

Maternal behaviour in gilts – The effect of genotype, social rearing environment and mixing after weaning



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List of abbreviations

WUR	Wageningen University and Research
SLU	Swedish University of Agricultural Sciences
SY	Swedish Yorkshire
DY	Dutch Yorkshire
QBA	Qualitative Behaviour Assessment
Std	Standard deviation
S.E.D.	Standard Error of Difference

Abstract

Maternal behaviour in pigs is influenced negatively by stress, and may therefore be linked to social behaviour of gilts (first litter) and sows that are kept in group housing during gestation. Gilts and sows in group housing are often aggressive towards each other, which causes stress and possibly injuries or even death. Measures to improve their social skills could lead to more positive social behaviour, which would reduce stress and could therefore lead to better maternal behaviour. Swedish Yorkshire gilts may have better social skills than Dutch Yorkshire gilts, because the Swedish genotype has been kept in and selected for group housing for a longer period of time. Furthermore, additional opportunities to practice social behaviour may improve later social skills. We studied three factors in a 2x2x2 factorial design, being genotype (Swedish versus Dutch Yorkshire), rearing environment (access farrowing pen allowing socialization with another litter versus control in standard farrowing pen) and group mixing (grouping after weaning with unfamiliar gilts or control grouped with sisters only). Sixty gilts were exposed to the treatments from birth and video recorded when their first litter of piglets was separated from them and after their piglets were returned. We analysed the first three minutes the gilt spent alone and the first three minutes after reunion with the piglets using the 'Qualitative Behaviour Assessment' (QBA) from the Welfare Quality® Assessment Protocol. Furthermore we recorded the occurrence of nursing during the first three minutes after reunion, the gilt's responsiveness towards the piglets during these three minutes and piglet growth until weaning. We analysed the QBA scores by Principle Component Analysis (PCA), which yielded two components for alone time and two for reunion time. None of the component scores were significantly affected by any of the three treatment factors or their interactions. Treatment factors also did not significantly affect piglet growth, responsiveness or the occurrence of nursing. However, low responsiveness did associate with a high likelihood of nursing during the first three minutes after reunion in a Chi-square test ($\chi^2=11.2$, $P=0.001$, $df=1$). Furthermore, an association between responsiveness and the first PCA component of the behaviour during reunion time indicated a more positive emotional state in low responsive gilts (independent samples t-test, equal variances assumed; $P<0.001$). Low responsive gilts were, among other things, less distressed than high responsive gilts. No conclusions about our treatment factors affecting maternal behaviour can be drawn, but this study does raise questions on what constitutes good maternal behaviour. High responsiveness is often seen as good maternal behaviour, though the stress that these gilts experience at separation from their piglets may affect their maternal behaviour negatively, at least on the short term.

1 Introduction

Maternal behaviour is an umbrella term used to describe all actions of a mother that are associated with raising her offspring. It is indispensable for the survival of new-born mammals, as the mother provides the offspring with food, warmth and protection. Maternal behaviour is therefore part of normal gilt (first litter) and sow behaviour, even after their domestication and further development for high production in intensive production systems. In a semi-natural environment, gestating sows of modern breeds were found to build a farrowing nest (Jensen, 1986). The nest was then used for farrowing and inhabited by the mother and piglets for at least one week. The nest provides warmth and protection against predation, and allows for bonding between mother and offspring. Even in barren environments, sows show a high motivation for nest building, which seems to be regulated not only externally but also internally, i.e. this motivation is present regardless of external nest-building stimuli (Hutson, 1992; Wischner et al., 2009). The high motivation is further demonstrated by sows still showing nest building behaviour after being presented with a prebuilt nest (Arey et al., 1991). This suggests that the behaviour itself, and not only the reaching of the end goal of an appropriate nest or farrowing environment, is important to reduce the expectant mother's motivation. In the semi-natural environment, the piglets left the nest together with their mother approximately seven to ten days after they were born (Jensen, 1986). Already before the piglets were four weeks old, they were seen to occasionally mix with piglets from other litters. As they got older, they socialized with piglets from other litters more and more frequently. They were not fully weaned until they were between 14 and 17 weeks old (Jensen, 1986). It was suggested that mingling with other litters before weaning is a natural socialization process, during which pigs form social relationships and acquire social skills (Petersen et al., 1989). This natural socialization process and the mother's maternal behaviour are often obstructed by management and housing conditions in production systems, as piglets may be weaned from three weeks of age and usually do not have access to piglets from other litters. Furthermore, standard housing rarely allows maternal behaviour like nest building, and housing in farrowing crates even vastly restricts sow and gilt movement (Blackshaw et al., 1994). Even though the expression of maternal behaviour is likely restricted by housing conditions, this does not seem to prevent bonding between the mother and the piglets. Sows were found to be restless and more vocal after their piglets were weaned, and they gave a clear response to the playback of piglet calls, as they appeared to start searching for them (Pajor et al., 1999).

Maternal behaviour in pigs and other mammals is influenced negatively by stress. In female mammals, stress during gestation and around birthing negatively affects for example the birthing process, lactation and bonding between mother and offspring (Leng et al., 2008). The development of optimal maternal behaviour may therefore be facilitated by reducing stress in gilts and sows. An important source of stress is the occurrence of aggression between gilts and sows that are group housed during gestation (Andersen and Bøe, 1999a; Anil et al., 2005; Engblom et al., 2007). Aggression between gilts and sows may be reduced by improving their social skills. The development of social skills is thought to be highly influenceable by early life experiences. Van Putten and Buré (1997) studied pigs that had been regrouped up to four times at 12, 14, 16 and 18 weeks of age. In comparison to a stable control group, the regrouped pigs fought significantly shorter during final regrouping at five months of age, and regrouped pigs had fewer injuries. Further research compared the behaviour of piglets that were able to socialize with piglets from other litters between 10 and 30 days of age with the behaviour

of piglets that were able to socialize within their own litter only, which formed the control group (D'Eath, 2005). Socialized piglets started fighting sooner than controls after mixing at 50 days of age. However, socialized piglets formed a stable hierarchy more quickly than controls. These studies suggest that both socialisation before weaning and regrouping of young pigs helps them to develop better social skills, and the improvement of social skills may indirectly improve maternal behaviour.

In addition to social behaviour being linked indirectly to maternal behaviour, the specific social behaviour of aggression towards conspecifics may be linked directly to later maternal behaviour. Løvendahl et al. (2005) studied variation in aggressive behaviour and maternal behaviour of sows. After confirmation of pregnancy, five to ten unfamiliar sows ($N_{\text{groups}}=137$; $N_{\text{sows-total}}=835$) were placed in a 30 m² testing area and aggression was scored during 30 minutes. Maternal behaviour was later scored as responsiveness to piglet distress calls when piglets were picked up by a person. Responsiveness regarded posture changes, signs of attention such as looking and in extreme cases attacking the person. Results indicated that less aggressive sows showed higher responsiveness. Higher responsiveness could be seen as better maternal behaviour, as for example a sow's responsiveness to a trapped piglet's distress call could prevent the piglet being crushed by the sow.

The aim of this study is to assess if specific measures that are thought to improve social skills also affect maternal behaviour. The broader research project 'Improving sow welfare in group housing systems – effect of genotype and rearing strategy on the behaviour of young gilts' is currently being executed at the Swedish University of Agricultural Sciences. It maps the effects of genotype and social environment (early in life and during gilt rearing) on the development of gilts' social skills and their performance in group housing systems later in life. The effect of genotype is studied by comparing the maternal behaviour of Dutch Yorkshire gilts (DY-gilts) with that of Swedish Yorkshire gilts (SY-gilts). SY-gilts have been kept in group housing for the full duration of gestation for roughly forty years. DY-gilts have been kept in individual stalls for decades, until gradual changes started roughly ten years ago. DY-gilts are now group housed during gestation, but still kept in individual stalls for a relatively long period around insemination. Due to this difference in the environment for which selection occurred, DY-gilts are expected to be less genetically adapted to group housing than SY-gilts, and thus DY-gilts are expected to experience more stress in group housing. The effect of social environment is studied by giving gilts access to piglets outside of their litter before weaning and by regrouping them after weaning, to provide them with additional opportunities to practice social behaviour early in life. We hypothesise that SY-gilts are better adapted to group housing than DY-gilts and that gilts that are given additional opportunities to practice social behaviour are better adapted to group housing. Furthermore, we hypothesise that these gilts therefore show better maternal behaviour, because they experience less stress in group housing. We expect the effects of the three factors to be additive, but the additional opportunities to practice social behaviour may affect DY-gilts more strongly than SY-gilts, which would cause interaction effects.

2 Literature review

2.1 Scientific background of the SLU research project

Common housing systems for gestating sows and gilts are either individual stalls or various types of group housing (McGlone et al., 2004). Individual stall housing used to be the most common in Europe, until consumer and political demands caused a transition from individual stall housing to group housing during the largest part of gestation. Gestating sows and gilts have been group housed in Sweden since the late 1980-ies (Einarsson et al., 2014), though in most other European countries this transition is more recent. The transition in other European countries followed changes in animal welfare legislation that were implemented in 2008 (EU Council Directive 2008/120/EU¹). For example, current Dutch legislation states that sows and gilts have to be housed in groups starting no later than four days after confirmation of pregnancy and that they must stay in group housing until one week before farrowing (Rijksdienst voor Ondernemend Nederland / Besluit houders van dieren²). In non-European countries, individual stall housing during gestation may still be the standard (McGlone, 2013; Kim et al., 2016).

Advantages and disadvantages of individual stall housing and group housing

Both individual stall housing and group housing during gestation have advantages and disadvantages. Individual stall housing reduces costs by allowing a higher stocking density and reducing labour (Marchant-Forde, 2009). Furthermore, individual stall housing facilitates management and easy adjustment of husbandry regime through precise monitoring of individual sows and gilts (Marchant-Forde, 2009). However, these individual stalls restrict the sows in movement and possibilities to perform social behaviour. Pigs cover large distances when they are let loose in large enclosures (Jensen, 1986) and restriction of movement is associated with reduced bone strength and reduced muscle weight in sows (Marchant and Broom, 1996). Furthermore, pigs are social animals, as they naturally live in family groups (Graves, 1984). Housing sows and gilts individually during gestation therefore interferes with the freedom to display normal behaviour, as issued in 1979 by the UK Farm Animal Welfare Council, through both restriction of movement and restriction of possibilities for social behaviour. Group housing allows both movement and social behaviour and may therefore be the preferred choice from an animal welfare perspective. However, group housing has disadvantages especially when the existing group composition is altered by introducing new gilts. Feral pigs generally do not accept unfamiliar individuals entering their family group and usually respond aggressively when this situation does occur (Graves et al., 1984). However, in current production systems new gilts have to be introduced into existing sow groups at some point, even when farmers aim to keep these groups as stable as possible. Especially the introduction of gilts into sow groups is associated with displays of aggression, though these displays may persevere also after introduction. The animals regularly inflict severe injuries onto each other during aggressive displays (Andersen and Bøe, 1999a; Anil et al., 2005) and injuries may be so severe that they require culling of the affected animal or result in its death. Despite Sweden's relatively long history with group housing, Swedish research has shown that 18% of

¹ Council Directive 2008/120/EC: laying down minimum standards for the protection of pigs. Official Journal of the European Union 2008/120/EC.

² Rijksdienst voor Ondernemend Nederland / Besluit houders van dieren: www.rvo.nl/onderwerpen/agrarisch-ondernemen/dieren/dierenwelzijn/welzijnseisen-voor-dieren/varkens

all group housed gilts that were culled after their first parity, were culled due to traumatic injuries (Engblom et al., 2007). Some of these animals were even found dead in their group pen.

Measures to reduce aggression behaviour in group housing

With group housing sows and gilts now being an obligation, measures to reduce aggressive behaviour and its consequences are imperative. Farmers need animals that are better adapted to group housing to minimize severe negative consequences. Common negative consequences of group housing are the result of aggressive behaviour between animals, which may be reduced by improving gilts' social skills through social training and genetic selection for appropriate social behaviour. Such improvements are the focus of the ongoing research project at the Swedish University of Agricultural Sciences (SLU), which maps the effects of social environment (early in life and after weaning) and genotype on the development of gilts' social behaviour and their performance in group housing systems later in life. This literature review consists of two parts that focus on two aspects of pigs' social behaviour. The first part is about agonistic behaviour, because that causes a large part of the problems that accompany the transition to group housing. The second part provides more information about maternal behaviour, because that is the focus of this thesis research.

