130.0±11.7	273.9±24.8	35.5	72.4	130.2
Piglet feed	19.8±2.8	286.2±21.7	596.3±36.5	16.7	163.8	249.3
Total feed	64.0±8.3	416.2±23.1	870.2±30.5	48.0	171.5	235.8
Milk	243.9±5.9	68.4±7.8	53.4±9.2	37.8	59.7	70.5
Feeding behaviour						
Contacting feed during sow feeding times (% of observed time)	7.1±0.9	24.9±1.8	21.3±1.8	5.6	14.0	13.7
Suckling behaviour						
The presence at the front and middle teats (no. of times)	4.5±0.4	3.8±0.4	1.8±0.3	2.8	2.9	1.7
The presence at the rear teats (no. of times)	1.7±0.3	1.6±0.4	0.9±0.2	2.6	2.7	1.6
Behaviours						
Positive behaviour (no. of times /hour)	13.9±1.1	13.6±1.2	9.1±1.0	8.9	9.1	6.9
Negative behaviour (no. of times /hour)	4.8±0.5	5.5±0.7	3.7±0.5	3.6	4.8	3.3
Explorative behaviour (no. of times /hour)	7.9±0.8	10.7±1.2	9.5±1.0	5.9	8.8	7.3
Ingestion (no. of times /hour)	5.9±1.1	14.2±1.5	10.6±1.3	6.9	11.3	9.4
Skin lesions (no.)	7.9±1.1	8.4±1.0	13.0±1.1	6.2	5.3	7.9

[1] Abbreviation: EBV: the estimated breeding value of BW gain from birth to slaughter.

Variables: (1) BW (kg): piglets were weighed on day 27 (week 2−4), 44 (week 4−6) and 58 (week 6−8); BW gain (g/day): the difference between BW on different days divided by numbers of days between both days, during days 14−27 (week 2−4), days 27−44 (week 4−6), days 44−58 (week 6−8); (2) Nutrient intake (g/day): Individual daily DM intake of sow feed and piglet feed was measured per focal piglet on days 25−26 (week 4), days 42−43 (week 6) and days 56−57 (week 8); (3) Contacting feed during sow feeding times (% of observations): contacting (i.e. sniffing or eating) sow feed or piglet feed during sow feed times on days 19−20 at 0800−0830 h and 1600−1630 h, and on days 40−43 and 54−57 at 1600−1630 h; (4) Suckling behaviour (on days 20, 41, 55 at 0900 h−1600 h): The presence at the front and middle teats (no. of times): the frequency of presence at front (first 2 pairs) and middle pairs of teats of each focal piglet in all suckling bouts during the day; Rear teats (no. of times): the frequency of presence at rear (last 2 pairs) of teats of each focal piglet in all suckling bouts during the day; (5) Behaviours (on day 19, 40, 54) : Positive behaviour (no. of times /hour): the frequency of play and nosing/nose contacting sows and piglets during the day; Negative behaviour (no. of times /hour): the frequency of manipulating sows and piglets, belly nosing, and aggressive behaviour during the day; Explorative behaviour (no. of times /hour):

exploring the system, i.e., nibbling, sucking or chewing on parts of the multi-suckling system such as iron bars, walls, door, floor, ropes, hessian sacks and straw; Ingestion behaviour (no. of times /hour): sniffing/nosing/eating sow feed and piglet feed, drinking during the day; (6) Skin lesions (no.): the number of fresh superficial and deep lesions on the whole body, except for ears and tails on day 27, 44 and 58.

3.3.2 Exploring variation in BW gain by multiple linear regression

In initial models containing all 12 variables (Supplementary Table S6), these variables jointly explained less than 45% of the variation in BW gain (week 2−4: 34.4%, week 4−6: 42.4%, week 6−8: 44.9%). In final models, after forward selection, the 5−6 retained variables explained 31.4%, 39.2% and 42.6% of the variation in BW gain in the consecutive periods (Fig. 5a, Table 3). In these models, BiW explained the highest percentage of variation (12.0%) in BW gain in week 2−4 compared to other variables and more than in week 4−6 (2.0%) and 6−8 (7.5%). Birthweight was positively related with BW gain in week 2−4 (regression coefficient (**β**)=0.1, *P*=0.001) and tended to be positively related with BW gain in week 6−8 (β=0.1, *P*=0.051). Dry matter intake of piglet feed explained the highest percentage of variation in BW gain in week 4−6 (15.1%) and 6−8 (25.9%) compared to other variables; it was positively related with BW gain in week 4−6 (β=0.1, *P*=0.008) and week 6−8 (β=0.2, *P*<0.001). Similarly with DM intake of piglet feed, ingestion behaviour also exceeded the impact of BiW in week 4−6 and it became the second important variable to explain variation in BW gain in that period (10.7%), but it explained very little variation in week 6−8 (0.5%); ingestion behaviour was positively related with BW gain in week 4−6 (β=1.5, *P*=0.036). Dry matter intake of sow feed was only retained in week 2−4 after forward selection in which it accounted for 3.9% of the variation in BW gain. It explained only little variation in week 4−6 (0.8%) and 6−8 (0.5%), and appeared unrelated with BW gain in each of the three periods. Presence at FM teats increasingly explained variation in BW gain with age, but explained less variation (2.4% in week 4−6 and 2.6% in week 6−8) than DM intake of piglet feed (15.1% in week 4−6 and 25.9% in week 6−8) and BiW in week 6-8 (7.5%); presence at FM teats tended to be negatively related with BW gain in week 4-6 (β=−6.0, *P*=0.083). Of the frequency of positive, explorative and negative behaviour, only positive behaviour was retained after forward selection in week 2−4 and accounted for 7.6% of the variation in BW gain. It explained only little variation in week 4−6 (1.5%) and 6−8 (0.5%); Of the frequency of behaviours, only positive behaviour was positively related with BW gain in week 2−4 (β=1.7, *P*=0.003), and no relationship was found in the other two periods. Contacting feed during sow feeding times explained the highest variation in BW gain in week 2−4 (4.1%) compared to week 4−6 (0.1%) and week 6−8 (1.7%) and it was positively related with BW gain in week 2−4 (β=1.8, *P*=0.020). The number of skin lesions explained little variation in BW gain in week 2−4 (1.0%) but more in week 4−6 (4.2%) and week 6−8 (2.5%) and it tended to be positively related with BW gain in week 4−6 (β=1.8, *P*=0.093). The estimated breeding value of BW gain explained the highest variation in BW gain in week 2−4 (3.7%) compared with week 4−6 (0.1%) and 6-8 (2.4%); EBV of BW gain explained relatively less variation in BW gain compared to BiW in each of the three periods; it only tended to be positively related with BW gain in week 2−4 (β=0.6, *P*=0.094). The results of the single linear regression are presented in Supplementary Table S4, largely confirming results of the multiple linear regression.

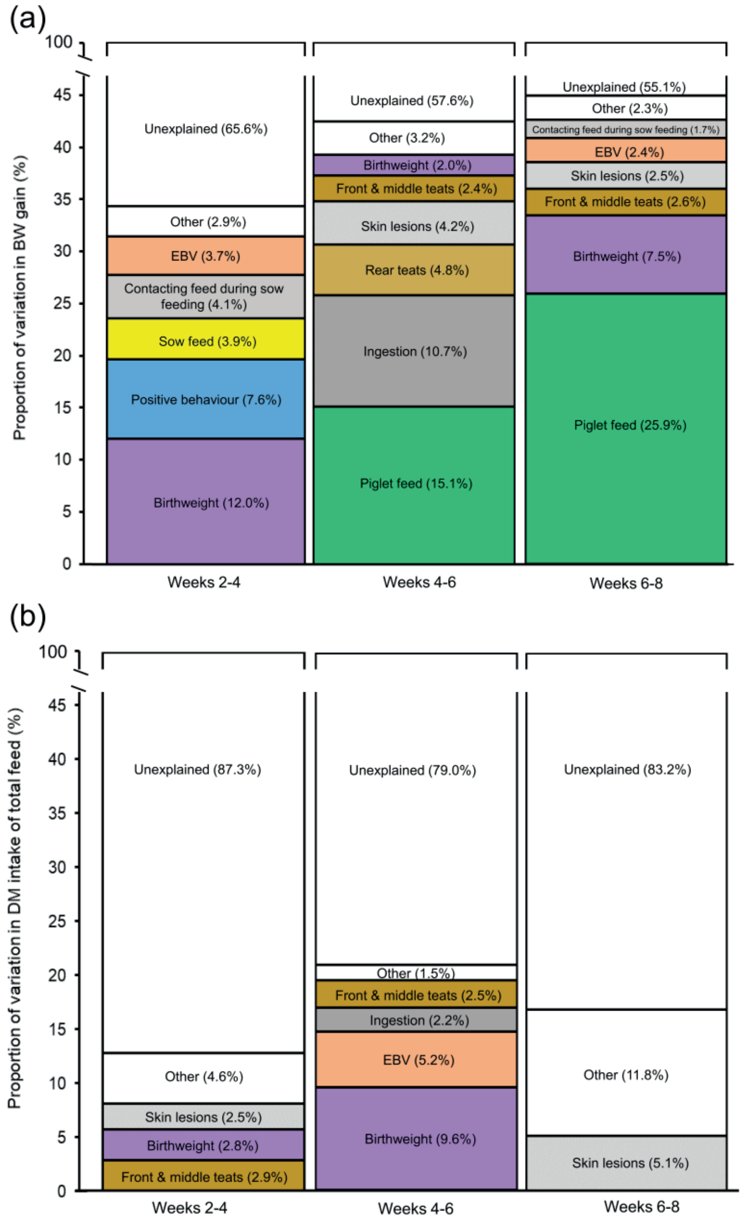


Figure 5. Stacked bar plot showing the percentage of variation in (a) BW gain and (b) DM intake of total feed (DFI) of focal piglets (n=60) explained by explanatory variables in week 2−4, 4−6 and 6−8 using multiple regression analysis. Variables with *P*<0.25 were remained in the model after forward selection. 'Other' indicates variables that were not remained after forward selection. The response variables were the residuals of BW gain and DFI corrected for sex and batch. The explanation of the variables can be found in Table 2.

Table 3. Multiple linear regression of BW gain and DM intake of total feed (DFI) of piglets on multiple explanatory variables in three periods during a 9-week lactation in the multi-suckling system after forward selection (*P* <0.25).

	BW gain						
			Forward selection			Multiple regression	
Period	Step	Explanatory Variables[2]	Partial R^2 (× 100)[3]	Model R^2 (× 100)[4]	*P* value selection	Partial Regression coefficient (β)[5]	*P* value of β
Week 2−4	1	Birthweight	12.0	12.0	0.007	0.1	0.001
	2	Positive behaviour	7.6	19.7	0.024	1.7	0.003
	3	DM intake of sow feed	3.9	23.6	0.096	-0.2	0.041
	4	Contacting feed during sow feeding	4.1	27.7	0.082	1.8	0.020
	5	EBV	3.7	31.4	0.094	0.6	0.094
Week 4−6	1	DM intake of piglet feed	15.1	15.1	0.002	0.1	0.008
	2	Ingestion	10.7	25.8	0.006	1.5	0.036
	3	Rear teats	4.8	30.6	0.057	-10.9	0.004
	4	Skin lesions	4.2	34.8	0.068	1.8	0.093
	5	FM teats	2.4	37.2	0.160	-6.0	0.083
	6	Birthweight	2.0	39.2	0.195	0.0	0.195
Week 6−8	1	DM intake of piglet feed	25.9	25.9	<.0001	0.2	<0.001
	2	Birthweight	7.5	33.4	0.016	0.1	0.051
	3	FM teats	2.6	35.9	0.149	10.5	0.133
	4	Skin lesions	2.5	38.5	0.145	3.1	0.071
	5	EBV	2.4	40.8	0.155	1.3	0.182
	6	Contacting feed during sow feeding	1.7	42.6	0.220	1.2	0.220

	DFI						
			Forward selection			Multiple regression	
Period	Step	Explanatory Variables[2]	Partial R^2 (× 100)[3]	Model R^2 (× 100)[4]	*P* value selection	Partial Regression coefficient (β)[5]	*P* value of β
Week 2−4	1	FM teats	2.9	2.9	0.198	-3.9	0.095
	2	Birthweight	2.8	5.7	0.197	0.0	0.131
	3	Skin lesions	2.5	8.1	0.226	-1.0	0.226
Week 4−6	1	Birthweight	9.6	9.6	0.017	0.2	0.076
	2	EBV	5.2	14.8	0.070	-2.2	0.128
	3	Ingestion	2.2	17.0	0.229	2.4	0.185
	4	FM teats	2.5	19.5	0.201	-9.0	0.201
Week 6−8	1	Skin lesions	5.1	5.1	0.086	6.2	0.086

[1] The response variables were the residuals of BW gain and DFI corrected for sex and batch.

[2] The explanation of the variables can be found in Table 2. Abbreviation: EBV: the estimated breeding value of BW gain from birth to slaughter; FM teats: the presence at the front and middle teats; Rear teats: the presence at the rear teats.

[3] Partial R^2 (× 100) is the partial squared correlation coefficient multiplied by 100, which represents the percentage of the variation in BW gain and DFI explained by the explanatory variables in the model.

[4] Model R^2 (× 100) is the cumulative squared correlation coefficient multiplied by 100, which is the R^2 from the previous variable plus the partial R^2 of the current explanatory variable and it represents the percentages of the variation in BW gain and DFI explained by having the identified explanatory variables in the model.

[5] The partial regression coefficient represents the effect of one explanatory variable when others were held constant. *P* values in bold are *P* values for forward selection < 0.25 and *P* values for partial regression coefficients < 0.10.

3.3.3 Exploring variation in DM intake of total feed by multiple linear regression

In initial models (Supplementary Table S6), 10 variables jointly explained less than 21% of the variation in DFI (week 2−4: 12.8%, week 4−6: 21.0%, week 6−8: 16.9%). In final models, after forward selection, the 1−4 retained variables explained 8.1%, 19.5% and 5.1% of the variation in DFI in the consecutive periods (Fig. 5b, Table 3). In these models, BiW explained the highest percentage (9.6%) of variation in DFI in week 4−6 compared to the other variables; and more than in week 2−4 (2.8%) and week 6−8 (2.8%). Also ingestion behaviour explained the highest variation in week 4−6 (2.2%) compared to week 2−4 (0.2%) and week 6−8 (0.4%). The variation of DFI explained by presence at FM teats gradually decreased over the three periods (week 2−4: 2.9%, week 4−6: 2.5%, week 6−8: 1.6%) and explained more variation in week 2−4 compared to other variables. The frequency of positive, explorative and negative behaviour and the percentage of contacting feed during sow feeding times explained less than 1.6% of variation in DFI in the three periods. The number of skin lesions explained the highest variation in DFI in week 6−8 (5.1%) compared to week 2−4 (2.5%) and 4−6 (0.2%) and it explained the highest variation in DFI in week 6−8 compared to the other variables. The estimated breeding value of BW gain explained the highest variation in DFI in week 4−6 (5.2%) compared to week 2−4 (2.0%) and week 6−8 (1.7%). In the multiple regression of DFI, in week 2−4, presence at FM teats tended to be negatively related with DFI (β=−3.9, *P* =0.095); in week 4−6, BiW tended to be positively related with DFI (β=0.2, *P*=0.076); in week 6−8, the number of skin lesions (β=6.2, *P*=0.086) and BiW (β=0.3, *P*=0.051) tended to be positively related with DFI, and presence at FM teats (β=−36.1, *P*=0.097) negatively. The results of the single linear regression are presented in Supplementary Table S4, mostly confirming the results of the multiple linear regression.

3.4 Discussion

In the current study, we attempted to explain variation in BW gain of piglets during a 9-week lactation in an MS system. Therefore, we measured nutrient intake, and several behavioural parameters in individual piglets of known genetic background and evaluated their contribution to BW gain. We also evaluated their contribution to a major contributor of BW gain, i.e. variation in feed intake. In initial models, these variables jointly explained 45% and 21% of the variation in BW gain and DFI, respectively.

3.4.1 Feed intake

Total DM intake of sow plus piglet feed, estimated using the dual marker approach (Tang et al., 2022) was 64, 416 and 870 g/day per piglet on days 25−26, 42−43, 56−57 respectively. To the best of our knowledge, it is the first quantitative estimation of feed intake obtained from individual piglets in an MS environment. These estimates are in range with earlier findings in week 4: 63 g/d (Pajor et al., 1991) and

66 g/d (Bøe and Jensen, 1995), on days 36−42: 439 g/d (Fraser et al., 1994), and on days 42−53: 851 g/d per pig (Fraser et al., 1994), and with earlier findings in an MS system: on days 28−33: 45 g/d and on days 63−68: 1220 g/d (Van Nieuwamerongen et al., 2017). Milk intake decreased over time, while feed intake increased over time, which is similar with semi-natural and group lactation conditions in which sows progressively reduce nursing frequency (Jensen and Recén, 1989; Van Nieuwamerongen et al., 2017) and piglets increasingly foraged for solid food (Newberry and Wood-Gush, 1986; Petersen et al., 1989).

3.4.2 Exploring variation in BW gain by multiple linear regression

Birthweight

Birthweight explained a relatively large portion of the variation in BW gain in week 2−4 (12.0%), only 2.3% in week 4−6 and 7.5% in week 6−8. Also in other studies, BiW was found to explain variation in BW and BW gain of piglets at week 3−6 p.p. (Lodge and McDonald, 1959; McBride et al., 1965; Fraser et al., 1979; Fraser et al., 1994). For instance, BiW explained 27.3% of the within-litter variation in BW of piglets at week 3 p.p. (Fraser et al., 1979) and explained around 6.5% of the variation of BW gain in week 5−6 p.p.(Fraser et al., 1994). A similar positive relationship between BiW and BW gain was found in piglets both before and after weaning (Pajor et al., 1991; Douglas et al., 2013; Huting et al., 2019; Van der Peet-Schwering et al., 2021). The physiological explanation of this effect includes a higher number of muscle fibres (Alvarenga et al., 2013) and a better developed digestive system (Pajor et al., 1991; Michiels et al., 2013) in high BiW piglets. This, in turn, could lead to an increase in the intake capacity of sow milk and dry feed. Moreover, higher BiW piglets have a greater ability to occupy and stimulate the best performing teats, thereby allowing them to ingest more milk (Scheel et al., 1977).

DM intake of feed

Feed intake explained variation in BW gain in especially middle and late lactation: only 3.9% of the variation in BW gain in week 2−4 was explained by DM intake of sow feed, while 15.1% and 25.9% of the variation was explained by DM intake of piglet feed in week 4−6 and 6−8, respectively. In addition, DM intake of sow feed was negatively related with BW gain in week 2−4, while DM intake of piglet feed was positively related with BW gain in week 4−6 and 6−8. It is a direct consequence of the increased DFI and reduced intake of milk, as the dependency of the piglets on solid feed increases with age, which probably was increased by the intermittent suckling strategy (Berkeveld et al., 2007) that started at 4 weeks of age. Dry matter intake of sow feed was limited as piglets could only access sow feed during sow feeding times, which likely explains the absence of a relationship between DM intake of sow feed and BW gain in each of the three periods.

The negative regression coefficient of DM intake of sow feed with BW gain in week 2−4 indicates a higher consumption of sow feed for slow growing piglets. This might be linked with a lower milk intake, as we found that the presence at FM teats which are normally considered to have good milk output tended to be negatively related with DFI in week 2−4. Thus, in early lactation, faster growing piglets seem to consume more milk, than slower growing piglets, who ingested more solid feed as compensation.

Interestingly, similar to DM intake of sow feed, contacting feed during sow feeding times explained more variation in BW gain in week 2−4 compared to week 4−6 and 6−8 and the regression coefficient was positive. This might be linked with floor feeding of the sows, which is thought to enhance feed intake of piglets by vertical social learning, especially in early lactation (Oostindjer et al., 2011). As piglet dependence from the sow decreases, this effect disappears.

Solid feed intake explained more variation in BW gain than BiW did in middle and late lactation. As this is the first study that quantitatively estimated feed intake of individual piglets, previous studies have not reported such relationships at piglet level, but have reported a similar difference at litter level (Lodge and McDonald, 1959). As DM intake of piglet feed explained a relatively high portion of variation in BW gain, especially in week 4−6 and 6−8, understanding which factors influence feed intake variation is helpful to design potential intervention strategies to reduce variation in BW gain.

Ingestion behaviour

In our study, ingestion behaviour was measured by recording the frequency of contacting feed during the day (from 0900 to 1600h). This variable hardly explained variation in BW gain and was not related with BW gain in week 2−4, likely because piglets mainly rely on milk. In week 4−6, ingestion behaviour was the second most important variable to explain variation in BW gain (10.7%) after DM intake of piglet feed (15.1%); while in week 6−8 it hardly explained variation in BW gain and was not related with BW gain. This might be linked with changes in feeding patterns from week 4−6 to week 6−8. In a review of Bus et al. (2021), feeding frequency generally reduces over time, while intake per visit increases over time in growing pigs. For piglets in semi-natural conditions, ingestion of significant quantities of solid food begins at 4−5 weeks, and increases considerably between 6 and 8 weeks (Petersen et al., 1989; Jensen, 1995). In our study, we observed that the mean ingestion frequency decreased in week 6−8 compared to week 4−6, and the feed intake per ingestion event increased in week 6−8 compared to week 4-6, where 64.8% of the piglets had a decreased ingestion frequency and 86.7% of the piglets had an increased feed intake per ingestion event (data not shown). This indicates that there is individual variation in meal patterns over time, confirming findings of van Erp

(2019). It also indicates that ingestion frequency alone does not accurately reflect daily feed intake and BW gain in late lactation.

Teat presence

The frequency of piglets being present at teats during suckling bouts was scored. In week 2−4, only a small percentage of the variation in BW gain was explained by both the presence at FM teats (0.1%) and the rear teats (0.5%). The presence at FM teats (4.0%) and rear teats (1.2%) explained more variation in BW gain in the single regression than in the multiple regression in week 2−4. Possibly, in the multiple regression part of the variation was attributed to BiW, as the presence at FM teats was significantly correlated with BiW (r=0.3, P=0.026). In the multiple regression, the presence at FM teats explained less variation in BW gain in week 2−4 than variation explained by BiW (12%). This is similar with previous findings (Fraser and Jones, 1975; Fraser et al., 1979). The presence at FM teats and at rear teats had a positive and negative regression coefficients though not significant in week 2−4 respectively. Similarly, piglets suckling front and middle teats had higher BW gain than those suckling rear teats before weaning (Kim et al., 2000; Huting et al., 2019). This could be associated with the difference in milk yield among teats (Skok et al., 2007). The reason could be that high BiW piglets tend to win more teat disputes which allows them to occupy teats with more milk yield (Scheel et al., 1977). Indeed, in our study in week 2−4 the correlation between BiW and FM teats was positive (r=0.3, P=0.026), while the correlation between BiW and rear teats was negative (r=−0.1, P=0.418). Therefore, split-weaning of heavy piglets may be a strategy that allows low BiW piglets access to productive teats, which might positively affect piglet homogeneity in later life.

In middle and late lactation, both of presence at FM and rear teats explained less variation in BW gain in week 4−6 (2.4% and 4.8%) and in week 6−8 (2.6% and 0.1%) compared to the DM intake of piglet feed in week 4−6 (15.1%) and week 6−8 (25.9%). Similarly, Lodge and McDonald (1959) found that 10% and 77% of the between-litter variation in BW of pigs at week 8 p.p. were explained by milk consumption and creep feed consumption to 8 weeks, respectively (Lodge and McDonald, 1959). This logically follows from the reduced dependency of the piglets on milk.

Behaviours

The frequency of positive behaviours (the sum of play and nosing behaviour) was retained in the multiple regression model after forward selection in week 2−4 and accounted for 7.6% of the variation in BW gain. Likely, play behaviour was the underlying cause of this effect, as it also showed a positive correlation with BW gain in the single regression in week 2−4. It corresponds to the finding by Brown et al. (2015) in piglets on days 0−27, and Šilerová et al. (2010), who found that the relationship persisted after weaning on day 39. Play can have both immediate and long-lasting benefits (Pellis et al., 2010). As play behaviour in early life benefits muscle and bone development (Fagen, 1976; Graham and Burghardt, 2010), stimulating play behaviour in early life for example by providing more enrichment materials (Yang et al., 2018) might have potential positive effects on BW gain that may be preserved in later life. Interestingly, positive (play) behaviour did not explain a sizeable portion of BW variation in later lactation. Relationships between negative and explorative behaviours and BW gain were not observed in this study.

Skin lesions

The number of skin lesions explained more variation in BW gain in week 4−6 (4.2%) and 6−8 (2.5%) compared to week 2−4 (1.0%) and the number of skin lesions tended to be positively related with BW gain in week 4−6. Similarly, a positive correlation between BW and lesion scores was found in post-weaned pigs after week 4 (Turner et al., 2006). Skin lesion score provides a more sensitive measure of the duration of aggression (Turner et al., 2006) and aggressive interactions (Yang et al., 2018) than observations on the frequency of aggressive behaviour. Indeed, it has been reported that heavier piglets are more involved in fighting, win more fights (D'Eath, 2002) and had more skin lesions (Yang et al., 2018).

In socially stable groups, the majority of aggressive behaviours occurs around the feeding area to obtain limited food resources (Hoy et al., 2012). In our study the piglet: feeding place ratio was fixed (6:1) which may explain the stronger relationship between skin lesions and BW gain with progressing age.

Estimated breeding value of BW gain from birth to slaughter

The genetic background of piglets affects their growth performance (Van der Peet-Schwering et al., 2013). In our study, the EBV for BW gain from birth to slaughter was assessed in all piglets. This parameter, however, hardly explained variation in BW gain in multiple and single regression analyses (week 2−4: 3.7%, 0.4%, week 4−6: 0.1%,1.0%, week 6−8: 2.4%, 0.2%) and only tended to be positively related with BW gain in week 2−4. It might be that a breeding value that estimates BW gain from birth to slaughter does not reflect BW gain in early life very well. In addition, genotype × environment interactions might play a role, as piglets were group housed and could interact with group mates. Also, in week 2−4, BiW explained 12.0% of the variation in BW gain. This might indicate that the genetic contribution to early life BW gain is relatively limited compared to birthweight.

3.4.3 Exploring variation in feed intake by multiple linear regression

Dry matter feed intake was explained for less than 21% by all factors considered in the three periods.

Birthweight

Birthweight explained 2.8%, 9.6% and 2.8% of variation in DFI in week 2−4, 4−6 and 6−8, respectively. In a conventional housing system, 9% of explained variation was found in piglets on days 10−28 p.p. (Pajor et al., 1991). Also, BiW tended to be positively related with DFI in week 4−6 and 6−8 and similar positive relationships between BiW and DFI were found in pigs before (Pajor et al., 1991; Huting et al., 2019) and after conventional weaning (Bérard et al., 2010; Huting et al., 2019), and in pigs which were kept with their mother in week 4−8 p.p. (Bøe and Jensen, 1995). In our study, BiW had limited effect on DFI in week 2−4 when milk intake dominates, and in this period the influence of BiW on BW gain is likely mediated through milk intake.

Teat presence

The presence at FM teats explained the highest portion of variation in DFI in week 2−4 (but still only 2.9%) compared to other variables. It tended to be negatively related with DFI in week 2−4 and 6−8 and had a negative regression coefficient in week 4−6. It indicates that piglets which are present more at FM teats ate less solid feed. Similarly in conventional housing systems, it was found that during week 3−4 before weaning, piglets that suckled at FM teats were less likely to eat creep feed compared to piglets that suckled rear teats (Huting et al., 2019).

Piglets that suckled more at FM teats, did so in consecutive periods: week 2−4 and 4−6 (r=0.73, P <0.001) and week 4−6 and 6−8 (r=0.48, P <0.001). Similarly, Barber et al. (1955) found that piglets with the highest milk intake during the first 3 weeks of lactation also had the highest milk intake during the following 5 weeks up to weaning at 8 weeks.

Behaviours

Similar to the multiple regression of BW gain, positive, negative and explorative behaviours explained little variation in DFI in the three periods. Previous studies showed that piglet feeding activity was associated with exploration around the trough (Delumeau and Meunier-Salaün, 1995) and stimulation of exploratory behaviour using specific feeders and feed sources increased creep feed intake of piglets (Kuller et al., 2010; Middelkoop et al., 2019). However, in those studies exploratory behaviour included nosing, sniffing and rooting on the feed and feeders, while not in current study.

Skin lesions

Skin lesions explained 5.1% of the variation in DFI in week 6−8 and tended to be positively related with DFI in week 6−8. It might be that piglets with a greater need to utilize the feeders involves in more fighting for food sources (Algers et al., 1990), and thus have more skin lesions and higher feed intake.

3.4.4 Unexplained variation and future research

In each of the three periods, more than 55% of the variation in BW gain and 79% of the variation in DFI remained unexplained. It is possible that the inherent variability associated with some explanatory variables contributed to this unexplained variation.

The accuracy of feed intake estimation, as described in Tang et al. (2022), was around 10−15% of the deviation from measured feed intake, which could possibly have contributed to this high unexplained variation. Further research to improve the accuracy of feed intake estimation is required. The inherent variability in behaviours remains unknown, as we only observed each focal piglet for 50 minutes per day for one day in each period. More days of behavioural observations are required.

Further investigations of other potentially influential factors are also required. Additional variables potentially contributing to the variation in BW gain and DFI of piglets include sow related variables including DFI and milk production of sows (Ramanau et al., 2004; Strathe et al., 2017); piglet related variables, including colostrum intake (Devillers et al., 2004), social rank (McBride et al., 1964; Bus et al., 2021), personality (O'Malley et al., 2019), digestive efficiency (Douglas et al., 2014; Gaillard et al., 2020), health status (Pastorelli et al., 2012; Van der Meer et al., 2020; Bus et al., 2021) and group size (Turner et al., 2003).

3.5 Conclusion

To conclude, a multiple regression analysis revealed that 19.7% of the individual variation in BW gain of piglets in a multi-suckling system during a 9-week lactation was explained by birthweight, play and nosing behaviour in week 2−4, and 15.1% and 25.9% by solid feed intake of piglets in week 4−6 and 6−8. It also revealed that 2.9% of the individual variation in DFI in week 2−4 was explained by the presence at front and middle teats, 9.6% by birthweight in week 4−6, and 5.1% by the number of skin lesions in week 6−8. Further investigation of other potentially influential factors is required.

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3.7 Supplemental materials

Supplementary Table S1. Ingredients and calculated nutrient composition of the sow diet during a 9-week lactation in a multi-suckling system.