2.2 Agonistic behaviour

Agonistic behaviour encompasses more than just aggressive behaviour, as it includes both causing and threatening to cause physical damage, and also seeking to reduce physical damage by showing submissive behaviour (McGlone, 1986). Agonistic behaviour between domestic pigs occurs mainly during periods after mixing groups or adding new animals to existing groups (Mount and Seabrook, 1993; Arey and Edwards, 1998), which will further be referred to as acute agonistic behaviour. Not only does the introduction of new animals increase agonistic behaviour between unfamiliar animals, it also increases agonistic behaviour between familiar animals in the existing group (Mount and Seabrook, 1993). The prevalence and severity of agonistic behaviour may decrease as quickly as one hour after mixing (Mount and Seabrook, 1993), but it is not completely minimized until a social hierarchy is established, which takes between two and ten days (Zurbrigg and Blackwell, 2005). Even though acute agonistic behaviour is caused by a natural need to form a social hierarchy, its severity makes it a significant welfare concern. Pigs regularly inflict severe injuries upon each other (Andersen and Bøe, 1999a; Anil et al., 2005), that can result in an animal's death in the most extreme cases (Engblom et al., 2007). Injuries reduce animal welfare due to the infliction of pain and agonistic behaviour causes stress regardless of physical consequences. In addition to reduced animal welfare, severe agonistic behaviour has economic consequences for the producer. These consequences include for instance costs that are associated with culling due to injuries, costs that are related to extra labour for caring for injured animals and costs that are associated with reduced reproductive function. Reduced reproductive function can be caused by fear and stress resulting from agonistic behaviour (Kongsted, 2004). Mendl et al. (1992) divided gestating gilts (N=37) into three groups based on their behavioural strategy in their social environment and found that the group with the highest stress levels produced piglets with the lowest average weight. Furthermore, stress hormones interfere with the precise concentrations of specific hormones that are needed to achieve ovulation and therefore stress may delay the onset of oestrus (Liptrap, 1973; Hennessy and Williamson, 1983). Delayed oestrus constitutes a delayed gestation and consequent lactation, and thus a reduced reproductive function.

These negative consequences of stress on reproductive function and pig welfare may occur not only as a result of acute agonistic behaviour, but also as a result of long-term agonistic behaviour that is not directly related to the event of group mixing. This long-term agonistic behaviour will be referred to as chronic agonistic behaviour. Main causes of chronic agonistic behaviour are unclear hierarchies or competition for resources.

Occurrence of agonistic behaviour

The occurrence of agonistic behaviour upon the mixing of previously unfamiliar pigs has been studied for decades. Meese and Ewbank (1973) studied groups of eight grower-finisher pigs (females and castrated males) aged between eight and thirty weeks. The pigs had not had any interaction with each other prior to the experiment. Upon their introduction into the pen, intense fighting and severe aggression occurred, mostly between pairs. During most fights, pigs attempted to bite each other in the ears or head. When one of the fighting pigs seemed to retreat, its opponent would continue to bite its rear and back legs. This continuation of attack even when the victim retreats stresses the need for pigs to learn to recognize submissive behaviour. Submissive behaviour may stop an ongoing attack if the victim displays it correctly and the attacker recognizes and accepts it as submission. Regardless, fighting diminished considerably after 24 hours and reached its apparent goal after 48 hours, namely the development of a stable social hierarchy. The highest-ranking pig had shown approximately 80% of all aggression that was shown during the first hour after mixing, after which its place in the hierarchy was clear. Spooler et al. (1996) studied pairs of gilts that were introduced into a large dynamic gilt and sow group. Gilts that had earlier been in the same subgroup recognized each other and showed less agonistic behaviour towards each other than towards unfamiliar individuals, including gilts from other groups that had been introduced during the same period. Arey (1999) performed research on adult sows and revealed that 93% of fights were between individuals which had not previously been housed together. Aggression between these sows declined rapidly after mixing and stabilized at a minimum seven days after mixing, which indicates the establishment of a social hierarchy. After the social hierarchy was formed, the sows appeared to remember each other and their place in the hierarchy over a long period of time, because there was no major disruption to social organization when sows were returned to their group after a six-week absence. These studies suggest that in order to reduce long-term agonistic behaviour, the composition of groups of pigs should be kept stable, to give them the opportunity to develop a social hierarchy. However, this is not entirely possible in gestating gilt and sow group housing. Even when a stable group is moved from gestation to farrowing and then back to gestation in the same composition, new gilts have to be added to the group at some point. Especially these new gilts seem to be at the receiving end of aggressive behaviour. Sow weight and parity was found to correlate positively with their social rank (Edwards et al., 1994; Arey and Edwards, 1998) and higher ranked sows were found to show more aggression than lower ranked sows (Elmore et al., 2011). This explains findings in groups of more than a hundred individuals, where gilts and second parity sows had more injuries than older sows (Hodgkiss et al., 1998). In order to protect these low parity individuals from severe consequences of agonistic behaviour, factors that may reduce aggression following mixing need to be considered. Spooler et al. (2009) concluded that aggression will never be fully eliminated from group housing systems, but that several factors influence the severity. These factors include but are not limited to early social environment, group composition,

procedure for introducing new gilts, space allowance, group size, feeding system and enrichment, all of which will be discussed in detail below.

Social environment and development of social behaviour

As suggested in the introduction, allowing piglets to socialize with piglets from other litters before weaning may improve the development of their social skills. Piglets that were kept in group farrowing systems reacted less strongly to the presence of unfamiliar piglets in a confrontation test than piglets that were kept in standard single litter pens (Hillman et al., 2003). Piglets (N=81) were individually confronted with three unfamiliar piglets for ten minutes. This confrontation occurred two days before weaning at four weeks of age and again two or three days after weaning. Piglets that were kept in a standard single litter pen reacted more strongly to the presence of unfamiliar piglets by spending more time near them ($P<0.05$) and being more active ($P<0.05$). Li and Wang (2011) also found piglets that were raised in group farrowing systems to be more tolerant of unfamiliar piglets. Piglets (N=216) from a group farrowing system and standard single litter pens were weaned at five weeks of age and mixed into groups of nine at eight weeks of age. Pigs were video recorded for 24 hours immediately after mixing and aggressive encounters were analysed. Fights were less frequent (1.5 versus 3.8 per hour; $P=0.002$) and shorter (19 versus 120 seconds per hour; $P=0.001$) among pigs that were raised in the group farrowing system. Bohnenkamp et al. (2013) had similar findings of fewer fights (2.1 versus 4.6 fights per pen and hour; $P<0.05$) and shorter fights (10.3 versus 18.8 seconds per pen and hour; $P<0.05$) among piglets that were raised in a group farrowing system compared to piglets that were raised in standard single litter pens. Piglets (N=240) were weaned and mixed into groups of 12 when they were 26 days old and continuously sampled for 40 hours.

The reduced aggression in pigs that were kept in group farrowing systems as piglets may be due to these systems providing more space and a more complex environment, which could enhance their development. Additional space and complexity for instance stimulates play behaviour (Bolhuis et al., 2005; Oostindjer et al., 2011), which is considered important for the (social) development of piglets. The presence of play behaviour may be used as an indicator of good welfare (Oliveira et al., 2009) and even though empirical evidence of the hypothesised functions of play behaviour is rare, it is thought to function by providing exercise and enhancing physical development (Fagen, 1981), allowing to test ones own abilities in a safe situation (Thompson, 1998) and facilitating the development of muscles and motor skills (Byers, 1977).

Adequate social development of piglets may lead to them adopting optimal behavioural strategies to deal with their social environment when mixed into a group. Mendl et al. (1992) discovered three different strategies in gilts dealing with changes in their social environment. Gestating gilts (N=37) were mixed into one large indoor pen when they were about nine months old and seven weeks into gestation. Mixing was done in four batches for practical reasons (each time another batch was added to the existing group), but none of the gilts were familiar with each other beforehand. The gilts' behaviour was observed at set times during the weeks after introduction. Agonistic interactions were recorded, and gilts were divided into three classes based on how often they were able to displace another gilt during an agonistic interaction. High success (HS) gilts displaced another gilt as many times as they were displaced, Low Success (LS) gilts sometimes displaced other gilts but were displaced more often themselves and No Success (NS) gilts never displaced other gilts. Aggression levels and scores for involvement in social interactions were highest in HS and LS gilts, despite the lack of success of LS gilts.

Furthermore, LS gilts had the highest cortisol levels of all three groups, indicating high stress levels in these gilts. NS gilts were involved in the lowest number of interactions, which they seemed to actively avoid. Successfully avoiding agonistic interactions likely requires good social skills, from both the avoiding individual and the 'attacking' individual, as the latter must recognize and react to the signals of the avoiding individual.

Group composition

Altering group composition to match sows of similar parities may decrease aggression following mixing. Hoy et al. (2009) found sow social rank to be closely related to parity, until after the fourth parity the sow's weight became the leading factor. Gilts and sows were mixed into groups of eight and gilts had an average rank of 6.4 ± 1.6 whereas tenth parity sows had an average rank of 2.1 ± 1.2 . Lower parity sows were at an increased risk of injury and the authors therefore suggested separate housing for lower and higher parity sows. This was confirmed by Li et al. (2012), who reported gestating gilts in gilt-only pens sustaining fewer injuries ($P=0.03$), gaining more body weight during gestation ($P=0.01$) and having a greater farrowing rate ($P=0.03$) than gilts in control pens with multiparous sows. Ison et al. (2014) compared gestating gilts that were housed in a gilt-only group with gestating gilts that were twice removed from their gilt-only group to be temporarily housed with older unfamiliar sows. Gilts that were housed with sows used the feeding stalls more and became less active. They altered their behaviour in order to avoid aggressive behaviour, but their lesion score ($P<0.001$) and salivary cortisol levels ($P<0.001$) remained higher, suggesting that they would benefit from being housed with other gilts only. In contradiction to these findings of aggression being related to parity and body weight, Bolhuis et al. (2005) studied grower-finisher pigs and found aggression to be related more to individual traits and personality. They subjected piglets to a backtest after weaning, during which the piglets were restrained for one minute while lying on their back. The number of escape attempts was used to classify piglets as either high resisting (HR) or low resisting (LR). In LR piglets, social rank was correlated with the level of aggression, thus more aggressive LR piglets successfully achieved a higher social rank. HR piglets appeared to be less flexible, as they were more aggressive regardless of whether or not that behaviour helped them to achieve a higher social rank. Even with aggression levels being related to individual traits and personality, separating gilts and low parity sows from high parity sows may still be useful, but aggression should also be evaluated individually.

Procedure for introducing new gilts into existing groups

Kennedy and Broom (1994) researched gradual familiarization of an existing group of sows with new gilts. They found sows to show less aggression when they were able to familiarize themselves with the new gilts in an adjacent pen for five days before the gilts were introduced into their pen. Another factor that may come into play is the introduction of a subgroup of gilts that are familiar with each other instead of the introduction of an individual gilt. Durrell et al. (2003) assigned 16 groups of four previously unfamiliar sows to either a 'pre-mixing' or 'direct introduction' treatment. Pre-mixed sows were housed together prior to being introduced into a dynamic group, whereas direct introduction sows were not housed together before being introduced into another dynamic group. During the first five weeks after mixing, 24-hour video recordings were taken on the first and fifth day of each week. Focal animal sampling (two periods of five minutes of each observation day) was used to analyse the behaviour of individual sows, and more specifically analyse aggressive encounters. All

sows formed separate subgroups within the larger group, regardless of whether they were familiar with each other (pre-mixed) or not (direct introduction). However, pre-mixing seems to be the preferred procedure, since there was less aggression within pre-mixed subgroups than within subgroups that formed after direct introduction. Furthermore, aggression between new sows and sows in the existing group was less severe when subgroups were pre-mixed.

Space allowance

Larger space allowance was found to associate with a lower prevalence of aggression in eight stable groups of six gestating sows that were housed in pens with four different sizes (Weng et al., 1998). Each group spent seven days in each pen size. The smallest pen provided 2.0 m² per sow, the second smallest 2.4 m², the second largest 3.6 m² and the largest pen provided 4.8 m² per sow. With an increase in pen size, sows spent less time sitting and standing inactive and more time rooting. Social interactions, including aggressive interactions decreased with an increase in pen size. Sows had an average of 816.8 social interactions per 46 hours (the last two days of the week, minus the two hours spent in the feeding stalls) when they were kept in the smallest pen and only 314.8 interactions per 46 hours when they were kept in the largest pen. Social interactions include for instance the aggressive interactions head-to-head-with-bite (22.3 per 46 hours in the smallest and 1.4 per 46 hours in the largest pen; P<0.01) and head-to-body-with-bite (40.3 per 46 hours in the smallest and 8.6 per 46 hours in the largest pen; P<0.001). The sows consequently had higher body lesion scores (combination of severity and incidence) after being housed in the smallest pen. The total lesion score was 326.8 after housing in the smallest pen and 129.3 after housing in the largest pen (P<0.001). Findings in grower-finisher pigs were similar, with pigs that were provided a low space allowance (50 kg/m²) having more lesions than pigs that were provided a high space allowance (32 kg/m²; Turner et al., 2000a). This study included groups of 80 pigs and groups of 20 pigs and regardless of group size, the number of skin lesions increased with a low space allowance (8.5 versus 10.1 S.E.D. 0.68). Even though additional space may reduce aggression and thus benefit pigs, providing more space than required is economically inefficient. The optimal space allowance considering both animal welfare and economics is still to be determined (Greenwood et al., 2014).

Group size

Group size is associated with total space allowance, since a minimum space allowance is required per animal. However, when researchers controlled for the space allowance per animal, sows in larger stable groups were still found to be less aggressive than sows in smaller stable groups (Broom et al., 1995). One explanation would be that a larger group's larger space allowance provides lower ranked animals with more opportunities to show submissive behaviours to avert an aggressive attack from higher ranked animals. Another explanation is that pigs alter their strategy with increasing group size and show more avoidance behaviour instead of agonistic behaviour (Turner and Edwards, 2000b). With increasing group size, animals will at some point no longer be able to recognize all other individuals. If they indeed do not recognize all other individuals, there is no point in fighting in order to establish their relative social status, because they will not recognize each other the next time they meet. This was confirmed in a later study, during which pigs were held in a group of either 20 or 80 individuals total (Turner et al., 2001). Six weeks later, the interactions between two pairs of pigs, i.e. one pair from each treatment or two pairs from the same treatment, were compared. Pigs that

originated from the larger groups displayed a lower prevalence of aggression (0.51 versus 0.90 aggressive acts per pig per minute S.E.D. 0.090) and initiated fewer fights (19.7 versus 34.6 initiations S.E.D. 3.62% of pigs, $P < 0.01$) than pigs that originated from smaller groups.