Ingredients, %					
Barley	4.0	Beet pulp	2.0	Salt	0.6
Maize	20.5	Beet molasses	2.0	L-lysine 50% VL	0.55
Wheat	26.0	Lucerne	15.0	L-threonine	0.12
Rapeseed meal	3.0	Fish oil	0.3	DL-methionine	0.05
Palm kernel expeller	2.8	Soybean oil	0.7	L-tryptophan (25%)	0.05
Soybean meal	7.8	Palm oil	2.6	Hiphos liquid	0.0074
Short meal	7.9	Limestone	0.9	Premix*	0.635
Soybean hulls	2.0	Monocalcium Phosphate	0.4		
Calculated nutrient composition, g/kg					
DM	891.9	Phosphorus	4.5		MJ/kg
CP	146.3	Digestible phosphorus	3.2	Net energy	9.4
Crude fat	62.9	Magnesium	3.0		
Crude fibre	86.1	Potassium	10.6		
Ash	66.2	Sodium	2.7		
Starch	339.8	Non starch polysaccharides	244.0		
Total sugars	44.5	Fermentable non starch polysaccharides	138.5		
Calcium	9.0				
Amino acids (g/kg)					
Apparent ileal digestible lysine	7.02	Apparent ileal digestible threonine	4.37		
Apparent ileal digestible methionine	2.19	Apparent ileal digestible tryptophan	1.38		
Apparent ileal digestible cystine	3.79	Apparent ileal digestible valine	4.73		

* Vitamin and mineral premix (per kg of feed): Vitamine A: 12000 IU, 25-hydroxychoecalcifercl: 50.0 µg, Vitamine E: 75.0 mg, Vitamine C: 0.0 mg, Vitamine B1: 2.0 mg, Vitamine B2: 10.0 mg, Vitamine B6: 6.0 mg, Vitamine B12: 0.1 mg, Cholinechloride: 250.0 mg, Biotine: 0.6 mg, L-carnitine: 50.0 mg, Iron: 411.6 mg, manganese: 115.8 mg, zinc: 130.1 mg, copper: 20.1 mg, iodine: 2.3 mg, selenium: 0.6 mg, niacin: 94.9 mg, folic acid: 0.7 mg, biotin: 0.8 mg.

Supplementary Table S2. Ingredients and calculated nutrient composition of the piglet pre-starter diet provided from 20 days of age during a 9-week lactation in a multi-suckling system.

Ingredients, %					
Barley	37.4	Sunflower meal	2.0	Calcium formate	0.75
Corn	0.0	Fish oil	0.5	L-lysine HCl	0.50
Soybean meal	6.3	Soja oil	1.0	DL-methionine	0.175
Processed corn	15.0	Fibre cell (lignocellulose)	1.0	L-threonine	0.195
Wheat	15.0	Premix (maize)	0.5	L-tryptophan	0.075
Whey powder sweet	7.5	Lime fine	0.1	L-valine	0.070
Soybeans full fat toasted	5.0	Mono calcium phosphate	0.5	Fumaric acid	1.00
Sugar beet pulp	2.0	Salt	0.25	Phyzyme	0.005
Potato protein	3.0	$NaHCO_3$	0.20		
Calculated nutrient composition, g/kg					
DM	885.8	ADF	46.6		MJ/kg

Crude ash	40.6	Calcium	6.0	Net energy	10.38
CP	164.4	Phosphorus, total	5.9	Linolic acid	18.0
Crude fat	47.2	Phosphorus, digestible	3.4		
Crude fibre	43.1	Magnesium	1.3		
Carbohydrates	581.5	Potassium	7.3		
Starch	392.6	Sodium	2.4		
Sugars	80.2	Chloride	4.8		
NDF	107.6	Base-excess (mg/kg)	157.9		
Amino acids (g/kg)					
Apparent ileal digestible lysine	10.38	Apparent ileal digestible threonine	6.57		
Apparent ileal digestible methionine	3.97	Apparent ileal digestible tryptophan	2.19		
Apparent ileal digestible cystine	2.15	Apparent ileal digestible valine	6.87		

Supplementary Table S3. Ingredients and calculated nutrient composition of the piglet starter diet provided from 37 days of age during a 9-week lactation in a multi-suckling system.

Ingredients, %					
Barley	43.8	Sunflower meal	3.0	Calcium formate	0.50
Corn	8.0	Fish oil	0.5	L-lysine HCl	0.45
Soybean meal	12.0	Soja oil	1.0	DL-methionine	0.15
Processed corn	0.0	Fibre cell (lignocellulose)	1.0	L-threonine	0.25
Wheat	16.0	Premix (maize)	0.5	L-tryptophan	0.055
Whey powder sweet	3.0	Lime fine	0.15	L-valine	0.040
Soybeans full fat toasted	5.0	Mono calcium phosphate	0.65	Fumaric acid	0.5
Sugar beet pulp	2.0	Salt	0.25	Phyzyme	0.005
Potato protein	1.0	$NaHCO_3$	0.20		
Calculated nutrient composition, g/kg					
DM	877.7	ADF	54.3		MJ/kg
Crude ash	42.3	Calcium	5.6	Net energy	10.09
CP	173.5	Phosphorus, total	6.3	Linolic acid	17.3
Crude fat	47.3	Phosphorus, digestible	3.6		
Crude fibre	49.2	Magnesium	1.5		
Carbohydrates	562.3	Potassium	7.8		
Starch	392.1	Sodium	2.1		
Sugars	55.4	Chloride	3.7		
NDF	119.8	Base-excess (mg/kg)	187.2		
Amino acids (g/kg)					
Apparent ileal digestible lysine	10.16	Apparent ileal digestible threonine	7.08		
Apparent ileal digestible methionine	3.70	Apparent ileal digestible tryptophan	2.12		
Apparent ileal digestible cystine	2.28	Apparent ileal digestible valine	6.70		

Supplementary Table S4. Results of the single linear regression analysis between BW gain or DM intake of total feed (**DFI**) of piglets and single explanatory variables in the three periods during a 9-week lactation in the multi-suckling system. The response variables were the residuals of BW gain and DFI corrected for sex and batch.

Explanatory variables[1]	Response variables					
	BW gain			DFI		
	Weeks 2−4	Weeks 4−6	Weeks 6−8	Weeks 2−4	Weeks 4−6	Weeks 6−8
Birthweight (kg)	0.1** (12.0)[2]	0.1* (7.1)	0.1* (8.3)	0.0 (1.3)	0.2* (9.1)	0.1 (2.4)
EBV (g/day)	-0.2 (0.4)	-0.5 (1.0)	0.3 (0.2)	0.3 (0.7)	-3.0* (7.6)	1.3 (0.8)
DM Intake of sow feed (g/day)	-0.1 (1.4)	0.1 (0.9)	-0.1 (4.6)	---	---	---
DM Intake of piglet feed (g/day)	-0.1 (0.3)	0.2** (16.7)	0.2** (25.9)	---	---	---
Contacting feed during sow feeding times (% of observed time)	1.1 (3.8)	0.4 (0.6)	0.1 (0.0)	-0.0 (0.0)	0.6 (0.3)	-0.1 (0.0)
FM teats (no. of times)	2.7 (4.0)	-0.8 (0.1)	7.1 (1.5)	-2.9 (2.9)	-7.2 (1.7)	-6.3 (0.4)
Rear teats (no. of times)	-1.6 (1.2)	-5.0 (3.7)	-8.4 (1.3)	2.6 (1.9)	6.1 (1.0)	-4.2 (0.1)
Skin lesions (no.)	0.5 (1.0)	2.0[tend] (5.2)	4.1* (7.5)	-0.7 (1.4)	3.7 (3.3)	6.2[tend] (5.1)
Positive behaviour (no. of times /hour)	1.1[tend] (5.6)	0.1 (0.0)	-2.5 (2.2)	0.2 (0.2)	-0.7 (0.2)	-2.3 (0.6)
Negative behaviour (no. of times /hour)	1.7 (2.4)	1.7 (1.4)	4.6 (1.8)	1.7 (1.6)	3.0 (0.8)	4.8 (0.6)
Explorative behaviour (no. of times /hour)	1.0 (2.4)	0.0 (0.0)	-0.3 (0.0)	0.6 (0.5)	-0.1 (0.0)	-1.4 (0.2)
Ingestion behaviour (no. of times /hour)	-0.2 (0.1)	2.1** (12.0)	0.2 (0.0)	-0.5 (0.8)	3.2[tend] (5.3)	-0.6 (0.1)
Play behaviour (no. of times /hour)	1.8* (7.9)	-0.4 (0.1)	4.2 (1.1)	0.3 (0.1)	-5.5 (2.8)	3.2 (0.2)
Nosing behaviour (no. of times /hour)	0.5 (0.5)	0.4 (0.1)	-5.3[tend] (6.0)	0.3 (0.1)	1.4 (0.4)	-4.7 (1.5)
Manipulative behaviour (no. of times /hour)	0.6 (0.2)	-1.9 (0.9)	5.1 (1.4)	3.4 (3.4)	6.4 (1.8)	3.4 (0.2)
Aggressive behaviour (no. of times /hour)	7.2* (8.3)	7.1*(9.2)	5.4 (0.6)	-0.8 (0.1)	0.0 (0.0)	11.2 (0.8)

[1] Abbreviation: EBV: the estimated breeding value of BW gain from birth to slaughter; FM teats: the presence at the front and middle teats; Rear teats: the presence at the rear teats.

Variables: (1) BW gain (g/day): the difference between BW on different days divided by numbers of days between both days, during days 14−27 (weeks 2−4), days 27−44 (weeks 4−6), days 44−58 (weeks 6−8); (2) Nutrient intake (g/day): Individual daily DM intake of sow feed and piglet feed was measured per focal piglet on days 25−26 (week 4), days 42−43 (week 6) and days 56−57 (week 8); (3) Contacting feed during sow feeding times (% of observations): contacting (i.e. sniffing or eating) sow feed or piglet feed during sow feed times on days 19−20 at 0800−0830 h and 1600−1630 h, and on days 40−43 and 54−57 at 1600−1630 h; (4) Suckling behaviour (on days 20, 41, 55 at 0900 h−1600 h): FM teats (no. of times): the frequency of presence at front (first 2 pairs) and middle pairs of teats of each focal piglet in all suckling bouts during the day; Rear teats (no. of times): the frequency of presence at rear (last 2 pairs) of teats of each focal piglet in all suckling bouts during the day; (5) Skin lesions (no.): the number of fresh superficial and deep lesions on the whole body, except for ears and tails on days 27, 44 and 58. (6) Behaviours (on days 19, 40, 54): Positive behaviour (no. of times /hour): the frequency of play and nosing/nose contacting sows and piglets during the day; Negative behaviour (no. of times /hour): the frequency of manipulating sows and piglets, belly nosing, and aggressive behaviour during the day; Explorative behaviour (no. of times /hour): exploring the system, i.e., nibbling, sucking or chewing on parts of the multi-suckling system such as iron bars, walls, door, floor, ropes, hessian sacks and straw; Ingestion behaviour (no. of times /hour): sniffing/nosing/eating sow feed and piglet feed, drinking during the day;

The first given value is the regression coefficient (β) and the value between brackets is the squared correlation coefficient (R^2) multiplied by 100, which represents the percentage of the variation in BW gain and DFI that were explained by single explanatory variables in the model. The significance level of β is indicated with * 0.01<*P*<0.05, ** *P*<0.01; 'tend' 0.05 <*P* <0.10.

Supplementary Table S5. Pearson and spearman correlations[1] among variables measured in piglets in three periods during a 9-week lactation in the multi-suckling system.

Method	Week					
Pearson	Weeks 2-4		BiW[2]	EBV	DM intake of sow feed	DM intake of piglet feed
		BiW	--	--	--	--
		EBV	-0.3*	--	--	--
		DM intake of sow feed	0.2	0.0	--	--
		DM intake of piglet feed	0.2	-0.3*	0.7**	--
	Weeks 4-6		BiW	EBV	DM intake of sow feed	DM intake of piglet feed
		BiW	--	--	--	--
		EBV	-0.3*	--	--	--
		DM intake of sow feed	0.0	0.2	--	--
		DM intake of piglet feed	0.2[tend]	-0.2 [tend]	-0.2	--
	Weeks 6-8		BiW	EBV	DM intake of sow feed	DM intake of piglet feed
		BiW	--	--	--	--
		EBV	-0.3*	--	--	--
		DM intake of sow feed	0.1	0.2	--	--
		DM intake of piglet feed	0.0	0.0	-0.6**	--

Spear-man	Weeks 2-4	BiW	EBV	Sow feed	Piglet feed	FM teats	Rear teats	Contacting feed during sow feeding times	Ingestion behaviour	Positive behaviour	Negative behaviour	Explorative behaviour	Skin lesions
	FM teats	0.3*	-0.1	-0.1	0.0	--	--	--	--	--	--	--	--
	Rear teats	-0.1	-0.2	0.0	0.0	-0.5**	--	--	--	--	--	--	--
	Contacting feed during sow feeding times	0.3 tend	-0.5**	0.0	0.2	0.2	0.1	--	--	--	--	--	--
	Ingestion behaviour	0.0	0.1	0.2 tend	0.2 tend	0.2	-0.1	0.0	--	--	--	--	--
	Positive behaviour	-0.1	0.0	0.1	0.1	0.1	-0.1	-0.2	0.2	--	--	--	--
	Negative behaviour	0.0	-0.2	0.0	-0.1	0.0	0.0	0.0	0.1	0.5**	--	--	--
	Explorative behaviour	0.1	0.1	0.0	0.2	0.1	0.0	-0.2	0.4**	0.4**	0.4**	--	--
	Skin lesions	0.1	0.0	0.2 tend	0.2	0.0	0.0	-0.1	0.4**	0.0	0.1	0.4**	--

Spear -man	Weeks 4-6	BiW	EBV	Sow feed	Piglet feed	FM teats	Rear teats	Contacting feed during sow feeding times	Ingestion behaviour	Positive behaviour	Negative behaviour	Explorative behaviour	Skin lesions
	FM teats	0.2	0.0	0.0	-0.2	--	--	--	--	--	--	--	--
	Rear teats	0.0	-0.3*	-0.2	0.1	-0.6**	--	--	--	--	--	--	--
	Contacting feed during sow feeding times	0.1	-0.1	0.3**	-0.1	0.0	-0.1	--	--	--	--	--	--
	Ingestion behaviour	0.3*	-0.2	0.2 tend	0.0	0.1	-0.1	0.1	--	--	--	--	--
	Positive behaviour	0.0	-0.2	-0.2	-0.1	0.0	0.3*	0.0	0.0	--	--	--	--
	Negative behaviour	0.1	-0.1	-0.1	0.1	0.0	0.1	0.0	0.1	0.7**	--	--	--
	Explorative behaviour	-0.1	0.0	0.1	-0.1	-0.1	0.1	0.2	0.2	0.6**	0.6**	--	--
	Skin lesions	0.3*	-0.2 tend	0.0	0.0	0.0	0.1	0.1	0.2tend	-0.1	-0.2	-0.1	--

Spear-man	Weeks 6-8	BiW	EBV	Sow feed	Piglet feed	FM teats	Rear teats	Contacting feed during sow feeding times	Ingestion behaviour	Positive behaviour	Negative behaviour	Explorative behaviour	Skin lesions
	FM teats	0.4**	0.0	0.4**	-0.3*	--	--	--	--	--	--	--	--
	Rear teats	0.1	-0.1	0.2 tend	-0.2	-0.2	--	--	--	--	--	--	--
	Contacting feed during sow feeding times	0.0	0.1	0.2 tend	-0.2	0.0	0.0	--	--	--	--	--	--
	Ingestion behaviour	0.0	0.0	0.3 tend	-0.1	0.5**	-0.1	0.0	--	--	--	--	--
	Positive behaviour	0.0	0.2 tend	0.1	-0.1	0.0	0.0	0.0	0.2	--	--	--	--
	Negative behaviour	0.0	0.3*	0.1	0.0	0.2	0.0	0.1	0.0	0.5**	--	--	--
	Explorative behaviour	-0.1	0.2	0.1	-0.1	-0.2 tend	-0.1	0.0	-0.2	0.6**	0.3**	--	--
	Skin lesions	0.1	-0.2	0.1	0.1	0.1	0.3*	-0.1	0.1	-0.1	0.1	0.0	--

[1] The significance level of *r* is indicated with * 0.01<*P*<0.05, ** *P*<0.01; 'tend' 0.05 <*P* <0.10.

[2] BiW: Birthweight (kg). The explanation of other variables was indicated in Supplementary Table S4.

Supplementary Table S6. Multiple linear regression of BW gain and DM intake of total feed (DFI) of piglets on multiple explanatory variables in three periods during a 9-week lactation in the multi-suckling system. The response variables were the residuals of BW gain and DFI corrected for sex and batch.

	BW gain						
			Forward selection			Multiple regression	
	Step	Explanatory Variables[1]	Partial R^2 (× 100)[2]	Model R^2 (× 100)[3]	*P* value selection	Partial Regression coefficient (β)[4]	*P* value of β
Weeks 2-4	1	Birthweight	12.0	12.0	0.007	0.1	0.004
	2	Positive behaviour	7.6	19.7	0.024	1.6	0.023
	3	DM intake of sow feed	3.9	23.6	0.096	-0.3	0.125
	4	Contacting feed during sow feeding	4.1	27.7	0.082	2.0	0.023
	5	EBV	3.7	31.4	0.094	0.8	0.095
	6	Skin lesions	1.0	32.4	0.386	0.6	0.342
	7	Negative behaviour	0.5	32.9	0.540	1.3	0.422
	8	Ingestion	0.5	33.4	0.547	-0.4	0.525
	9	Rear teats	0.5	33.8	0.551	-0.5	0.824
	10	DM intake of piglet feed	0.3	34.2	0.621	0.3	0.577
	11	FM teats	0.1	34.3	0.788	0.7	0.763
	12	Explorative behaviour	0.1	34.4	0.804	-0.3	0.804
Weeks 4-6	1	DM intake of piglet feed	15.1	15.1	0.002	0.1	0.015
	2	Ingestion	10.7	25.8	0.006	1.5	0.055
	3	Rear teats	4.8	30.6	0.057	-12.6	0.005
	4	Skin lesions	4.2	34.8	0.068	2.0	0.085
	5	FM teats	2.4	37.2	0.160	-6.8	0.081
	6	Birthweight	2.0	39.2	0.195	0.0	0.189
	7	Positive behavior	1.5	40.7	0.262	1.7	0.226
	8	Explorative behaviour	0.7	41.4	0.441	-1.0	0.412
	9	DM intake of sow feed	0.8	42.2	0.421	0.1	0.435
	10	EBV	0.1	42.3	0.731	0.2	0.763
	11	Contacting feed during sow feeding	0.1	42.4	0.781	-0.2	0.787
	12	Negative behaviour	0.0	42.4	0.990	0.0	0.990
Weeks 6-8	1	DM intake of piglet feed	25.9	25.9	<.0001	0.2	0.004
	2	Birthweight	7.5	33.4	0.016	0.1	0.056
	3	FM teats	2.6	35.9	0.149	5.4	0.613
	4	Skin lesions	2.5	38.5	0.145	2.9	0.134
	5	EBV	2.4	40.8	0.155	1.4	0.185
	6	Contacting feed during sow feeding	1.7	42.6	0.220	1.5	0.175
	7	Positive behaviour	0.5	43.1	0.509	-2.5	0.312
	8	DM intake of sow feed	0.5	43.6	0.514	-0.1	0.427
	9	Ingestion	0.5	44.1	0.515	1.6	0.400
	10	Negative behaviour	0.5	44.5	0.538	2.3	0.644

	11	Explorative behaviour	0.3	44.8	0.651	0.9	0.710
	12	Rear teats	0.1	44.9	0.772	-3.1	0.772

	DFI						
			Forward selection			Multiple regression	
	Step	Explanatory variables	Partial R^2 (× 100)	Model R^2 (× 100)	*P* value selection	Partial Regression coefficient (β)	*P* value of β
Weeks 2-4	1	FM teats	2.9	2.9	0.198	-3.2	0.306
	2	Birthweight	2.8	5.7	0.197	0.0	0.127
	3	Skin lesions	2.5	8.1	0.226	-1.0	0.282
	4	Negative behaviour	1.5	9.6	0.343	1.9	0.390
	5	EBV	2.0	11.6	0.280	0.6	0.297
	6	Rear teats	0.5	12.0	0.602	1.3	0.690
	7	Explorative behaviour	0.3	12.4	0.655	0.6	0.623
	8	Ingestion	0.2	12.6	0.712	-0.3	0.712
	9	Contacting feed during sow feeding	0.1	12.7	0.786	0.3	0.799
	10	Positive behavior	0.0	12.8	0.954	-0.1	0.954
Weeks 4-6	1	Birthweight	9.6	9.6	0.017	0.1	0.129
	2	EBV	5.2	14.8	0.070	-2.3	0.154
	3	Ingestion	2.2	17.0	0.229	2.0	0.293
	4	FM teats	2.5	19.5	0.201	-10.5	0.283
	5	Rear teats	0.5	20.1	0.557	-4.2	0.704
	6	Skin lesions	0.2	20.2	0.756	1.2	0.694
	7	Positive behaviour	0.1	20.3	0.786	-2.2	0.561
	8	Negative behaviour	0.6	20.9	0.533	4.0	0.534
	9	Contacting feed during sow feeding	0.1	21.0	0.860	-0.3	0.867
	10	Explorative behaviour	0.0	21.0	0.989	0.0	0.989
Weeks 6-8	1	Skin lesions	5.1	5.1	0.086	6.8	0.091
	2	EBV	1.7	6.8	0.317	2.8	0.220
	3	Birthweight	2.8	9.6	0.202	0.3	0.051
	4	FM teats	1.6	11.2	0.323	-36.1	0.097
	5	Rear teats	2.9	14.0	0.190	-29.4	0.163
	6	Explorative behaviour	1.6	15.6	0.331	-4.5	0.349
	7	Negative behaviour	0.6	16.2	0.565	6.3	0.542
	8	Ingestion	0.4	16.6	0.630	2.2	0.571
	9	Contacting feed during sow feeding	0.2	16.8	0.714	0.8	0.713
	10	Positive behavior	0.1	16.9	0.838	-1.0	0.838

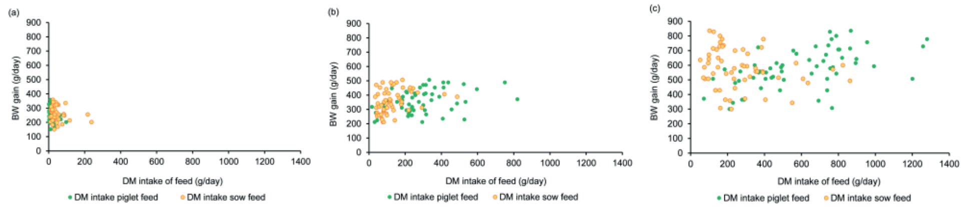
[1] The explanation of variables was indicated in Supplementary Table S4.

[2] Partial R^2 (× 100) is the partial squared correlation coefficient multiplied by 100, which represents the percentage of the variation in BW gain and DFI explained by the explanatory variables in the model.

[3] Model R^2 (× 100) is the cumulative squared correlation coefficient multiplied by 100, which is the R^2 from the previous variable plus the partial R^2 of the current explanatory variable and it represents the percentages of the variation in BW gain and DFI explained by having the identified explanatory variables in the model.

[4] The partial regression coefficient represents the effect of one explanatory variable when others were held constant.

Supplementary Figure S1



Scatter plots showing DM intake of piglet and sow feed versus BW gain of individual piglets in (a) weeks 2−4, (b) weeks 4−6 and (c) weeks 6−8 in the multi-suckling system. BW gain (g/day): the difference between BW on different days divided by numbers of days between both days, during days 14−27 (weeks 2−4), days 27−44 (weeks 4−6), days 44−58 (weeks 6−8); Individual daily DM intake of sow feed and piglet feed was measured per focal piglet on days 25−26 (week 4), days 42−43 (week 6) and days 56−57 (week 8).

Supplementary Material S1. Approximation of DM intake of piglets from incomplete data

Piglets with no faecal samples during the faecal collection period (8, 3, 2 pigs in weeks 4, 6 and 8) were omitted from the calculation. Piglets refusing more than 50% of the C32 boluses (5, 8 and 6 piglets in weeks 4, 6 and 8, respectively) were also omitted from the calculation.

Due to variable mixing of digesta and variable passage rates, the excretion rate of the reference marker may not be constant, hence leading to over or under representation of the reference marker in spot faecal samples, and leading to erroneous estimates of feed intake (see also (Tang et al., 2022)). For this reason, feed intake estimates were carefully checked, and considered unrealistic in the case that estimated feed intake of an individual piglet exceeded 2, 3 or 4 times the calculated energy requirements for maintenance (Blok et al., 2015) in weeks 4, 6 and 8, respectively. This resulted in unrealistic feed intake estimates of 1, 6, and 3 piglets in weeks 4, 6 and 8, respectively. For these piglets and for piglets refusing more than 50% of the C32 bolus per week (5, 8 and 6 piglets in weeks 4, 6 and 8, respectively), feed intake was estimated based on the linear regression between the intake of total feed and the concentration of in-feed marker in faeces of the focal piglets with successful feed intake estimates. The feed intake of these piglets was then calculated from this relationship using the concentration of in-feed marker in faeces of these piglets. To maximize use of data during the multiple regression analyses, for piglets with no faecal samples, feed intake was assumed the average intake of the remaining pigs in that period.

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Chapter 4: Effects of timing of grouping and split-weaning on growth performance and behaviour of piglets in a multi-suckling system

Tianyue Tang [a, b], Walter Gerrits [a], Nicoline Soede [b], Carola van der Peet-Schwering [c], Inonge Reimert [b*]

[a] Animal Nutrition Group, Department of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[b] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[c] Wageningen Livestock Research, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
* Corresponding author. E-mail address: inonge.reimert@wur.nl (Inonge Reimert)

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Abstract

Variation in body weight (**BW**) of piglets is a drawback for successful implementation of multi-suckling (**MS**) systems. The current study investigated the combination of two intervention strategies, i.e. the timing of grouping and split-weaning, aiming to improve the BW gain of low birthweight (**BiW**) piglets in an MS system and thereby reduce the BW variation at weaning on day 48 postpartum (*p.p.*). Eight batches of 5 sows with their litters were divided into 4 control (**CTRL**) and 4 treatment (**TREAT**) batches. In each litter, the second lowest (**LBW**) and highest (**HBW**) BiW piglets from both sexes were selected as focal piglets. CTRL piglets were grouped on day 8 *p.p.* and no split-weaning was applied; TREAT piglets were grouped on day 13 *p.p*. and the three heaviest non-focal piglets per litter were split-weaned on day 35 *p.p*. Behaviour and feed intake were measured in focal piglets, and BW and mortality were measured in all piglets. Results showed that: (1) Throughout lactation there were no differences in BW or BW gain between CTRL and TREAT, nor were BiW × treatment interactions found. (2) After grouping, there were no obvious differences between CTRL and TREAT in feeding and suckling behaviours on day 18, damage scores on snout, ear or tail and skin lesions on day 27, nor were BiW × treatment interactions found. (3) After split-weaning, in week 6, piglets in TREAT tended to consume less feed than CTRL (*P*=0.072). LBW piglets in TREAT consumed numerically less feed and spent numerically less time contacting feed during the day than CTRL piglets. In week 6, there was a significant BiW × treatment interaction in DM milk intake (*P*=0.030), caused by a higher milk intake of TREAT-LBW piglets compared with CTRL-LBW piglets. In week 6, TREAT piglets tended to be present more at front and middle teats (*P*=0.052), tended to have lower snout damage scores (*P*=0.084) and had lower restlessness scores during suckling bouts (*P*=0.028) than CTRL piglets. (4) Piglet crushing of all piglets in TREAT tended to be higher during the period when TREAT piglets were not yet grouped i.e. on days 9-14 than CTRL (*P*=0.087). To conclude, split-weaning of the heavy piglets increased milk intake particularly of low BiW piglets but this did not lead to a reduction in BW variation at weaning, as the increased milk intake was largely compensated for by a simultaneous decrease in feed intake.

Keywords

Co-mingling, Partial weaning, Piglet homogeneity, Suckling behaviour, Feeding behaviour, Group lactation

4.1 Introduction

Multi-suckling (**MS**) systems for sows and piglets have been developed as an alternative for conventional single litter housing systems to improve animal welfare. However, in such systems, BW variation of piglets at weaning was observed to be large (Thomsson et al., 2016; Van Nieuwamerongen et al., 2017). Our recent study in an MS system found that variation in BW gain in week 2-4 was mainly explained by birthweight and in week 4-8 by piglet feed intake (Tang et al., 2022b). The early stage of pigs' life is crucial as it can influence pigs' lifetime performance (Zeng et al., 2019). As low birthweight (**BiW**) piglets have the potential to compensate during their lifetime growth (Douglas et al., 2013), specific interventions might support these piglets to catch up. The current study aims to improve piglet homogeneity through improving performance of low BiW piglets in an MS system, by using a combination of two intervention strategies, one applied in early and one in late lactation.

The first intervention strategy applied in early lactation, is to delay the age at grouping of litters, from days 6-8 p.p. applied in earlier studies (Van Nieuwamerongen et al., 2017)(Tang, et al. 2022b) to days 13-14 p.p. Previous studies in group housing systems revealed that early grouping non-littermates during lactation, i.e. on day 5 p.p. helped piglets to stimulate social skills and reduce fighting in the post-weaning period after day 26 (Bohnenkamp et al., 2013). In addition, unacquainted pre-weaning piglets that were shortly placed together were reported to fight shorter and had fewer injuries at younger age (Pitts et al., 2000). However, grouping at a young age might lead to a higher occurrence of cross-suckling, when teat order and mother-piglet bond are not fully established (Vanheukelom et al., 2012; Downing, 2015). Cross-suckling can cause stress to piglets owing to teat competition and can result in a negative effect on piglet BW gain in MS systems (Olsen et al., 1998). Moreover, grouping at a young age might have a more negative effect on smaller piglets, as they have disadvantages in settling new dominance hierarchies and competing for milk with cross-sucklers. Later grouping has the potential to reduce the occurrence of cross-suckling (Dybkjær et al., 2003; Nicolaisen et al., 2019), as it allows piglets to develop the tight bonds with their mother prior to the socialization with other sows and their litters (Nicolaisen et al., 2019), which might especially benefit smaller piglets. Later grouping was also proposed to reduce the rate of mortality in MS systems (Dybkjær et al., 2003; Verdon et al., 2020), the reason could be that the reduced cross-suckling and reduced interrupted nursing helps to reduce restlessness of sows and thus leads to less crushing. Later grouping was even reported to have the potential for improving piglet homogeneity shortly after grouping in one study (Verdon et al., 2020): piglets grouped on day 14 p.p. had less variation in weaning BW on day 26 p.p. compared with piglets grouped on day 10 p.p.(Verdon et al., 2020). However in this study the reason of the reduced variation was not clear. In contrast, in one study no differences in variation of BW at weaning at week 6 were found when piglets housed in group lactation system from 7,14 or 21 days of age (Thomsson et al., 2016). In the current study, we hypothesized that later grouping will strengthen mother-piglet bond, and therefore increase teat presence and growth performance of low birthweight (**LBW**) piglets, and will also reduce mortality during lactation. As a communal feeding area is available for sows and piglets in our MS system (Tang et al, 2022b), the strengthened mother-piglet bond might also help small piglets to stay close to their mother during sow feeding times and thus may help them to adapt to solid feed earlier and increase their feed intake.

The second intervention, applied in late lactation, is split-weaning of the heaviest piglets per litter two weeks before final weaning. Split-weaning is thought to increase milk intake and pre-weaning performance for the remaining BW of piglets (Pluske and Williams, 1996; Vesseur et al., 1997). We expect that split-weaning could reduce the competition at the udder and thereby enable especially LBW piglets to have an increased milk consumption and thus increased growth rate. In addition, in our previous study in MS system (Tang et al., 2022b), a stronger relationship between skin lesions and BW gain, between skin lesions and solid feed intake with progressing age from week 4 to week 8 was found; feed competition was speculated to be larger from week 4 to week 6 as piglet: feeding place ratio was fixed (6:1). We expected that split-weaning could reduce the competition in the feeding area and thereby increasing the feed intake of LBW piglets. Several traits, including individual DM intake of feed and milk, suckling behaviours, feeding behaviours, damage scores on snout, ear and tail and skin lesions, and mortality were measured to evaluate the effect of the two interventions on BW and variation in BW in an MS system.

4.2 Materials and Methods

4.2.1 Animals, housing and experimental design

The experiment was conducted at the facilities of Swine Innovation Centre Sterksel in the Netherlands from January 23rd until November 4th, 2020 and was approved by the Animal Care and Use committee of Wageningen University & Research (Wageningen, the Netherlands). Eight consecutive batches of five multiparous sows (Topigs 20) and their litters (Tempo × Topigs 20) were kept per batch in a MS system (Fig. 1a–f) during a 7 week-lactation, with four control (**CTRL**) (batch 1, 4, 5, and 8) and four treatment (**TREAT**) (batch 2, 3, 6, and 7) batches. In total, eight batches of five sows (parity: 3.6 ± 0.2) with 160 focal piglets were studied. In each litter, two boars and two gilts with the second lowest and highest BiW within sex were selected as focal piglets on day 14 postpartum (**p.p.**). Sows within a batch were distinguished by different colours of marker sprays.

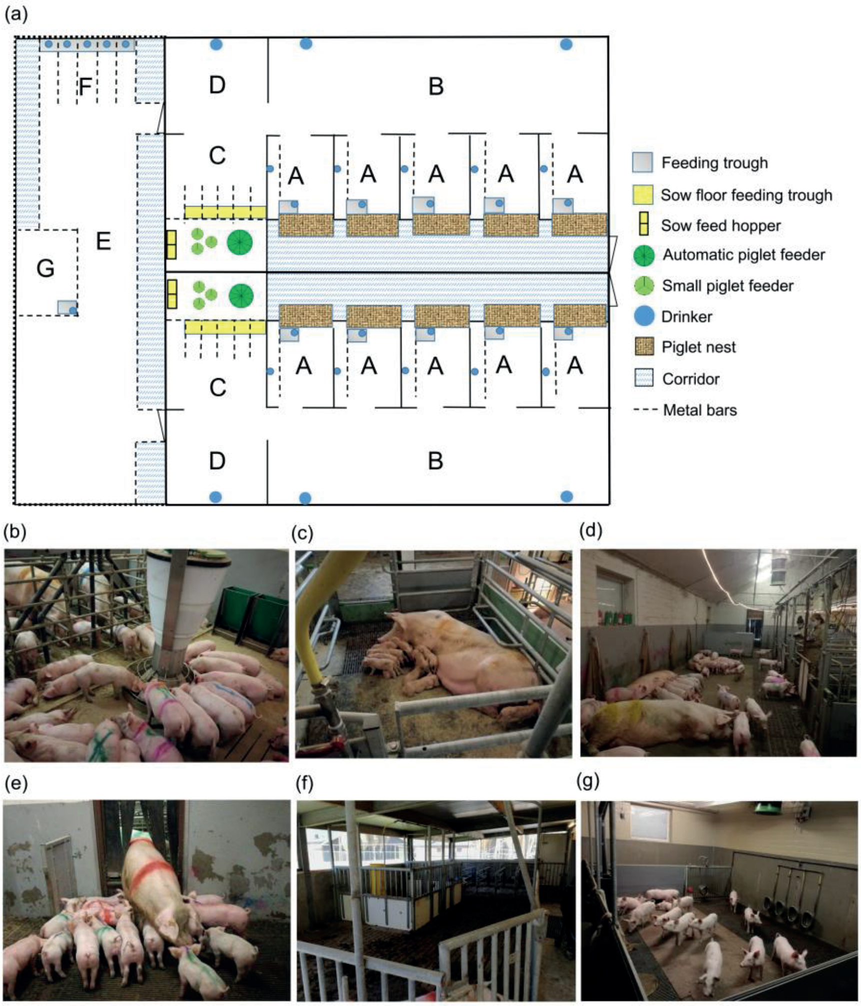


Figure 1. (a) Layout of the multi-suckling (MS) system for sows and piglets consisting of two MS units and an intermittent suckling (IS) area. Each MS unit contained 5 farrowing pens with piglet nests (A), a communal MS area which included a lying area (B), a feeding area (C) and a dunging area (D). Connected to the MS unit was the IS area (E), which included feeding stalls for sows (F) and a boar pen (G). (b) Communal feeding area. (c) Farrowing pen. (d) Communal MS area. (e) The area between MS area and IS area. (f) IS area. (g) Rearing department.

The MS system was previously described by Tang et al. (2022b). The MS system consisted of two MS units and one Intermittent Suckling (**IS**) area (Fig 1a). Each MS unit contained five farrowing pens (2.2 x 3.2 m), and a communal MS area including a lying area (11.1 x 2.80 m), a feeding area (4.2 x 3.3 m) and a dunging area (2.8 x 3.3 m). In each farrowing pen, there were a heated nest for the piglets (0.65 x 1.6 m), a feeding trough with a drinking nipple for the sows, and a water nipple for the piglets. Two extra drinking bowls were available for the sows and piglets in the lying area and in the dunging area. The feeding area contained five feeding places for the sows and a surrounding area which was accessible only to the piglets. In the five feeding places there was a stainless steel feeding trough on the floor with five feeding places, separated by horizontal metal bars, which was accessible to both sows and piglets. In the surrounding area, there were three small round feeders (diameter: 28 cm) (used until day 35 p.p.), a sensor-controlled automatic piglet feeder containing ten feeding places (Rondomat, Fancom B.V., the Netherlands) (used from day 28 p.p.) and two feed hoppers with sow feed (used from day 28 p.p.) to enable piglets access to sow feed both during the day and night.

One week before farrowing (Fig. 2), five sows were moved to the MS system, balanced for parity and expected farrowing date. Sows were only locked up in a crate in the farrowing pen in the first 3 days p.p. both during the day and night to prevent piglet crushing. Within 24–48 h p.p., litter sizes were standardized to 14 or 15 piglets per litter (average: 14.3 ± 0.1 piglets), based on the number of functional teats per sow. Piglets were ear-tagged within 24 h p.p. Piglets received an iron injection within 3 days p.p. and were vaccinated on day 19 p.p., but were not castrated nor tail-docked. Sows were able to access the communal area again from day 4 p.p. while piglets could not by setting a piglet barrier. On days 8–9 p.p. (average: 8.2 ± 0.1) in CTRL batches and on days 13–14 p.p. (average: 13.4 ± 0.1) in TREAT batches, piglets were also able to access the communal area by removing the piglet barrier. On days 28–34 p.p., forced IS was conducted by transferring sows to the IS area for 10 h/day (07:00 – 17:00 h), during which there was a boar in the boar pen to stimulate oestrus. On days 35–49 p.p., voluntary IS was applied, during which no boar was present in the IS area, and sows could freely choose to access both the MS and IS area by setting a flexible partition between the two areas which sows could step over while piglets could not. On day 35 p.p. in TREAT batches, the three heaviest non-focal piglets in each litter with a total number of 15 piglets/batch were split-weaned and transferred to a rearing department (4.1 x 2.6 m) until 7 weeks of age (Fig. 1g). One sow in TREAT batch 7 was seriously wounded during days 27–34 p.p. after being bitten by piglets, after which both the sow and her whole litter were removed from the MS system on day 35 p.p. and the piglets were not housed in the rearing department either. One litter in TREAT batch 6 only had 5 piglets on day 35 p.p., therefore no piglets in this litter were split-weaned on day 35 p.p. Piglets in CTRL and piglets remaining in the MS system in TREAT were weaned on day 48 p.p.

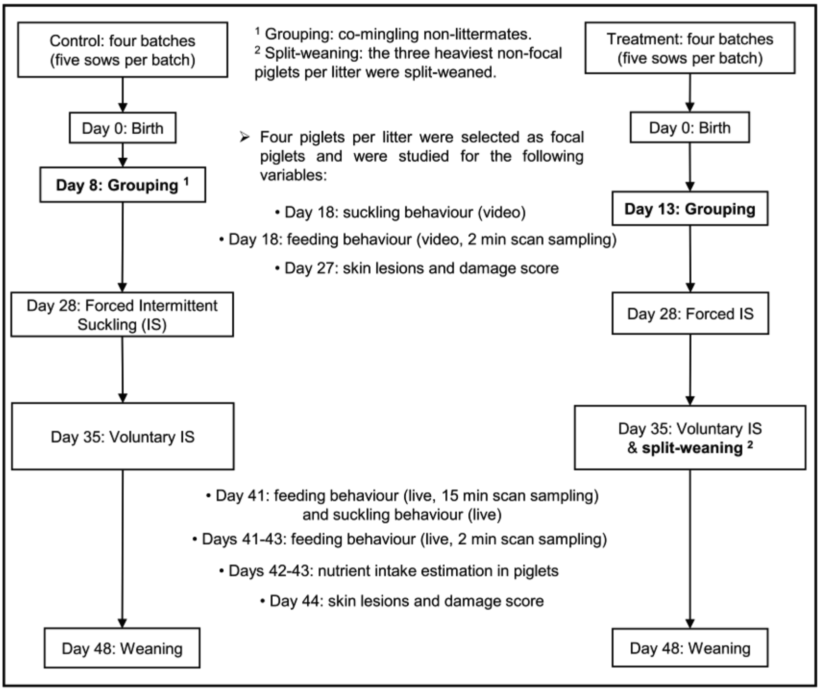


Figure 2. Time schedule of experiment setup of sows and piglets in control and treatment batches during a 7-week lactation in the multi-suckling system.

Provision of enrichment materials, temperature settings and the lighting schedule were as described in Tang et al. (2022b). In the rearing department, two hessian sacks and four ropes were provided and two handfuls of straw were provided twice/day; the temperature was set at 28 °C on day 35 and 24 °C on days 36–48; artificial lighting was on at 07:00 – 18:00 h.

4.2.2 Feeding regime

The ingredient and calculated nutrient composition of sow and piglet diets are described in (Tang et al, 2022b). Sows were fed twice daily at 08:00 and 16:00 h, with 3.0 kg/day of sow diet before farrowing and the amount gradually increased up to 8.5 kg/day on day 15 p.p. Before farrowing, sows were fed in the MS feeding area in the morning and in the farrowing pen in the afternoon. In the first 3 days p.p., sows were fed in the farrowing pen due to the confinement. On days 4–27, sows were fed in the MS feeding area. On days 28–34 during forced IS, sows were fed in the IS area at 07:30 h and in the MS feeding area at 17:30 h. On days 35–48, sows were fed in the MS feeding area at 08:00 and 16:00 h. Sows had *ad libitum* access to water via drinking bowls and nipples.

From 2 days p.p. until grouping, large feed pellets (8 mm diameter) were spread twice/day for piglets in the farrowing pen on the floor behind the metal bars of the crate to prevent sows accessing the feed (CTRL: on days 2–9 p.p., TREAT: on days 2–14 p.p.). After grouping, large pellets were provided in the part of the MS feeding area which was only accessible by the piglets for another 3 days, i.e. piglets in CTRL and TREAT received large pellets up to 12 and 17 days of age, respectively. After grouping, piglets could access sow feed in the MS feeding area from the floor feeding trough during sow feeding times from grouping until day 48 p.p. and from the two sow feed hoppers on days 21–48 p.p. Piglets in TREAT were also fed with sow feed in a small round feeder in the farrowing pen on days 9–14 p.p. before grouping. Piglets received a weaner diet from day 9 p.p.; this diet was provided to piglets in CTRL in the MS feeding area and to piglets in TREAT in one small round feeder in the farrowing pen until grouping, and in two small round feeders in the MS feeding area after grouping. On days 20–21 p.p. the weaner diet was mixed with a pre-starter diet, after which only the pre-starter diet was provided. On days 37−38 p.p. the pre-starter diet was mixed with a starter diet, after which only starter diet was provided until weaning at day 48 p.p. Piglets had *ad libitum* access to water via drinking bowls and nipples.

After grouping till day 35 p.p., piglet feed was provided in three small round feeders in the MS feeding area, which were re-filled twice daily. Sow feed was provided in two sow feed hoppers in the MS feeding area on days 21–48 p.p. which were replenished daily. Piglet feed was provided in the automatic piglet feeder on days 28–48 p.p.

In the rearing department on days 35–48 p.p., sow and piglet feed were provided separately via a feed hopper consisting of two compartments. For piglet feed, the pre-starter diet was provided on days 35–36 p.p. and was mixed with the starter diet on 37–38 p.p. On days 39–48, piglets were given only starter diet. The sow feed was provided on days 35–48 p.p. Four drinking nipples were provided.

4.2.3 Measurements

BW

Piglets were weighed on day 0, 6, 27, 35 and 48 p.p. Within-batch SE, SD and CV in BW and BW gain were calculated.

Video and live observations of feeding and suckling behaviours

Focal piglets within each batch were marked for recognition of low and high BiW class one day before the day for behavioural observation from video, and were marked for individual recognition before live observations.

Feeding behaviour during sow feeding times and during the day

Feeding behaviour of the piglets during sow feeding times was scored from video on day 18 p.p., and live on days 41–43 p.p. using 2-min instantaneous scan sampling at 08:00–08:30 h and 16:00–16:30 h. Feeding behaviour during the day was scored live on day 41 p.p. using 15-min instantaneous scan sampling at 08:30h–16:00h.

During these times, every 2 min (during sow feeding times) or 15 min (during the day) it was observed whether a piglet was in the feeding area, contacting (i.e. sniffing or eating) sow feed or contacting (i.e. sniffing or eating) piglet feed. For live observations, the percentage of time spent on contacting sow feed and piglet feed was calculated per piglet; for video observations, it was calculated per high or low BiW piglet.

Suckling behaviour

Suckling behaviour was scored from video at 08:30–16:00 h on day 18 p.p., and live at 08:30-16:00 h on day 41 p.p. A nursing bout was scored as 'unsuccessful' and excluded from analysis when a new nursing bout began within 20 min after a previous nursing bout (Weary et al., 2002), and no milk let-down was noted. The frequency of presence at the front pair (first two pairs), middle pair or rear pair (last two pairs) of teats were recorded. For live observations, the frequency of presence at teats at both their own mother and cross-suckling sows was calculated per piglet during the 7.5 h; the frequency of presence at alien teats i.e. the teats of cross-suckling sows was calculated per piglet during the 7.5 h as well. For video observations, the frequency of presence at teats at both their own mother and cross-suckling sows was calculated per high or low BiW piglet during the 7.5 h. For video observations, focal piglets can only be recognized as high or low BiW piglet without individual recognition, therefore the specific recognition of presence at alien teats was not available. The frequency of presence at front and middle teats (**FM teats**) were summed into one variable for further analysis.

Suckling behaviour could not be observed from video for batch 3 due to technical problems, therefore batch 3 was not taken into account for further video analysis. The suckling related variable, i.e. number of successful suckling bouts per sow during 7.5 h (no.), and cross-suckling related variables including percentage of successful suckling bouts involving cross-sucklers per sow during 7.5 h (%), percentage of piglets involved in cross-suckling at least once per litter (%), percentage of non-permanent cross-sucklers per litter (i.e. focal piglets that were present both at their own mother and alien sows during suckling bouts) (%), percentage of permanent cross-sucklers per litter (i.e. focal piglets that were present only at alien sows during suckling bouts and never present at their own sows) (%) were calculated. Restlessness of sows and piglets during suckling bouts could be used to reflect the extent of disruption of suckling by foreign piglets after grouping of litters (Olsen et al., 1998). Restlessness scores of sows and piglets for each suckling bout during 7.5 h were scored separately from 0–2, from calm to a lot of disturbance (Supplementary Table 1). Restlessness score of sows was calculated as the average value of all successful suckling bouts during 7.5 h per sow, and restlessness score of piglets was calculated as the average value of all successful suckling bouts during 7.5 h per litter.

Skin lesions, damage score and mortality

On days 27 and 44 p.p., the number of skin lesions was counted per piglet by visual assessment as the number of fresh lesions on the whole body, except for ears and tails. These skin lesions are regarded as a proxy for aggressive behaviour given and received (Turner et al., 2006). The damage on snout, ears, and tail were scored from 0–3, from no damage to the presence of a wound or erosion (adapted from (Van Nieuwamerongen et al., 2015)) (Supplementary Table 1). Snout damage can be regarded as a reflection of head knocking (Van Nieuwamerongen et al., 2015) and fighting for teats during suckling bouts. The damage on ears and tails can be regarded as a measure of oral manipulation from other pigs (Van Nieuwamerongen et al., 2015). The averaged ear and snout damage score for left and right were used for further analysis.

Piglet mortality were calculated after litter standardisation i.e. 24–48 h p.p. until weaning. In order to test the effect of interventions on mortality, stillborn piglets and piglets that died before cross-fostering were excluded from the data set. Piglet mortality was calculated as the percentage of piglet mortality per litter (%) and the percentage of crushed piglets per litter (%).

Estimation of individual feed and milk intake

Individual DM intake of the focal piglets of sow feed and piglet feed on days 42–43 p.p. was measured using the dual alkane method (Tang et al., 2022a). Dotriacontane (**C32**) was considered as a reference marker and was administered to the piglets for three times/day on days 42–43 (60 mg/d). C32 was melted on a small amount of feed in a forced air oven and was mixed with lemonade syrup to make ~2.0 g/bolus containing 20 mg of C32. Piglets had a habituation period on days 36, 37 and 40, during which piglets were given boluses without marker twice a day. The number of boluses eaten by the piglets was recorded. Hentriacontane (**C31**) and hexatriacontane (**C36**) were considered as in-feed markers for the sow and piglet diets, respectively. C31 was provided via the inclusion of 15% alfalfa in the sow feed, and C36 was melted on soybean meal in a forced air oven followed by mixing it into the piglet feed. This provided around 40 mg/kg of C31 in sow feed and 160 mg/kg of C36 in piglet feed. On day 44, two spot faecal samples were collected from each focal piglet at 08:30 and 12:30 h. N-alkanes in faecal and feed samples were measured by gas chromatography (Smit et al., 2005).

DM intake of the sow feed and piglet feed in each piglet were calculated for days 42–43 using eq. [1]:

Estimated intake of piglet or sow feed (g/day)=

$$\frac{\left(\frac{\text{concentration of in-feed marker in faeces (mg/kg)}}{\text{concentration of reference marker C32 in faeces (mg/kg)}} \times \text{daily intake of reference marker C32 (mg/day)}\right)}{\text{concentration of in-feed marker in diet (mg/kg)}} \times 1000$$

[1]

Milk intake was calculated using eq. [2], assuming fixed feed conversion ratios (**FCR**) of converting DM feed intake into BW gain of 1.5 g/g, and assuming a fixed efficiency of converting fresh milk into BW gain of 4.89 g/g (Theil et al., 2002). Resulting negative estimates were replaced by 0, assuming these piglets did not consume sow milk anymore (CTRL: 13, TREAT: 7 piglets; therein, in CTRL 2 high birthweight (**HBW**) piglets and 11 low birthweight (**LBW**) piglets; in TREAT 3 HBW piglets and 4 LBW piglets).

Estimated intake of milk (g/day)=

(BW gain (g/day) – intake of total feed (g/day) / FCR (g/g)) × 4.89 [2]

DM intake of milk was then calculated assuming a DM content of 19 % (Hurley, 2015). The complete procedures for the calculation of nutrients intake can be found in Supplementary Text 1.

4.2.4 Statistics

Statistical analyses were conducted with SAS 9.4. The effects of treatment (i.e. grouping day plus split-weaning vs. control), BiW class (HBW vs. LBW) and their interaction on multiple response variables based on piglet level were analysed by analysis of variance. For response variables based on piglet level, (1) For response variables piglet BW, BW gain, DM intake of feed and milk, live feeding behaviour, live suckling behaviour, damage scores on snout, ear and tail and skin lesions, fixed effects included treatment, BiW class and their interaction; the random effect was sow nested within batch. (2) For video feeding behaviour and video suckling behaviour where piglets could only be distinguished into high and low BiW class within batch, fixed effects included treatment, BiW class and their interaction; the random effect included batch only. When the interaction between treatment and BiW class was significant, it was further investigated with post hoc pairwise comparisons using the differences of the least square means among four types of focal piglets (CTRL-HBW, CTRL-LBW, TREAT-HBW, TREAT-LBW).

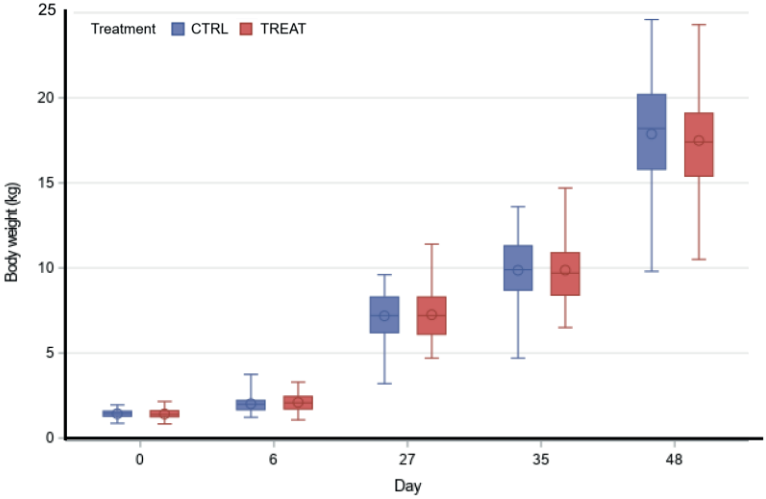
For response variables based on sow or batch level, (1) For suckling related variables, number of successful suckling bouts per sow during 7.5 h, percentage of successful suckling bouts involving cross-sucklers per sow during 7.5 h, percentage of piglets involved in cross-suckling at least once per litter, percentage of non-permanent cross-sucklers per litter, percentage of permanent cross-sucklers per litter, average restlessness scores of sows and piglets of all successful suckling bouts during 7.5 h per sow, percentage of piglet mortality per litter and percentage of crushed piglets per litter, the fixed effect was treatment; the random effect included batch only. (2) For homogeneity related variables, within-batch SE and SD included treatment as fixed effect, and within-batch CV included treatment as fixed effect as well as batch as random effect.

(1) For continuous variables (i.e. BW, BW gain, DM intake of feed and milk, damage score on snout and ear, average restlessness scores of sows of all successful suckling bouts during 7.5 h per sow, average restlessness scores of piglets of all successful suckling bouts during 7.5 h per litter, and within-batch SE and SD in BW and BW gain), the normality of model residuals was checked using PROC UNIVARIATE. The distribution of residuals in the models with DM intake of milk and restlessness scores of sows as response variables was not normal, therefore these variables was converted using log (1+N) before analysis in PROC MIXED. For the other continuous variables, the distribution of residuals was normal, therefore PROC MIXED was used. (2) For proportions, i.e. proportion of time spent on contacting sow feed and piglet feed during sow feeding times and during the day, percentage of successful suckling bouts involving cross-sucklers per sow during 7.5 h, percentage of piglets involved in cross-suckling at least once per litter, percentage of non-permanent cross-sucklers per litter, percentage of permanent cross-sucklers per litter, percentage of piglet mortality per litter, percentage of crushed piglets per litter, within-batch CV in BW and BW gain which were in the range of 0–1, PROC GLIMMIX with a beta distribution and logit link function was used; when the proportion was equal to 0 and 1, it was converted to 0.0000001 and 0.9999999 before analysis, respectively, to accommodate a beta distribution. (3) For count data, i.e. the frequency of presence at teats, skin lesions and number of successful suckling bouts per sow during 7.5 h, PROC GLIMMIX with Laplace approximation, Poisson distribution and log link function were initially used. In models where no evidence of overdispersion was present, i.e. the values of Pearson Chi-Square / DF were smaller than one (Stroup et al., 2018), Poisson distribution was used; when overdispersion was detected, a negative binomial distribution was used as an alternative for the Poisson distribution. (4) For categorial data, i.e. damage score on tail, PROC GLIMMIX with a multinomial distribution and cumulative logit link function was used. Statistical significance was set at $P<0.05$ and data are presented as mean ± SEM.

4.3 Results

4.3.1 The effect of two intervention strategies on piglet and sow traits

During the 7 weeks of lactation, as piglets grow older, variation in BW increased in both CTRL and TREAT for both focal piglets (Fig. 3) and all piglets (Supplementary Fig.1). There was no significant difference in within-batch SE, within-batch SD or within-batch CV in BW, or BW gain between CTRL and TREAT in either focal piglets or all piglets during lactation (Supplementary Table 2).



	Day 0 CTRL	Day 0 TREAT	Day 6 CTRL	Day 6 TREAT	Day 27 CTRL	Day 27 TREAT	Day 35 CTRL	Day 35 TREAT	Day 48 CTRL	Day 48 TREAT
Max	2.0	2.2	3.7	3.3	9.6	11.4	13.6	14.7	24.6	24.3
Min	0.9	0.8	1.2	1.1	3.2	4.7	4.7	6.5	9.8	10.5
Mean	1.4	1.4	2.0	2.1	7.2	7.3	9.9	9.9	17.9	17.5
Median	1.4	1.4	2.0	2.1	7.2	7.2	9.9	9.7	18.2	17.4
N	80	79	80	79	79	79	79	75	78	74
SEM	0.0	0.0	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3

Figure 3. Boxplot showing the BW of focal piglets during a 7-week lactation in the multi-suckling system at five weighing times, with indicating minimum, 25th percentile values of BW, median, 75th percentile values of BW, maximum of BW of piglets. The hollow circle in each box indicates mean values of BW of piglets. CTRL: piglets were grouped on days 8−9 p.p. and no split-weaning was applied; TREAT: piglets were grouped on days 13−14 p.p. and the three heaviest non-focal piglets per litter were split-weaned on day 35 p.p.

As shown in Table 1, in early lactation, no significant differences were found between CTRL and TREAT in the variables measured up to day 35, i.e. BW, BW gain, percentage of time spent on contacting feed during sow feeding times and the frequency of presence at teats on day 18, or damage scores and skin lesions on day 27.

Table 1. BW, BW gain, nutrients intake, feeding behaviours, suckling behaviours, and damage scores of snout, ears and tails, and skin lesions of focal piglets (n=160) in control (CTRL) and treatment (TREAT) batches, and in two birthweight (BiW) classes of piglets, i.e. high birthweight (HBW) and low birthweight (LBW) piglets during a 7-week lactation in the multi-suckling system. CTRL: piglets were grouped on days 8–9 p.p. and no split-weaning was applied; TREAT: piglets were grouped on days 13–14 p.p. and the three heaviest non-focal piglets per litter were split-weaned on day 35 p.p.

Variables[1]	Mean ± SEM Treatment		BiW class[2]		*P*		
	CTRL	TREAT	HBW	LBW	Treat-ment	BiW class	Inter-action
BW (kg)							
Day 0	1.4 ± 0.0	1.4 ± 0.0	1.6 ± 0.0	1.3 ± 0.0	0.774	**<0.001**	0.653
Day 6	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	1.9 ± 0.1	0.524	**<0.001**	0.533
Day 27	7.2 ± 0.2	7.3 ± 0.2	7.7 ± 0.2	6.8 ± 0.2	0.825	**<0.001**	0.655
Day 35	9.9 ± 0.2	9.9 ±0.2	10.4 ± 0.2	9.4 ± 0.2	0.969	**<0.001**	0.756
Day 48	17.9 ± 0.3	17.5 ± 0.3	18.7 ± 0.3	16.7 ± 0.4	0.479	**<0.001**	0.746
BW gain (g/day)							
Days 0–27	213 ± 5	216 ± 6	225 ± 5	204 ± 6	0.777	**0.001**	0.675
Days 27–35	335 ± 9	332 ± 8	341 ± 9	326 ± 8	0.852	0.194	0.838
Days 35–48	618 ± 14	586 ± 18	641 ± 14	563 ± 17	0.230	**<0.001**	0.742
Days 0–35	241 ± 5	242 ± 6	251 ± 5	232 ± 5	0.906	**0.004**	0.767
Days 0–48	343 ± 7	335 ± 7	357 ± 6	321 ± 7	0.495	**<0.001**	0.753
DM intake (g/day) on days 42-43							
Piglet feed	329 ± 21	261 ± 21	313 ± 22	278 ± 20	0.120	0.173	0.594
Sow feed	173 ± 8	178 ± 10	180 ± 9	170 ± 9	0.773	0.331	0.131
Total feed	502 ± 20	439 ± 21	494 ± 22	449 ± 20	**0.072**	0.103	0.296
Milk	135 ± 12	190 ± 14	183 ± 15	141 ± 11	**0.039**	**0.027**	**0.030**
Feeding behaviour during sow feeding times (%) on day 18							
Contacting piglet feed	9.2 ± 2.4	10.5±4.2	10.9 ±4.1	8.8 ± 2.5	0.855	0.412	0.732
Contacting sow feed	9.4 ± 2.2	7.4 ± 1.6	11.1 ± 2.1	5.7 ± 1.2	0.582	**0.050**	0.923
Contacting total feed	18.6 ± 2.8	18.0 ± 4.2	22.1 ± 3.5	14.5 ± 2.9	0.858	**0.059**	0.914
Feeding behaviour during sow feeding times (%) on days 41-43							
Contacting piglet feed	8.5 ± 0.9	5.3 ± 0.8	6.7 ± 0.9	7.1 ± 0.9	**0.072**	0.304	0.254
Contacting sow feed	10.0 ± 0.8	9.4 ± 0.8	10.1 ± 0.9	9.3 ± 0.7	0.613	0.412	0.271
Contacting total feed	18.4 ± 1.2	14.7 ± 1.2	16.8 ± 1.2	16.4 ± 1.2	0.169	0.829	0.660
Feeding behaviour during the day (%) on day 41							
Contacting piglet feed	5.6 ± 0.5	3.2 ± 0.5	4.4 ± 0.5	4.5 ± 0.5	**0.005**	0.792	0.537
Contacting sow feed	4.9 ± 0.6	5.6 ± 0.5	4.8 ± 0.5	5.7 ± 0.6	0.303	0.192	0.591
Contacting total feed	10.5 ± 0.8	8.9 ± 0.6	9.2 ± 0.7	10.2 ± 0.8	0.298	0.266	0.910
Suckling behaviour: the presence at teats (no./7.5h) on day 18							
Front and middle teats	3.5 ± 0.4	3.5 ± 0.5	4.3 ± 0.5	2.7 ± 0.4	0.843	**0.021**	0.347
Rear teats	1.5 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	1.3 ± 0.3	0.891	0.735	0.162
Total teats	5.0 ± 0.5	4.8 ± 0.6	5.7 ± 0.6	4.0 ± 0.5	0.960	**0.036**	0.944
Suckling behaviour: the presence at teats (no./7.5h) on day 41							
Front and middle teats	3.9 ± 0.3	5.0 ± 0.4	4.5 ± 0.3	4.4 ± 0.4	**0.052**	0.731	0.693
Rear teats	2.1 ± 0.3	1.4 ± 0.2	1.6 ± 0.3	1.8 ± 0.3	0.151	0.816	0.625

Total teats	6.0 ± 0.2	6.4 ± 0.3	6.2 ± 0.3	6.2 ± 0.3	0.471	0.964	0.871
Alien teats	1.4 ± 0.3	2.0 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	0.177	0.793	0.447
Damage score on day 27							
Snout	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.146	0.172	0.678
Ear	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.868	0.516	0.784
Tail	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.221	0.283	0.697
Damage score on day 44							
Snout	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	**0.084**	0.612	0.439
Ear	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.913	0.521	0.545
Tail	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.934	0.509	0.400
Skin lesions on day 27							
	3.5 ± 0.4	4.9 ± 0.6	4.8 ± 0.6	3.6 ± 0.5	0.216	**0.080**	0.226
Skin lesions on day 44							
	8.4 ± 0.8	8.0 ± 0.7	8.9 ± 0.9	7.6 ± 0.6	0.932	0.161	0.472

[1] Variables: (1) Contacting feed during sow feeding times (% of observations): contacting (i.e. sniffing or eating) sow feed or piglet feed during sow feeding times on day 18 (video observation), on days 41–43 (live observation) using 2-min instantaneous scan sampling at 08:00–08:30 h and 16:00–16:30 h. For live observations, the percentage of time spent on contacting feed was calculated per piglet; for video observations, it was calculated per high or low BiW piglet. (2) Contacting feed during the day (% of observations): contacting (i.e. sniffing or eating) sow feed or piglet feed during the day on day 41 (live observation) using 15-min instantaneous scan sampling at 08:30h–16:00h; it was calculated per piglet. (3) Suckling behaviour on day 18 (video observation) at 08:30 h−16:00 h: the frequency of presence at teats per (high or low BiW) piglet during 7.5 h; suckling behaviour on day 41 (live observation) at 08:30h−16:00h: the frequency of presence at teats per piglet during 7.5 h. Front and middle teats: front (first two pairs) and middle pairs of teats at both their own mother and cross-suckling sows; Rear teats: last two pairs of teats at both their own mother and cross-suckling sows; Total teats: the sum of front, middle and rear teats at both their own mother and cross-suckling sows. Alien teats on day 41: teats of cross-suckling sows. (4) The damage scores on ear and snout were averaged from left and right sides. (5) Skin lesions were counted as the number of fresh lesions on the whole body, except for ears and tails.