Feeding system

High production sows in intensive production systems are usually food restricted, which makes it probable that they feel unsatiated even right after feeding (Lawrence and Illius, 1989). This food restriction may cause aggressive food competition, which is why the type of feeding system affects the number of aggressive encounters. Gestating sows in group housing were indeed found to show severe aggression during feeding, even more than during the day that they were mixed (Kirkwood and Zanella, 2005). Individual feeding stalls provide sows with the best protection against aggressive behaviour. When that is not possible, full body partitions can be used to decrease aggression to some degree (Andersen et al., 1999b). Aggression around feeding is chronic and may therefore be even more important to decrease than the aggression that occurs around mixing.

Environmental enrichment

Environmental enrichment was defined by Newberry (1995) as an 'improvement in the biological functioning of captive animals resulting from modifications to their environment'. Aggression levels in weaner pigs (Beattie et al., 1996; Blackshaw et al., 1997) and grower-finisher pigs (Schaefer et al., 1990; Beattie et al., 2000; Olsen et al., 2002) were found to be lower in environments where enrichment was present. Very little research has focused on the effects of enrichment on aggression between sows and gilts and results are contradictory (Greenwood et al., 2014). Enrichment might provide a distraction and thus decrease aggression, but it could also increase aggression when it is a limited resource that animals compete to obtain. The use of straw is generally considered to improve animal welfare (Tuytens, 2005) and when used as bedding (thus not a limited resource), it was found to reduce aggression following mixing (Jensen et al., 2000). Greenwood et al. (2014) suggests further research before being able to suggest enrichment in addition to straw or as an alternative to straw when the use of straw is not an option.

2.3 Maternal behaviour

Maternal behaviour is an umbrella term used to describe all actions of a mother that are associated with raising her offspring. It is indispensable for the survival of new-born mammals, as the mother provides the offspring with food, warmth and protection. Maternal behaviour is therefore part of normal gilt (first litter) and sow behaviour, even after their domestication and further development for high production in intensive production systems.

Maternal behaviour in semi-natural environments

One of the earliest maternal behaviours in gilts and sows is nest building behaviour. When four gestating gilts and one gestating sow of modern breeds were released in a semi-natural environment, they were found to build nests consisting of rooted out shallow holes which they filled with grass (Jensen, 1986). Even in barren environments, sows show a high motivation for nest building, which seems to be regulated not only externally but also internally, i.e. this motivation is present regardless of external nest-building stimuli (Hutson, 1992; Wischner et al., 2009). The high motivation is further

demonstrated by sows still showing nest building behaviour after being presented with a prebuilt nest (Arey et al., 1991). This suggests that the behaviour itself, and not only the reaching of the end goal of an appropriate nest or farrowing environment, is important to reduce the expectant mother's motivation. Around two days after the gilts and sow finished their nest, they used it for farrowing (Jensen, 1986). During farrowing, the gilts and sow touched and sniffed the new-born piglets and changed positions several times. During the first five days after farrowing, the piglets were nursed with an average frequency of 1.3 nursings per hour. Both the mother and piglets remained in the nest for at least the first two days after farrowing, after which the mother briefly left the nest for the first time. Thereafter, the frequency with which the mother left the nest increased gradually. The piglets started following the mother for the shortest excursions, which were less than twenty meters away from the nest. After approximately seven to ten days, the piglets followed their mother further away, after which neither the mother nor the piglets returned to the nest. As the piglets got older, they gradually explored areas further away from their mothers. The piglets were not fully weaned until they were between 14 and 17 weeks old.

Nest building in modern housing systems

To be able to build a nest, sows need not only adequate material, but also sufficient space to move around. Crates likely do not provide sufficient space to move around. Andersen et al. (2014) found crated sows (N=23) to show significantly less nest building behaviour than loose housed sows (N=21), and crated sows to show more behaviour indicating frustration, even though both were presented with adequate material. Hansen et al. (2017) also found crated gilts (N=19) and sows (N=21) to show less nest building behaviour than loose housed gilts (N=20) and sows (N=20). These loose housed gilts and sows were loose housed during the entire experimental period, whereas the crated gilts and sows were loose housed upon entering the farrowing pen, but then confined in a crate from two days before until four days after farrowing. However, the difference in nest building behaviour, did not seem to affect farrowing parameters (Hansen et al., 2017). There were no significant differences in the farrowing parameters of total number of piglets per litter, total duration of farrowing, interval between births or number of stillborn piglets.

Stress around farrowing affects the farrowing process, lactation and bonding

Despite the lack of effect on farrowing parameters, giving sows adequate nest building material and the opportunity to move freely in loose housing is beneficial from an animal welfare point of view. Jarvis et al. (2001) found the plasma cortisol concentration, which indicates an animal's stress level, to increase during the last 48 hours until farrowing in both crated (N=7) and loose housed (N=7) second parity sows. However, plasma cortisol concentrations were higher in crated than in loose housed sows and crated sows were more restless. Oliviero et al. (2008) confirmed crated sows to have higher plasma cortisol concentrations than loose housed sows. These findings imply reduced welfare due to increased stress around farrowing in crated sows compared to loose housed sows. Stress and restlessness around farrowing will, in addition to reducing welfare, influence the oxytocin concentration of the farrowing and lactating sow or gilt. Oxytocin is a hormone that positively affects farrowing and lactation, and the development of appropriate maternal behaviour (Leng et al., 2008). Oliviero et al. (2008) found crated sows (N=18) to have lower concentrations of oxytocin than loose housed sows (N=20), and farrowing to last on average 93 minutes longer in crated sows. In lactating

mammals, a neurohormonal reflex dependent on oxytocin mediates the milk release into the udder ducts and teat canal. Folley and Knaggs (1966) found that sows show a peak in oxytocin before milk release, thus stress and lower oxytocin levels during a prolonged farrowing may negatively affect early lactation in crated sows. Since oxytocin is also important for bonding and the development of appropriate maternal behaviour (Leng et al., 2008), crated or otherwise stressed sows may bond less with their piglets and show poor maternal behaviour.

The vicious circle of stress during gestation and/or lactation

Prenatal stress in gestating mammals is thought to influence the stress sensitivity of the offspring by influencing the responsiveness of their hypothalamic-pituitary-adrenal axis (Andrews and Matthews, 2004; Anisman et al., 1998; De Kloet et al., 1998; Meaney, 2001; Weinstock, 2001; Welberg and Seckl, 2001). The hypothalamic-pituitary-adrenal axis is a complex system that among other things controls the reaction to stress. The effect of prenatal stress on the stress sensitivity of the offspring was studied by Jarvis et al. (2006). Eight month old gilts (N=36) were inseminated and then divided into three groups. Gilts in the first group formed the control group, the second group was stressed by mixing the gilts twice while they were in their second trimester of gestation and the third group was stressed by mixing them twice while they were in their third trimester of gestation. After the gilts farrowed, three of their female piglets were selected. Two out of three were euthanised when they were 60 days old. Their brain tissue was collected and piglets from stressed gilts were found to show increased expression of brain regions that regulate the production and release of corticotropin-releasing hormone (CRH). Increases in CRH are associated with the induction of negative behavioural states such as fear and anxiety (Johnson et al., 1994). The third piglet of each litter was regrouped and inseminated. Gilts that were themselves farrowed by stressed mothers were more restless during their own farrowing and more responsive towards piglets that approached their head. Restlessness and responsiveness during farrowing is associated with increased savaging behaviour (Ahlstrom et al., 2002; Jarvis et al., 2004). Jarvis et al. (2006) confirmed that gilts that were farrowed by stressed mothers showed more savaging behaviour in the form of biting their own piglets than gilts that were farrowed by non-stressed mothers.

Poor maternal behaviour – Savaging

A clear example of poor maternal behaviour is savaging, which encompasses all piglet-directed aggression by the mother gilt or sow. This threatens piglet welfare and may result in increased piglet mortality. Therefore, savaging has both animal welfare and economic consequences. Sows and gilts that later show savaging behaviour appear to be more restless and more investigative towards piglets during farrowing (Ahlstrom et al., 2002; Jarvis et al., 2004). Hansen et al. (2017) video recorded the behaviour of gilts (N=19) during and after farrowing and retrospectively categorized gilts as either savagers (N=4) or non-savagers (N=15). They confirmed that gilts that savaged after farrowing were more restless during farrowing. Restlessness was characterized by an increased frequency of posture changing, increased walking around the pen, and increased investigation of piglets. The apparent causal relation between the sow or gilt being stressed and restless during farrowing and that sow or gilt savaging after farrowing emphasizes the need to reduce stress around farrowing.

Poor maternal behaviour – Piglet crushing

Another example of poor maternal behaviour is piglet crushing. Weber et al. (2007) found that more piglets were crushed in pens with loose housed sows (N=18,824 litters on 173 farms; 0.62 piglets per litter crushed) than in pens with crated sows (N=44,837 litters on 482 farms; 0.52 piglets per litter crushed). However, the number of piglets that died for other reasons was higher in pens with crated sows, which resulted in no differences in the average total piglet mortality. Another factor that may influence the occurrence of crushing is individual sow behaviour, which was studied by Wechsler and Hegglin (1997). Loose housed sows (N=11) and their litters were video recorded during farrowing and the following ten days. Five sow behaviours that are likely to increase the number of piglets are:

- The average frequency of lying down.
- The average frequency of rolling onto her side from a more vertical lying position.
- The percentage of all lying down events in which the sow flopped straight down, as opposed to lying down in a slower and more controlled manner.
- The percentage of all lying down events in which the sow lied down on the side where the most piglets were present.
- The average percentage of all piglets of a litter that were within half a meter from the sow when she started to lie down.

The scores for these behaviours were used to calculate an average rank for each sow to represent the quality of her maternal behaviour, and higher scores resulted in a lower average rank. Furthermore, the responsiveness of the sows to playbacks of piglet distress calls was recorded. The playbacks lasted one minute, and sows were considered responsive when they showed a posture change from a lying position to a sitting or standing position. As a control, sows were on other occasions played a recording of bird calls. Both the rank for the quality of maternal behaviour (based on the five summed up sow behaviours) and the responsiveness to piglet distress calls were negatively correlated with the percentage of crushed piglets.

3 Material and methods

3.1 Experimental design

Three factors likely influencing social behaviour were studied in a 2x2x2 factorial design. These factors were genotype, early rearing environment and group mixing. Firstly, the effect of genotype was studied by comparing the maternal behaviour of Dutch Yorkshire gilts (DY-gilts) with that of Swedish Yorkshire gilts (SY-gilts). The sows that were used to produce the experimental gilts were 100% SY or at least 50% DY. The boars were either 100% SY or 100% DY, resulting in the experimental gilts being 100% SY or at least 75% DY. Secondly, the effect of early social rearing environment was studied by keeping half of the gilts with their mother sow and litter mates in a standard farrowing pen as controls (control pen) and half of the gilts with their mother sow and litter mates, but with additional access to the sow and the piglets in the neighbouring pen through a pop-hole (access pen). The additional social access was available from when the gilts were two weeks old and ended at weaning when they were five weeks old. See Appendix 1 for a lay-out of the access pen. Thirdly, the effect of group mixing was studied by regrouping half of the gilts with unfamiliar gilts after weaning (mixed group) and keeping the other half with only sisters (intact group). All gilts were housed in groups of four and mixed groups originated from two different litters (always two gilts from each litter). Gilts were ten weeks old at the time of regrouping and remained in their group of four until they were moved into the farrowing pens one week before expected farrowing.

3.2 Animals, housing and management

The gilts that were used in this study originated from 28 litters that were divided into seven batches indicated A to G. Four gilts from each litter were randomly selected to be included in the study, resulting in an original total of 112 gilts. This report contains data on gilts from batches A to E, resulting in a total of 65 gilts. However, five gilts from these batches did not have the correct genotype due to the correct sperm being unavailable at the moment of the sow's insemination. Consequently, these gilts could not be classified as either SY-gilt or DY-gilt and were therefore excluded from all analyses, resulting in a total of 60 gilts ($N_A=10$, $N_B=12$, $N_C=11$, $N_D=12$ and $N_E=15$) included in the analyses.

Housing of all experimental gilts and their siblings during the lactation period was in individual loose-housed farrowing pens together with their mother sow. All pens had the same design with a slatted-floor dung area, a concrete lying area and a heated corner (by heat lamp) to which only the piglets had access. The total pen area was 6 m² (see appendix 1). Mother sows were given dry feed (standard commercial sow feed) two times per day from farrowing until ten days after farrowing. Thereafter and until weaning they were given feed three times per day. The ratios were individual and based on weight and body condition (measured as back fat thickness with ultra-sound). Farrowing was in batches, with one batch kept in one section and each batch farrowing within one week. The mother sows were given an excessive amount of long straw (i.e thick bedding in the entire pen) one week before farrowing, and then additional straw was provided by hand (long straw) and from machine (cut straw) daily until weaning. Amounts were not measured, different mother sows and their piglets used different amounts. Piglets were given a commercial dry piglet feed in a feeding automat in the piglet corner from two weeks of age. Temperature was constant and regulated through floor heating and ventilation. The experimental gilts received the same treatment as their mothers at the time of their own first farrowing.