[2] BiW class: HBW and LBW focal piglets. In each litter, two boars and two gilts with the second lowest and highest BiW within sex were selected as focal piglets on day 14 postpartum (p.p.).

As shown in Table 1, after split-weaning on day 35, no significant interaction between treatment and BiW class was found, except for DM intake of milk on days 42–43 (Treatment × BiW class interaction: P=0.030, Fig. 4b). The results of the pairwise comparisons among the four types of focal piglets (CTRL-HBW, CTRL-LBW, TREAT-HBW, TREAT-LBW) showed that TREAT-LBW piglets drank more milk than CTRL-LBW piglets (182 ± 16 g/day vs. 103 ± 14 g/day, P=0.004), but there was no difference between TREAT-HBW and CTRL-HBW piglets (197 ± 23 g/day vs. 169 ± 18 g/day, P=0.828). In addition, although there was no significant interaction between treatment and BiW class, it was noted that the effect of treatment on total feed intake on days 42–43 was more pronounced for LBW piglets (CTRL: 493 ± 29 g/day, TREAT: 401 ± 27 g/day) than for HBW piglets (CTRL: 512 ± 29 g/day, TREAT: 476 ± 33 g/day); similarly, the effect of treatment on the frequency of presence at front and middle teats during 7.5 h on day 41 was more pronounced for LBW piglets (CTRL: 3.8 ± 0.5, TREAT: 5.0 ± 0.5) than for HBW piglets (CTRL: 4.1 ± 0.4, TREAT: 5.0 ± 0.5). The detailed description of BW, BW gain and DM intake of nutrients in four types of focal piglets (CTRL-HBW, CTRL-LBW, TREAT-HBW, TREAT-LBW) are shown in Fig. 4a and Fig. 4b, respectively. The detailed description of all measured variables in four types of focal piglets are shown in Supplementary Table 3.

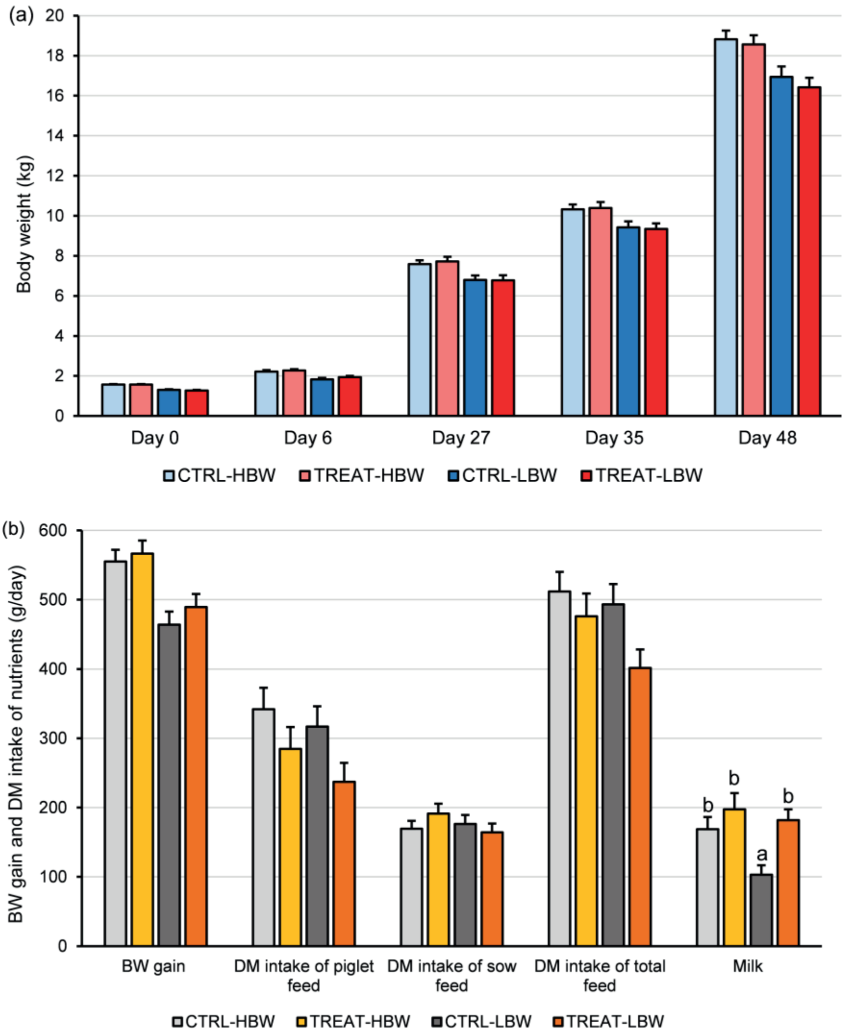


Figure 4. (a) Bar chart showing the BW in four types of focal piglets, i.e. high birthweight piglets in control (CTRL-HBW), high birthweight piglets in treatment (TREAT-HBW), low birthweight piglets in control (CTRL-LBW) and low birthweight piglets in treatment (TREAT-LBW) during a 7-week lactation in the multi-suckling system at five weighing times. (b) Bar chart showing BW gain on days 35–48 (g/day), DM intake of nutrients (g/day) on days 42–43 in four types of focal piglets during a 7-week lactation in the multi-suckling system. ^{a,b} values with different letters differ significantly within nutrient intake or BW gain among four types of focal piglets (*P* <0.05). CTRL: piglets were grouped on days 8–9 p.p. and no split-weaning was applied; TREAT: piglets were grouped on days 13–14 p.p. and the three heaviest non-focal piglets per litter were split-weaned on day 35 p.p.

During sow feeding times on days 41–43, TREAT piglets tended to spend less time on contacting piglet feed than CTRL piglets (5.3 ± 0.8% vs. 8.5 ± 0.9%) (*P*=0.072). On day 41 during the day, TREAT piglets spent less time on contacting piglet feed than CTRL piglets (3.2 ± 0.5% vs. 5.6 ± 0.5%) (*P*=0.005). Correspondingly, TREAT piglets tended to consume less feed than CTRL piglets (439 ± 21 g/day vs. 502 ± 20 g/day) on days 42–43 (*P*=0.072). On day 41, TREAT piglets tended to present more often at front and middle teats per 7.5 h during suckling bouts than CTRL piglets (5.0 ± 0.4 vs. 3.9 ± 0.3) (*P*=0.052). In addition, on days 42–43, 9.3% of piglets in TREAT (HBW: 4%, LBW: 5.3%) and 16.9% of piglets in CTRL (HBW: 2.6%, LBW: 14.3%) did not consume milk. On day 44, TREAT piglets tended to have lower damage scores on snout than CTRL piglets (0.3 ± 0.0 vs. 0.4 ± 0.1) (*P*=0.084). No other significant differences were found between CTRL and TREAT (Table 1).

As shown in Supplementary Table 4, there were no differences between CTRL and TREAT in the number of successful suckling bouts per sow during 7.5 h either on day 18 (mean: 8.8 ± 0.4) or day 41 (mean: 5.5 ± 0.3) and in the percentage of successful suckling bouts involving cross-sucklers per sow during 7.5 h on day 41 (mean: 75.9 ± 6.2 %). The percentage of piglets involved in cross-suckling at least once per litter was higher in TREAT than that in CTRL (68.0 ± 5.3 % vs. 37.1 ± 6.6%, *P*=0.020). The percentage of non-permanent cross-sucklers per litter (i.e. piglets which were present both at their own mother and alien sows during sucking bouts) was higher in TREAT than that in CTRL (62.7 ± 5.5% vs. 27.1 ± 5.8%, *P*=0.008). The percentage of permanent cross-sucklers per litter (i.e. piglets which were only present at alien sows and never present at their own mother during suckling bouts) did not differ between the two groups (mean: 7.7 ± 2.3 %). The restlessness score of piglets on day 41 in TREAT was lower than that CTRL (0.3 ± 0.1 vs. 0.8 ± 0.1, *P*=0.028), but there were no differences in restlessness scores of sows (mean of CTRL and TREAT: 0.1 ± 0.0) and piglets (mean: 0.5 ± 0.0) on day 18 or restlessness scores of sows on day 41 (mean: 0.1 ± 0.0) between the two groups.

As shown in Supplementary Table 4, no difference in percentage of piglet mortality per litter was found between CTRL and TREAT during the entire lactation. In total, 68.4% and 80.6% of deaths in CTRL and TREAT were due to crushing. The percentage of crushed piglets per litter tended to be higher in TREAT than in CTRL groups on days 9-14 (7.3 ± 1.7% vs. 2.9 ± 1.1 %; *P*=0.087), i.e. during the period in which the TREAT piglets were not yet grouped; but over the complete period on days 0-48, neither the 5% higher crushing, nor the 3% higher piglet mortality in TREAT was statistically different from CTRL.

4.3.2 Comparison between low and high birthweight piglets

As shown in Table 1, there was a significant effect of BiW class on BW and BW gain during all periods examined, with HBW piglets performing better, except for the period on days 27–35 in which the difference in BW gain was not significant.

The difference in BW gain on days 0–27 (difference: 21 g/day, *P*=0.001) and on days 0-35 (difference: 19 g/day, *P*=0.004) was in line with the observed percentage of time spent on contacting total feed (difference: 7.6%, *P*=0.059) and sow feed (difference: 5.4%, *P*=0.050) during sow feeding times on day 18. Moreover, it was in line with the observed teat presence of piglets on day 18, where HBW piglets suckled more often from front and middle teats during 7.5 h (difference: 1.6, *P*=0.021) and from total teats (difference: 1.7, *P*=0.036) than LBW piglets. Skin lesions on day 27 were higher in HBW than in LBW piglets (difference: 1.2, *P*=0.080), while damage scores on snout, ear and tail on day 27 were similar in HBW and LBW piglets.

After split-weaning on day 35, HBW piglets had a higher BW gain on days 35–48 than LBW piglets (difference: 78 g/day, *P*<0.001) likely because of both a numerical increase in total feed intake (difference: 45 g/day, *P*=0.103) and an increase in milk intake (difference: 42 g/day, *P*=0.027) on days 42–43. No other significant differences were found between HBW and LBW piglets.

4.4 Discussion

The aim of the present study was to investigate if performance and behaviours of HBW and LBW piglets during a 48 day lactation in an MS system differed in view of the two intervention strategies, i.e. later grouping of litters at day 13 p.p. instead of day 8 p.p. and reducing litter size by split-weaning of the three heaviest non-focal piglets from each litter at day 35. These interventions aimed to improve the performance, particularly of LBW piglets and reduce the performance variation at weaning.

4.4.1 Later grouping

The first intervention strategy delayed the age of grouping with other litters in the MS system from day 8 p.p. to day 13 p.p. With later grouping, we aimed to strengthen the sow-piglet bond. It was expected that a stronger sow-piglet bond would increase the presence at teats of LBW piglets during suckling bouts, reduce cross-suckling, increase the frequency of contacting feed during sow feeding times, and thereby increase the milk and feed intake of especially smaller piglets. However, we found that later grouping did not help to improve the growth performance, suckling behaviour and feeding behaviour of LBW piglets after grouping.

In our study, grouping at either day 8 or 13 did not affect BW, BW gain or within-batch variation in BW or BW gain after grouping, nor was there an interaction between treatment and high vs low BiW piglets. Consistently, in conventional housing, Salazar et al. (2018) did not observe differences in BW or BW gain during days 0–35 between piglets co-mingled at day 14 p.p. and day 7 p.p.; Van Kerschaver et al. (2021) found no differences between piglets co-mingled at day 13 p.p. and day 8 p.p. in their BW gain, creep feed intake and within-litter variation in BW after grouping. In MS systems, Thomsson et al. (2016) found no differences between piglets co-mingled at day 7,14 and 21 p.p. in their BW and within-litter variation in BW at weaning on day 44. However, one study with MS systems found that piglets grouped on day 7 had a lower BW gain on days 6–26 than those grouped on day 14 p.p. (Verdon et al., 2020). In the current study, no differences were found in growth performance of LBW piglets before day 35 between early and later grouping batches, possibly indicating later grouping does not improve the growth performance of LBW piglets.

In early lactation, piglets are mainly reliant on sow milk for their development. The occurrence of cross-suckling after grouping can have detrimental consequences for milk intake and performance of piglets (Dybjaer et al., 2001) owing to increased competition at the udder (Olsen et al., 1998), missing of milk injection when fighting for teats, disrupted teat order (Wattanakul et al., 1997a) and disrupted nursing which diminishes the sow's motivation to nurse (Pedersen et al., 1998; Wattanakul et al., 1998). We expected that later grouping would strengthen the sow-piglet bond, reduce cross-suckling, snout damage scores, restlessness of sows and piglets during suckling bouts and increase the presence at teats of especially LBW piglets.

However, in the current study, no effect of grouping age was observed on teat presence, the number of successful suckling bouts, snout damage scores at day 27 and restlessness of sows and piglets during suckling bouts on day 18. Similarly, other studies did not find the influence of age at grouping on the number of successful suckling bouts in few days after grouping (Thomsson et al., 2016; Verdon et al., 2019). The reason could be that grouping has been applied 10 and 4 days before day 18 in early and late grouping batches respectively, and the effects of later grouping on these indicators might have disappeared. Grouping piglets disturbed suckling patterns only on the day of grouping (Wattanakul et al., 1997b), and restlessness scores of sows and piglets increased within 1 week after grouping, but more organised nursing bouts resumed after around 1 week (van Nieuwamerongen, 2017).

It was found that piglets can learn feeding behaviours from sows by observation and participation, which is called 'vertical social learning' (Jensen, 1988; Oostindjer et al., 2011). We expected that later grouped piglets have a better mother-piglet bond, stay more close to their mother, and thereby spend more time contacting feed during sow feeding times by observing and learning from sows to eat. However, in the current study, later grouping did not affect the percentage of time spent on contacting solid feed during sow feeding times on day 18, nor was there an interaction between treatment and BiW class. It might be that TREAT piglets stay close to their mother waiting for suckling bouts during sow feeding times rather than contacting feed.

Reciprocal fighting commonly occurs after grouping of litters for establishing a new dominance hierarchy, often causing skin lesions (Pitts et al., 2000; Turner et al., 2006; Van Kerschaver et al., 2021). It was reported that unacquainted pre-weaning piglets that were shortly placed together were reported to fight shorter and had fewer injuries at younger age (Pitts et al., 2000). We expected that later grouping piglets have more skin lesions than early grouping piglets. However, in the present study, no differences in fresh skin lesions on the piglets' body were found between treatment groups on day 27, probably indicating that there was no difference in aggressive behaviour on day 27 anymore. In another study, it was found that piglets performed frequent aggressive behaviours resulting in skin lesions within 1 day after grouping on day 10 p.p. (D'Eath, 2005). As grouping had been applied 14 and 19 days earlier respectively in CTRL and TREAT, it might be that aggressive behaviours have disappeared on day 27. Similar results were also found by (Van Kerschaver et al., 2021) that on day 20 no differences in skin lesions were observed between piglets co-mingled on day 8 and day 13 p.p. An earlier measurement of skin lesions shortly after grouping needs to be taken for the further investigation. On the other hand, it was reported that in semi-natural conditions, when encountering unfamiliar piglets for the first time, the majority of interactions was peaceful and consisted mainly of nose-to-nose contacts while the frequency of aggressive behaviour was low (Petersen et al., 1989). Therefore, it could also be that MS system are more close to the semi natural rearing conditions than conventional housing, piglets may have shown minimal aggressive behaviour after grouping which thus led to minimal skin lesions and no difference between the groups at day 27.

In the current study, the proportion of crushing tended to be higher in TREAT than CTRL during the period when TREAT piglets were not yet grouped on days 9–14. This higher crushing might be caused by that in later grouping batches, sows have to nurse their piglets in the loose farrowing pens during days 9–14 when these piglets did not have access to communal MS area. These piglets have less free moving space compared to early grouping piglets, which probably increased the difficulty for escaping from nursing sows in the process of lying down. But over the entire lactation, piglet mortality, due to crushing or other causes, did not differ between early and later grouping batches.

To summarise, later grouping had little effect on most parameters measured in our study. Later grouping does appear to increase crushing shortly after grouping, even though piglet mortality during the entire lactation on days 0–48 was not statistically different between early and later grouping batches. We could not confirm our hypothesis that later grouping helps to improve the growth performance of LBW piglets post grouping.

4.4.2 Split-weaning

The second intervention strategy was split-weaning of the three heaviest non-focal piglets per litter on day 35 in the TREAT group. The remaining piglets in TREAT and all piglets in CTRL were weaned on day 48. We expected that split-weaning could reduce the competition at the udder and in the feeding area, thereby enabling especially LBW piglets to have an increased milk consumption and solid feed intake, and thus accelerated growth.

During week 6 and 7 after split-weaning, there was no difference in BW, BW gain and within-batch variation in BW or BW gain between CTRL and TREAT. But as expected, we observed a significant higher milk intake and a tendency of decreased snout damage in TREAT piglets on days 42–44, compared with CTRL piglets. In addition, restlessness scores of piglets during suckling bouts on day 41 in TREAT were lower than CTRL. This might be due to the decreased competition at the udder and availability of more functional teats. Moreover, on days 42–43, TREAT piglets tended to be more present at the front and middle teats, while numerically less present at rear teats than CTRL piglets, possibly due to their moving from teats with a lower milk yield to teats with a higher milk yield after split-weaning. A similar finding was observed by Pluske and Williams (1996) that following split-weaning, the remaining piglets used the anterior teats previously occupied by the split-weaned piglets. The front and middle part of the udder is known to have a higher milk production than the rear part of the udder (Skok et al., 2007).

We expected that split-weaning could reduce the competition at the feeder and increase feed intake of piglets due to a lower number of piglets in the MS area. Unexpectedly, no difference in skin lesions on day 44 was found between CTRL and TREAT. TREAT piglets did not have an increased feed intake on days 42–43; inversely, TREAT piglets had a tendency of reduced feed intake and a numerically lower percentage of time contacting feed both during the day and during sow feeding times after split weaning. This may suggest that TREAT piglets seem to transfer their interest from feed to milk when more productive teats are available. The unchanged BW gain in TREAT piglets compared to CTRL piglets was probably a results of that the increased milk intake was compensated for by the reduced feed intake. Moreover, no differences were found in BW and BW gain on days 35–48 in LBW piglets between CTRL and TREAT groups after split-weaning. But interestingly, we found that after split-weaning, the increased amount of milk intake, the increased presence at front and middle teats and the reduced amount of feed intake was more obvious for LBW piglets than for HBW piglets. Likewise, the increased milk intake in LBW piglets was seemingly compensated for by a reduced feed intake, resulting in no treatment effects on BW gain of LBW piglets. Some previous studies indicated that piglets mainly rely on milk in week 0–3 (Barber et al., 1955) (Abraham and Chhabra, 2004) and on solid feed for growth from week 4 onwards (Tang et al, 2022b). Therefore, an earlier split-weaning age before day 35 may be more helpful to increase milk intake and thus the growth rate of LBW piglets. As LBW piglets have a lower BW than their HBW counterparts, further research in understanding the nutrient requirement of piglets from different BW rather than their real biological age might help to adopt more effective measures to increase growth rate.

On day 41, cross-suckling occurred frequently in both CTRL and TREAT, observed on average in 75.9 % of all successful suckling bouts per sow. However, cross-suckling seems to occur more often in TREAT, as the percentage of piglets involved in cross-suckling at least once per litter was higher in TREAT than in CTRL (68.0% vs. 37.1%). In addition, on day 41, the proportion of non-permanent cross sucklers per litter (i.e. piglets which were present both at their own mother and alien sows) was higher in TREAT than in CTRL (62.7% vs. 27.1%). It could be that as the split-weaning strategy reduces the total number of piglets in the MS system, more empty positions of functional teats were available for piglets, which results in a higher number of non-permanent cross-sucklers trying to attain milk from foreign mothers, especially when their own mother was in the IS area, or when their own mother has a low milk production (Olsen et al., 1998). This could also explain the increased milk intake in TREAT compared with CTRL. According to Algers and Jensen (1991), milk production is influenced by intensity of teat stimulation. If so, TREAT sows might have had lower milk production as fewer piglets remained in the MS system to stimulate teats after split-weaning. The reason that TREAT piglets still achieved a higher milk intake could be that they transferred to more productive teats or became cross-sucklers. It is interesting to see that on day 41 the permanent cross-sucklers per litter (i.e. cross-sucklers which were never present at their own mother) (5.3 % vs. 10.0 %) in TREAT was numerically lower than that in CTRL. There might be an interaction between later grouping and split-weaning on cross-suckling, as later grouping seems to help to reduce cross-suckling, while split-weaning seems to stimulate cross-suckling. The two intervention strategies might also have interaction effects on other measured variables and cannot be separated, as these two interventions were imposed on the same group of piglets.

4.5 Conclusion

The aim of current study was to investigate the effect of later grouping of litters and split-weaning on performance, nutrient intake, suckling and feeding behaviour of focal piglets during a 7-week lactation in a multi-suckling system to improve the BW gain of LBW piglets and thereby reduce the BW variation at weaning. We found that grouping litters on days 13 –14 instead of days 8 – 9 did not affect their BW gain, within batch variation in BW gain, suckling and feeding behaviours after grouping. Piglet crushing was higher on days 9–14 in later grouping piglets when piglets were not yet grouped, compared to early grouping piglets; but no difference in piglet mortality was found between the two treatments during the entire lactation. We could not confirm our hypothesis that later grouping helps to improve the growth performance of LBW piglets post grouping. After split-weaning of three heaviest non-focal piglets on day 35, no differences were found in growth performance or piglet homogeneity between the two groups. But split-weaning did appear to reduce the competition at the udder, as snout damages and restlessness scores of piglets during suckling bouts were reduced, and milk intake and presence at front and middle teats increased. The treatment did not favour the LBW piglets, with the exception of milk intake. The milk intake and presence at front and middle teats in TREAT piglets was increased in particular in LBW piglets, but this was seemingly compensated for by a reduction in feed intake and therefore likely not reflected in an increased BW gain and reduced BW variation. Further research is needed for example changing the age of split-weaning in order to reduce BW variation in MS systems.

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4.7 Supplementary materials

Supplementary Table 1. The restlessness scoring protocol of sows and piglets during suckling bouts and the damage scoring protocol on snout, ear and tail of piglets in the multi-suckling system.

		Score	Description
Restlessness scores	Sows	0	Calm: sow keeps lying down with her head on the floor. If a sow stands during suckling; sows stands still on one spot and keeps her head still
		1	Little restless: sow lift her head up a few times, but doesn't stop the nursing. If a sows stands during nursing; sow looks around.
		2	A lot of disturbance: sow stands up, sits or lies on her belly/udder and ends the nursing before the of milk yield. If a sow stands during suckling; the sow starts to walk before the milk yield.
	Piglets	0	Calm: piglets stay calm during the suckling period.
		1	Little restless: piglets fight for the teats.
		2	A lot of disturbance: piglets change teat/position at the udder the whole time
Damage scores	Snout	0	No fresh facial wounding
		1	1–3 fresh superficial scratches
		2	4–6 fresh superficial scratches
		3	>6 fresh superficial scratches/deep lesions/erosion
	Ear	0	No damage
		1	Small fresh bite marks are visible, ear is intact
		2	Small and medium-sized wound, ear is intact
		3	Severe wound, part ear is missing/necrosis
	Tail	0	No damage visible
		1	The tail lacks its hair partially or completely
		2	Small damages/bite marks are visible. These individual bite marks have the size of a pinhead
		3	Clearly visible wound

Supplementary Table 2. Homogeneity in BW and BW gain of all piglets (n=376 on day 48, after excluding split weaned piglets) and focal piglets (n=152 on day 48) in two treatment batches, i.e. control (CTRL) and treatment (TREAT) batches during a 7-week lactation in the multi-suckling system. CTRL: piglets were grouped on days 8–9 p.p. and no split-weaning was applied; TREAT: piglets were grouped on days 13–14 p.p. and the three heaviest non-focal piglets per litter were split-weaned on day 35 p.p.

All piglets	Treatment			Focal piglets	Treatment		
	CTRL	TREAT	*P*		CTRL	TREAT	*P*
Within-batch SE in BW (kg)				Within-batch SE in BW (kg)			
Day 0	0.0	0.0	0.956	Day 0	0.0	0.1	0.680
Day 6	0.1	0.1	0.901	Day 6	0.1	0.1	0.711
Day 27	0.2	0.2	0.517	Day 27	0.3	0.3	0.798
Day 35	0.2	0.2	0.744	Day 35	0.4	0.4	0.752
Day 48	0.4	0.4	0.388	Day 48	0.7	0.7	0.709
Day 35 (excluding split-weaned piglets in TREAT)							
	0.2	0.3	0.534				
Day 48 (excluding split-weaned piglets in TREAT)							
	0.4	0.5	0.618				
Within-batch SE in BW gain (g/day)				Within-batch SE in BW gain (g/day)			
Days 0–27	6.7	5.8	0.444	Days 0–27	10.3	10.5	0.915
Days 27–35	9.8	9.9	0.830	Days 27–35	16.8	15.5	0.538
Days 35–48	17.0	18.3	0.650	Days 35–48	26.0	33.0	0.319
Days 0–35	6.6	6.1	0.577	Days 0–35	10.3	10.7	0.833
Days 0–48	8.6	7.6	0.354	Days 0–48	13.5	14.2	0.757
Days 35–48 (excluding split-weaned piglets in TREAT)							
	17.0	20.0	0.422				
Days 0–48 (excluding split-weaned piglets in TREAT)							
	8.6	9.0	0.697				
Within-batch SD in BW (kg)				Within-batch SD in BW (kg)			
Day 0	0.3	0.3	0.905	Day 0	0.2	0.2	0.705
Day 6	0.5	0.5	0.949	Day 6	0.4	0.4	0.702
Day 27	1.5	1.4	0.472	Day 27	1.3	1.4	0.796
Day 35	1.9	1.7	0.463	Day 35	1.7	1.7	0.892
Day 48	3.2	2.7	0.179	Day 48	3.0	3.0	0.846
Day 35 (excluding split-weaned piglets in TREAT)							
	1.9	1.7	0.288				
Day 48 (excluding split-weaned piglets in TREAT)							
	3.2	2.8	0.214				
Within-batch SD in BW gain (g/day)				Within-batch SD in BW gain (g/day)			
Days 0–27	50.5	43.6	0.396	Days 0–27	45.7	46.7	0.908
Days 27–35	73.8	71.7	0.703	Days 27–35	74.7	67.0	0.419
Days 35–48	126.6	131.6	0.812	Days 35–48	114.3	143.3	0.372
Days 0–35	49.6	44.1	0.339	Days 0–35	45.8	46.0	0.982
Days 0–48	63.9	54.0	0.167	Days 0–48	59.4	60.7	0.885
Days 35–48 (excluding split-weaned piglets in TREAT)							
	126.6	123.6	0.895				
Days 0–48 (excluding split-weaned piglets in TREAT)							
	63.9	55.1	0.216				

All piglets	Treatment			Focal piglets	Treatment		
	CTRL	TREAT	*P*		CTRL	TREAT	*P*
Within-batch CV in BW (%)				Within-batch CV in BW (%)			
Day 0	19.2 ± 1.1	19.5 ± 1.3	0.895	Day 0	15.2 ± 1.1	16.4 ± 2.7	0.692
Day 6	24.3 ± 2.4	23.1 ± 3.4	0.780	Day 6	21.7 ± 2.8	19.2 ± 3.5	0.605
Day 27	21.5 ± 1.7	18.4 ± 1.7	0.250	Day 27	18.4 ± 1.8	19.1 ± 1.9	0.810
Day 35	19.1 ± 1.1	17.5 ± 1.5	0.398	Day 35	17.2 ± 1.8	17.6 ± 1.8	0.867
Day 48	18.0 ± 1.0	16.0 ± 1.3	0.286	Day 48	16.5 ± 2.0	17.4 ± 0.8	0.709
Day 35 (excluding split-weaned piglets in TREAT)							
	19.1 ± 1.1	17.9 ± 1.4	0.504				
Day 48 (excluding split-weaned piglets in TREAT)							
	18.0 ± 1.0	16.6 ± 1.3	0.406				
Within-batch CV in BW gain (%)				Within-batch CV in BW gain (%)			
Days 0–27	24.2 ± 2.5	19.9 ± 1.8	0.199	Days 0–27	21.5 ± 2.6	21.5 ± 1.6	0.996
Days 27–35	22.4 ± 1.2	22.4 ± 1.5	0.988	Days 27–35	22.4 ± 2.4	20.2 ± 0.9	0.408
Days 35–48	20.9 ± 1.7	24.2 ± 4.3	0.491	Days 35–48	18.6 ± 2.7	24.7 ± 4.9	0.313
Days 0–35	20.8 ± 1.5	18.4 ± 1.3	0.269	Days 0–35	19.0 ± 2.3	19.0 ± 1.5	1.000

Days 0–48	18.8 ± 1.3	16.6 ± 1.3	0.288	Days 0–48	17.4 ± 2.4	18.1± 0.8	0.771
Days 35–48 (excluding spilt-weaned piglets in TREAT)							
	20.9 ± 1.7	21.8 ± 4.2	0.838				
Days 0–48 (excluding spilt-weaned piglets in TREAT)							
	18.8 ± 1.3	17.1 ± 1.4	0.402				

Supplementary Table 3. Descriptive values for variables in four types of focal piglets: i.e. in two treatment batches, i.e. control (CTRL) and treatment (TREAT) batches, and in two birthweight (BiW) classes of piglets, i.e. high birthweight (HBW) and low birthweight (LBW) piglets during a 7-week lactation in the multi-suckling system. CTRL: piglets were grouped on days 8–9 p.p. and no split-weaning was applied; TREAT: piglets were grouped on days 13–14 p.p. and the three heaviest non-focal piglets per litter were split-weaned on day 35 p.p.