Experimental gilts and their siblings were weaned at five weeks of age, by removing their mother sow from the pen. The piglets remained in the pen for an additional five weeks, until they were ten weeks old. After weaning cut straw was provided daily. Amounts were again not measured, different groups of piglets used different amounts of straw.

At ten weeks of age, experimental gilts were moved into pens in the growing stable. All gilts were divided into groups of four, either originating from one litter or originating from two litters (two gilts from each litter). Pens were 1.80 x 3.20 meters, of which 1.80 x 2.20 concrete floor and 1.80 x 1.00 slatted floor, thus 5.8 m² in total. Straw was provided daily by hand (long straw) and from machine (cut straw). Amounts were not measured, but straw was available at all times. Gilts were given a commercial dry sow feed three times a day.

At twenty weeks of age the groups of four gilts were moved into pens (one group of four per pen) in the sow stable. Pens were 2.00 x 3.25 meters, of which 2.00 x 2.05 concrete floor and 2.00 x 1.20 slatted floor, thus 6.5 m² in total. The pens had a deep straw bedding covering the whole pen. Additional straw (unmeasured amounts) was provided when necessary. Gilts were given a commercial dry sow feed two times a day, in an individual stall. They were inseminated when they were six months old, with sperm obtained from Hampshire boars. All gilts were inseminated with sperm from the same breed of boar, regardless of their genotype, rearing environment or group mixing. Approximately one week before their expected farrowing date, they were moved into the farrowing pen.

3.3 Video recording

Gilts were video recorded when their piglets got their first iron injection at approximately five days of age. For each gilt, video recording started when a staff member started to collect the piglets. The piglets were then moved from the pen and given an iron injection and an ear tag at a treatment station in the hallway near the sections with farrowing pens, not outside of hearing distance. Video recording continued while the gilts were without their piglets and for at least three minutes after all piglets were returned. The piglets were separated from the gilt for at least 11 minutes, even if their treatment was completed before 11 minutes had passed. Some piglets were separated from the gilt for more than 11 minutes, due to treatment taking longer or due to unforeseen circumstances. Gilts could be disturbed by normal husbandry activities in the stables and by piglet distress calls from the treatment station or from neighbouring pens during any part of video recording. All disturbances occurred randomly and are not accounted for in the following analyses.

3.3 Video analysis

Information on the number of piglets that was present with the gilt at the moment of iron injection and the piglets' exact age in days was obtained from the research facility administration. Videos were analysed by one and the same person. Piglet activity at the beginning of each video was noted as the majority of piglets being 'inactive' (lying or sitting), 'active' (standing or walking/running) or 'nursing'. Gilt activity at the beginning of each video was noted as 'inactive' (lying or sitting) or 'active' (standing or walking). The location of the litter at the beginning of piglet collection was noted as either 'completely in the piglet corner' or 'at least one piglet in the gilt pen'. Lastly it was noted if the collecting staff member entered the gilt pen or only the piglet corner.

The videos were then divided into three parts: collection time, alone time and reunion time. Collection time began immediately at the start of the video and ended when the staff member and all

piglets had left the pen. Reunion time began when the first piglet was returned and lasted three minutes. Alone time was the time between collection and reunion. From video, the gilt's behaviour during the first three minutes of alone time was scored following the 'Qualitative Behaviour Assessment for sows, piglets and grower pigs' (QBA) from the Welfare Quality® Assessment protocol for pigs (see Appendix 2). The QBA was found to be a repeatable, objective and valid measure of animal behavioural responses in multiple experimental set-ups (reviewed by Fleming et al., 2016). Only the first three minutes of the alone time were taken into account for the QBA, because after three minutes the gilt's behaviour stabilised. The QBA was filled in a second time for the same gilt for the full three-minute reunion time. Furthermore, a binary yes or no indicated whether or not the gilt had nursed the piglets during the three-minute reunion time. Lastly, the gilt's responsiveness towards the piglets during the three-minute reunion was indicated on a scale from one to ten. Responsiveness is defined as 'the quality of having a reaction to something or someone, especially a quick or positive reaction'³, in this case towards the piglets. High responsiveness was here characterized by a gilt's repeated vocalization, looking at her piglets and actively seeking physical contact with her piglets. Responsiveness was set to binary for analyses, with 0 indicating low scores (≤ 3) and 1 indicating high scores (≥ 4). The cut-off was chosen by selecting the two values that showed the largest difference between the number of gilts for each value.

3.4 Data on piglet growth

On some occasions, piglets were moved from their birth litter to another litter, for example because their birth litter was too big for the gilt to nurse or because most piglets had died in the litter they were moved to. This moving of piglets between litters is called piglet adoption. Information on piglet adoption was obtained, to select only gilts that did not adopt piglets from other gilts and did not have piglets adopted from them by other gilts. For these gilts, information was obtained on the number of piglets born and weaned, the piglet weight at birth and weaning and the exact age in days at the moment of the second weighing. There were no strict guidelines for when a gilt was or was not involved in piglet adoption. The aim was to have the litter size and body weight as even as possible, ideally the litter size would be between 10 and 12 piglets. Treatment factors were not taken into account to decide between which gilts piglets were moved.

3.5 Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics 24th edition. Firstly, independent samples t-tests were used to determine differences in numbers of total born and live born piglets between gilts of the two genotypes. These and following data are presented as means with standard deviation, unless indicated otherwise.

Secondly, behaviour scores from the QBA were analysed by Principle Component Analysis (PCA) and each QBA (alone time and reunion time) yielded two principal components. PCA component scores were calculated from original QBA element scores with loadings as weighing factors and loadings $> |0.4|$ were considered significant. The first two principal components were selected because together they represented all twenty original element scores. The component scores were then used as the dependent variables in separate analyses of variance (ANOVA). Genotype, rearing environment, pen mixing and their interaction effects were used as the independent variables. In case of significant

³ <https://dictionary.cambridge.org/dictionary/english/responsiveness>

three-way interactions, Tukey corrections were used to account for multiple pairwise comparisons between treatment groups.

Thirdly, chi-square frequency tests were used to test for associations between responsiveness and the occurrence of nursing, between responsiveness and any of the three treatment factors or between the occurrence of nursing and any of the three treatment factors.

Fourthly, independent samples t-tests were used to determine differences in the behaviour of low responsive and high responsive gilts. This was done by comparing the average PCA component scores during alone time and during reunion time.

Lastly, the average growth per piglet for each gilt was analysed with analysis of covariance (ANCOVA). To confirm that piglet mortality between birth and weaning depended on the number of live born piglets and not the treatment factors, piglet mortality was used as the dependent variable in an ANCOVA. Genotype, rearing environment and pen mixing were used as independent variables (including interaction effects) and the number of live born piglets was used as a covariate. Only the covariate was significant and therefore the number of piglets at weaning and weighing and their exact age in days at weaning and weighing were then the only covariates used in a second ANCOVA. The average growth per piglet for each gilt was the dependent variable and the independent variables were the genotype, rearing environment and group mixing including interaction effects. The average growth per piglet was used as the dependent variable in a third ANCOVA with the occurrence of nursing as the independent variable and in a fourth ANCOVA with the binary responsiveness score as the independent variable. In both cases, the number of weaned piglets and their age at weaning were used as covariates. In case of significant three-way interactions, Tukey corrections were used to account for multiple pairwise comparisons between treatment groups.

4 Results

4.1 Descriptive statistics

In the complete data sample (N=60), 42% (N=25) was of the Swedish genotype (SY-gilts) and 58% (N=35) was of the Dutch genotype (DY-gilts). Fifty-seven percent (N=34) was kept in control pens and 43% (N=26) in access pens. Fifty-three percent (N=32) was kept in intact groups and 47% (N=28) in mixed groups. The number of gilts in each of the factor combinations is presented in Table 1, together with the total number of piglets, the number of live born piglets, and the number of piglets at the moment of the iron injection for each of the treatment factor combinations.

Table 1 – The occurrence of each of the three factor combinations of genotype, rearing environment and group mixing for 60 gilts together with the total number of piglets, the number of live born piglets, and the number of iron injected piglets for each of the treatment factor combinations.

<i>Genotype</i>	<i>Rearing environment</i>	<i>Group mixing</i>	<i>Number of gilts</i>	<i>Number of piglets (mean±Std)</i>	<i>Number of live born piglets (mean±Std)</i>	<i>Number of piglets iron injected (mean±Std)</i>
SY-gilt	Control pen	Intact group	8	14.63±3.82	12.25±2.61	10.25±2.32
DY-gilt	Control pen	Intact group	11	14.82±2.44	13.82±2.96	12.18±2.96
SY-gilt	Access pen	Intact group	3	11.67±4.04	11.33±4.51	11.33±3.22
SY-gilt	Control pen	Mixed group	9	13.11±4.17	12.78±4.06	12.00±2.60
SY-gilt	Access pen	Mixed group	5	15.00±2.83	12.80±2.49	12.00±2.35
DY-gilt	Control pen	Mixed group	6	16.00±4.47	15.17±4.26	13.00±1.79
DY-gilt	Access pen	Intact group	10	15.00±1.49	14.50±1.58	12.40±2.12
DY-gilt	Access pen	Mixed group	8	14.50±3.63	14.13±3.64	12.38±2.33

The mean total number of piglets born (stillborn or alive) per gilt was 14.50 (N=60, range 3-23, Std 3.29) and did not differ between the two genotypes in an independent samples t-test (P=0.165, equal variances assumed). The mean number of piglets per SY-gilt was 13.80 (N=25, range 3-21, Std 3.74) and per DY-gilt this was 15.00 (N=35, range 8-23, Std 2.87). However, the mean number of piglets that was born alive was 13.53 (N=60, range 3-22, Std 3.20) and this did differ between the two genotypes in an independent samples t-test (P=0.024, equal variances assumed). The mean number of live piglets per SY-gilts was 12.44 (N=25, range 3-17, Std 3.23) and per DY-gilt this was 14.31 (N=35, range 7-22, Std 2.98). Some of the live born piglets died or were culled before the first iron injection, or adopted by or from other sows or gilts. Therefore, the number of piglets at the moment of the first iron injection differed from the number of live born piglets for the majority of the gilts. The mean number of piglets per gilt at the moment of the first iron injection was 11.89 (N=60, range 6-16, Std 2.44). The mean age of the piglets at the moment of the first iron injection was 4.18 days (N=60, range 2-7, Std 1.23).

In 17% (N=10) of the cases the collecting staff member did not enter the gilt pen, because all piglets were in the piglet corner and could be collected from there. In 3% (N=2) of cases the collecting staff member entered the gilt pen, despite all piglets being in the piglet corner. In the remaining 80% (N=48) of the cases, at least one piglet was in the gilt pen during collection, causing the collecting staff member to have to enter the gilt pen. In 20% (N=12) of the cases, the piglets were nursing when

collection started, in 57% (N=34) of the cases the piglets were inactive but not nursing and in the remaining 23% (N=14) of the cases the piglets were active. The gilt was inactive in 83% (N=50) and active in 17% (N=10) of the cases. Collection time lasted on average 107 seconds (range 27-187 seconds, Std 36.6) and alone time 838 seconds (range 670-1650 seconds, Std 135.8). The reunion time (starting when the first piglet was returned) lasted three minutes for all gilts, though there were differences in how long it took to return all piglets to the pen. On average this took 34 seconds (range 16-54 seconds, Std 9.49). All mentioned differences were unrelated to the treatment factors and must be kept in mind, as they are not accounted for in the following analyses.

4.2 Behaviour during alone time and reunion time

Principal Component Analysis (PCA) of the twenty elements in the 'Qualitative Behaviour Assessment for sows, piglets and grower pigs' (QBA) from the Welfare Quality® Assessment protocol for pigs (see Appendix 1) yielded two principal components for both the first three minutes of alone time and the three minutes of reunion time (see Table 2). For the first component for both PCA's, the elements with the strongest positive loadings were relaxed, calm and content and the elements with the strongest negative loadings were agitated, frustrated, irritable and distressed. For the second component there was more variation between the two PCA's. The strongest positive loadings for the second component of alone time were for playful, positively occupied and inquisitive. The strongest positive loading for the second component of reunion time was for sociable and the strongest negative loadings were for bored and apathetic.

Table 2 – Principle Component Analysis (PCA) results for the twenty elements from the 'Qualitative Behavioural Assessment for sows, piglets and grower pigs' (QBA) from the Welfare Quality® Assessment protocol for pigs. Piglets were collected from the farrowing pen and kept separated from the gilt for at least 11 minutes. The gilt's behaviour was video recorded and judged following the QBA for the first three minutes following separation (alone time) and the three minutes following reunion (reunion time). QBA scores for each element on a scale from 0 to 125 were analysed by two separate Principle Component Analyses. Presented are the loadings >|0.4| and percentages of variation explained by the main components.

<i>Variation explained</i>	<i>First three minutes of alone time</i>		<i>Three minutes of reunion time</i>	
	56%	18%	48%	21%
<i>Active</i>	-0.66	0.58	-0.62	0.54
<i>Relaxed</i>	0.93		0.91	
<i>Fearful</i>	-0.86		-0.82	
<i>Agitated</i>	-0.93		-0.92	
<i>Calm</i>	0.94		0.88	
<i>Content</i>	0.92		0.91	
<i>Indifferent</i>	0.66	-0.57	0.50	-0.66
<i>Frustrated</i>	-0.93		-0.90	
<i>Friendly</i>	0.89		0.77	
<i>Bored</i>	0.67	-0.45		-0.70
<i>Playful</i>		0.82		0.58
<i>Positively occupied</i>		0.74		0.41
<i>Lively</i>	-0.55			0.54

<i>Inquisitive</i>		0.76	-0.50	0.64
<i>Irritable</i>	-0.90		-0.92	
<i>Calmless/Uneasy</i>	-0.91		-0.90	
<i>Sociable</i>		0.44		0.72
<i>Apathetic</i>	0.45	-0.63		-0.73
<i>Happy</i>	0.88		0.58	0.53
<i>Distressed</i>	-0.91		-0.92	

First three minutes of alone time

Genotype, rearing environment and group mixing were used as independent variables in two separate three-way analyses of variance (ANOVA) with the first or second component scores as dependent variables. The average scores for both the first and second PCA component for the eight treatment groups are presented in Figure 1. For the first component scores neither the main effects of genotype ($P=0.222$), rearing environment ($P=0.624$) and group mixing ($P=0.974$) nor the interaction effects were significant (P -values two-way interactions 0.479 to 0.708 and three-way interaction 0.668). The main effects of genotype ($P=0.597$), rearing environment ($P=0.545$) and group mixing ($P=0.428$) were also not significant for the second component scores and neither were the interaction effects (P -values two-way interactions 0.163 to 0.583 and three-way interaction 0.210).

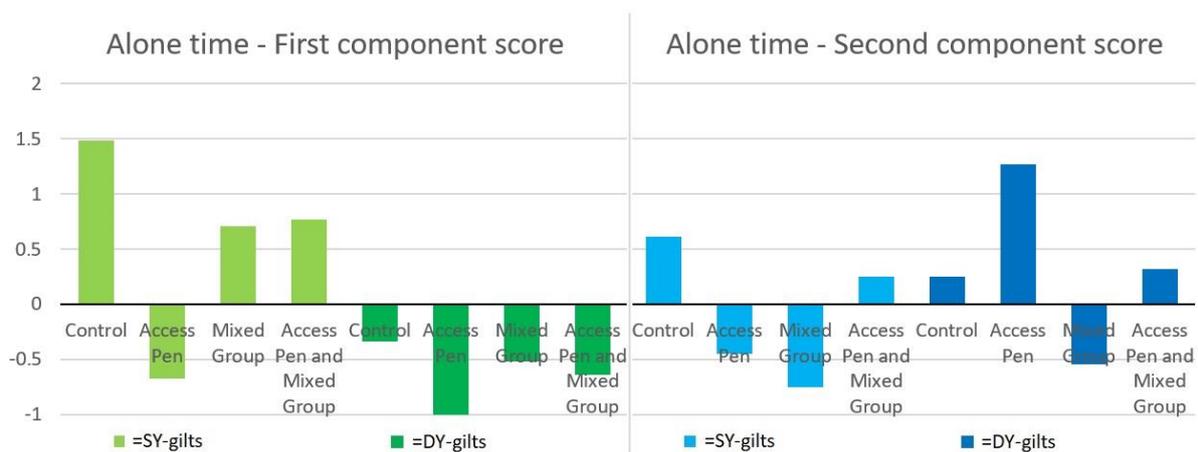


Figure 1 – Average PCA component scores for Swedish and Dutch Yorkshire gilts (SY-gilts and DY-gilts respectively) that were housed in either an access pen or a control pen as piglets and that were either mixed or not mixed after weaning. The gilt's piglets were separated from them and the gilt's behaviour was scored during the first three minutes of alone time. The behaviour scores were analysed with PCA. Three way ANOVA's with genotype, rearing environment and group mixing as independent variables including interaction effects revealed no significant differences in the PCA component scores.

Three minutes of reunion time

Again, both PCA component scores were used as dependent variables in two separate three-way ANOVA's with genotype, rearing environment and group mixing as independent variables. The average scores for both component scores for the eight treatment groups are presented in Figure 2. Neither the main effects of genotype ($P=0.517$), rearing environment ($P=0.161$) and group mixing ($P=0.125$) nor the two-way interaction effects (P -values 0.261 to 0.828) were significant for the first component scores. The three-way interaction effect was significant ($P=0.038$), but no significant pairwise differences were found.

The main effects of genotype ($P=0.613$), rearing environment ($P=0.780$) and group mixing ($P=0.095$) were also not significant for the second component scores and neither were the interaction effects (P -values two-way interactions 0.065 to 0.728 and three-way interaction 0.852). No significant differences were found, but there was a trend for group mixing as a main effect ($P=0.095$). Gilts that were not group mixed scored slightly lower than gilts that were group mixed (-0.44 ± 2.06 versus 0.50 ± 1.93), indicating that group mixed gilts were more sociable and less apathetic and bored. There was also a trend for the two-way interaction between genotype and rearing environment ($P=0.065$). SY-gilts that were housed in control pens scored slightly higher than SY-gilts that were housed in access pens (0.55 ± 2.11 versus -0.13 ± 1.98). In contrast, DY-gilts that were housed in control pens scored slightly lower than DY-gilts that were housed in access pens (-0.85 ± 2.09 versus 0.34 ± 1.84). This trend indicates that SY-gilts were more sociable and less apathetic and bored if they were housed in control pens, but that this was the case for DY-gilts that were housed in access pens.

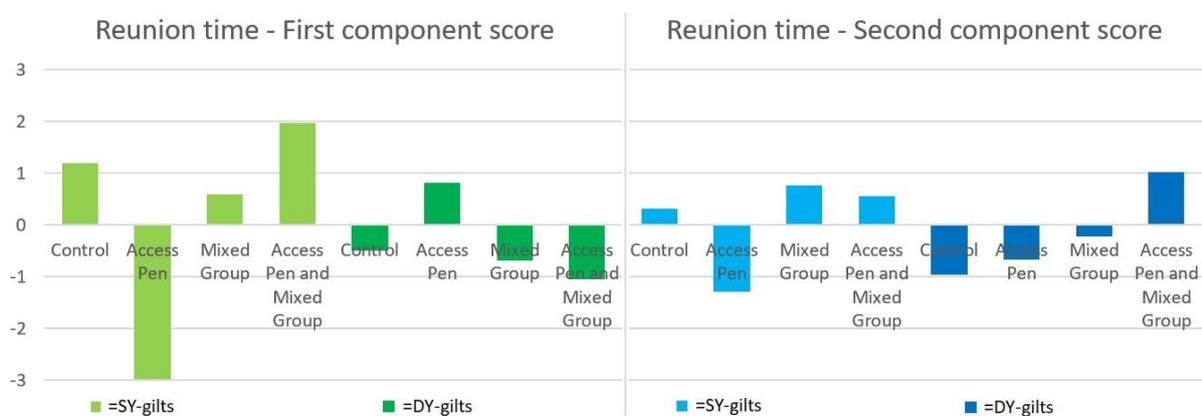


Figure 2 – Average PCA component scores for Swedish and Dutch Yorkshire gilts (SY-gilts and DY-gilts respectively) that were housed in either an access pen or a control pen as piglets and that were either mixed or not mixed after weaning. The gilt's piglets were separated from them for at least 11 minutes, the gilt's behaviour was scored during the three minutes after reunion with the piglets time and the behaviour scores were analysed with PCA. Three way ANOVA's with genotype, rearing environment and group mixing as independent variables including interaction effects revealed no significant differences in the PCA component scores.

4.3 Responsiveness and the occurrence of nursing

Nursing occurred within three minutes after reunion with the piglets in 23% ($N=14$) of the gilts, the remaining 77% ($N=46$) did not nurse within three minutes after reunion. Forty-seven percent of gilts was low responsive ($N=28$) and 53% ($N=32$) was high responsive towards her piglets during the three directly after reunion. Low responsiveness was associated with a high likelihood of nursing in a Chi-square test (Table 3; $\chi^2=11.19$, $P=0.001$, $df=1$, $N=60$).

Table 3 – The occurrence of nursing by low responsive and high responsive gilts upon return of their piglets after at least 11 minutes of separation. Gilts were divided into groups of low and high responsiveness towards their piglets after reunion and for each gilt it was noted whether or not she nursed her piglets within three minutes after reunion. Counts represent gilts and Chi-square residuals (all $> |2|$) are between brackets ($\chi^2=11.19$, $P=0.001$, $df=1$, $N=60$).

	<i>No nursing</i>		<i>Nursing</i>		<i>Total</i>
<i>Low responsiveness</i>	16	(-3.3)	12	(3.3)	28
<i>High responsiveness</i>	30	(3.3)	2	(-3.3)	32
<i>Total</i>	46		14		60

Chi-square tests were also used to test for associations between nursing and any of the three factors genotype, rearing environment and group mixing. As previously described, in the total sample 23% (N=14) of gilts nursed within three minutes of reunion with the piglets and 77% (N=46) did not. Eight out of 25 SY-gilts nursed (32%) versus six out of 35 DY-gilts (17%), but this difference was not significant ($\chi^2=1.799$, $P=0.180$, $df=1$, $N=60$). Ten out of 34 gilts that were housed in control pens nursed (29%) versus four out of 26 gilts that were housed in access pens (15%), but this difference was not significant ($\chi^2=1.621$, $P=0.203$, $df=1$, $N=60$). Seven out of 32 gilts that were kept in an intact group nursed (22%) versus seven out of 28 gilts that were group mixed (25%), but this difference was not significant ($\chi^2=0.082$, $P=0.775$, $df=1$, $N=60$).

Lastly, chi-square tests were used to test for associations between responsiveness and any of the three factors genotype, rearing environment and group mixing. As previously described, 47% (N=28) of gilts was low responsive and 53% (N=32) was high responsive. Fourteen out of 25 SY-gilts were high responsive (56%) versus 18 out of 35 DY-gilts (51%), but this difference was not significant ($\chi^2=1.122$, $P=0.726$, $df=1$, $N=60$). Seventeen out of 34 gilts that were housed in control pens were high responsive (50%) versus 15 out of 26 gilts that were housed in access pens (58%), but this difference was not significant ($\chi^2=0.350$, $P=0.554$, $df=1$, $N=60$). Eighteen out of 32 gilts that were kept in an intact group were high responsive (56%) versus 14 out of 28 gilts that were group mixed (50%), but this difference was not significant ($\chi^2=0.234$, $P=0.628$, $df=1$, $N=60$).

4.4 Behaviour during alone time and reunion time in relation to nursing and responsiveness

The binary responsiveness score was then used to compare the previously obtained PCA component scores during alone time and reunion time between gilts that were either low responsive or high responsive towards their piglets upon reunion. See Figure 3 for an overview of the PCA component scores during alone time and reunion time.

First three minutes of alone time

The first PCA component score did not differ significantly between low and high responsive gilts in an independent samples t-test, though there was a trend for low responsive gilts scoring higher than high responsive gilts ($P=0.069$, equal variances assumed; 0.83 ± 2.80 versus -0.73 ± 3.64). Low responsive gilts also scored higher on the second component score (0.61 ± 2.28 versus -0.53 ± 1.33) and this difference was significant in an independent samples t-test ($P=0.026$, equal variances not assumed). The first component score had the strongest positive loadings for relaxed, calm and content, and the strongest negative loadings for agitated, frustrated and distressed. The second component score had the strongest positive loadings for playful, positively occupied and inquisitive and the strongest negative loadings for indifferent, bored and apathetic. Low responsive gilts scoring higher on both component scores thus indicates a more positive emotional state in low responsive gilts during alone time.

Three minutes of reunion time

Both PCA component scores that represent the behaviour during reunion time differed significantly between low and high responsive gilts in independent samples t-tests (both $P < 0.001$, equal variances assumed). However, on the first component low responsive gilts scored higher (1.50 ± 2.14 versus -1.32 ± 3.22 in high responsive gilts), whereas on the second component score high responsive gilts scored higher (0.90 ± 1.42 versus -1.03 ± 2.17 in low responsive gilts). Similar to during alone time, the first component score had the strongest positive loadings for relaxed, calm and content, and the strongest negative loadings for agitated, irritable and distressed. This first component therefore indicates a more positive emotional state in low responsive gilts. However, the second component score of the reunion time had strong positive loadings for elements that are different from those that are represented in the second component score of the alone time. The second component score of the reunion time had the strongest positive loading for sociable and the strongest negative loadings for bored and apathetic. High responsive gilts are thus more sociable and less bored and apathetic during the first three minutes after reunion with their piglets.