Variables [1]			CTRL		TREAT	
			HBW	LBW	HBW	LBW
BW (kg)		Day 0	1.6 ± 0.0	1.3 ± 0.0	1.6 ± 0.0	1.3 ± 0.0
		Day 6	2.2 ± 0.1	1.8 ± 0.1	2.3 ± 0.1	1.9 ± 0.1
		Day 27	7.6 ± 0.2	6.8 ± 0.2	7.7 ± 0.2	6.8 ± 0.2
		Day 35	10.3 ± 0.2	9.4 ± 0.3	10.4 ± 0.3	9.3 ± 0.3
		Day 48	18.8 ± 0.4	16.9 ± 0.5	18.6 ± 0.5	16.4 ± 0.5
BW gain (g/day)		Days 0–27	223 ± 7	203 ± 8	228 ± 8	204 ± 8
		Days 27–35	342 ± 13	329 ± 12	341 ± 13	323 ± 10
		Days 35–48	654 ± 19	582 ± 20	628 ± 21	544 ± 28
		Days 0–35	250 ± 7	232 ± 8	252 ± 8	231 ± 7
		Days 0–48	359 ± 9	326 ± 10	354 ± 9	316 ± 10
DM intake (g/day)	Days 42–43	Piglet feed	342 ± 31	317 ± 29	285 ± 31	237 ± 27
		Sow feed	170 ± 11	176 ± 13	191 ± 14	164 ± 13
		Total feed	512 ± 29	493 ± 29	476 ± 33	401 ± 27
		Milk	169 ± 18	103 ± 14	197 ± 23	182 ± 16
Feeding behaviour during sow feeding times (%)	Day 18	Contacting piglet feed	2.5 ± 0.7	1.3 ± 0.4	2.0 ± 0.5	1.0 ± 0.3
		Contacting sow feed	2.0 ± 0.9	1.7 ± 0.5	2.4 ± 1.5	1.8 ± 1.0
		Contacting total feed	4.5 ± 1.0	3.0 ± 0.4	4.4 ± 1.2	2.8 ± 1.2
	Days 41–43	Contacting piglet feed	8.6 ± 1.4	8.3 ± 1.3	4.7 ± 1.0	5.9 ± 1.1
		Contacting sow feed	9.8 ± 1.3	10.1 ± 1.0	10.5 ± 1.2	8.3 ± 1.1
		Contacting total feed	18.4 ± 1.8	18.5 ± 1.6	15.2 ± 1.7	14.2 ± 1.7
Feeding behaviour during the day (%)	Day 41	Contacting piglet feed	5.8 ± 0.7	5.5 ± 0.6	3.0 ± 0.7	3.4 ± 0.7
		Contacting sow feed	4.3 ± 0.7	5.4 ± 0.9	5.3 ± 0.7	5.9 ± 0.6
		Contacting total feed	10.1 ± 1.0	10.9 ± 1.2	8.4 ± 0.9	9.4 ± 0.9
Suckling behaviour: the presence at teats (no. of times)	Day 18	Front and middle teats	4.6 ± 0.7	2.5 ± 0.4	4.0 ± 0.8	3.0 ± 0.7
		Rear teats	1.3 ± 0.3	1.7 ± 0.4	1.6 ± 0.5	0.9 ± 0.3
		Total teats	5.8 ± 0.8	4.1 ± 0.6	5.6 ± 1.0	3.9 ± 0.8
	Day 41	Front and middle teats	4.1 ± 0.4	3.8 ± 0.5	5.0 ± 0.5	5.0 ± 0.5
		Rear teats	1.8 ± 0.4	2.3 ± 0.5	1.4 ± 0.3	1.3 ± 0.3
		Total teats	5.9 ± 0.3	6.0 ± 0.4	6.4 ± 0.4	6.4 ± 0.5
		Alien teats	1.3 ± 0.4	1.6 ± 0.4	2.1 ± 0.4	1.9 ± 0.4
Damage score	Day 27	Snout	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
		Ear	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
		Tail	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
	Day 44	Snout	0.5 ± 0.1	0.4 ± 0.1	0.3 ±0.1	0.3 ±0.1
		Ear	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
		Tail	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Skin lesions	Day 27	Total lesions	4.4 ± 0.8	2.7 ± 0.4	5.1 ± 1.0	4.6 ± 0.8

Day 44	Total lesions	9.4 ± 1.4	7.4 ± 0.9	8.4 ± 1.0	7.7 ± 0.9

[1] Variables: (1) Contacting feed during sow feeding times (% of observations): contacting (i.e. sniffing or eating) sow feed or piglet feed during sow feeding times on day 18 (video observation), on days 41–43 (live observation) using 2-min instantaneous scan sampling at 08:00–08:30 h and 16:00–16:30 h. For live observations, the percentage of time spent on contacting feed was calculated per piglet; for video observations, it was calculated per high or low BiW piglet. (2) Contacting feed during the day (% of observations): contacting (i.e. sniffing or eating) sow feed or piglet feed during the day on day 41 (live observation) using 15-min instantaneous scan sampling at 08:30h–16:00h; it was calculated per piglet. (3) Suckling behaviour on day 18 (video observation) at 08:30 h–16:00 h: the frequency of presence at teats per (high or low BiW) piglet during 7.5 h; suckling behaviour on day 41 (live observation) at 08:30h–16:00h: the frequency of presence at teats per piglet during 7.5 h. Front and middle teats: front (first two pairs) and middle pairs of teats at both their own mother and cross-suckling sows; Rear teats: last two pairs of teats at both their own mother and cross-suckling sows; Total teats: the sum of front, middle and rear teats at both their own mother and cross-suckling sows. Alien teats on day 41: teats of cross-suckling sows. (4) The damage scores on ear and snout were averaged from left and right sides. (5) Skin lesions were counted as the number of fresh lesions on the whole body, except for ears and tails.

Supplementary Table 4. Suckling related behaviours, restlessness scores of sows and piglets during suckling bouts and piglet mortality in control (CTRL) and treatment (TREAT) batches during a 7-week lactation in the multi-suckling system. CTRL: piglets were grouped on days 8–9 p.p. and no split-weaning was applied; TREAT: piglets were grouped on days 13–14 p.p. and the three heaviest non-focal piglets per litter were split-weaned on day 35 p.p.

Type of variables			Mean ± SEM		*P*
			Treatment		
		Day	CTRL	TREAT	
Suckling related behaviours [1]	Number of successful suckling bouts per sow during 7.5 h (no.)	18	8.4 ± 0.4	9.4 ± 0.6	0.316
		41	5.6 ± 0.5	5.4 ± 0.5	0.841
Cross-suckling related variables	Percentage of successful suckling bouts involving cross-sucklers per sow during 7.5 h (%)	41	68.3 ± 9.5	83.8 ± 7.6	0.661
	Percentage of piglets involved in cross-suckling at least once per litter (%)	41	37.1 ± 6.6	68.0 ± 5.3	**0.020**
	Percentage of non-permanent cross-sucklers per litter [2] (%)	41	27.1 ± 5.8	62.7 ± 5.5	**0.008**
	Percentage of permanent cross-sucklers per litter [3] (%)	41	10.0 ± 3.4	5.3 ± 3.1	0.485
Restlessness scores [4]	Restlessness scores of sows	18	0.1 ± 0.0	0.2 ±0.1	0.600
		41	0.2 ± 0.1	0.0± 0.0	0.204
	Restlessness scores of piglets	18	0.4 ± 0.1	0.5 ± 0.1	0.403
		41	0.8 ± 0.1	0.3 ± 0.1	**0.028**
Mortality[5]	Percentage of piglet mortality per litter (%)	2–8	11.9 ± 2.9	12.5 ± 2.2	0.859
		9–14	4.3 ± 1.3	8.0 ± 1.7	0.187
		2–14	16.2 ± 3.2	20.5 ± 3.4	0.253
		15–48	4.2 ± 1.4	2.8 ± 1.1	0.765
		2–48	20.1 ± 3.4	23.3 ± 3.6	0.528
	Percentage of crushed piglets per litter (%)	2–8	8.4 ± 2.2	10.4 ± 2.1	0.525

	9–14	2.9 ± 1.1	7.3 ± 1.7	**0.087**
	2–14	11.3 ± 2.5	17.7 ± 3.3	0.101
	15–48	2.5 ± 0.9	1.1 ± 0.8	0.312
	2–48	13.7 ± 2.7	18.8 ± 3.4	0.129

[1] Suckling behaviour was scored from video at 08:30–16:00 h on day 18, and live at 08:30–16:00 h on day 41.

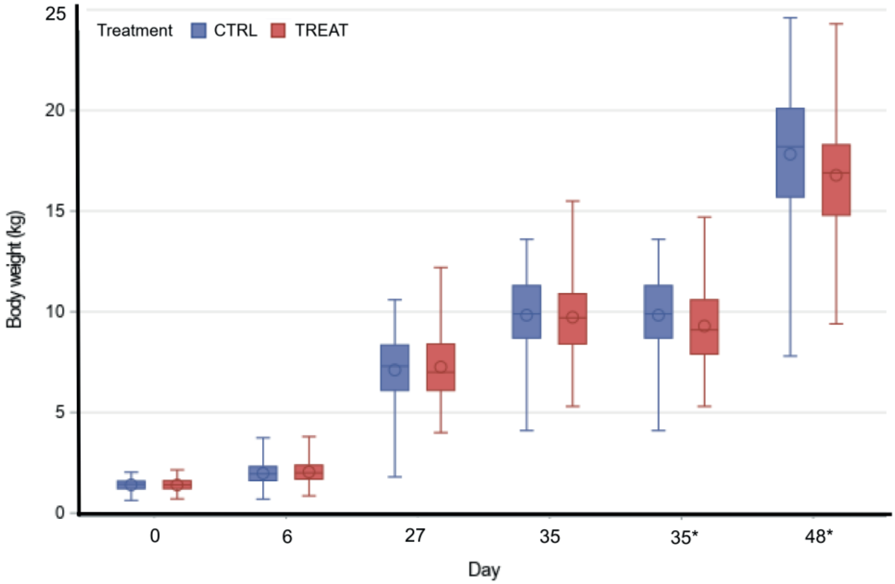
[2] Non-permanent cross-sucklers: focal piglets that present both at their own mother and cross-suckling sows during suckling bouts;

[3] Permanent cross-sucklers: focal piglets that present only at cross-suckling sows and never present at their own mother during suckling bouts;

[4] The detailed restlessness scoring protocol of sows and piglets during suckling bouts can be found in Supplementary Table 1. Restlessness scores of sows: average restlessness scores of sows of all successful suckling bouts during 7.5 h per sow; restlessness scores of piglets: average restlessness scores of piglets of all successful suckling bouts during 7.5 h per litter.

[5] Mortality: piglet mortality were calculated after litter standardisation i.e. 24–48 h p.p. until weaning. In order to test the effect of interventions on mortality, stillborn piglets and piglets that died before cross-fostering were excluded from the data set. Piglet mortality was calculated as the percentage of piglet mortality per litter (%) and the percentage of crushed piglets per litter (%).

Supplementary Figure 1



	0		6		27		35		35*		48*	
Max	2.0	2.2	3.7	3.8	10.6	12.2	13.6	15.5	13.6	14.7	24.6	24.3
Min	0.6	0.7	0.7	0.9	1.8	4.0	4.1	5.3	4.1	5.3	7.8	9.4
Mean	1.4	1.4	2.0	2.0	7.1	7.3	9.8	9.7	9.8	9.3	17.8	16.8
Median	1.4	1.4	2.0	2.0	7.3	7.0	9.9	9.7	9.9	9.1	18.2	16.9
N	283	287	266	273	232	225	227	209	227	155	225	151
SEM	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2

Boxplot showing the body weight (BW) of all piglets during a 7-week lactation in the multi-suckling system at five weighing times, with indicating minimum, 25th percentile values of BW, median, 75th percentile values of BW, maximum of BW of piglets. The hollow circle in each box indicates mean values of BW of piglets. CTRL: piglets were grouped on days 8–9 p.p. and no split-weaning was applied; TREAT: piglets were grouped on days 13–14 p.p. and the three heaviest non-focal piglets per litter were split-weaned on day 35 p.p. Day 35: the split-weaned piglets were included in the box in TREAT groups. Day 35* and day 48*: the split-weaned piglets were excluded from the boxes in TREAT groups.

Supplementary Text 1. The estimation of dry matter intake of piglets from incomplete data

Piglets with no faecal samples during the faecal collection period (3 and 4 pigs in CTRL and TREAT) were omitted from the calculation. Piglets refusing more than 50% of the C32 boluses (7 and 3 piglets in CTRL and TREAT) were also omitted from the calculation.

Due to variable mixing of digesta and variable passage rates, the excretion rate of the reference marker may not be constant, hence leading to over or under representation of the reference marker in spot faecal samples, and leading to erroneous estimates of feed intake (see also (Tang et al., 2022)). For this reason, feed intake estimates were carefully checked, and considered unrealistic in the case that estimated feed intake of an individual piglet exceeded 3 times the calculated energy requirements for maintenance (Blok et al., 2015). This resulted in unrealistic feed intake estimates of 12 and 3 piglets in CTRL and TREAT, respectively. For these piglets and for piglets refusing more than 50% of the C32 bolus on days 42–43, feed intake was estimated based on the linear regression between the intake of total feed and the concentration of in-feed marker in faeces of the focal piglets with successful feed intake estimates. The feed intake of these piglets was then calculated from this relationship using the concentration of in-feed marker in faeces of these piglets.

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Chapter 5: Effects of birthweight of piglets in a multi-suckling system on mortality, growth rate, catchup-growth, feed intake and behaviour.

T. Tang [a, b], W. J. J. Gerrits [a], C. M. C. van der Peet-Schwering [c], N. M. Soede [b] and I. Reimert [b]

[a] Animal Nutrition Group, Department of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[b] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[c] Wageningen Livestock Research, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands

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Abstract

Birthweight (**BiW**) plays an important role in explaining variation in BW gain of piglets in an multi-suckling (**MS**) system which aims to improve animal welfare. However, some low BiW piglets were found to show compensatory growth, indicating that BiW is not the sole predictor of postnatal BW gain. As BiW was found to have effect on feed intake in the MS system, it may also explain variation in other piglet traits. This study aims to investigate the relationships between BiW and several piglet traits including feed and milk intake, behaviours, skin lesions and mortality up to day 44 postpartum (**p.p.**) during lactation, and to investigate which piglet traits can predict whether a piglet has the ability to catch-up or not. Data were pooled from two experiments, using a total of 55 sows and 180 focal piglets. Focal piglets were divided into 4 groups based on their BiW class (high BiW (**HBW**) vs. low BiW (**LBW**)) and growth rate class (fast vs. slow). Within BiW class, piglets were defined as fast growing when their BW on day 44 p.p. was equal to or exceeded the median BW of their litter, others were defined as slow growing. Results showed that: (1) Piglets with a low BiW less than 1.1 kg were more prone to be at risk of death during lactation. (2) Birthweight was positively related with BW gain on days 0-44 p.p., number of sucklings, correspondingly to milk intake, and to a lesser extent to the intake of sow feed measured in week 6. Birthweight was also positively related with skin lesions measured in week 4. (3) We found no indications that fast-growing, LBW piglets differed from fast growing, high HBW piglets. However, fast growing piglets differed from slow growing piglets, regardless of their BiW class; for piglets born both small and big, fast growing piglets tended to eat more feed, were present less often at teats of alien sows and have more skin lesions measured in week 6, compared to slow growing piglets. Hence, our study confirm the previously published link between BiW and BW gain of piglets and provide insight into the traits that are affected by BiW throughout lactation in an MS system. Our study provides little insight into the mechanisms of catchup growth in an MS environment, but increases insight into the way that fast growing piglets differ from slow growing piglets, regardless of their BiW class.

Keywords

Group lactation, Piglet uniformity, Preweaning survival, Compensatory growth, light piglets

Implications

We studied the relationships between birthweight and piglet traits in a multi-suckling system, and studied which traits can predict whether a low birthweight piglet has the ability to catch-up or not. We found that birthweight was positively related with number of sucklings, milk intake, feed intake, and skin lesions. Our study provides little insight into the mechanisms of catchup growth. However, for piglets born both small and big, fast growing piglets tended to eat more feed, were present less often at teats of alien sows and had more skin lesions.

5.1 Introduction

Genetic selection for increased litter size during the last decades has coincided with a higher proportion of low birthweight (**BiW**) piglets within litters, and thus increased within litter variation in BiW (Moreira et al., 2020; Knol et al., 2022), which consequently results in an increased pre-weaning mortality (Ward et al., 2020; Knol et al., 2022). The heterogeneity in weight gradually increases as piglets grow older (Paredes et al., 2012), which negatively affects production efficiency in current all-in-all-out pig production systems (Douglas et al., 2014).

During the last decades, various housing systems have been designed that aim to be closer to the natural living conditions of pigs. One example of such system is a multi-suckling (**MS**) system, in which several sows and their litters are housed together during a longer lactation (Van Nieuwamerongen et al., 2017; Thomsson et al., 2018). As consequences of BW uniformity for subsequent performance have usually been studied in conventional housed pigs, the current study aims to explore this for an MS system.

In conventional housing, variation in BiW was found to account for substantial variation in BW or BW gain of piglets (Lodge and McDonald, 1959; Thompson and Fraser, 1986; Fraser et al., 1994). For example, BiW was significantly correlated with BW gain during days 0-23 after birth (r=0.392) (Muns et al., 2013). It was found that BiW explained 30%-40% of the within-litter variation in BW of piglets at 2 weeks of age (Thompson and Fraser, 1986), 34% of the individual variation in BW of piglets at 21 days of age (Wiegert et al., 2017), and 20% of the between-litter variation in BW of piglets at 8 weeks of age (Lodge and McDonald, 1959). In our previous research in an MS system, we explored factors affecting variation in BW gain of piglets which included BiW, behaviours, genetics, skin lesions and feed intake (Tang et al., 2022a). We found that BiW, ingestive behaviour and piglet feed intake were important piglet traits which influenced piglet BW gain variation during lactation. In addition, we found that BiW also played an important role in explaining variation in feed intake during middle lactation in the MS system. In addition to its effect on feed intake, BiW may also play a role in explaining variation in other piglet parameters.

Many studies in conventional housing have shown that BiW affects pre-weaning mortality, as piglets that died before weaning were lighter at birth (Milligan et al., 2002; Quiniou et al., 2002; Hales et al., 2013; Knol et al., 2022). For example, Knol et al. (2022) found a curvilinear relationship between individual BiW and lactation survival during lactation, with the highest survival at the highest birthweight; piglets with BiW above 0.9 kg have >70% survival, while piglets with BiW below 0.7 kg have < 25% survival. Hales et al. (2013) found that piglets that survived till weaning had a higher BiW than piglets that died before weaning (1.5 vs. 1.2 kg, *P*<0.001). As piglet mortality during 0-4 weeks of age before weaning was found to be higher in an MS system compared with conventional housing (Van Nieuwamerongen et al., 2015), the question arises how this was related to BiW. Therefore, the first aim of the current study was to investigate the relationships between BiW and piglet traits including growth performance, nutrient intake, behaviours and skin lesions, and to study the relationship between BiW and piglet mortality, up to day 44 of lactation in an MS system.

The majority of literature suggests an important role of BiW for postnatal growth, with piglets born light more likely to have a lower BW throughout their life (Quiniou et al., 2002; Rehfeldt and Kuhn, 2006; Beaulieu et al., 2010). However, it has been found that some low birthweight piglets are able to show compensatory growth, whereas others are not (Paredes et al., 2012; Douglas et al., 2013). For example, Douglas et al. (2013) found that piglets with a BiW below the average BiW of the population were able to achieve a subsequent BW that was at or above the average weight of the population for that age. In addition, it was proposed that the impact of BiW on growth rate of piglets decreases with increasing age (Madsen and Bee, 2015). This suggests that BiW is not the sole predictor of postnatal growth performance (Douglas et al., 2016). In conventional housing, the characteristics that make low birthweight piglets have the ability to compensate growth have been explored, for example morphology characteristics such as body mass index and abdominal circumference (Douglas et al., 2016). Therefore, the second aim of the current study was to investigate which piglet traits can predict whether a piglet has the ability to catch-up or not, by comparing various piglet traits between catch-up piglets and piglets that do not catch-up.

5.2 Material and Methods

5.2.1 Animals and experimental design

Data were utilized from two studies which were conducted in an MS system, i.e. Study 1 (Tang et al., 2022a) and Study 2 (Tang et al., 2022 (submitted)), at Swine Innovation Center, Sterksel, the Netherlands.

The main experimental design and general management will be described briefly. As we collected specific data of the piglets in week 6 p.p. in both studies, the animal management in week 6 will be described in more detail. Other details can be found in the previous publications (Tang et al., 2022 (submitted); Tang et al., 2022a).

In total, 55 multiparous sows (Topigs 20) were used in 3 batches of 5 sows in Study 1 (parity 3.9 ± 0.4) and 8 batches of 5 sows in Study 2 (parity 3.6 ± 0.2). In each litter, 4 focal piglets (Tempo x Topigs 20) were selected based on their BiW, which were the piglets with the second lowest and second highest BiW for both the boars and gilts. In Study 2, treatments were applied, having 4 control (**CTRL**) batches and 4 treatment (**TREAT**) batches. In TREAT of Study 2, interventions were applied aimed at improving the BW gain of small piglets, by later grouping of the piglets at days 13-14 postpartum (**p.p.**) and split-weaning on day 35 p.p. In both studies, sows and focal piglets were marked to be individually distinguished.

The MS system consisted of two MS units and one Intermittent Suckling (**IS**) area. Each MS unit consisted of five farrowing pens connected to a communal MS area including a lying area, a feeding area and a dunging area. Each farrowing pen had a feeding trough with a drinking nipple for the sows, a water nipple for the piglets, and a piglet nest. In the lying and dunging area of the system, three drinking bowls were accessible to both sows and piglets. In the feeding area, there were five feeding places for the sows which were also accessible for piglets, and one piglet feeding area in front of the sow feeding places which was only accessible to the piglets. This piglet feeding area contained three round small feeders (used until day 35 p.p.) containing 9 feeding places and a sensor-controlled automatic piglet feeder containing 10 feeding places (used from day 28 p.p.). In study 2, two extra feed hoppers with sow feed (used from day 21 p.p.) containing 4 feeding places were provided in the piglet feeding area to enable piglets access to sow feed both during the day and night.

Per batch, 5 pregnant sows were introduced into the MS system one week before the expected farrowing date. Sows were restrained between bars in a temporary cate within the farrowing pens from one night before the expected farrowing date until day 3 p.p. to prevent piglet crushing. From day 4 p.p. onwards, the bars were opened to create loose housing. Sows could move freely in and out the farrowing pens in the days before farrowing and from day 4 p.p. Piglets were given access to the whole system (i.e. grouped) on day 9.1 ± 0.1 p.p. in Study 1 and, dependent on treatment on day 8.2 ± 0.1 p.p. in CTRL and day 13.4 ± 0.1 p.p. in TREAT in Study 2. The averaged grouping age of Study 1 and 2 was day 10.3 ± 0.3 p.p. On days 28 - 34 p.p., sows were separated from the piglets for 10 h/d (07:00h -17:00 h) by bringing the sows to the IS area, in which the sows had contact with a boar. From day 35 p.p. onwards, sows could choose to be in the IS area at all times, whereas piglets could only stay in the MS unit. All piglets were weaned on days 64 and 48 p.p. in Study 1 and 2, respectively. The exception was made for TREAT in Study 2, where the three heaviest non-focal piglets within each litter were weaned and transferred to a rearing department on day 35 p.p. (i.e. split-weaning).

5.2.2 General housing and feeding management

Piglets were ear tagged within 1 day p.p. Litter sizes were standardized between 24 and 48 h p.p. (Study 1: 13.8 ± 0.3 piglets/litter; Study 2: 14.3 ± 0.1 piglets/litter) according to the number of functional teats available per sow. Piglets were not tail docked, teeth resected or castrated. On days 0-38 p.p., piglets firstly received large creep feed pellets (Study 1: from day 2 p.p. until 2 days after grouping; Study 2: from day 2 p.p. until 3 days after grouping), thereafter a weaner diet (on days 9-21 p.p.), pre-starter diet (on days 20-38 p.p.) and starter diet (from days 37 p.p. onwards). In Study 2, TREAT piglets also received sow feed in the farrowing pens on days 9-14 p.p. In both studies, from day 39 p.p. onwards, only starter diet was provided via the automatic piglet feeder in the piglet feeding area until weaning.

Sows were fed twice daily at 0800 and 1600 h. From voluntary IS (day 35 p.p.) onwards, in Study 1, sows were fed in the IS area at 0800 h and were floor fed in the MS feeding area at 1600 h; in Study 2, sows were fed in the MS feeding area both at 0800 and 1600 h. Water was available ad libitum for sows and piglets. In Study 1 and 2, piglets could eat sow feed together with the sows during sow feeding times; additionally in Study 2, sow feed was available at all times in sow feed hoppers in the piglet feeding area.

Enrichment materials were provided throughout the lactation period. In the MS units, the enrichment included 2 hessian sacks in each farrowing pen during farrowing as nesting materials, 2 handfuls of long straw from day 2 p.p. in each farrowing pen, 5 hessian sacks with 5 ropes which were hung on the wall in the lying area and were regularly replaced. In the IS area, the enrichment included 1 rope and 2 hessian sacks for the sows, and 1 metal chain and 1 rope for the boar.

5.2.3 Data collection from Study 1 and Study 2

BW

Body weight of piglets in both studies was assessed on days 0, 27 and 44 p.p. Body weight of piglets in Study 2 was additionally determined on day 35 p.p.

Feeding behaviour during sow feeding times and during the day

Feeding behaviour of the piglets during sow feeding times was scored live using 2-min instantaneous scan sampling on days 40−43 p.p. at 1600-1630 h in Study 1, and days 41-43 p.p. at 0800-0830 h and 1600-1630 h in Study 2. Feeding behaviour of the piglets during the day was scored live on day 41 p.p. at 0830-1600 h, using 30-min and 15-min instantaneous scan sampling in Study 1 and 2, respectively.

During these observation periods, for each focal piglet it was noted whether a piglet was in the feeding area, contacting (i.e. sniffing or eating) sow feed or contacting (i.e. sniffing or eating) piglet feed. From these observations, the percentage of time spent on contacting sow feed and piglet feed was calculated per piglet.

Suckling behaviour

In both studies on day 41 p.p. at 0830-1600 h, the frequency of presence at teats of each focal piglet in all successful suckling bouts (at biological mother and other sows), on either the front (the first two pairs of teats), the rear (the last two pairs of teats) or the middle teats (the remaining teats) was determined. An unsuccessful nursing bout was defined when it started within 20 min after a previous nursing bout (Weary et al., 2002) and with no milk let-down. The unsuccessful bouts were subsequently excluded from the analysis. The frequency of presence at teats at both their own mother and cross-suckling sows was calculated per piglet during the 7.5 h of observations; the frequency of presence at alien teats i.e. the teats of cross-suckling sows was calculated per piglet during the 7.5 h of observations as well. The frequency of presence at front and middle teats (**FM teats**) were summed into one variable for further analysis.

Skin lesions

On day 44 p.p., the number of skin lesions was counted per piglet by visual assessment as the number of fresh lesions on the whole body, except for ears and tails. Scoring was performed according to Tang et al. (2022b). Skin lesions can be regarded as a proxy for aggressive behaviour given and received (Turner et al., 2006).

Individual nutrient intake

The dual marker method (Tang et al., 2022b) was used to measure individual DM intake of sow feed and piglet feed on days 42-43 p.p. The dual marker included two types of markers, i.e. a reference marker and an in-feed marker. N-alkanes C31 and C36 were considered as in-feed markers for the sow and piglet diets, respectively, with a concentration of 40-50 mg/kg C31 in sows diets and 160-170 mg/kg C36 in piglets diets. C31 was provided via the inclusion of 15% alfalfa in the sow feed. C36 was melted on soybean meal in a forced air oven followed by mixing it into the piglet feed. The reference marker C32 was provided to the piglets on days 42-43 p.p. via a feed bolus for 3 times/day at 0830 h, 1430 h and 2030 h, with each bolus (~2.0 g) containing 20 mg of C32. On day 44 p.p., two spot faecal samples were collected from each focal piglet at 0830 h and 1230 h. N-alkanes in faecal and feed samples were measured by gas chromatography (Smit et al., 2005).

DM intake of sow feed and piglet feed in each piglet was calculated for days 42-43 p.p. using eq. [1]:

$$\text{Estimated intake of piglet or sow feed (g/day)}$$

$$= \frac{\left(\frac{\text{concentration of in-feed marker in faeces (mg/kg)}}{\text{concentration of reference marker C32 in faeces (mg/kg)}} \times \text{daily intake of reference marker C32 (mg/day)}\right)}{\text{concentration of in-feed marker in diet (mg/kg)}} \times 1000$$

[1]

Milk intake was calculated using eq. [2], assuming fixed feed conversion ratios (**FCR**) of converting DM feed intake into BW gain of 1.5 g/g, and assuming a fixed efficiency of converting fresh milk into BW gain of 4.89 g/g (Theil et al., 2002).

$$\text{Estimated intake of milk (g/day)}$$

(BW gain (g/day) – intake of total feed (g/day) / FCR (g/g)) × 4.89

[2]

DM intake of milk was then calculated assuming a DM content of 19% (Hurley, 2015). The complete procedures for the calculation of nutrient intake can be found in (Tang et al., 2022a; Tang et al., 2022 (submitted)). In Study 1, BW gain was calculated on days 27-44 p.p., while in Study 2 BW gain was calculated on days 35-44 p.p.

Piglet mortality

For all piglets in the MS system, the percentage of piglet mortality per litter (%) and the percentage of crushed piglets per litter (%) on days 0-8 p.p., days 0-26 p.p. and on days 0-44 p.p. were calculated. The number of dead and crushed piglets per litter were also calculated. Stillborn piglets were excluded.

All piglets in the MS system were then grouped into 14 BiW categories, ranging from 0.7 to 2.0 kg in 0.1 kg intervals. Piglets with BiW below 0.7 and over 2.0 kg were placed into the 0.7 and 2.0 kg BiW class, respectively. There were 14 and 9 piglets born with BiW below 0.7 and over 2.0 kg, respectively. Piglet mortality (dead piglets/total number of alive piglets on day 0 p.p. x 100%) within each BiW class was calculated for two periods, i.e. days 0-26 and 27-44 p.p. Stillborn piglets were excluded.

5.2.4 The definition of fast and slow growing piglets

Focal piglets were divided into four groups based on their BiW class and growth rate class. BiW class: high BiW (**HBW**) focal piglets and low BiW (**LBW**) focal piglets. Growth rate class (fast vs. slow): Within each BiW class, focal piglets were defined as fast growing when their BW on day 44 p.p. was equal to or exceeded the median BW of their litter; other focal piglets were identified as slow growing. LBW-fast growing piglet was also called a catch-up low BiW piglet.

5.3 Statistics

Statistical analyses were conducted with SAS 9.4. Data were merged from Study 1 and 2. The variables of DM milk intake (g/day) and contacting piglet feed during the day (% of observations) were the residuals of the original values corrected for treatment effect in Study 2, as these were the only variables significantly affected by treatment among all measured variables in Study 2.