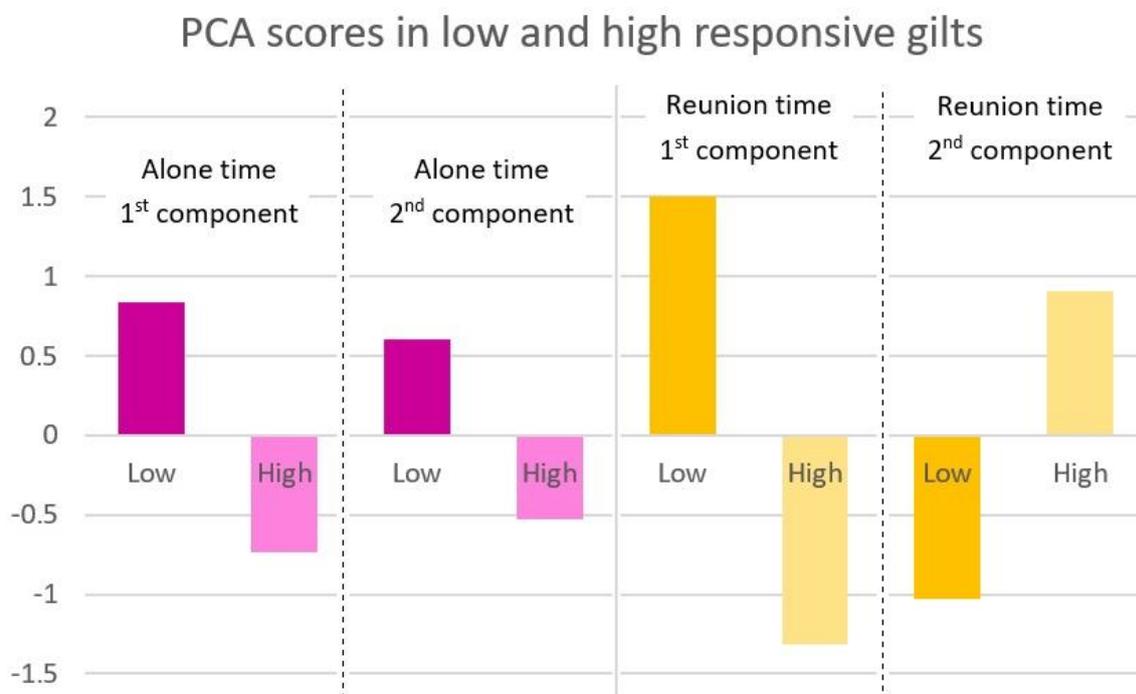


Figure 3 – Average PCA component scores for low and high responsive gilts. Alone time constitutes the first three minutes after gilts had their piglets separated from them and reunion time constitutes reunion with the piglets after at least 11 minutes of separation. Within a component score, low and high responsive gilts differed significantly from each other in independent samples t-tests, except for the first component of the alone time.

4.5 Piglet growth from birth to weaning

In the analyses of piglet growth from birth to weaning, gilts that adopted piglets from other gilts or had piglets adopted from them by other gilts were excluded ($N=20$). Forty gilts remained for analysis, of which 43% ($N=17$) were SY-gilts and 58% ($N=23$) were DY-gilts. Forty-eight percent ($N=19$) was kept in control pens and 53% ($N=21$) was kept in access pens. Fifty percent ($N=20$) was kept in intact groups and 50% ($N=20$) in mixed groups. All eight combinations of the three factors are present

in this subsample and the occurrence of each of the three factor combinations is presented in Table 4, together with the number of live born piglets, the number of weaned piglets and the average piglet growth for each of the treatment factor combinations.

Table 4 – The occurrence of each of the three factor combinations of genotype, rearing environment and group mixing for 40 gilts that did not adopt piglets from other gilts and did not have other gilts adopt piglets from them, together with the number of live born piglets, the number of weaned piglets and the average piglet growth for each of the treatment factor combinations.

<i>Genotype</i>	<i>Rearing environment</i>	<i>Group mixing</i>	<i>Number of gilts</i>	<i>Number of live born piglets (mean±Std)</i>	<i>Number weaned (mean±Std)</i>	<i>Average piglet growth in kg (mean±Std)</i>
SY-gilt	Control pen	Intact group	5	13.40±2.30	10.60±1.67	9.83±2.34
DY-gilt	Control pen	Intact group	4	13.50±1.73	11.00±3.56	10.18±1.56
SY-gilt	Access pen	Intact group	2	13.50±3.54	12.00±4.24	11.07±3.78
SY-gilt	Control pen	Mixed group	7	14.14±1.95	12.14±1.95	9.51±2.40
SY-gilt	Access pen	Mixed group	3	14.00±1.73	11.67±3.22	7.68±2.67
DY-gilt	Control pen	Mixed group	3	16.67±4.62	11.00±1.73	12.66±2.69
DY-gilt	Access pen	Intact group	9	14.33±1.58	12.00±1.94	9.84±1.67
DY-gilt	Access pen	Mixed group	7	15.14±2.41	11.86±2.55	8.69±1.99

The mean number of piglets born (stillborn or alive) per gilt was 14.98 (N=40, range 11-23, Std 2.34) and the number of live born piglets per gilt was 14.34 (N=40, range 11-22, Std 2.26). Piglet mortality (culled or found dead in pen) before weaning and weighing at approximately five weeks of age was on 2.73 piglets per gilt (N=40, range 0-9, Std 2.10). The piglet mortality was influenced by litter size (P=0.002), with a higher number of live born piglets being associated with higher piglet mortality. Piglet mortality was not associated with any of the three treatment factors or their interactions (P-values 0.229 to 0.712). These findings indicate that piglet mortality depends on the number of live born piglets, rather than on any of the treatment factors. It was therefore concluded that it would be reasonable to disregard piglet mortality and instead use the number of weaned (and thus weighed) piglets and their exact age in days as covariates in a second ANCOVA to determine the weight gain of the piglets that survived until weaning.

In this second ANCOVA the mean weight gain per piglet for each gilt (mean weight gain 9.72 kilogram, range 4.59-15.19, Std 2.29) was influenced by the covariates weaning age (P<0.001; mean 34 days, range 19-40, Std 4.69) and the number of piglets at weaning (P=0.003; mean 11.63 piglets, range 6-15, Std 2.25). A higher average weight gain associated with a higher weighing age and lower number of piglets. The weight gain was not significantly affected by genotype (P=0.163), rearing environment (P=0.248) or group mixing (P=0.900), nor by any of their interaction effects (P-values two-way interactions 0.097 to 0.974 and three-way interaction 0.950). There was a trend for the interaction between genotype and rearing environment (P=0.097). For SY-gilts the mean weight gain per piglet was 9.64±2.27 kilogram for gilts that were kept in control pens and 9.03±3.26 kilogram for gilts that were kept in access pens. For DY-gilts the mean weight gain per piglet was 11.24±2.32 kilogram for gilts that were kept in control pens and 9.34±1.85 kilogram for gilts that were kept in access pens.

In a third and fourth ANCOVA the occurrence of nursing and the binary responsiveness score were used as independent variables to test their influence on the mean weight gain per piglet. The

occurrence of nursing was not significant ($P=0.975$), but both of the covariates were (number of piglets at weaning $P=0.004$ and weaning age $P<0.001$). The responsiveness also did not significantly affect piglet growth ($P=0.105$), but both covariates did (number of piglets at weaning $P=0.002$ and weaning age $P<0.001$). Though not significant ($P=0.105$), piglets from low responsive gilts had a slightly higher mean weight gain (10.43 ± 2.70) than piglets from high responsive gilts (9.20 ± 1.82).

5 Discussion

Maternal behaviour in gilts is linked to social behaviour in multiple ways, and therefore improving gilts' social behaviour may improve their maternal behaviour. We studied the effect of three treatment factors that are expected to improve social skills (and thus social behaviour) on maternal behaviour. These factors were genotype, early social rearing environment (access to another litter or control) and group mixing after weaning. We scored the behaviour of the gilts while their first litter of piglets was briefly separated from them and upon their reunion. Surprisingly, these behaviour scores were unrelated to any of our treatment factors of genotype, rearing environment and group mixing. Even though there were no significant findings, there were two trends in one of the component scores of the gilt's behaviour during the three minutes after reunion with the piglets. The first indicated group mixed gilts to be slightly more sociable and less apathetic and bored than gilts that were kept in groups with sisters only after weaning ($P=0.095$). Some positive effect of group mixing on maternal behaviour may thus exist, since 'sociable' in this context is based on sociability between the gilt and her piglets. The second trend was an interaction between genotype and rearing environment ($P=0.065$). This interaction indicated these higher scores for sociable and lower scores for apathetic and bored in Dutch Yorkshire gilts (DY-gilts) that were housed in access pens when they were piglets, but in Swedish Yorkshire gilts (SY-gilts) that were housed in control pens. Interestingly, there was also a trend for an interaction between genotype and rearing environment that was not directly related to the gilts' behaviour, but regarded the average growth of their piglets ($P=0.097$). The average weight gain per piglet was 20.3% higher in DY-gilts that were housed in control pens versus those that were housed in access pens, and only 6.8% higher in SY-gilts that were housed in control pens versus those that were housed in access pens. SY-gilts and DY-gilts may be affected differently by the other treatment factors of rearing environment and group mixing, due to the SY-gilts naturally having better social skills and thus not 'needing' the extra training of social skills as much as DY-gilts. However, the two trends indicating that the rearing environment with additional access to another litter affects the behaviour of SY-gilts and the piglet growth of both genotypes negatively is unexpected and can presently not be explained. In addition to the treatment factors of genotype, rearing environment and group mixing not significantly affecting gilt behaviour during separation from and after reunion with their piglets, the treatment factors also did not affect whether or not the gilts nursed their piglets within three minutes after reunion, nor their responsiveness towards their piglets during these three minutes.

Perhaps the lack of effect of the treatment factors on the studied aspects of maternal behaviour is due to the gilts generally feeling relaxed in the loose housed farrowing pen with straw bedding. The use of straw is generally considered to improve animal welfare (Tuytens, 2005), and so is loose housed farrowing in comparison to crated farrowing (Andersen et al., 2014). Such circumstances may have caused all gilts to experience good welfare and low stress levels, regardless of their previous exposure to the treatments. The treatments may, in other words, not have significantly decreased stress levels, because stress levels were already low. Another reason for possibly low stress levels is that the gilts were kept with other gilts only during gestation and not mixed into sow groups until after their first farrowing. This prevents stress that is normally seen in dynamic gilt and sow groups, of which the composition often changes. The introduction of (an) unfamiliar animal(s) into an existing group causes aggression towards the unfamiliar animal(s), but also between familiar animals of the existing group (Mount and Seabrook, 1993). Li et al. (2012) found gilts in gilt-

only groups to gain more weight and sustain fewer injuries than gilts that were mixed into sow groups. Ison et al. (2014) also found that gilts sustained fewer injuries in gilt-only groups and that the salivary cortisol levels of these gilts were lower than those of gilts in sow groups. The effects of the treatments on social behaviour possibly do not come into play until the gilts are mixed into sow groups after their first farrowing. If that is the case, any possible effects on maternal behaviour cannot be seen until the second farrowing or later, thus outside the scope of this thesis research.

Unfortunately, it is at this point unknown whether or not the treatment factors had any effect on social behaviour. The research project is ongoing at the Swedish University of Agricultural Sciences and analyses on adult social behaviour have not yet been performed. If no effect of the treatment factors on social behaviour is found after further analysis, the same reasoning can be followed of all gilts experiencing good welfare and low stress levels regardless of previous exposure to the treatments, due to adequate housing and management. If an effect of the treatment factors on social behaviour is found, there are two possible explanations for the lack of effect on maternal behaviour. Either there is no link between social behaviour and maternal behaviour, or we did not detect the effect on maternal behaviour. It seems unlikely that there is no link between social behaviour and maternal behaviour. Improved social behaviour likely decreases stress and stress affects maternal behaviour, like bonding with offspring and lactation, negatively (Leng et al., 2008). Furthermore, stress in gestating mother sows or gilts reduces the stress resistance of the offspring (Jarvis et al., 2006), thus creating a vicious circle.

If an effect of the treatment factors on social behaviour is found, it is therefore likely that we did not detect the effect on maternal behaviour, for which there are multiple possible explanations. As described earlier, possible effects on maternal behaviour may not be seen until the second farrowing. To detect any treatment effect on maternal behaviour, we may need to study the gilts' behaviour during the second or third farrowing and lactation period. Furthermore, the method of behavioural analysis might have to be adjusted in future research. The separation from the piglets started with a staff member from the research facility entering the gilt pen, which rarely happens and might not have happened at all since the birth of the piglets. The event of a staff member entering the pen may elicit changes in behaviour, that are unrelated to the removal of and consequent separation from the piglets. Alternative methods could include long-term observation of the gilt and her piglets being undisturbed. Behaviours to be scored and analysed could include social interactions between the gilt and her piglets. Other data to be included could be causes of piglet mortality and the gilt's weight loss corrected for food intake. In overly stressed gilts, the weight loss might not be entirely reflected in piglet growth. Another explanation for not detecting treatment effects on maternal behaviour may be that complications in standardizing the circumstances and procedure around the separation likely increased the difficulty of finding significant differences in behaviour. Standardization renders animals within an experiment more homogenous by equalizing circumstances and procedures, which makes it easier to compare them and to detect relevant differences (Van Zutphen et al., 2001). There was much variation in for instance piglet age at the moment of the iron injection, collection time and age at weaning and weighing. Furthermore, gilts were often disturbed by normal husbandry activities in the stables and by piglet distress calls from the treatment station or from neighbouring pens, which may have influenced their behaviour. Even though variation and disturbance occurred randomly, these factors likely make it much harder to detect significant differences and are therefore a point of attention for future research.