The effect of sex (boars vs. gilts) and birthweight (continuous) and their interaction on multiple response variables including BW gain, nutrient intake, feeding and suckling behaviour and skin lesions were analyzed by analysis of covariance using the General Linear Models (GLM) procedure. Study was also included in the GLM models as a fixed effect. The interaction between study and sex, and the interaction between study and birthweight were initially included in the GLM models as well, but were removed from the final models as these did not reach statistical significance (*P*>0.05).

The effects of BiW class (HBW vs. LBW), growth rate class (fast vs. slow) and their interaction on multiple response variables were analyzed by analysis of variance. Study was also included in the models as a fixed effect. The interaction between study and BiW class, and the interaction between study and growth rate class were initially included in the models as well, but were removed from the final models as these did not reach statistical significance (*P*>0.05). Batch nested within study was included as random effect. When the interaction between BiW class and growth rate class was significant, it was further investigated with post hoc pairwise comparisons using the differences of the least squares means among four types of focal piglets (HBW-fast, HBW-slow, LBW-fast, LBW-slow). For continuous response variables, i.e. BW, BW gain and DM intake of feed and milk, the normality of model residuals was checked using PROC UNIVARIATE. The distribution of residuals in the model with DM intake of milk as response variable was not normal; therefore, DM milk intake was converted using log (1+N) before analysis in PROC MIXED. For the other continuous variables, the distribution of residuals was normal and PROC MIXED was used. For proportional response variables, i.e. proportion of time spent on contacting sow feed and piglet feed during sow feeding times and during the day which were in the range of 0-1, PROC GLIMMIX with a beta distribution and logit link function was used; when the proportion was equal to 0 and 1, it was converted to 0.0000001 and 0.9999999 before analysis, respectively, to accommodate a beta distribution. For count response variables, i.e. the frequency of presence at teats and skin lesions, PROC GLIMMIX with Laplace approximation, Poisson distribution and log link function were initially used. In models where no evidence of overdispersion was present, i.e. the values of Pearson Chi-Square / DF were smaller than one (Stroup et al., 2018), Poisson distribution was used; When overdispersion was detected, a negative binomial distribution was used as an alternative for the Poisson distribution. Statistical significance was set at *P*<0.05 and tendency was set at 0.05<*P*<0.10. Data are presented as mean ± SEM.

5.4 Results

5.4.1 Birthweight and mortality

The percentage of piglet mortality per litter (%) and the percentage of crushed piglets per litter (%) during days 0-8 p.p. were 14.4 ± 1.8% and 9.9 ± 1.4%, respectively, during days 0-26 p.p. were 20.5 ± 2.2% and 14.5 ± 1.8%, respectively, and during days 0-44 p.p. were 21.6 ± 2.2% and 14.9 ± 1.9%, respectively. The number of dead and crushed piglets per litter during days 0-8 p.p. were 2.3 ± 0.3 and 1.6 ± 0.2, respectively, during days 0-26 p.p. were 3.2 ± 0.4 and 2.3 ± 0.3, respectively, and during days 0-44 p.p. were 3.4 ± 0.4 and 2.3 ± 0.3, respectively.

Number of dead and alive piglets in different BiW class on days 0-26, days 27-44 up to day 44 of lactation was summarized in Supplementary Figure 1. As shown in Figure 1, there was a curvilinear relationship between piglet mortality (%) and BiW, where mortality were higher for low BiW piglets. Mortality on days 0-26 p.p. and 0-44 p.p. declined and plateaued when BiW increased above 1.0 kg. Of all piglets that died during days 0-44 p.p., 68.3 % of the piglets died during days 0-8 p.p.; 68.5 % of the piglets died during days 0-8 p.p. was due to crushing. 68.8 % of the piglets died during days 0-44 p.p. was due to crushing.

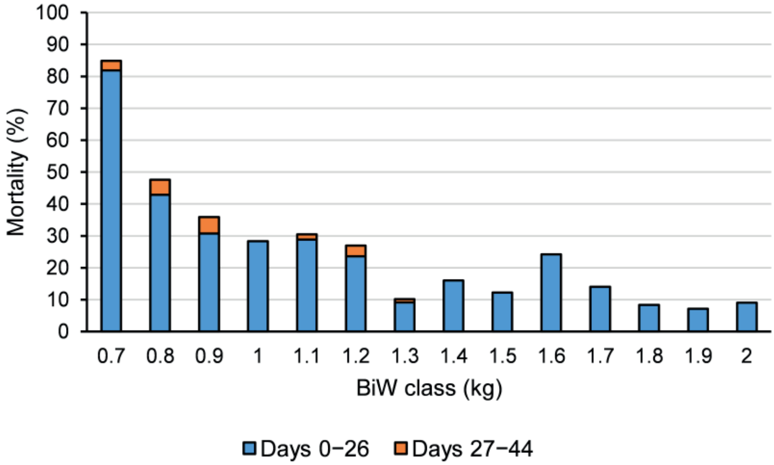

Figure 1. Piglet mortality (%) in different birthweight (BiW) classes on days 0-26 p.p. and 27-44 p.p. in all piglets up to day 44 of lactation in the multi-suckling system. Piglet mortality was calculated as the number of dead piglets divided by the number of both dead and alive piglets within BiW class. Piglets with BiW <0.7 and >2.0 kg are included in the 0.7 and 2.0 kg BiW class, respectively. Stillborn piglets were excluded from the data set and piglets which were cross-fostered in the MS system were included in the data set. Data were merged from two animal studies conducted in the MS system. In Study 1, piglets were grouped on days 8-9 p.p. In Study 2, piglets in control groups were grouped on days 8-9 p.p. and no split-weaning was applied; piglets in treatment groups were grouped on days 13-14 p.p. and the three heaviest non-focal piglets per litter were split-weaned on day 35 p.p. Previously we found no treatment effect on most of the measured variables of piglets in Study 2.

5.4.2 Birthweight and piglet traits

As shown in Table 1, in all piglets, BiW (kg) was positively related with BW gain (g/day) during days 0-27 p.p. (β_{BiW}=102 g/kg of BiW per day, P<0.001), 27-44 p.p. (β_{BiW} =110 g/kg of BiW per day, P<0.001) and 0-44 p.p. (β_{BiW} =102 g/kg of BiW per day, P<0.001). Likewise, in focal piglets, BiW was positively related with BW gain on days 0-27 p.p. (β_{BiW} =85 g/kg of BiW per day, P<0.001), 27-44 p.p. (β_{BiW} =103 g/kg of BiW per day, P<0.001) and 0-44 p.p. (β_{BiW}=91 g/kg of BiW per day, P<0.001), and this relation was similar for both sexes.

Other parameters were available for focal piglets only. During days 42-43 p.p., BiW tended to be positively related with DM intake of sow feed (β_{BiW} =44 g/kg of BiW per day, P=0.065) and milk intake (β_{BiW} =102 g/kg of BiW per day, P<0.001). Birthweight was not related with DM intake of piglet feed, DM intake of total feed, the percentage of time spent on contacting feed during sow feeding times on days 40-43 p.p., or contacting total feed during the day on day 41 p.p. Both the percentage of time spent on contacting sow feed during the day and piglet feed during the day on day 41 p.p. were affected by BiW in gilts (β_{BiW}=4.6 %/kg of BiW, P=0.038 and β_{BiW}= –3.4 %/kg of BiW, P=0.081, respectively) (interaction effect: P=0.041 and P=0.055, respectively), but not in boars. Birthweight was positively related with the frequency of presence at front and middle teats (β_{BiW}=2.3 no./kg of BiW per 7.5 h, P=0.009) and total teats on day 41 p.p. (β_{BiW}=2.8 no./kg of BiW per 7.5 h, P<0.001), but it was not related with the frequency of presence at rear teats or at alien teats. Birthweight was positively related with skin lesions on day 27 p.p. (β_{BiW} =4.3 no./kg of BiW, P =0.012).

Table 1. Results of the linear regression analysis between response variables and birthweight (BiW, kg) in all piglets and focal piglets[1] up to day 44 of lactation in the multi-suckling (MS) system.

		Mean ± SEM	β_{BiW} [2]	*P*	
				BiW	Sex
Response variables [3] for all piglets (n=621 on day 44)					
BW gain (g/day)	Days 0-27	214 ± 2	102	**<0.001**	0.153
	Days 27-44 [4]	398 ± 4	110	**<0.001**	**0.024**
	Days 0-44 [5]	285 ± 2	102	**<0.001**	**0.027**
Response variables for focal piglets (n=214 on day 44)					
BW gain (g/day)	Days 0-27	216 ± 3	85	**<0.001**	0.270
	Days 27-44	412 ± 6	103	**<0.001**	0.904
	Days 0-44	291 ± 4	91	**<0.001**	0.549
Nutrient DM intake (g/day) on days 42-43	Sow feed	163 ± 6	44	**0.065**	0.793
	Piglet feed	293 ± 12	5	0.918	0.832
	Total feed	456 ± 13	50	0.354	0.925
	Milk	137 ± 7	102	**<0.001**	0.420
Feeding behaviour during sow feeding times (%) on days 40-43	Contacting sow feed	11.9 ± 0.7	1.3	0.609	0.221
	Contacting piglet feed	7.1 ± 0.5	2.7	0.205	0.832
	Contacting total feed	18.9 ± 0.8	4.0	0.235	0.410
Feeding behaviour during the day (%) on day 41	Contacting sow feed [6]	4.7 ± 0.3	**---**	0.278	**0.035**
	Contacting piglet feed [7]	4.6 ± 0.3	**---**	0.481	**0.045**
	Contacting total feed	9.3 ± 0.5	0.8	0.672	0.968
Suckling behaviour: the presence at teats (no./7.5h) on day 41	Front and middle teats	4.3 ± 0.2	2.3	**0.009**	0.883
	Rear teats	1.7 ± 0.2	0.5	0.524	0.898
	Total teats	6.0 ± 0.2	2.8	**<0.001**	0.746
	Alien teats	1.4 ± 0.2	-0.3	0.705	0.467
Skin lesions (no.)	Day 27	5.2 ± 0.4	4.3	**0.012**	0.105
	Day 44	8.3 ± 0.5	3.3	0.108	0.746

[1] Focal piglets: On day 14 postpartum (p.p.), in each litter, the surviving second highest and lowest BiW piglets from both sexes were selected as focal piglets.

[2] β_{BiW}: the regression coefficient of BiW, which was averaged over both sexes.

[3] The explanation of measured variables is further explained in the text.

[4] BW gain of all piglets on days 27-44: least squares means ± SEM: boars: 379 ± 5 g/day, gilts: 394 ± 5 g/day.

[5] BW gain of all piglets on days 0-44: least squares means ± SEM: boars: 276 ± 3 g/day, gilts: 285 ± 3 g/day.

[6] Contacting sow feed during the day: *P* (sex x BiW interaction)=0.041; Boars: Mean ± SEM: 4.9 ± 0.5 (%), $\beta_{BiW=}$ -1.4, *P*=0.466; Gilts: Mean ± SEM: 4.4 ± 0.5 (%), $\beta_{BiW=}$ 4.6, *P*=0.038.

[7] Contacting piglet feed during the day: *P* (sex x BiW interaction)=0.055; Boars: Mean ± SEM: 4.4 ± 0.4 (%), β_{BiW}= 1.6, *P*=0.357; Gilts: Mean ± SEM: 4.9 ± 0.4 (%), $\beta_{BiW=}$ -3.4, *P*=0.081.

5.4.3 The effect of growth rate class and BiW class on piglet traits

Table 2 shows the piglet traits as affected by BiW class (high BiW vs. low BiW) and the growth rate class (fast vs. slow). For BW and BW gain, the interaction between BiW class and growth rate class was not significant (Table 2). BiW class only significantly affected BW on day 27 p.p., with HBW piglets having a higher BW on day 27 p.p. than LBW piglets (7.7 ± 0.1 vs. 6.8 ± 0.1 kg, P=0.016). BiW class did not affect BW on day 44 p.p., nor BW gain on days 0-27, 27-44 or 0-44 p.p.

None of the nutrient intake parameters were different among the four types of piglets. For feeding behaviours, the interaction between BiW class and growth rate class tended to be significant only for the variable contacting piglet feed during sow feeding times on days 40-43 p.p. (P=0.071), where HBW-fast piglets tended to spend more time on contacting piglet feed during sow feeding times than LBW-fast piglets (7.5 ± 0.8 vs. 6.6 ± 1.1 %, P=0.065). Suckling behaviours, i.e. the frequency of presence at teats were not different among the four types of piglets. For skin lesions, the interaction between BiW class and growth rate class tended to be significant on day 27 p.p. (P=0.084), where within HBW piglets, HBW-fast piglets had more skin lesions than HBW-slow piglets (5.7 ± 0.7, vs. 3.9 ± 1.2, P=0.010), while this difference did not exist within LBW piglets.

For the effect of growth rate class, fast growing piglets from both the high and low BiW class on average had a higher BW on day 27 p.p. (7.8 ± 0.1 vs. 6.6 ± 0.1, P<0.001), and day 44 p.p. (15.4 ± 0.2 vs. 12.8 ± 0.2, P<0.001), and a higher BW gain on days 0-27 p.p. (233 ± 4, vs. 195 ± 5, P<0.001), days 27-44 p.p. (448 ± 8 vs. 367 ± 8, P<0.001) and days 0-44 (316 ± 4, vs. 261 ± 5, P<0.001) compared to slow growing piglets. Correspondingly, fast growing piglets tended to have a higher total feed intake on days 42-43 p.p. (485 ± 18 vs. 420 ± 17, P=0.068). They tended to spend less time on contacting sow feed during the day on day 41 p.p. (4.5 ± 0.5 vs. 4.9 ± 0.5, P=0.095). Fast growing piglets were present less often at alien teats on day 41 p.p. (1.1 ± 0.2 vs. 1.8 ± 0.3, P=0.010), and had more skin lesions on day 44 p.p. (9.0 ± 0.6 vs. 7.4 ± 0.4, P=0.047) than slow growing piglets.

Table 2. BW, BW gain, nutrient intake, feeding behaviours, suckling behaviours, skin lesions and genetics in four types of focal piglets[1] i.e. HBW-fast, HBW-slow, LBW-fast and LBW-slow up to day 44 of lactation in the multi-suckling (MS) system.

	Mean ± SEM Piglet type [2]				*P*		
Variables [3]	HBW-fast	HBW-slow	LBW-fast	LBW-slow	BiW class [2]	Growth rate class [2]	Inter-action
No. of alive piglets on day 44							
	89	18	29	78	---	---	---
BW (kg)							
Day 0 [4]	1.6 ± 0.0	1.7 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	---	---	---
Day 27	7.9 ± 0.1	7.1 ± 0.3	7.5 ± 0.2	6.5 ± 0.1	**0.016**	**<0.001**	0.579
Day 44	15.6 ± 0.2	12.9 ± 0.4	14.8 ± 0.4	12.8 ± 0.2	0.151	**<0.001**	0.693
BW gain (g/day)							
Days 0-27	234 ± 4	200 ± 12	228 ± 8	194 ± 5	0.416	**<0.001**	0.868
Days 27-44	453 ± 9	353 ± 16	431 ± 17	371 ± 9	0.609	**<0.001**	0.529
Days 0-44	319 ± 5	255 ± 8	307 ± 9	262 ± 5	0.604	**<0.001**	0.537
Nutrient DM intake (g/day) on days 42-43							
Sow feed	171 ± 9	159 ± 25	175 ± 16	150 ± 9	0.800	0.157	0.382
Piglet feed	326 ± 21	252 ± 38	275 ± 32	272 ± 19	0.461	0.198	0.497
Total feed	497 ± 21	411 ± 33	450 ± 34	423 ± 20	0.436	**0.068**	0.764
Milk [5]	165 ± 14	87 ± 14	145 ± 19	113 ± 10	0.277	0.536	0.484
Feeding behaviour during sow feeding times (%) on days 40-43							
Contacting sow feed							
	12.1 ± 1.0	13.0 ± 3.0	10.8 ± 1.9	11.7 ± 1.0	0.469	0.715	0.625
Contacting piglet feed [6]							
	7.5 ± 0.8^{y}	6.3 ± 1.7^{xy}	6.6 ± 1.1^{x}	7.1 ± 0.8^{xy}	0.584	0.901	**0.071**
Contacting total feed							
	19.6 ± 1.2	19.2 ± 3.8	17.4 ± 2.2	18.7 ± 1.3	0.472	0.744	0.378
Feeding behaviour during the day (%) on day 41							
Contacting sow feed							
	4.4 ± 0.5	4.3 ± 1.5	5.0 ± 1.1	5.0 ± 0.6	0.733	**0.095**	0.944
Contacting piglet feed							
	4.7 ± 0.5	4.0 ± 1.0	4.3 ± 0.7	4.8 ± 0.5	0.875	0.391	0.550
Contacting total feed							
	9.0 ± 0.7	8.2 ± 1.9	9.1 ± 1.4	9.9 ± 0.8	0.723	0.402	0.827
Suckling behaviour: the presence at teats (no./7.5h) on day 41							
Front and middle teats	4.7 ± 0.3	4.1 ± 0.6	4.3 ± 0.6	4.0 ± 0.4	0.679	0.512	0.826
Total teats	6.1 ± 0.2	6.4 ± 0.5	6.3 ± 0.4	5.8 ± 0.3	0.665	0.859	0.614
Alien teats	1.2 ± 0.2	2.0 ± 0.7	0.8 ± 0.3	1.8 ± 0.3	0.383	**0.010**	0.833
Skin lesions (no.)							
Day 27 [7]	5.7 ± 0.7^{b}	3.9 ± 1.2^{a}	6.8 ± 1.3^{ab}	4.5 ± 0.6^{a}	0.667	**0.039**	**0.084**
Day 44	9.0 ± 0.7	7.1 ± 1.4	9.2 ± 1.4	7.5 ± 0.9	0.424	**0.047**	0.518

[1] Focal piglets: On day 14 postpartum (p.p.), in each litter, the surviving second highest and lowest birthweight piglets from both sexes were selected as focal piglets.

[2] Piglet type: Focal piglets were divided into four groups based on their birthweight (BiW) class and growth rate class. BiW class: high BiW (HBW) focal piglets and low BiW (LBW) focal piglets. Growth rate class (fast vs. slow): Within each BiW class, focal piglets were defined as fast growing when their BW on day 44 p.p. was equal to or exceeded the median BW of their litter; other focal piglets were identified as slow growing.

[3] The explanation of measured variables is further explained in the text. In a row, a,b values with different letters differ significantly ($P < 0.05$); x,y values with different letters tend to be different ($0.05 < P < 0.10$).

[4] No statistics were performed for BiW.

[5] The least squares means ± SEM of DM milk intake were 4.3 ± 0.1, 3.9 ± 0.3, 4.0 ± 0.2, 3.9 ± 0.2 for HBW-fast, HBW-slow, LBW-fast, LBW-slow piglets, respectively (the model was parameterized using a lognormal distribution).

[6] The least squares means ± SEM of contacting piglet feed during sow feeding times (%) were -2.4 ± 0.1, -2.7 ± 0.2, -2.8 ± 0.2, -2.5 ± 0.1 % for HBW-fast, HBW-slow, LBW-fast, LBW-slow, respectively (the data was parameterized of the beta distribution).

[7] The least squares means ± SEM of skin lesions (no.) on day 27 were 1.7 ± 0.3, 1.2 ± 0.3, 1.4 ± 0.3, 1.4 ± 0.3 for HBW-fast, HBW-slow, LBW-fast, LBW-slow, respectively (the model was parameterized using a negative binomial distribution).

5.5 Discussion

The first aim of the current study was to investigate the relationships between BiW and several piglet traits including nutrient intake, feeding and suckling behaviours and skin lesions, which have been shown to influence the variation in BW gain of piglets in an MS system (Tang et al., 2022a). In addition, we studied the relationship between BiW and piglet mortality, up to day 44 of lactation. We found increased mortality during lactation in piglets with a birthweight less than 1.1 kg. Birthweight was positively related with number of sucklings, correspondingly to milk intake, and to a lesser extent to the intake of sow feed. In addition, it was positively related with skin lesions. As some low birthweight piglets seem to have the ability to compensate their growth while some cannot, the second aim of the current study was to investigate what characteristics affect the ability of low birthweight piglets to catchup. We found no indications that fast-growing, LBW piglets differ from fast growing, HBW piglets. However, our study increases insight into the way that fast growing piglets differ from slow growing piglets, regardless of their BiW class. For piglets born both small and big, fast growing piglets tended to eat more feed, were present less often at teats of alien sows and had more skin lesions, compared to slow growing piglets.

5.5.1 Birthweight and mortality

In the current study, we found that the percentage of piglet mortality per litter (%) during days 0-8 p.p. and 0-44 p.p. were 14.4 % and 21.6 %, respectively. It was bit lower than the mortality in the study of Thomsson et al. (2016), who found 17% (days 0-7) and 27% (days 0-44) in an MS system where litters were grouped after week 1 p.p. In the current study, the number of dead piglets per litter during days 0-8 p.p. and 0-26 p.p. was 2.3 and 3.2, respectively, which was comparable with observations in the MS system by Van Nieuwamerongen et al. (2015). The high mortality in MS system compared to conventional housing (10-15%) (Kirkden et al., 2013; Heuß et al., 2019; Vande Pol et al., 2021) was also observed by Verdon et al. (2020), and is likely caused by the increased crushing in farrowing pens before grouping (Van Nieuwamerongen et al., 2015; Verdon et al., 2020).

In the current study, we found that the majority (68.3 %) of the piglets dying occurred during days 0-8 p.p. which is in agreement with Thomsson et al. (2016); and of these, the majority (68.5%) was due to crushing, which was in agreement with observations by Van Nieuwamerongen et al. (2015). Also in conventional housing, it was reported that during lactation on days 0-25 p.p., more than half of the mortality (57.3%) occurred during days 0-4 p.p. and the majority of these were due to crushing (67.4%) (Marchant et al., 2000).

In the current study, we observed higher mortality of low BiW piglets. Similarly, several studies both in conventional housing and loose farrowing found that piglet mortality during lactation was negatively related with BiW (Pedersen et al., 2011;

Hales et al., 2013; Muns et al., 2013). Furthermore, we found that the relationship between total piglet mortality up to day 44 p.p. and BiW is curvilinear, where mortality rate declined and plateaued when BiW increased above 1.0 kg. Knol et al. (2022) also found a curvilinear relationship between individual BiW and lactation survival, with the survival increased and plateaued above around 1.2 kg. Marchant et al. (2000) found a curvilinear relationship between BiW and survival rate as well, and they found that only 28% of piglets weighing less than 1.1 kg at birth survived after 7 days. It was shown that piglets weighing less than 1.1 kg are particularly at risk in a cold environment (Herpin et al., 2004; Kammersgaard et al., 2011). It could be that low BiW piglets have a reduced thermoregulation ability (Kammersgaard et al., 2011; Vanden Hole et al., 2018) due to a greater surface to body mass ratio (Herpin et al., 2002), and are less able to compete for teats during suckling. Therefore, these low birthweight piglets stay longer close to the sow to minimize heat loss (Kammersgaard et al., 2011), and spend more time near the sow for stimulating the udder (Fraser, 1990; Weary et al., 1996), which increases the risk of being crushed by the sow. It was found that a large proportion of crushed piglets had an empty stomach (Andersen et al., 2011; Hales et al., 2013). For example, 48% and 21% of crushed piglets were found to have no milk in their stomach which died on days 0-1 and days 2-26 p.p., respectively (Hales et al., 2013).

In summary, piglets with a BiW less than 1.1 kg have increased risk for mortality during lactation. Of all dead piglets on days 0-44 p.p., the majority of them dying on days 0-8 p.p. due to crushing. To reduce piglet mortality in the MS system, further interventions before grouping should be considered, including reducing crushing before grouping, ensuring enough heat protection and milk intake for especially piglets with lower BiW.

5.5.2 Birthweight and piglet traits

For the piglets that survived till day 44 p.p. in the current study, BiW was positively related with BW gain on days 0-27 p.p., 27-44 p.p. and the total period on days 0-44 p.p. Similarly, in conventional housing, it was found that BiW was significantly correlated with BW gain during days 0-23 p.p. pre-weaning (r=0.392) (Muns et al., 2013), and was significantly related with BW at the end of the nursery phase at week 10 p.p. (Paredes et al., 2012).

We investigated the underlying relations of BiW and BW gain by investigating several piglet traits, i.e. intake of nutrients and behavioural characteristics and we found that both the intake of sow feed, the intake of milk and the level of skin lesions were related with BiW. For intake of sow feed in week 6, we found that a 100 g increase in BiW was related with a 4.4 g/day increase in intake of sow feed and a numerical increase of total feed intake of 5.0 g/day. The relatively high contribution of sow feed intake could be related to the floor feeding of the sows, possibly enhancing intake of sow feed of piglets by maternal learning (Oostindjer et al., 2011). This effect of maternal learning might be more obvious on piglets with higher BiW, as they are stronger and might attack smaller piglets to get away from the sow feeding area. Similar to the general relationship between intake of solid feed and BiW in the current study, Van der Peet-Schwering et al. (2013) also found that piglets with a high BiW (averaged 1.56 kg) ate more from weaning up to 5 weeks after weaning in conventional housing, compared to piglets with a low BiW (averaged 1.13 kg) (feed intake: 0.66 vs. 0.60 kg/day per pig, P<0.001). Piglets with a higher BiW have a higher number of muscle fibres at birth (Alvarenga et al., 2013) which may result in higher muscle accretion and higher feed intake (Van der Peet-Schwering et al., 2021). It might also be that piglets with a higher BiW have a better developed digestive system which allows them to ingest more feed (Michiels et al., 2013).

For milk intake measured in week 6, we found that a 100 g increase in BiW was related with a 10 g/day increase in DM milk intake; BiW was also positively related with the frequency of presence at sucklings and specifically at the front and middle teats, measured in week 6. It was proposed in conventional housing that the competitive disadvantage for teats of the smaller piglets compared to the large ones remains throughout lactation (Andersen et al., 2011). The lower competence of piglets with lower BiW might make them miss more suckling bouts than other piglets, which may explain the positive relationship between BiW and the frequency of presence at sucklings. Front and middle teats were shown to produce higher quantities of milk (Gill and Thomson, 1956; Fraser et al., 1985; Skok et al., 2007) and higher concentrations of immunoglobulin in colostrum (Ogawa et al., 2014) than do rear teats. For example, front and middle teats produced 41 g milk/suckling during weeks 1-4 p.p., while rear teats produced 31 g milk/suckling (Skok et al., 2007). Possibly, piglets with higher BiW are more successful in competing for more productive teats, thus having an advantage in obtaining nutritional and immunological components. As a result, they may grow faster and healthier than piglets with lower BiW which suckle the rear teats throughout lactation. It was shown that the degree of mammary development during lactation is dependent on the extent of suckling intensity by piglets (Hurley, 2001). As sow grunting appears to attract piglets to the front teats (Skok et al., 2007) and piglets with higher BiW tend to win more fights and occupy the front teats (Hartsock et al., 1977), these piglets may have more intensive massage towards the front teats, hence stimulating higher milk yield of the front teats (Farmer, 2019).

We found that a 100 g increase in BiW was related with an increase of 0.4 skin lesions counted on day 27 p.p. The scoring of skin lesion has been used as an indicator for aggressive behaviour given and received (Turner et al., 2006; Guevara et al., 2022). Skin lesions could be caused by the reciprocal fighting activity among unfamiliar non-littermates immediately after grouping (Van Kerschaver et al., 2021) and competitive aggressive behaviour towards food (Bernardino et al., 2016). As grouping has been applied for 14-19 days until day 27 p.p., the skin lesions on day 27 p.p. are more possibly caused by competitive behaviour for food. In Study 1 and 2, on day 27 p.p., the piglet: feeding place ratio was 7:1 and 4:1, respectively, which probably provides the competitive environment for piglets. In addition, as the body size of piglets becomes bigger with increased age, the competition among piglets towards teats might also be bigger with increased age until day 27 p.p. It might be that the increased nutrient requirements of high BiW piglets causes them to fight for nutrient resources both in feeding area and around the teats during suckling bouts, thus having more skin lesions on day 27 p.p.

In summary, we confirm the previously published link between BiW and BW gain of piglets and provide insight into the traits that are affected by BiW throughout lactation in an MS system. We found that BiW was positively related with number of sucklings, correspondingly to milk intake, and to a lesser extent to the intake of sow feed. In addition, BiW was positively related with skin lesions.

5.5.3 The effect of growth rate class and BiW class on piglet traits

As discussed above, BiW was positively related with subsequent growth performance throughout lactation in the MS system. Previously, low BiW piglets have been shown capable of showing catch-up growth (Paredes et al., 2012; Douglas et al., 2013). We therefore investigated which pig traits affected piglets with low BiW to become a catchup-piglet. We divided all focal piglets into four groups, with the combination of BiW class (high vs. low) and growth rate class (fast vs. slow); therein, fast and slow growing piglets were defined within each BiW class when their BW on day 44 p.p. exceed or did not exceed the median value of their litter. Interactions between BiW class (high vs. low) and growth rate class (fast vs. slow growing) would then be indicative for catchup piglets in the current data. We found that the interaction between BiW class (high vs. low) and growth rate class (fast vs. slow) was only present for the piglet traits contacting piglet feed during sow feeding times and skin lesions. For most of the piglet traits including BW gain, feed and milk intake, feeding behaviour, suckling behaviour and skin lesions, neither the interaction between BiW class (high vs. low) and growth rate class (fast vs. slow), nor the effect of BiW class alone was present, indicating that the catchup growth effects were similar for high and low BiW piglets. Effects of growth rate class existed on some piglet traits, i.e. BW gain on days 0-27, 27-44 and 0-44 p.p., total feed intake, the frequency of presence at teats of alien sows and skin lesions. Therefore, our study demonstrated that the majority of the piglet traits measured are not specifically affecting LBW piglets; instead, for piglets born both small and big, fast growing piglets ate more feed, were present less often at alien teats and had more skin lesions.

In the current study, we found that the interaction between BiW class and growth rate class was only present for the traits contacting piglet feed during sow feeding times and skin lesions. HBW-fast piglets tended to spend more time on contacting piglet feed during sow feeding times than LBW-fast piglets, and HBW-fast piglets had more skin lesions than HBW-slow piglets. However, for these two traits, there were no differences between LBW-fast and LBW-slow piglets, and the reason is not clear.

The effect of growth rate class existed on some piglet traits, i.e. BW gain, feed intake, contacting sow feed during the day, skin lesions and the frequency of presence at teats of alien sows. In the current study, fast growing piglets had a higher total feed intake than slow growing piglets in week 6. Similarly, other studies also reported that feed intake plays an important role for growth rate of piglets after week 4 (Paredes et al., 2014; Van Nieuwamerongen et al., 2017). In the current study, fast growing piglets had more skin lesions than slow growing piglets in week 6. Similarly, Turner et al. (2006) also found a positive correlation between BW and lesions scores in post-weaned pigs after week 4. It was reported that in socially stable groups, the majority of aggressive behaviours occurs around the feeding area to obtain limited food resources (Hoy et al., 2012). In the current study, the limited piglet: feeding place ratio in week 6 (6:1 in Study 1 and 4:1 in Study 2) might provide a competitive environment for piglets, especially during the synchronization period when groups of piglets are eating at the same time in the piglet feeding area (Nielsen et al., 2016). It might be that in week 6 these fast growing piglets tend to have a higher motivation to feed (Valros et al., 2021), have more competition at the feeder (Algers et al., 1990; Valros et al., 2021), and are therefore involved in more mutual aggressions with other piglets, leading to skin lesions (Yang et al., 2018).

In the current study, fast growing piglets tended to spend less time on contacting sow feed during the day than slow growing piglets. However, as previously mentioned, we found a positive relationship between BiW and intake of sow feed, the reason of which is not clear. It indicates that the growth rate does not correspond to BiW, and the percentage of time spent on contacting sow feed during the day does not correspond to intake of sow feed. For the latter, it might be that feeding behaviour alone does not accurately reflect feed intake in late lactation (Tang et al., 2022a), as generally the ingestion frequency decreased and intake per meal increased with the increased age of piglets (Bus et al., 2021).

In the current study, fast growing piglets were present less often at teats of alien sows than slow growing piglets. It was reported that piglets tend to become a cross-suckler when it has to compensate for the low milk yield of its own mother by cross-suckling an alien sow with a higher milk yield (Olsen et al., 1998). It could be that due to the better ability to compete for teats, fast growing piglets are able to fulfil their need for milk already from their own mother, thus were present less often at teats of alien sows. After starting IS, for the slow growing piglets, especially when their own mother was in the IS area, or had a low milk yield, they are more likely to become cross-sucklers. However, as milk yield of sows reaches the peak in week 4 and decreases afterwards (Quesnel et al., 2015), these cross-sucklers probably could not even get enough milk from alien sows in week 6 thus leading to a reduced growth rate. It was previously reported that the IS strategy increased the dependency of the piglets on solid feed (Berkeveld et al., 2007). Therefore, it could also be that fast growing piglets might have higher dependency on solid feed than slow growing piglets due to the more mature digestive system, thus they might have less possibility to become cross-sucklers in week 6.

5.6 Conclusion

The current study confirms previously published links between birthweight and survival, and the links between birthweight and BW gain of piglets and provides insight into the traits that are affected throughout lactation in an MS system. Increased mortality was observed in piglets with a BiW below 1.1 kg. Birthweight was positively related with number of sucklings, correspondingly to milk intake, and to a lesser extent to the intake of sow feed. In addition, it was positively related with skin lesions. We found no indications that fast-growing, LBW piglets differed from fast growing, HBW piglets and hence, our study provides little insight into the mechanisms of catchup growth in an MS environment. However, our study increases insight into the way that fast growing piglets differ from slow growing piglets, regardless of their BiW class. For piglets born both small and big, fast growing piglets tended to eat more feed, were present less often at teats of alien sows and had more skin lesions, compared to slow growing piglets.

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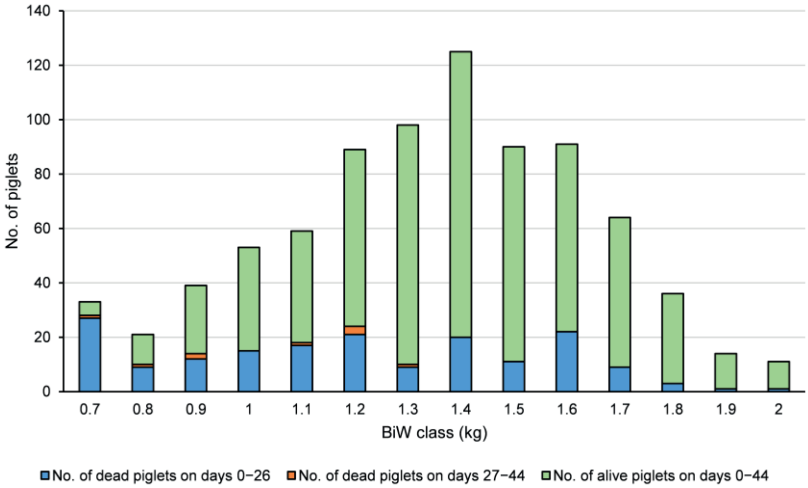
5.8 Supplementary materials



Supplementary Figure 1. Number of dead and alive piglets in different birthweight (BiW) classes on days 0-26 p.p., days 27-44 p.p. up to day 44 of lactation in the multi-suckling system. Piglets with BiW <0.7 and >2.0 kg are included in the 0.7 and 2.0 kg BiW class, respectively. Stillborn piglets were excluded from the data set and piglets which were cross-fostered in the MS system were included in the data set. Data were merged from two animal studies conducted in the MS system. In Study 1, piglets were grouped on days 8-9 p.p. In Study 2, piglets in control groups were grouped on days 8-9 p.p. and no split-weaning was applied; piglets in treatment groups were grouped on days 13-14 p.p. and the three heaviest non-focal piglets per litter were split-weaned on day 35 p.p. Previously we found no treatment effect on most of the measured variables in Study 2.

Chapter 6: General Discussion

6.1 Introduction

In recent years, integral group housing systems, or multi-suckling (**MS**) systems for lactating sows and their litters have been developed, mimicking the natural living conditions of pigs, as a solution to welfare problems of conventional farrowing pens. However, one of the problems associated with these MS systems is the large variation in growth rate of the piglets, a problem that is hampering introduction of such systems in commercial practice. The main aim of this thesis was to improve the feasibility of an MS system which can be used in the current all-in-all-out pig production systems, by reducing the variation in growth rates of individual piglets in the MS system during lactation. Variation in growth rate is discussed in **Chapter 1**. The first investigation of this thesis (**Chapter 2**) aimed to develop a dual marker method for the estimation of individual feed intake in piglets, for use in complex housing systems. A protocol was suggested with recommendations for frequency of administration of reference markers and faecal sampling strategy, which was used for the measurement of individual feed intake of piglets in the MS system in subsequent studies presented in this thesis. The second investigation of this thesis (**Chapter 3**) studied to what extent different factors including nutrient intake, behavioural traits and genetic background of piglets contribute to variation in growth rate of piglets in an MS system. We found that variation in BW gain of piglets was mainly explained by birthweight (**BiW**) (12%) and positive behaviour (play and nosing) (7.6%) in week 2-4, and by variation in solid feed intake in week 4-6 (15.1%) and 6-8 (25.9%). Additionally, we found that variation in feed intake was explained by piglet's teat presence (2.9%) in week 2-4, by its birthweight (9.6%) in week 4-6, and by its number of skin lesions (5.1%) in week 6-8. A remarkable finding of this study was that more than 55% of the variation in BW gain remained unexplained by the parameters studied. Based on the findings of this study, interventions were designed to reduce variation in growth rate of piglets in MS systems. The third investigation of this thesis (**Chapter 4**) studied the combination of two intervention strategies, i.e. later grouping of non-littermates (from day 8 to day 13 postpartum (**p.p.**)) and split-weaning the 3 heavy piglets on day 35 p.p., both aiming to improve the BW gain of specifically low birthweight (**LBW**) piglets and thereby reducing the BW variation at weaning on day 48 p.p. Several piglet traits including individual feed intake, the frequency of presence at sucklings, feeding behaviours, skin lesions and damage scores were investigated. However, we found that later grouping did not improve the growth performance, suckling behaviour and feeding behaviour of LBW piglets after grouping. Split-weaning increased milk intake, particularly of LBW piglets, but this did not lead to an increase in BW of LBW piglets and a reduction in BW variation at weaning, as the increased milk intake was largely compensated for by a simultaneous decrease in feed intake of these LBW piglets. We also found that piglet mortality per litter was high (around 22%) on days 2-48 p.p. Based on the findings in Chapter 3 that BiW plays an important role in explaining variation in growth rate of piglets during early lactation and in explaining variation in feed intake of piglets during middle lactation in the MS system, we hypothesized that BiW may also play a role in explaining variation in other piglet parameters in addition to feed intake, which probably further influences how these piglet parameters affect BW gain. In addition, based on the findings in Chapter 4 that piglet mortality was high in the MS system, the question arises how this was related to BiW. Therefore, the fourth investigation of this thesis (**Chapter 5**) studied the relationship between BiW and piglet traits including nutrient intake, behaviours, skin lesions as well as piglet mortality up to day 44 of lactation in the MS system, by utilizing the data from Chapter 3 and Chapter 4. As some low BiW piglets were able to show catchup growth in later life, we also investigated which characteristics these piglets had. We found that mortality of piglets with a BiW less than 1.1 kg was higher during lactation. In the surviving piglets birthweight was positively related with number of sucklings and correspondingly with milk intake, and to a lesser extent to the intake of sow feed. In addition, birthweight was positively related with the skin lesion score. We found no indications that fast-growing LBW piglets differed from fast growing HBW piglets and hence, Chapter 5 provides little insight into the mechanisms of catchup growth in an MS environment. However, Chapter 5 increased insight into the way that fast growing piglets differ from slow growing piglets, regardless of their BiW class. For piglets born both small and big, fast growing piglets tended to eat more feed, were present less often at teats of alien sows and had a higher skin lesion score, compared to slow growing piglets. As in Chapter 3, more than 55% of the variation in BW gain of piglets in early, middle and late lactation in the MS system remained unexplained, and in Chapter 4, the two interventions to reduce variation in BW of piglets had no obvious effects on variation in BW gain, there are many more possible interventions that can be considered for this aim. In this Chapter (**Chapter 6**), interventions that potentially contribute to a reduced piglet growth rate variation in the MS system are therefore discussed from a broader perspective, integrating the findings in this thesis to findings in literature. The sequence of potential interventions will be discussed in chronological order of events after parturition. The development of changes in nutrient intake from sow milk vs. solid feed over time is visualized in Figure 1 and is an important determinant of the success of interventions aiming at a reduction in the variation of BW gain. As milk and feed intake play an important role in the growth rate of piglets in early and late lactation, respectively, suckling behaviour (e.g. milk intake, teat presence at sucklings) related interventions are discussed firstly, followed by feeding behaviour (e.g. feed intake, the presence in the feeding area) related interventions and other interventions. Thereafter, cross-suckling strategies and piglet mortality, two important features of the MS system, will be discussed. The scientific, societal and pig production relevance of this thesis are discussed throughout this Chapter.

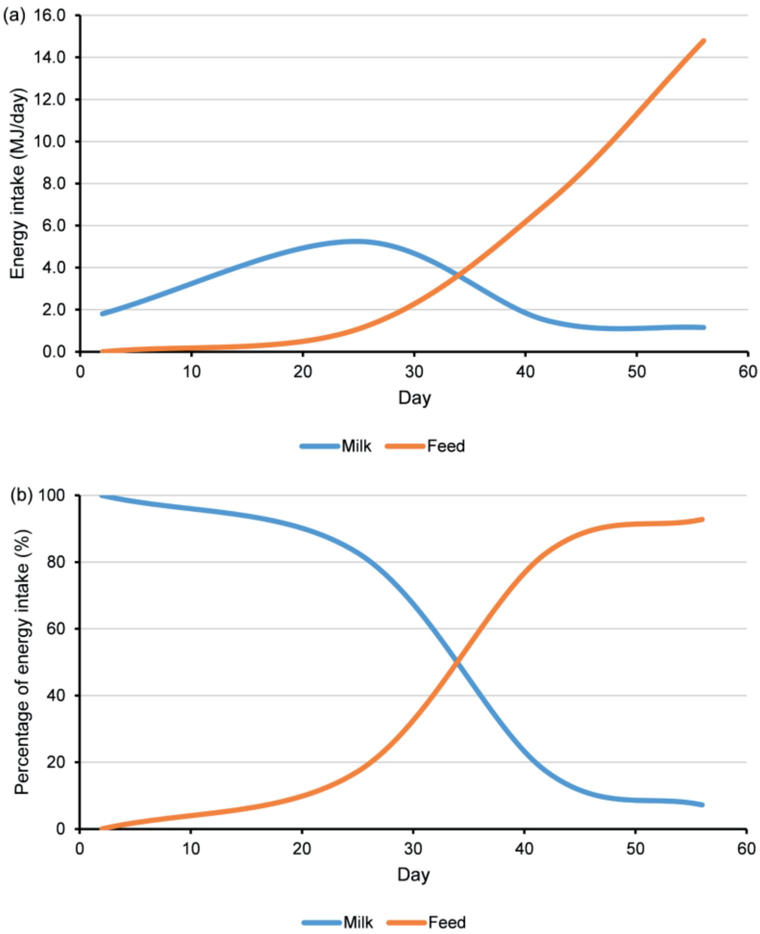


Figure 1. Energy intake (MJ/day) (a) and percentage of energy intake (%) (b) in piglets on days 0-60 after birth reared in the multi-suckling system (based on Chapter 3).

6.2 Interventions to reduce variation in growth rate of piglets in MS systems

Several studies showed that the lactation period has the greatest potential in reducing the birthweight associated growth rate variation among individual piglets compared to the later age period after weaning (Hawe et al., 2020). Catchup growth is more likely to occur at early age (Blavi et al., 2021). As the absolute BW variation increases with increasing age (Paredes et al., 2012), it seems most advantageous to steer interventions in early age. In the following sub-chapters, interventions aiming to reduce variation in BW gain of piglets during the lactation period in MS systems will be discussed, in chronological order of events after parturition, as well as considering the relative importance among these interventions.

The management regimes throughout lactation in MS systems are also described, as the success of interventions for reducing variation in BW of piglets are affected by how these management regimes are applied. Using the MS system that was conducted in this thesis as an example, generally, a group of sows are moved to the MS system one week before expected farrowing, and are restricted in individual farrowing crates on days 0-3 p.p. Litter sizes are standardized on days 1-2 p.p. by cross-fostering, based on the number of functional teats per sow. Sows are allowed to enter the MS area before farrowing and again from day 4 p.p. Litters are allowed to enter the MS area, i.e., grouping of litters, either on days 8-9 p.p. for early grouping or on days 13-14 p.p. for late grouping. Subsequently, to allow gradual weaning, intermittent suckling (**IS**) is applied, i.e. forcedly separating sows from piglets for several hours/day from day 28 p.p., and allowing sows to voluntarily leave the MS area from day 35 p.p. until weaning. Moreover, split-weaning is applied to reduce variation in within-batch BW of piglets on day 35 p.p. As the interventions are interspersed with these management regimes applied in MS systems, the different timing of applying these regimes as well as other regimes that could be used in MS systems, with the potential pros and cons will also be discussed.

6.2.1 Suckling related interventions for piglets

Variation in milk intake could influence the variation in BW gain of piglets (Skok et al., 2007). Therefore, reducing the variation in milk intake could help to improve BW gain uniformity of piglets. High BiW piglets were found to drink more milk than low BiW piglets, because, within the first week p.p. when the teat order was established, high BiW piglets more occupied front teats which were more productive than other teats (Hartsock et al., 1977). It was reported that during weeks 1-4 p.p., front and middle teats produced 41 g of milk/suckling, which was significantly higher than 31 g of milk/suckling produced by rear teats (Skok et al., 2007). As milk intake is related with the BW gain of piglets, helping small piglets to drink more milk could help to improve their BW gain and thus reduce variation in growth performance of piglets in the MS system, especially during early lactation when piglets rely more on milk for growth than on solid feed.

(1) Within 3 days p.p.

The earliest suckling related intervention for piglets could be applied directly after birth. Firstly, split-suckling, or called split-nursing, can be performed. This procedure temporarily removes heavy piglets that already acquired sufficient colostrum for a few hours, which gives light piglets better access to teats and reduces within-litter CV of BW at weaning in conventional housing (Donovan and Dritz, 1996; Donovan and Dritz, 2000). However, this strategy is often laborious and therefore not easily feasible to apply in commercial farms where labour is expensive. Secondly, reducing the number of piglets per litter can be considered, as for example sibling competition for access to teats can be reduced which benefits small piglets. Ocepek et al. (2017)

studied the relationship between litter size (ranging from 5 to 20 piglets) and piglet traits including suckling behaviour and growth performance; they found that larger litters had higher CV in BW at weaning on day 35 p.p., lower BW at weaning and more piglets without a functional teat at milk let-down. It was reported that low BiW piglets (0.9-1.0 kg) in small litters (8 piglets/sow) had higher BW gain and missed fewer nursings on days 0-21 p.p. compared to low BiW piglets in large litters (12 piglets/sow) (Deen and Bilkei, 2004). In Chapter 3 and 4, litter sizes were standardized into 14 piglets/sow, based on the number of functional teats per sow. To maximize utilizing the functional teats of sows in MS systems and maximize total number of weaned piglets in MS systems for practical pig production, litter size of 8 piglets/sow seems to be low. In addition, due to genetic selection for large litters in pig production (Kemp et al., 2018), rearing the excess piglets which are removed from MS systems remains a realistic problem. Although piglets can start to drink milk replacer from day 3 p.p. when they are removed from the sow (Theil and Jørgensen, 2016; Frei et al., 2018; Schmitt et al., 2019) and they may even have a higher growth rate than sow reared piglets on days 3-19 p.p. before weaning (Vergauwen et al., 2017), such removing deprives the behavioural need of piglets to suckle sows and against the original aim of the MS system that mimic the natural living conditions of piglets. In semi-natural conditions, piglets remain the piglet nest within the first week p.p. and thus they rely solely on milk from the sow to cover their need for nutrients (Jensen, 1986); in addition, piglets still suckle the sow even when they are able to eat solid food after day 28 p.p.(Petersen et al., 1989). A litter size which is slightly lower than the number of function teats can be recommended, for example 12-13 piglets/sow in the MS system used in this thesis.

(2) Day 3 p.p. until grouping

The regime for sows to get away from farrowing pens

In this thesis, sows were allowed to enter the MS area from day 4 p.p., while piglets were allowed to enter this MS area from day 8 p.p. in Chapter 3 and days 8-13 p.p. in Chapter 4. Similarly, in Bohnenkamp et al. (2013), sows were allowed to enter the MS area from day 2 p.p. until piglet grouping on day 5 p.p.; in the study of van Nieuwamerongen (2017), sows were allowed to enter the MS area from day 4 p.p. until piglet grouping on day 8 p.p. While in some other studies in MS systems, individual sow and her offspring was reared together in individual farrowing pens for 2 weeks p.p. before entering the MS system, without opportunity for sows to get away from piglets few days after farrowing (Thomsson et al., 2016). These two types of regimes in MS systems have both strengths and drawbacks.

Providing sows with the opportunity to get away from piglets few days after farrowing in this thesis mimics better the natural living conditions of pigs, that sows only stay with the piglets during the first few days and then start leaving the nest to seek food and piglets follow sows 1-2 weeks p.p. (Stangel and Jensen, 1991). However, in this thesis, we noticed that some sows went to the wrong farrowing pen to nurse after allowing them to get away from day 4 p.p. Similarly, van Nieuwamerongen (2017) also observed that before grouping (litters were grouped on day 8 p.p.), sows occasionally look inside another farrowing pen. As the sow-piglet bond establishes within 3 days p.p. and teat order becomes stable after 1 week p.p. (Hemsworth et al., 1976; Puppe and Tuchscherer, 1999), this early timing for sows to get away seems to hinder the process of establishing a strong sow-piglet bond and a stable teat order, which could lead to increased number of cross-sucklers. In addition, this wrong nursing towards other litters might lead to the consequences that her own piglets missed some suckling bouts, which may negatively affect the BW gain of this litter and may increase between-litter variation in BW gain of piglets in MS systems. In another MS system where housing individual sows and piglets together for 2 weeks p.p. (Thomsson et al., 2016), it seems to help strengthen sow-piglet bond, but on the other hand the freedom of movement and social contact with other sows are limited for sows during this period.

After grouping, the effects of cross-suckling on piglet growth performance and uniformity are not straightforward (Van Nieuwamerongen et al., 2014). The details of cross-suckling will be discussed in Section 3, as this section mainly focuses on the day before grouping. However, it can be speculated that the timing of allowing sows to get away and enter MS area before grouping, or entirely without providing opportunity for sows to get away might have an influence on the extent of cross-suckling after grouping and hence piglet growth uniformity in MS systems.

(3) Grouping of litters

In semi natural conditions, sows and piglets re-joined the family group on days 7-14 p.p. (Jensen and Redbo, 1987; Jensen, 1988; Stangel and Jensen, 1991). The grouping date in natural conditions can be regarded as reference to be applied in MS systems. However, slight changes of this should be made, as the outdoor environment conditions and the construction of family groups (several sows with progeny born in different periods) in natural conditions differ from that of the MS systems used for practical pig production.

In Chapter 4, we compared the effect of different grouping days of piglets on variation in BW gain of piglets and found that deferred grouping from day 8 to day 14 p.p. did not influence uniformity of BW of piglets. As it was observed that the younger piglets were occasionally forced away from the teats by older cross-suckling piglets (Maletınská and Špinka, 2001; van Nieuwamerongen, 2017), later grouping age might help small piglets to fend for themselves at teats, especially as variation in litter age exists in MS groups (van Nieuwamerongen, 2017).

However, grouping at later age might have issues regarding aggression among piglets. It was reported that unacquainted pre-weaning piglets that were shortly placed together fought shorter and had fewer injuries at younger age (Pitts et al., 2000). Grouping of litters on days 5-12 p.p. has been recommended, as grouping at later age might increase the frequency of aggressive behaviour among piglets (D'Eath, 2005; Weary et al., 2008; Kutzer et al., 2009).

It is noticed that in this thesis, the barriers for piglets between farrowing pens was solid iron plate rather than slatted iron bar (Figure 2), which hinders nose contact between different litters before grouping. If allowing them to have nose contact across the slatted iron bar before grouping, the negative effect of aggression among piglets due to late grouping might be reduced. Under this new rearing condition, it is speculated that the later grouping day could help small piglets fend for themselves at teats thus leading to better growth uniformity in MS systems compared to early grouping day.

Figure 2. The multi-suckling system used in Chapter 3 and 4.

(4) After grouping

Intermittent suckling

Intermittent suckling (**IS**), i.e. separating sows from piglets temporarily for several hours per day, mimics the natural conditions of pigs where sows increased their time spend away from their offspring. It is a form of gradual weaning that can induce an oestrus of sows during lactation (Soede et al., 2012) and can improve the adaptation of piglets to weaning (Berkeveld et al., 2009). In conventional housing, IS stimulated pre-weaning feed intake of piglets, as well as improving postweaning feed intake and growth rate of piglets (IS from day 22 p.p., weaned on day 29 p.p. (Turpin et al., 2016a)). In the MS system, Van Nieuwamerongen et al. (2017) suggested that after applying IS on day 28 p.p. piglets likely compensated for a decreased nursing frequency with an increased feed intake. In Chapter 3 and 4 in this thesis, IS was also applied on day 28 p.p. In conventional housing, the effect of duration and staring time of applying IS on piglet performance before weaning have been investigated. However, no investigations regarding the effect of duration and staring time of applying IS on the variation in BW gain of piglets were made in MS systems.

Studying the effects of delaying the start of IS, Turpin et al. (2016b) found that in conventional housing, applying IS on days 28-35 p.p., when compared with days 21-28 p.p., increased feed intake of the piglets on days 27-34 p.p., leading to a higher BW gain on days 30-34 p.p. but numerically increased variation in BW gain on days 30-34 p.p. before weaning. In MS systems, applying IS at an early age before day 28 p.p. might also reduce the growth performance at weaning, possibly related to emotional stress due to early maternal separation (Kanitz et al., 2002). In addition, the effects on milk intake are likely larger when applying IS at a younger age (See Figure 1). As in MS systems where several sows and litters are housed together and cross-suckling could occur, which is different from conventional housing, it is unclear which type of piglets would suffer more from IS when applying IS in early age before day 28 p.p. On the one hand, light piglets have a less mature digestive system for solid feed (Pluske et al., 2003) and are more dependent on milk for their growth before day 28 p.p., which might suffer more from IS compared to heavy piglets. In addition, in Chapter 3 and 4 where IS was applied on day 28 p.p., we observed that when sows returned to the MS area during the IS period, piglets were restless and crowded around sows, which could increase the risk of trampling and increase restlessness during subsequent suckling, which was also observed by (van Nieuwamerongen, 2017). The restlessness of piglets occurred around sucklings could be even worse when IS is applied before day 28 p.p., which could be especially harmful for small piglets. On the other hand, due to the ‘compensatory feeding hypothesis’ that piglets having a lower ability to compete for teats visit feeder more often (Middelkoop et al., 2019a), light piglets might more get used to solid feed before IS, therefore heavy piglets might have more stress after applying IS compared to light piglets.

For the duration of IS, Berkeveld et al. (2009) found that in conventional housing when weaning occurred at the same age on day 33 p.p., piglets applied with IS on days 26-33 p.p. had a slightly lower feed intake on days 26-33 p.p. before weaning, compared to piglets applied with IS on days 19-33 p.p.. The BW of piglets at weaning did not differ between the two groups, but BW variation of piglets at weaning was slightly smaller in the group where IS was applied for a shorter time. It seems that applying shorter IS (1 week vs. 2 week) before weaning helps to reduce the variation of BW of piglets at weaning. As MS systems are different from conventional housing, it is also unclear the effect of duration of IS regimes on the variation in BW of piglets at weaning in MS systems, which needs further investigation.

Split-weaning

In conventional housing, split-weaning generally takes place one week i.e. on days 21-22 p.p. before the normal weaning age i.e. on day 28-29 p.p., by removing the heaviest half of the litter from sows (Pluske and Williams, 1996; Vesseur et al., 1997; Abraham, 2020). Split-weaning later than day 21-22 p.p. was also reported in conventional housing. For example, it was reported that BW gain on days 28-70 p.p. of light piglets was higher from groups in which the heaviest half of the litters was split-weaned on day 28 p.p. and the remaining piglets were weaned on day 56 p.p., compared to the BW gain of light piglets from groups in which no split-weaning was applied and all piglets were weaned on day 56 p.p. (Abraham and Chhabra, 2004). In Chapter 4, we applied a split-weaning intervention by removing three heavy piglets from each litter on day 35 p.p., as one of the approaches to reduce teat competition for light piglets. No improvement of uniformity of piglets in BW gain was found, as the increased milk intake was largely compensated for by a simultaneous decrease in feed intake. Possibly, it is worth studying the effect of IS at earlier age before day 35 p.p. and studying the effect of removing more piglets than 3 piglets in Chapter 4 on the growth uniformity of piglets in MS systems. As the majority of piglets could be able to ingest solid feed from week 2-3 p.p. (Middelkoop, 2020) and sow milk production reaches its peak in week 3-4 p.p. (Theil et al., 2012), split-weaning of the piglets that eat a large amount of solid feed rather than the heavy piglets, as well as remaining piglets which are still in need of milk with sows might also be an option to be investigated. It is proposed that the latest split-weaning age could be on around day 28 p.p. The earliest split weaning age is on day 21 p.p. which is the minimum weaning age required by EU legislation.

Weaning

Different weaning ages were used in MS systems, which may have different consequences on growth performance and thus the uniformity of piglets. In literature, weaning age applied in MS systems have either led to a ‘short lactation’ with weaning on for example day 26 p.p. (Bohnenkamp et al., 2013; Grimberg-Henrici et al., 2019; Verdon et al., 2020), day 27 p.p.(Lange et al., 2021) or day 28 p.p. (Kutzer et al., 2009; de Ruyter et al., 2017; Greenwood et al., 2019), or to a ‘long lactation’ with weaning on for example day 34 p.p. (Nicolaisen et al., 2019), day 35 p.p. (Schrey et al., 2019), day 44 p.p. (Thomsson et al., 2016) or day 63 (Van Nieuwamerongen et al., 2017).

A short weaning age at 28 days of age or earlier would not only compromise piglet welfare due to a growth check and less adaptation time for solid feed, but also violates the aim of MS systems which is to mimic a smooth transition from milk to solid feeds (Widowski et al., 2008).

An extended lactation in MS systems from 4 to 9 weeks helps piglets to cope better with transitions than piglets weaned abruptly in week 4 (Van Nieuwamerongen et al., 2017). Therefore, in this thesis in Chapter 3, we also used an extended lactation with weaning days on day 64 p.p. in week 9. However, we adjusted the weaning age into week 7, i.e. on day 48 p.p. in Chapter 4. This is because, in Chapter 3, a large proportion of piglets (43.1%) had zero milk intake on days 56-57 p.p. In addition, suckling by heavy piglets on days 56-57 p.p. might cause more teat damage for sows due to the developed teeth of piglets. However, weaning at week 7 might also have drawbacks for the efficiency of pig production. In the study of (van Nieuwamerongen, 2017), piglets were weaned on day 63 p.p. One reason was that piglets could be relocated directly from the MS system to the finishing unit, and thus eliminating the extra relocation to the nursery facility during week 4-9 p.p. (van Nieuwamerongen, 2017). By doing so an extra change in living conditions and the related stress could be avoided (van Nieuwamerongen, 2017). However, on the other hand, BW variation in MS systems at weaning is proposed to be larger when piglets are weaned in week 9 compared to week 7.

6.2.2 Feeding related interventions for piglets

(1) Ways to stimulate early feeding behaviour towards solid feed

Previous studies have indicated the importance of early feeding, as in conventional housing, feed and water intake during lactation were positively correlated with feed intake after weaning (Carstensen et al., 2005; Berkeveld et al., 2007; Sulabo et al., 2010). Several approaches were proposed to stimulate early feeding, one of which is called ‘vertical maternal learning’ (Oostindjer et al., 2011). In both Chapter 3 and 4, we applied this approach by providing piglets with the opportunity to eat with sows together in the communal feeding area after grouping (Oostindjer et al., 2011), which could stimulate early feeding of piglets. However, in Chapter 4, it was found that there was no relationship between time spent on contacting feed of piglets and time spent on being present in the feeding area of sows, during sow feeding times on both day 18 p.p. and days 42-43 p.p.

During the first week p.p. before grouping, piglets were confined in the farrowing pens and therefore were close to the sows, which could be a good time for piglets to learn from sows to eat solid feed. In both Chapter 3 and 4, the sow trough and sow drinker in the farrowing pen was however not accessible to the piglets. This withholds piglets the opportunity to learn from their mother (Figure 3a). Making the sow trough and drinker accessible to piglets in farrowing pens, reduced the percentage of non-eaters compared to the control groups pre-weaning (10% vs. 22%) (van der Peet-Schwering et al., 2021). However, feed intake is generally low until day 18 p.p. which was reviewed by (Middelkoop, 2020). Nonetheless, it is still good to provide piglets with more opportunity to receive feed information from sows during the first week of lactation, as feed information transfer from sows to piglets already starts in the placenta (Oostindjer et al., 2009). The aromas of sow feed are transferred via amniotic fluid (Oostindjer et al., 2009), which is even earlier than the first week p.p. It can be achieved for example by installing some extra feeding places close to the sow trough, and making the sow trough accessible to piglets as much as possible (Figure 3b). In addition, in both Chapter 3 and 4, there was only one small round feeder with 3 feeding places available for piglets in each farrowing pen before grouping, while there were 14-15 piglets. The number of piglets: feeding place ratio was high (5:1) in farrowing pens, which should be reduced in order to reduce competition of contacting feed for small piglets, as piglets tend to synchronize their activity including feeding activity (Nielsen et al., 2016). These strategies might help to increase the proportion of eaters, thus helps to reduce piglet performance variation in the MS system.