The present research showed no significant effects of our treatment factors of genotype, early social rearing environment and group mixing, but there were significant findings that were unrelated to these treatment factors. Gilts that nursed their piglets within three minutes after being reunited with them showed a higher responsiveness towards their piglets during these three minutes ($P=0.001$). Furthermore, gilts with low and high responsiveness showed significant differences in their PCA component scores. During the three minutes of reunion time, low responsive gilts scored higher on the first and second PCA component (both $P<0.001$). A higher score on the first component indicates a more positive emotional state, as it had the strongest positive loadings for relaxed, calm and content, and the strongest negative loadings for agitated, irritable and distressed. The second component score has the strongest positive loading for sociable and the strongest negative loadings for bored and apathetic. High responsive gilts are thus more sociable and less bored and apathetic during the first three minutes after reunion with their piglets, which is a logical finding. The finding of high responsive gilts being, among other things, more distressed raises questions on what constitutes good maternal behaviour. High responsiveness, regarding for instance the gilt or sow responding to piglet distress calls and actively seeking contact with piglets, is often seen as good maternal behaviour. Piglet distress calls are for instance heard when the gilt or sow traps a piglet under her when she lies down. Some gilts and sows are very responsive and will get up in response to these calls, whereas others do not react at all. Expectedly, high responsiveness to piglet distress calls was found to associate with fewer trapped piglets being crushed (Wechsler and Hegglin, 1997). However, we found the likelihood of a gilt nursing her piglets within three minutes after reunion to be lower in high responsive gilts, and more importantly, these gilts were more distressed during these first three minutes after reunion. The link between gilts being stressed and them not immediately nursing is expected. Even though the lactation period attenuates the stress response in mammals (Stern et al., 1973; Higuchi et al., 1989; Lightman and Young 1989; Lightman 1992), lactation is affected negatively when the mother does experience stress (Leng et al., 2008). We measured an acute effect of a stressor causing high responsive gilts to postpone nursing, but these gilts could generally be stressed more easily throughout the whole lactation period. If this is the case, these gilts may nurse their piglets less often or provide less milk per nursing, which would result in reduced piglet growth. Furthermore, through emotional contagion (Reimert et al., 2013), piglets may get stressed due to their mother being stressed. This could also contribute to reduced piglet growth and the negative emotional state would have welfare implications. Though inconclusive and not significant, two arguments for high responsive gilts possibly being more stressed in general can be presented based on current data. The first is that the average weight gain of piglets from low responsive gilts was 13% higher than that of piglets from high responsive gilts ($P=0.105$). The second is that high responsive gilts scored lower on the first PCA component score that represented their behaviour during the first three minutes of alone time (after separation from their piglets; $P=0.069$). This score had a strong negative loading for distressed and other elements that reflect a negative emotional state. High responsive gilts are therefore more distressed not only during reunion time, but also slightly more distressed already during alone time.

6 Conclusion

No conclusions about our treatment factors affecting maternal behaviour can presently be drawn, due to a lack of significant findings. Perhaps present trends may become more meaningful after adding the analyses of the gilts in the batches that were not included in this thesis research. Otherwise, the lack of effect of the treatment factors could be due to analysis of the first farrowing instead of a later farrowing, disturbances during video recording that decreased standardization, or all gilts experiencing good welfare regardless of treatment factors. This study does raise questions on what constitutes good maternal behaviour. High responsiveness is often seen as good maternal behaviour, though the stress that these gilts experience at separation from their piglets and possibly even throughout the whole lactation may affect their maternal behaviour negatively.

References

- Ahlstrom S., Jarvis S., Lawrence A.B. (2002). Savaging gilts are more restless and more responsive to piglets during the expulsive phase of parturition. *Applied Animal Behaviour Science*, Volume 76, Pages 83-91.
- Andersen I.L., Bøe K.E. (1999a). Straw bedding or concrete floor for loose-housed pregnant sows: consequences for aggression, production and physical health. *Acta Agriculturae Scandinavica*, Volume 49, Issue 3, Pages 190-195.
- Andersen I.L., Bøe K.E., Kristiansen A.L. (1999b). The influence of different feeding arrangements and food competition at feeding in pregnant sows. *Applied Animal Behaviour Science*, Volume 65, Pages 91-104.
- Andersen I.L., Vasdal G., Pedersen L.J. (2014). Nest building and posture changes and activity budget of gilts housed in pens and crates. *Applied Animal Behaviour Science*, Volume 159, Pages 29-33.
- Andrews M.H., Matthews S.G. (2004). Programming of the hypothalamo–pituitary–adrenal axis: serotonergic involvement. *Stress*, Volume 7, Pages 15-27.
- Anisman H., Zaharia M.D., Meaney M.J., Merali Z. (1998). Do early-life events permanently alter behavioral and hormonal responses to stressors? *International Journal of Developmental Neuroscience*, Volume 16, Pages 149-164.
- Anil L., Sukumarannair S.A., Deen J., Baidoo S.K., Wheaton J.E. (2005). Evaluation of well-being, productivity, and longevity of pregnant sows housed in groups in pens with an electronic sow feeder or separately in gestation stalls. *American Journal of Veterinary Research*, Volume 66, Pages 1630-1638.
- Arey D.S., Petchey A.M., Fowler V.R. (1991). The preparturient behaviour of sows in enriched pens and the effect of pre-formed nests. *Applied Animal Behaviour Science*, Volume 31, Pages 61-68.
- Arey D.S., Edwards S.A. (1998). Factors influencing aggression between sows after mixing and the consequences for welfare and production. *Livestock Production Science*, Volume 56, Pages 61-70.
- Arey D.S. (1999). Time course for the formation and disruption of social organization in group-housed sows. *Applied Animal Behaviour Science*, Volume 62, Issue 2, Pages 199-207.
- Beattie V., Walker N., Sneddon I. (1996). An investigation of the effect of environmental enrichment and space allowance on the behaviour and production of growing pigs. *Applied Animal Behaviour Science*, Volume 48, Pages 151-158.
- Beattie V., O'Connell N., Moss B. (2000). Influence of environmental enrichment on the behaviour, performance and meat quality of domestic pigs. *Livestock Production Science*, Volume 65, Pages 71-79.
- Blackshaw J.K., Blackshaw A.W., Thomas F.J., Newman F.W. (1994). Comparison of behaviour patterns of sows and litters in a farrowing crate and a farrowing pen. *Applied Animal Behaviour Science*, Volume 39, Issues 3-4, Pages 281-295.

- Blackshaw J.K., Thomas F.J., Lee J-A. (1997). The effect of a fixed or freetoy on the growth rate and aggressive behaviour of weaned pigs and the influence of hierarchy on initial investigation of the toys. *Applied Animal Behaviour Science*, Volume 53, Pages 203-212.
- Bohnenkamp A.L., Traulsen I., Meyer C., Müller K., Krieter J. (2013). Comparison of growth performance and agonistic interaction in weaned piglets of different weight classes from farrowing systems with group or single housing. *Animal*, Volume 7, Pages 309-315.
- Bolhuis E.J., Schouten W.G., Schrama J.W., Wiegant V.M. (2005). Individual coping characteristics, aggressiveness and fighting strategies in pigs. *Animal Behaviour*, Volume 69, Pages 1085-1091.
- Broom D.M., Mendl M.T., Zanella A.J. (1995). A comparison of the welfare of sows in different housing conditions. *Animal Science*, Volume 61, Pages 369-385.
- Byers J.A. (1977). Terrain preferences in the play behavior of Siberian ibex kids (*Capra ibex sibirica*). *Zeitschrift für Tierpsychologie*, Volume 45, Pages 199-209
- D'Eath R.B. (2005). Socialising piglets before weaning improves social hierarchy formation when pigs are mixed post-weaning. *Applied Animal Behaviour Science*, Volume 93, Pages 199-211.
- De Kloet E.R., Vreugdenhil E., Oitzl M.S., Joels M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocrine Reviews*, Volume 19, Pages 269-301.
- Durrell J.L., Beattie V.E., Sneddon I.A., Kilpatrick D. (2003). Pre-mixing as a technique for facilitating subgroup formation and reducing sow aggression in large dynamic groups. *Applied Animal Behaviour Science*, Volume 84, Pages 89-99.
- Edwards S.A., Mauchline S., Marston G.C., Stewart A.H. (1994). Agonistic behaviour amongst newly mixed sows and the effects of pen design and feeding method. *Applied Animal Behaviour Science*, Volume 41, Issues 3-4, Page 272
- Einarsson S., Sjunnesson Y., Hultén F., Eliasson-Selling L., Dalin A-M., Lundeheim N., Magnusson U. (2014). A 25 years experience of group-housed sows—reproduction in animal welfare-friendly systems. *Acta veterinaria scandinavica*, 56:37.
- Elmore M.R.P., Garner J.P., Johnson A.K., Kirkden R.D., Richert B.T., Pajor E.A. (2011). Getting around social status: motivation and enrichment use of dominant and subordinate sows in a group setting. *Applied Animal Behaviour Science*, Volume 133, Pages 154-163.
- Engblom L., Lundeheim N., Dalin A-M., Andersson K. (2007). Sow removal in Swedish commercial herds. *Livestock Science*, Volume 106, Pages 76-86.
- Fagen R. (1981). *Animal Play Behavior*. Oxford University Press, New York, NY, pp. 3-69.
- Fleming P.A., Clarke T., Wickham S.L., Stockman C.A., Barnes A.L., Collins T., Miller D.W. (2016). The contribution of qualitative behavioural assessment to appraisal of livestock welfare. *Animal Production Science*, Volume 56, Issue 10, Pages 1569-1578.
- Folley S.J., Knaggs G.S. (1966). Milk-ejection activity (oxytocin) in the external jugular vein blood of the cow, goat and sow, in relation to the stimulus of milking or suckling. *Journal of Endocrinology*, Volume 34, Pages 197-214.
- Graves H. (1984). Behavior and ecology of wild and feral swine (*Sus scrofa*). *Journal of Animal Science*, Volume 58, Pages 482-492.

- Greenwood E.C., Plush K.J., Van Wettere W.H.E.J., Hughes P.E. (2014). Hierarchy formation in newly mixed, group housed sows and management strategies aimed at reducing its impact. *Applied Animal Behaviour Science*, Volume 160, Pages 1-11.
- Hansen C.F., Hales J., Weber P.M., Edwards S.A., Moustsen V.A. (2017). Confinement of sows 24 h before expected farrowing affects the performance of nest building behaviours but not progress of parturition. *Applied Animal Behaviour Science*, Volume 188, Pages 1-8.
- Hennessy D.P., Williamson P. (1983). The effects of stress and of ACTH administration in hormone profiles, oestrus and ovulation in pigs. *Theriogenology*, Volume 20, Issue 1, Pages 13-26.
- Higuchi T., Negoro H., Arita J. (1989). Reduced response of prolactin and catecholamine to stress in the lactating rat. *Journal of Endocrinology*, Volume 122, Pages 495-498.
- Hillmann E., Von Hollen F., Bünger B., Todt D., Schrader L. (2003). Farrowing conditions affect the reactions of piglets towards novel environment and social confrontation at weaning. *Applied Animal Behaviour Science*, Volume 81, Pages 99-109.
- Hodgkiss N.J., Eddison J.C., Brooks P.H., Bugg P. (1998). Assessment of the injuries sustained by pregnant sows housed in groups using electronic feeders. *Veterinary Record*, Volume 143, Pages 604-607.
- Hoy S., Bauer J., Borberg C., Chonsch L., Weirich C. (2009). Investigations on dynamics of social rank of sows during several parities. *Applied Animal Behaviour Science*, Volume 121, Pages 103-107.
- Hutson G.D. (1992). A comparison of operant responding by farrowing sows for food and nest-building materials. *Applied Animal Behaviour Science*, Volume 34, Issue 3, Pages 221-230.
- Ison S., Donald R.D., Jarvis S., Robson S., Lawrence A., Rutherford K. (2014). Behavioral and physiological responses of primiparous sows to mixing with older, unfamiliar sows. *Journal of Animal Science*, Volume 92, Pages 1647-1655.
- Jarvis S., Van der Vegt B.J., Lawrence A.B., McLean K.A., Deans L.A., Chirnside J., Calvert S.K. (2001). The effect of parity and environmental restriction on behavioural and physiological responses of pre-parturient pigs. *Applied Animal Behaviour Science*, Volume 71, Pages 203-216.
- Jarvis S., Reed B.T., Lawrence A.B., Calvert S.K., Stevenson J. (2004). Peri-natal environmental effects on maternal behaviour, pituitary and adrenal activation, and the progress of parturition in the primiparous sow. *Animal Welfare*, Volume 13, Issue 2, Pages 171-181.
- Jarvis S., Moinard C., Robson S.K., Baxter E., Ormandy E., Douglas A.J., Seckl J.R., Russell J.A., Lawrence A.B. (2006). Programming the offspring of the pig by prenatal social stress: Neuroendocrine activity and behaviour. *Hormones and Behavior*, Volume 49, Pages 68-80.
- Jensen P. (1986). Observations on the maternal behaviour of free-ranging domestic pigs. *Applied Animal Behaviour Science*, Volume 16, Pages 131-142.
- Jensen K.H., Sørensen L.S., Bertelsen D., Pedersen A.R., Jørgensen E., Nielsen N., Vestergaard K. (2000). Management factors affecting activity and aggression in dynamic group-housing systems with electronic sow feeding: a field trial. *Animal Science* Volume 71, Pages 535-545.
- Johnson R.W., Vonborell E.H., Anderson L.L., Kojic L.D., Cunnick J.E. (1994). Intracerebroventricular injection of corticotropin-releasing hormone in the pig - Acute effects