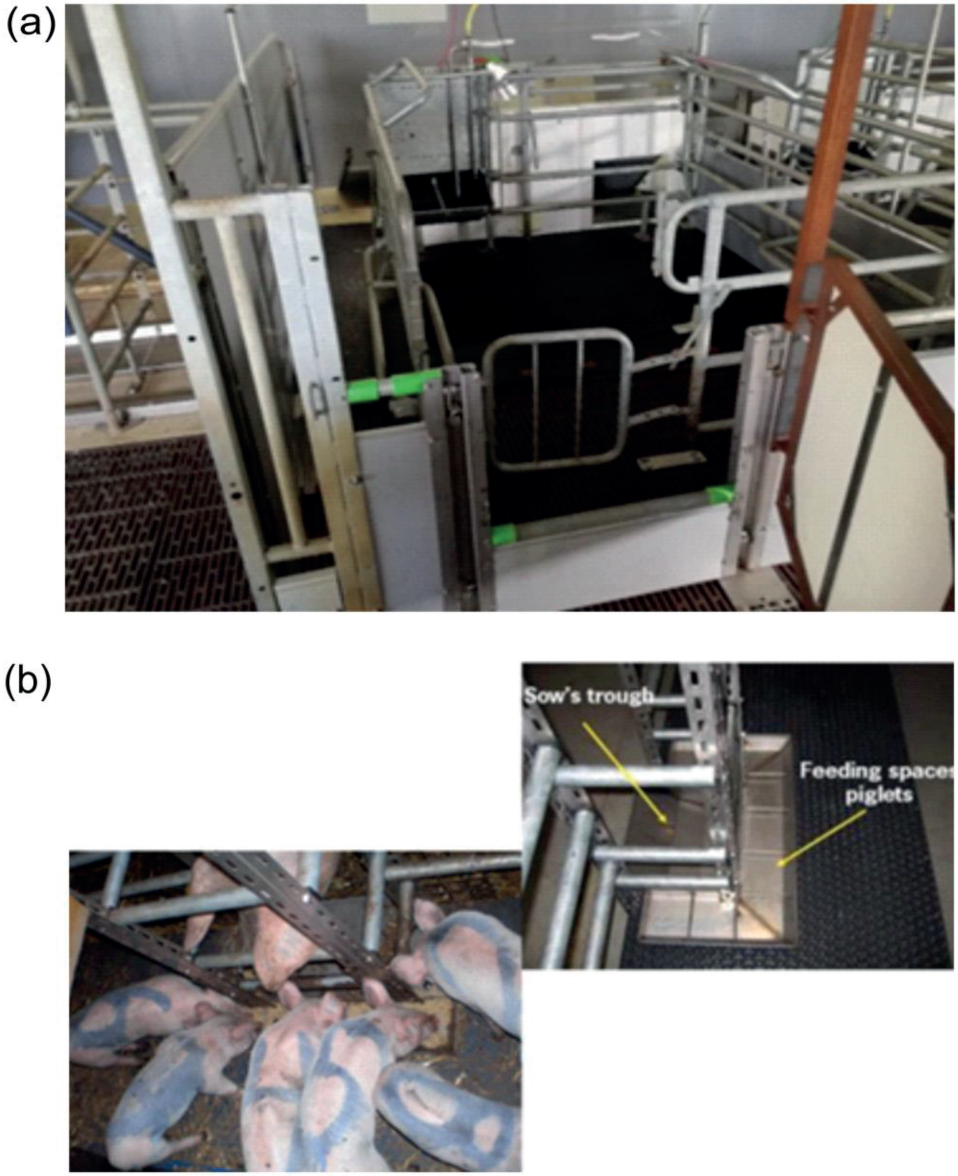


Figure 3. (a) Farrowing pen used in Chapter 3 and 4. One sow trough was installed inside the pen which was only accessible to the sow. (b) Proposed extra feeding spaces which are close to the sow's trough (cited from Center of Animal Welfare and Adaptation, Wageningen University & Research).

(2) Improving diet diversity to increase the percentage of eaters

In semi-natural conditions, piglets spend most of their active time on foraging and eating, such as rooting, grazing and nosing. Their diet is inherently diverse, including nuts, fungi, leaves, resin, grasses, etc. (Ballari and Barrios‐García, 2014). From a few days p.p., they have already started to explore their surroundings and forage for other food items apart from sow milk. Their feeding behaviour is divided into 2 phases: “appetitive phase” (foraging) and “consummatory phase” (eating) (Berridge, 2004). Providing piglets with a more diverse diet was reported to stimulate their feeding behaviour by giving them more opportunity to explore (Middelkoop et al., 2019b). However, the provision of dietary diversity did not enhance the proportion of piglets within a litter that consumed creep feed or their growth performance before weaning (Middelkoop et al., 2018). It is also proposed that early feed intake, rather than its composition, determines the rate of adaptation of piglets to solid feeds after weaning (Spreeuwenberg et al., 2001). In Chapter 3 and 4, piglets were provided with large creep feed pellets (8 mm diameter) from day 2 p.p. until 2-3 days after grouping, which could be regarded as a strategy to increase the diet diversity and feeding behaviour of piglets. However it is unknown whether these large pellets could increase feed intake in small piglets more than large piglets, and it is not clear whether this strategy would help to reduce variation in feed intake of piglets in MS systems.

(3) Supplementing nutrients for lightweight piglets

In conventional housing systems, provision of supplemental nutrients, for example, using milk replacers, only to the lightweight litters could be used to reduce variation in BW of piglets at weaning. Wolter et al. (2002) performed a study with a 2×2 factorial design with BiW (heavy vs. light) and liquid milk replacer (supplemented vs. unsupplemented on days 0-21 p.p.) during lactation, and found that piglets supplemented with milk replacer on days 3-21 p.p. had higher BW at weaning on day 21 p.p. than piglets unsupplemented with milk replacer. However, the authors also found that that milk replacer had a greater effect on weaning weight of heavy BiW piglets compared with that of the light BiW piglets. In the study of Kobek-Kjeldager et al. (2020a), it was found that when light and heavy piglets were reared together in one farrowing units and had the same access to milk replacer, piglets having lower competence fighting for teats could compensate with milk replacer; however, the authors also found that within litter, milk replacer was mainly used by the larger piglets and as a supplement to increase BW gain of piglets having higher competence fighting for teats. Translating these observations to a multi-suckling unit, it would appear that provision of a milk replacer to a group of piglets with both heavy and light piglets would likely benefit heavy piglets more so than light piglets. Offering milk replacer only to light piglets could work to reduce BW gain variation, however this would require (too) expensive electronic facilities.

(4) Making sow feed available both during the day and night for sows and piglets to better access feed

The feed intake of sows has been observed to be positively related with the BW gain of the litter (Strathe et al., 2017). It was reported that there existed variation in individual feed intake and milk production of sows (Ramanau et al., 2004; Strathe et al., 2017), which could potentially influence the within-litter variation in milk intake and thus variation in BW gain of piglets in an MS system. Therefore, interventions to reduce variation in feed intake and milk production of sows in the MS system could be considered. The estimation of individual feed intake of sows is not available in the current MS system, as the 5 feeding places in the floor trough were both accessible for all sows and piglets. In Chapter 4, the individual feeding behaviour of sows in week 6 was recorded, as a reflection of their individual feed intake variation. The percentage of time sows spent in the feeding area during sow feeding times ranged from 0 to 97.9% (average: 64.4%), while during the day ranged from 0 to 25.8% (average: 9.8%), which indicated a large variation among sows. It was observed that in the MS system some sows ate later after others left the feeding area during sow feeding times, and some sows wandered back to the feeding area seeking for sow feed during the day. It was also observed that some sows showed aggressive behaviour towards other sows when stay close to them in the feeding area, probably because of the difference in social rank. The sows which ate later probably could not get enough feed, as sow feed was only available during sow feeding times and could be eaten both by the sows present earlier in the feeding area and by the piglets. Therefore, to make sow feed ad libitum for sows during the day and night in the floor trough could help especially low rank sows ingest enough sow feed and thus reducing the variation in BW gain of piglets in the MS system.

In this thesis, it was found that there existed big variation among individual piglets regarding the time spent on contacting total feed during the day in the feeding area. In Chapter 3, the percentage of time spent on contacting feed ranged from 0 to 20% in week 2-4 (mean ± SEM: 2.7 ± 0.5%), from 0-31% (mean ± SEM: 8.2 ± 1.0 %) in week 4-6 and 0-25% (mean ± SEM: 7.8 ± 0.9 %) in week 6-8; in Chapter 4, it ranged from 0 to 32% in week 4-6 (mean ± SEM: 9.7 ± 0.5%). It was observed that sometimes the feeding area was crowded (Figure 4), which could explain this big variation, as the crowded piglet feeding area might be detrimental for especially small piglets to eat or contact feed. In our MS system, in Chapter 3, a stronger relationship was found between skin lesions and BW gain with progressing age throughout a 9-week lactation. It was speculated that as the piglet: feeding place was fixed (6:1), the presence of the increased body size of piglets in the feeding area probably make the feeding area more and more crowded and thus increased the feed competition among piglets. It would be especially harmful for small piglets and low rank piglets and would reduce piglet uniformity. As piglets highly synchronized their feeding behaviour when group housed (Nielsen, 1999), it is proposed that making sow feed available also in the sow feeding floor during the day and night might help small piglets to eat more feed especially when piglets are eating together.

(a)

(b)

Figure 4. Piglet feeding area during the day (non-sow feeding time) on day 41 p.p. in the MS system (based on Chapter 3), during which no sow feed was provided in the feeding trough on the floor. (a) Piglets are eating together; (b) Piglets are sleeping together.

6.3 Cross-suckling

Typically, in MS systems, cross-suckling increases from zero before grouping to potentially high proportions after IS and close to weaning. In Chapter 4, in week 6, cross-suckling occurred frequently; it was observed in average 75.9 % of all successful suckling bouts per sow. Cross-suckling might have both pros and cons for piglets and sows. It could provide benefits for sows, as alien piglets could help to remove and utilize extra milk from sows with more milk production to prevent waste of milk (Roulin, 2002). It also benefits the growth of piglets especially for those have a low milk intake from their own mother (Olsen et al., 1998). However, the extent of this benefits might depend on the difference between the number of functional teats and litter size in MS systems. In addition, the benefits might be dynamic and might even disappear at some stages. In this thesis, litter size was standardized to 14-15 piglets per litter within 24-48 h p.p., which was close to the number of functional teats of the sows. At the beginning of lactation, cross-suckling might enhance teat competition and be harmful for small piglets. Cross-suckling could also result in restlessness and disrupted nursing, which potentially has negative consequences for all piglets. After the peak of milk production in week 3-4 (Theil et al., 2012), the activity of cross-suckling after week 4 might help some piglets to fulfil the behavioural need to suckle from sows when their own mother is in IS area, however on the other hand it might reduce their time spent on eating solid feed; cross-suckling activity at week 4 at this stage thus might be a drawback for some piglets.

The extent of cross-suckling could be influenced by the ways of management regimes that applied in MS systems. Although the cons of cross-suckling may outweigh the pros, two approaches to reduce cross-suckling are discussed here. Firstly, synchronizing suckling of nursing sows may help to reduce cross-suckling. This may be favoured by a smaller group size of sows (Maletınská and Špinka, 2001; Illmann et al., 2005). Some studies even recommended a group size of a maximum of 8 sows to prevent cross-suckling (Aubel et al., 2011), which needs further exploration. Secondly, as the sow-piglet bond establishes within 3 days p.p. and teat order becomes stable after 1 week p.p. (Hemsworth et al., 1976; Puppe and Tuchscherer, 1999), allowing sows to get away before day 4 p.p. might increase the number of cross-sucklings after piglets enter the group, compared with allowing the sows to get away later. In semi-natural conditions, individual sows walked distances of several hundred meters from the family group for farrowing and nursing (Jensen, 1986), while in this thesis in Chapter 3 and 4, five farrowing pens were close to each other. Due to the limited space for MS system for economic reasons, we could not extend the distance between individual pens to prevent sows go to the wrong pens or prevent them to disturb each other. When allowing sows to get away from day 4 p.p., a further improvement would be to make individual pens more recognizable for sows to avoid them to go to wrong pens.

6.4 Piglet mortality

As discussed in Chapter 5 and in Van Nieuwamerongen et al. (2014), piglet mortality in MS systems is usually higher than in conventional housing (Van Nieuwamerongen et al., 2014). In this thesis, piglets crushing occurred in two peaks. One in the first week of lactation when piglets were still in the farrowing pen and the second one during the first few days after grouping. The majority of mortality in the first week was due to crushing. Efforts have been made to reduce crushing by restricting sows in farrowing cates within the first 3 days p.p., however this violates the aim of MS systems of which is to accommodate the natural behaviour of pigs. In Chapter 5, it was found that low BiW piglets have a higher risk to be crushed. It is evident that the high mortality of the low BiW piglets influences piglet uniformity and also litter size at weaning in MS systems. In general, piglet mortality is a concern from a welfare and thus societal acceptability perspective.

It was noticed that in Chapter 3 and 4, when piglets were still restricted in the loose farrowing pens, the area which was only accessible to piglets is limited. Enlarging this piglet area probably could reduce pre-grouping mortality, as piglets could have more space to escape when sows are lying down. This would, however, also increase the cost of the MS system as more space is required. In Chapter 5, it was noticed that low BiW and crushing were the two main reasons for piglet mortality before grouping. Light piglets are much more likely to rest close to the sows to prevent heat loss, which increases the risk of being crushed (Kammersgaard et al., 2011). Also, in this thesis, the piglet nest was in front of the sow. Further improvement could be made by installing a piglet nest parallel to the sow and installing heat lamps parallel to the sow, which helps to attract piglets to go to the warm area after suckling bouts, and shorten the walking distance between sows and the warm area for piglets. In addition, light piglets spend more time near the sow for stimulating the udder after nursing bouts (Fraser, 1990; Weary et al., 1996), which also increases the risk of being crushed. Providing litters with milk replacer in farrowing pens could improve the survival of piglets especially for those missed nursing bouts (Kobek-Kjeldager et al., 2020b). However, early mortality before piglets learned to drink milk replacer poses a challenge.

In addition to BiW and crushing, piglet mortality is also related to many other factors, for example sow related factors including mothering ability of the sow (Knol et al., 2022) and litter size (Weary et al., 1998; Weber et al., 2009; Ocepek et al., 2017). It was found that lactation survival of piglets was correlated with the estimated breeding value for mothering ability of sows in both conventional and MS systems (Dunkelberger et al., 2019). In addition, in this thesis, we observed a large variation in piglet crushing per sow (mean ± SEM: 9.9 ± 1.4%, min: 0%, max: 44.4%) and overall mortality per sow (mean ± SEM: 14.4 ± 1.8 %, min: 0%, max: 50.0%) on days 0-8 p.p. As in Chapter 5, litter sizes were standardized to 14-15 piglets per sow, the variation in piglet mortality per sow might be more likely linked with variation in mothering abilities of sows rather than the size of the litters. This variation in piglet mortality among sows might also be linked with variation in sows' physical conditions, for example poor leg condition or a large body size. For litter size, Ocepek et al. (2017) studied the relationship between litter size (ranging from 5 to 20 piglets) and piglet mortality before weaning at day 35 p.p. in conventional housing; they found that larger litter size had a lower proportion of nursings with milk let down and an increased risk of mortality due to starvation and crushing. Therefore, to increase survival of piglets, selecting sows with higher mothering ability for MS systems and using sows with a relatively lower litter size (for example, 12-13 piglets/sow) might be options for further research. In addition, in order to improve piglet BW gain uniformity in MS systems, it is also important to reduce variation in piglet mortality among sows, therefore choosing sows with similar body conditions and similar mothering ability are needed.

6.5 Conclusions of this thesis

Main findings

- A dual marker method was developed using alkanes as poorly digestible markers. It was demonstrated that using pooled spot samples of faeces of individual piglets, absolute prediction errors of 10%-15% of simultaneously measured intakes of multiple nutrient resources of these piglets in a complex housing system are feasible using this dual marker technique.
- Variation in growth rate of piglets in a multi-suckling (MS) housing system was mainly explained by piglet's birthweight (12%) and positive behaviour (play and nosing) (7.6%) in weeks 2−4, and by its solid feed intake in weeks 4−6 (15.1%) and 6-8 (25.9%). More than 55% of the variation in BW gain remained unexplained.
- Later grouping of the 5 litters in the MS system from day 8 to day 13 p.p. does not help to reduce the variation in BW at weaning on day 48.
- The introduction of split-weaning of the 3 heavy piglets on day 35 p.p. in MS systems increases milk intake of particularly the remaining low BiW piglets, but this does not lead to a reduction in BW variation at weaning, as the increased milk intake is largely compensated for by a simultaneous decrease in feed intake.
- Birthweight of piglets is positively related with the number of sucklings, milk and feed intake, and negatively with mortality.
- For piglets born both small and big, fast growing piglets tend to eat more feed, have more skin lesions and are present less often at teats of alien sows.
- The absence of interactions between birth weight class and growth rate class illustrates the limited capacity for low birth weight piglets to catch up with their littermates.

Recommendations

- With the first 3 days p.p., reducing the litter size into a number slightly lower than the number of functional teats likely contributes to reduced variation in body weight of piglets at weaning;
- During the first week p.p., before grouping, when piglets are confined in the farrowing pens, providing piglets with opportunities to learn from sows to contact sow feed is recommended;
- Making sow feed available both during the day and night for sows and piglets to better access feed is recommended;
- Weaning at 7 weeks of age in a MS system allows a gradual transition without having the disadvantages of too heavy piglets suckling their dams;

Future perspectives

- The effect of the starting time and duration of intermittent suckling on the variation in growth rate of piglets in MS systems deserves further study.

- Effects of split-weaning strategies, e.g. before day 35 p.p., by removing more than 3 piglets per litter or the split-weaning of good eaters, on the growth uniformity of piglets in MS systems need further research.
- The effect of diet diversity on growth uniformity of piglets in MS systems need further research.
- Although the MS system generally benefits pig welfare and accommodates natural behaviour of pigs, piglet growth performance is quite variable and piglet mortality in the system is rather high. These issues hamper the introduction of such systems in commercial practice.

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Summary

Summary

In recent years, integral group housing systems, or multi-suckling (**MS**) systems for lactating sows and their litters have been developed, mimicking the natural living conditions of pigs, as a solution to welfare problems of conventional farrowing pens. However, one problem associated with these MS systems is a large variation in growth rate of piglets, a problem that is hampering the introduction of such systems in commercial practice. The main aim of this thesis was to study causes of variation in growth rate of piglets in the MS system during lactation and possibilities to reduce this variation.

For effectively designing strategies to reduce growth rate variation, it is crucial to understand the underlying factors that cause this variation. In **Chapter 1**, potential factors which affect variation in growth rate of piglets in MS systems are discussed, focusing on piglet related characteristics, such as birthweight (**BiW**), intake of milk and feed, intake associated behaviours and other behaviours.

As feed intake of piglets are crucial for the variation in growth rate of piglets, **Chapter 2** explored the use of a novel method, the 'dual alkane method' to estimate individual feed intake of piglets. In this study, twelve 6.5-week-old individually housed male pigs were offered a diet containing chromium chloride ($CrCl_3$) and the alkane hexatriacontane (C36) as in-feed markers. For three days in a row either once (R1), three times (R3) or five times (R5) daily the piglets orally received a reference marker, either ytterbium chloride ($YbCl_3$) or the alkane dotriacontane (C32). Faecal collections, both total and spot samples were taken on days 2-4. Individually measured feed intake was recorded daily, and was compared to predicted feed intake using the ratio of the dual marker pairs (Yb:Cr and C32:C36). Based on the obtained prediction errors for feed intake with the different procedures, it was recommended to dose the alkane reference marker three times per day for 2 days (days 1-2) and analyse pooled faecal spot samples collected on days 3 and 4. This technique was subsequently used to estimate intake of piglet feed, sow feed and thereby milk intake in two experiments in the MS system (Chapter 3 and 4). The MS system at Pig Innovation Center Sterksel housed 5 sows and their piglets, during a 9 week (Chapter 3) or 7 week (Chapter 4) lactation. The sows could move freely in the system, except for days 0-3 postpartum (**p.p.**) after farrowing, when they were restricted in individual farrowing pens. Litters were allowed to enter the MS area, i.e., grouping of litters, on days 8-9 p.p. or on days 13-14 p.p. (see Chapter 4). Subsequently, to allow gradual weaning, intermittent suckling (**IS**) was applied, i.e. forcedly separating sows from piglets for several hours/day from day 28 p.p., and allowing sows to voluntarily leave the MS area from day 35 p.p. until weaning. Piglet weight was assessed for all piglets at different timepoints, but other piglet characteristics were assessed for 4 piglets, the focal piglets, in each litter. These were the piglets with the second lowest (**LBW**) and second highest (**HBW**) birthweight from both sexes.

Chapter 3 was conducted in the MS system to study piglet related factors that might explain variation in growth rate and DM intake of solid feed (**DFI**) of piglets in three different periods during a 9-week lactation. These piglet related factors included BiW, milk intake, intake of sow feed, intake of piglet feed, behavioural traits and their genetic background. A total of 15 sows and 60 focal piglets across three batches were studied. Multiple linear regression analysis with forward selection was conducted on growth rate and DFI after correcting for piglet sex and batch, using the piglet related factors as explanatory variables. In this study, both growth rate and DFI varied between piglets. For example, in week 4-6, BW gain was 360 ± 11 (mean ± SEM) g/day, intake of sow feed was 130 ± 12 g/day and intake of piglet feed was 286 ± 22 g/day. The variables in the models explaining growth rate and DFI jointly explained less than 45% and 21% of the variation in growth rate and DFI, respectively. Variation in growth rate of piglets was mainly explained by BiW (12%) and the frequency of positive behaviour (play and nosing) (7.6%) in week 2-4, and by intake of piglet feed in week 4-6 (15.1%) and week 6-8 (25.9%). Additionally, we found that variation in DFI was explained by piglet's teat presence (2.9%) in week 2-4, by its BiW (9.6%) in week 4-6, and by its number of skin lesions (5.1%) in week 6-8. A remarkable finding of this study was that more than 55% of the variation in growth rate of piglets remained unexplained by the parameters studied.

Chapter 4, the 2nd study conducted in the MS system, studied the combination of two intervention strategies aimed at improving the growth rate of specifically low birthweight (LBW) piglets and thereby reducing the growth rate variation at weaning on day 48 p.p. These interventions consisted of later grouping of piglets (from day 8 p.p. to day 13 p.p.) and split-weaning the heaviest three non-focal piglets on day 35 p.p. Eight batches of 5 sows with their litters were divided into 4 control (CTRL) and 4 treatment (TREAT) batches. Behaviour and feed intake were measured in focal piglets, and body weight (**BW**) and mortality were measured in all piglets. Results showed that: (1) Throughout lactation there were no differences in BW or growth rate between CTRL and TREAT. (2) After grouping, there were no obvious differences between CTRL and TREAT in feeding and suckling behaviours on day 18 p.p., damage scores on snout, ear or tail and skin lesions on day 27 p.p. (3) After split-weaning, in week 6, TREAT piglets tended to be present more at front and middle teats, tended to have lower snout damage scores and had lower restlessness scores during suckling bouts than CTRL piglets. Also, TREAT-LBW piglets had a higher milk intake than CTRL-LBW piglets. To conclude, later grouping did not improve growth rate, suckling behaviour or feeding behaviour of LBW piglets after grouping. Split-weaning increased milk intake, particularly of LBW piglets, but this did not lead to an increase in BW of LBW piglets or a reduction in BW variation at weaning, as the increased milk intake was largely compensated for by a simultaneous decrease in feed intake of these LBW piglets. Thus, we were not able to reduce the growth rate variation in the MS system using these two interventions.

Based on the findings in Chapter 3 that BiW plays an important role in explaining variation in growth rate of piglets during early lactation and in explaining variation in feed intake of piglets during middle lactation in the MS system, we hypothesized that BiW may also play a role in explaining variation in other piglet parameters. In addition, based on the findings in Chapter 4 that piglet mortality was high in the MS system, the question arises how this was related to BiW. Therefore, in **Chapter 5**, we studied the relationship between BiW and piglet traits including nutrient intake, nutrient intake associated behaviours, skin lesions as well as piglet mortality up to day 44 of lactation in the MS system, by utilizing the data from Chapter 3 and Chapter 4. As some low BiW piglets were able to show catchup growth in later life, we also investigated which characteristics these piglets had. We found that mortality of piglets with a BiW less than 1.1 kg was highest. In the surviving piglets BiW was positively related with number of sucklings and correspondingly with milk intake, and to a lesser extent to the intake of sow feed. In addition, BiW was positively related with the number of skin lesions. To evaluate catchup growth, the focal piglets were divided into 4 groups based on their within-litter BiW class (high BiW (HBW) vs. low BiW (LBW)) and their growth rate (piglets were defined as fast-growing when their BW on day 44 p.p. was equal to or exceeded the median BW of their litter, others were defined as slow-growing (fast vs. slow)). We found no piglet characteristics that differed between fast-growing LBW piglets and fast-growing HBW piglets. Thus, our study provides little insight into the mechanisms of catchup growth in an MS environment. However, insight was gained into how fast-growing piglets differ from slow-growing piglets, regardless of their BiW class. For piglets born both small and big, fast-growing piglets tended to eat more feed, were present less often at teats of alien sows and had more number of skin lesions, compared to slow-growing piglets. As in Chapter 3, more than 55% of the variation in growth rate of piglets in early, middle and late lactation in the MS system remained unexplained, and in Chapter 4, the two interventions aiming to reduce variation in BW of piglets had no obvious effects on variation in growth rate, other possible interventions that can be considered for this aim were discussed in **Chapter 6**. Recommendations are given on preferred litter size, further stimulation of feeding pre-grouping, increased availability of sow feed for both sows and piglets, and preferred lactation length in the MS system. Future research should be aimed at further reducing piglet mortality, clarifying the role of cross-suckling for piglet performance and on optimizing the use of intermittent suckling and split-weaning.

Finally, although the MS system generally benefits pig welfare and accommodates natural behaviour of pigs, piglet growth performance is quite variable and piglet

mortality in the system is rather high. These issues hamper the introduction of such systems in commercial practice.

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About the Author

Tianyue Tang (唐天悦) was born on April 4th, 1994 in Zhengzhou City, Henan Province, China. After graduated from Zhengzhou No.1 High School in 2012, she studied animal science in Henan University of Science and Technology in Luoyang City during 2012–2016. She then went to South China Agricultural University in Guangzhou City for master study majoring in pig nutrition during 2016–2018, under the supervision of Jinping Deng, Baichuan Deng, Chengquan Tan, etc. in Yulong Yin's Lab. During 2017-2018, she conducted animal experiment for master thesis in Institute of Subtropical Agriculture, Chinese Academy of Sciences in Changsha City, under the supervision of Tiejun Li. In Aug 2018, she obtained scholarship from Oversea Study Program of Guangzhou Elite Project. In Oct 2018, she started as a PhD candidate in Animal Nutrition Group and Adaptation Physiology Group in Wageningen University & Research in the Netherlands, under the supervision of Walter J.J. Gerrits, Nicoline M. Soede, Inonge Reimert and Carola M.C. van der Peet-Schwering. During her PhD study, she focuses on both animal nutrition and applied animal behaviour science.

(Contact: tianyue.tang1994@outlook.com; 877211636@qq.com)

List of Publications

Tang, T., van der Peet-Schwering, C.M.C., Soede, N.M., Laurenssen, B.F.A., Bruininx, E.M.A.M., Bos, E.J., Gerrits, W.J.J., 2022. A dual marker technique to estimate individual feed intake in young pigs. Animal 16, 100451.

Tang, T., Gerrits, W.J.J., Reimert I., van der Peet-Schwering, C.M.C., Soede, N.M., 2022. Variation in piglet body weight gain and feed intake during a 9-week lactation in a multi-suckling system. Animal 16, 100651.

Tang, T., Gerrits, W.J.J., Soede, N.M., van der Peet-Schwering, C.M.C., Reimert I. 2023. Effects of timing of grouping and split-weaning on growth performance and behaviour of piglets in a multi-suckling system. (Applied Animal Behaviour Science, accepted)

Tang, T., Gerrits, W.J.J., van der Peet-Schwering, C.M.C., Soede, N.M., Reimert I. 2023. Effects of birthweight of piglets in a multi-suckling system on mortality, growth rate, catchup-growth, feed intake and behaviour. (Animals, accepted)

Tang, T., Zhai, Z., Tan, C., Deng, B., Deng, J., 2018. Review of cysteamine on swine nutrition. Chinese Journal of Animal Nutrition 30, 1647-1654.

Deng, B., Long, H., **Tang, T.**, Ni, X., Chen, J., Yang, G., Zhang, F., Cao, R., Cao, D., Zeng, M., Yi, L., 2019. Quantitative Structure-Activity Relationship Study of Antioxidant Tripeptides Based on Model Population Analysis. International Journal of Molecular Sciences 20, 995.

Deng, B., Ni, X., Zhai, Z., **Tang, T.**, Tan, C., Yan, Y., Deng, J., Yin, Y., 2017. New quantitative structure–activity relationship model for angiotensin-converting enzyme inhibitory dipeptides based on integrated descriptors. Journal of agricultural and food chemistry 65, 9774-9781.

Tan, C., Zhai, Z., Ni, X., Wang, H., Ji, Y., **Tang, T.**, Ren, W., Long, H., Deng, B., Deng, J., 2018. Metabolomic profiles reveal potential factors that correlate with lactation performance in sow milk. Scientific reports 8, 1-9.

Wang, H., Ji, Y., Yin, C., Deng, M., **Tang, T.**, Deng, B., Ren, W., Deng, J., Yin, Y., Tan, C., 2018. Differential analysis of gut microbiota correlated with oxidative stress in sows with high or low litter performance during lactation. Frontiers in microbiology 9, 1665.

WIAS Training and Supervision Plan (TSP)[1] of Tianyue Tang

	ECTS[2]
The Basic Package	
WIAS Introduction Day, 2018	0.3
Scientific Integrity, 2020	0.6
Ethics and Animal Sciences, 2021	0.8
Introduction course on Personal Effectiveness, 2021	1.2
Disciplinary Competences	
PhD literature survey, 2019	6
Design of Experiments, 2019	0.8
Statistics for the Life Sciences, 2019	2.0
Genotype by environment interaction, uniformity and resilience, 2020	1.5
Modelling and optimization with GAMS, 2020	1.3
BHE-31306-Applied Animal Behaviour and Welfare (master course), 2021	6
Introduction to R and R studio, 2022	0.9
Professional Competences	
Adobe Indesign Essential Training, 2019	0.6
High Impact Writing in Science, 2019	1.3
Survival Guide to Peer Review, 2019	0.3
Research Data Management, 2020	0.5
Critical thinking and Augumentation, 2020	0.3
Scientific Artwork, 2020	0.6
Brain training, 2020	0.3
Searching and Organising Literature for PhD, 2020	0.6
Supervising BSc and MSc thesis students, 2020	0.6
The Final Touch, 2021	0.6
Career Orientation, 2021	1.5
Presentation Skills	
WIAS Annual Conference 2021, oral, Wageningen	1
WIAS Annual Conference 2022, poster, Wageningen	1

The 15th International Symposium on Digestive Physiology of Pigs 2022, poster, Rotterdam	1
Teaching competences	
Supervising 3 MSc students (major: Iris van den Belt, Anna-Maria Koch, Kristel Mulder), 2021	3
Supervisor for Introduction to Animal Science (BSc course), 2019-2020	2
Supervisor for Principles of Animal Nutrition (BSc course), 2022	1
Total (minimum 30 credits)*	**38**

[1] Completed in fulfilment of the requirements for the educational certificate of the Wageningen Institute of Animal Science (WIAS).

[2] One ECTS credit equals a study load of 28 hours.

Colophon

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