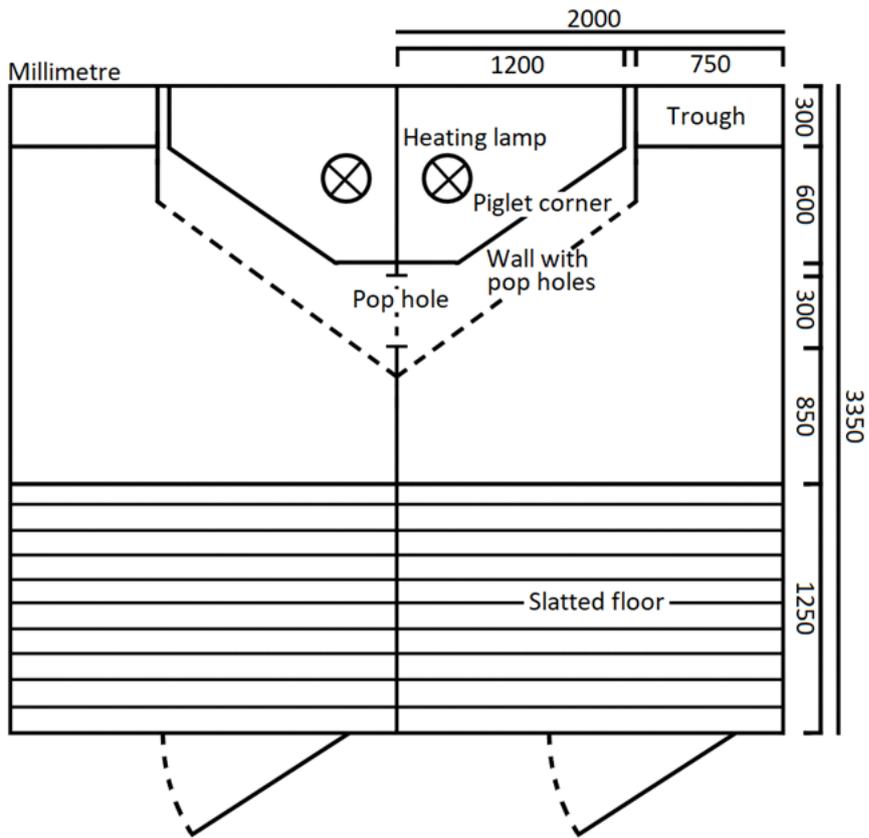
on behavior, adrenocorticotropin secretion, and immune suppression. *Endocrinology*, Volume 135, Pages 642-648.

- Kennedy M.J., Broom D.M. (1994). A method of mixing gilts and sows which reduces aggression experienced by gilts. *Proceedings of the 45th Annual Meeting of the EAAP*, 5-8 September 1994, Page 333.
- Kim K.H., Hosseindoust A., Ingale S.L., Lee S.H., Noh H.S., Choi Y.H., Jeon S.M., Kim Y.H., Chae B.J. (2016). Effects of Gestational Housing on Reproductive Performance and Behavior of Sows with Different Backfat Thickness.
- Kirkwood R., Zanella A. (2005). Title: Influence of Gestation Housing on Sow Welfare and Productivity.
- Kongsted A.G. (2004). Stress and fear as possible mediators of reproduction problems in group housed sows: a review. *Acta Agriculturae Scandinavica, Section A: Animal Science* 54, Pages 58-66.
- Lawrence A.B., Illius A.W. (1989). Methodology for measuring hunger and food needs using operant conditioning in the pig. *Applied Animal Behaviour Science*, Volume 24, Pages 273-285.
- Leng G., Meddle S.L., Douglas A.J. (2008). Oxytocin and the maternal brain. *Current Opinion in Pharmacology*, Volume 8, Issue 6, Pages 731-734.
- Li Y., Wang L. (2011). Effects of previous housing system on agonistic behaviors of growing pigs at mixing. *Applied Animal Behaviour Science*, Volume 132, Pages 20-26.
- Li Y.Z., Wang L.H., Johnston L.J. (2012). Sorting by parity to reduce aggression toward first-parity sows in group-gestation housing systems. *Journal of Animal Science*, Volume 90, Pages 4514-4522.
- Lightman S.L. (1992). Alternations in hypothalamic-pituitary responsiveness during lactation. *Annals of the New York Academy of Sciences*, Volume 652, Pages 340-346.
- Lightman S.L., Young W.S. (1989). Lactation inhibits stress-mediated secretion of corticosterone and oxytocin and hypothalamic accumulation of corticotropin releasing factor and enkephalin messenger ribonucleic acid. *Endocrinology*, Volume 124, Pages 2358-2364.
- Liptrap R.M. (1973). Oestrogen Excretion by Sows with Induced Cystic Ovarian Follicles. *Research in Veterinary Science*, Volume 15, Issue 2, Pages 215-219.
- Løvendahl P., Damgaard L.H., Nielsen B.L., Thodberg K., Su G., Rydhmer L. (2005). Aggressive behaviour of sows at mixing and maternal behaviour are heritable and genetically correlated traits. *Livestock Production Science*, Volume 93, Issue 1, Pages 73-85.
- Marchant J.N., Broom D.M. (1996). Effects of dry sow housing conditions on muscle weight and bone strength. *Animal Science*, Volume 62, Pages 105-113.
- Marchant-Forde J.N., (2009). Chapter 1 Introduction to the Welfare of Pigs in *The Welfare of Pigs*. Springer, Page 9.
- McGlone J.J. (1986). Agonistic Behavior in Food Animals: Review of Research and Techniques. *Journal of Animal Science*, Volume 62, Issue 4, Pages 1130-1139.
- McGlone J.J., Von Borell E.H., Deen J., Johnson A.K., Levis D.G., Meunier-Salaun M., Morrow J., Reeves D., Salak-Johnson J.L., Sundberg P.L. (2004). Review: Compilation of the Scientific Literature Comparing Housing Systems for Gestating Sows and Gilts Using Measures of Physiology, Behavior, Performance, and Health. *Professional Animal Scientist*, Volume 20, Pages 105-107.

- McGlone J.J. (2013). Review: Updated scientific evidence on the welfare of gestating sows kept in different housing systems. *Applied Animal Science*, Volume 29, Issue 3, Pages 189-198.
- Meaney M.J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience*, Volume 24, Pages 1161-1192.
- Meese G.B., Ewbank R. (1973). The establishment and nature of the dominance hierarchy in the domesticated pig. *Animal behaviour*, Volume 21, Pages 326-334.
- Mendl M., Zanella A.J., Broom D.M. (1992). Physiological and reproductive correlates of behavioural strategies in female domestic pigs. *Animal Behaviour*, Volume 44, Pages 1107-1121.
- Mount N.C., Seabrook M.F. (1993). A study of aggression when group housed sows are mixed. *Applied Animal Behaviour Science*, Volume 36, Pages 377-383.
- Newberry R.C. (1995). Environmental enrichment: increasing the biological relevance of captive environments. *Applied Animal Behaviour Science*, Volume 44, Pages 229-243.
- Oliveira A.F.S., Rossi A.O., Silva L.F.R., Lau M.C., Barreto R.E. (2009). Play behaviour in nonhuman animals and the animal welfare issue. *Journal of Ethology*, Volume 28, Pages 1-5.
- Oliviero C., Heinonen M., Valros A., Hälli O., Peltoniemi O.A.T. (2008). Effect of the environment on the physiology of the sow during late pregnancy, farrowing and early lactation. *Animal Reproduction Science*, Volume 105, Pages 365-377.
- Olsen A., Simonsen H., Dybkjaer L. (2002). Effect of access to rough age and shelter on selected behavioural indicators of welfare in pigs housed in a complex environment. *Animal Welfare*, Volume 11, Pages 75-87.
- Oostindjer M., Van den Brand H., Kemp B. and Bolhuis J.E. (2011). Effects of environmental enrichment and loose housing of lactating sows on piglet behaviour before and after weaning. *Applied Animal Behaviour Science*, Volume 134, Pages 31-41.
- Pajor E.A., Weary D.M., Fraser D., Kramer D.L. (1999). Alternative housing for sows and litters 1. Effects of sow-controlled housing on responses to weaning. *Applied Animal Behaviour Science*, Volume 65, Pages 105-121.
- Petersen H.V., Vestergaard K., Jensen P. (1989). Integration of Piglets into Social Groups of FreeRanging Domestic Pigs. *Applied Animal Behaviour Science*, Volume 23, Pages 223-236.
- Reimert I., J.E. Bolhuis, Kemp B., Rodenburg B. (2013). Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & Behavior*, Volume 109, Pages 42-50.
- Schaefer A., Salomons M., Tong A., Sather A., Lepage P. (1990). The effect of environment enrichment on aggression in newly weaned pigs. *Applied Animal Behaviour Science*, Volume 27, Pages 41-52.
- Spoolder H.A.M., Burbidge J.A., Lawrence A.B., Simmins P.H., Edwards S.A. (1996). Individual behavioural differences in pigs: intra and inter-test consistency. *Applied Animal Behaviour Science*, Volume 49, Pages 185-198.
- Spoolder H.A.M., Geudeke M.J., Van der Peet-Schwering C.M.C., Soede N.M. (2009). Group housing of sows in early pregnancy: A review of success and risk factors. *Livestock Science* Volume 125, Pages 1-14.
- Stern J.M., Goldman L., Levine S. (1973). Pituitary-adrenal responsiveness during lactation in rats. *Neuroendocrinology*, Volume 12, Pages 179-191.

- Thompson K.V. (1998). Self assessment in juvenile play. In: Bekoff M., Byers J.A. (Eds.), *Animal Play: Evolutionary, Comparative, and Ecological Perspectives*. Cambridge University Press, Cambridge, MA, pp. 183-204.
- Turner S.P, Ewen M., Rooke J.A., Edwards S.A. (2000a). The effect of space allowance on performance, aggression and immune competence of growing pigs housed on straw deep-litter at different group sizes. *Livestock Production Science*, Volume 66, Issue 1, Pages 47-55.
- Turner S.P., Edwards S.A. (2000b). Housing in large groups reduces aggressiveness of growing pigs. *Proceedings of the 51st Annual Meeting of the European Association for Animal Production*, 21–24 August 2000, The Hague, The Netherlands.
- Turner S.P., Horgan G.W., Edwards S.A. (2001). Effect of social group size on aggressive behaviour between unacquainted domestic pigs. *Applied Animal Behaviour Science* Volume 74, Pages 203-215.
- Tuytens F.A.M. (2005). The importance of straw for pig and cattle welfare:a review. *Applied Animal Behaviour Science*, Volume 92, Pages 261-282.
- Van Putten G., Buré R.G. (1997). Preparing gilts for group housing by increasing their social skills. *Applied Animal Behaviour Science*, Volume 54, Pages 173-183.
- Weber R., Keil N.M., Fehr M., Horat R. (2007). Piglet mortality on farms using farrowing systems with or without crates. *Animal Welfare*, Volume 16, Pages 277-279.
- Wechsler B., Hegglin D. (1997). Individual differences in the behaviour of sows at the nest-site and the crushing of piglets. *Applied Animal Behaviour Science*, Volume 51, Pages 39-49.
- Weinstock M. (2001). Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Progress in Neurobiology*, Volume 65, Pages 427-451.
- Welberg L.A.M., Seckl J.R. (2001). Prenatal stress, glucocorticoids and the programming of the brain. *Journal of Neuroendocrinology*, Volume 13, Pages 113-128.
- Weng R.C., Edwards S.A., English P.R. (1998). Behaviour, social interactions and lesion scores of group-housed sows in relation to floor space allowance. *Applied Animal Behaviour Science*, Volume 59, Pages 307-316.
- Wischner D., Kemper N., Krieter J. (2009). Nest-building behaviour in sows and consequences for pig husbandry. *Livestock Science*, Volume 124, Pages 1–8.
- Zurbrigg K., Blackwell T. (2005). Injuries, lameness, and cleanliness of sows in four group housing gestation facilities in Ontario. *Journal of Swine Health and Production*, Volume 14, Issue 4, Pages 202-206.
- Van Zutphen L.F., Baumans V., Beynen A.C. (2001). *Principles of Laboratory Animal Science*, Revised Edition. A contribution to the humane use and care of animals and to the quality of experimental results. Elsevier.

Appendix 1



Illustrated by Andersson (2019)

Appendix 2

Page: _____
Assessor: _____
Date: _____
Farm: _____

Qualitative Behaviour Assessment for sows, piglets and growing pigs

Please observe the animals in the unit for 10-20 minutes, and then assess their behavioural expression ('body language') by scoring the following terms:

Visual Analogue Scale VAS for Qualitative Behaviour Assessment (please be sure that the lines of the QBA measures are 125 mm)

Please observe the animals in the unit for 10-20 minutes, and then assess their behavioural expression ('body language') by scoring the following terms:

Active	Min. _____	Max. _____
Relaxed	Min. _____	Max. _____
Fearful	Min. _____	Max. _____
Agitated	Min. _____	Max. _____
Calm	Min. _____	Max. _____
Content	Min. _____	Max. _____
Indifferent	Min. _____	Max. _____
Frustrated	Min. _____	Max. _____
Friendly	Min. _____	Max. _____
Bored	Min. _____	Max. _____
Playful	Min. _____	Max. _____
Positively occupied	Min. _____	Max. _____
Lively	Min. _____	Max. _____
Inquisitive	Min. _____	Max. _____
Irritable	Min. _____	Max. _____

