LEARNING HOW TO EAT LIKE A PIG:

FACILITATING VERTICAL INFORMATION TRANSFER TO REDUCE WEANING PROBLEMS IN PIGLETS.

Marije Oostindjer

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Learning how to eat like a pig: facilitating vertical
information transfer to reduce weaning problems
in piglets.

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ABSTRACT

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Piglets in commercial husbandry face a challenge around weaning, because they are weaned abruptly and at a rather young age. Many weanling piglets are poorly adapted to ingest solid food, often resulting in a period of underfeeding leading to several health and welfare problems in the immediate postweaning period. The aim of this thesis was to explore whether providing piglets with more opportunities to learn from their mother about what, how and where to eat increases food intake before and after weaning and consequently can reduce health and welfare problems after weaning. Three ways of learning that could probably be improved in current pig husbandry were chosen: 1) direct learning from the sow through cues derived from observation and eating together, 2) learning in an enriched environment and 3) learning from flavour cues in the maternal diet. Piglets that were allowed to explore novel food items before weaning in the presence of the sow were less reluctant to explore and ingest these novel food items than in her absence. Being able to interact more and eat together with the sow also had positive effects on food intake before weaning and resulted in less damaging behaviour and more play behaviour after weaning. Piglets learn effectively through both observation of the sow and participation with the sow while she eats, and they use information from both the location and the type of food eaten. Enrichment of the environment before weaning positively affected growth and development of feeding-related behaviours before weaning and food intake after weaning. Providing enrichment after weaning increased growth and feed efficiency and decreased diarrhoea and stress-related behaviours. Allowing piglets to learn from flavours in the diet of the sow both before birth and during lactation and subsequently re-exposing piglets to this flavour at weaning increased their growth and food intake and reduced the incidence of diarrhoea and stress-related behaviours. These effects are the result of reduced weaning stress due to the presence of the familiar flavour in the post weaning environment, rather than the result of an increased intake of similarly flavoured food. In conclusion, information from the sow plays a critical role in the development of independent feeding. Improving opportunities for sow-piglet information transfer enhances the ability of piglets to adapt to the postweaning situation, which consequently reduces weaning-related problems.

CONTENTS

01

GENERAL INTRODUCTION

The challenge of weaning

Young animals face a major challenge in the period around weaning when they have to make the transition from drinking milk to eating solid food and have to start selecting appropriate food items to include in their diet. Some food items will be non-nutritious or even toxic and many animals show some level of food neophobia, i.e. a reluctance to try unfamiliar food items, to prevent harmful consequences of eating unfamiliar food types (Rozin and Vollmecke 1986). Trial-and-error learning can be used to learn what should be included in the diet (i.e. sampling every food item that is encountered), but it is not only dangerous, but time and energy consuming as well. It is therefore more efficient to use information from experienced conspecifics on what, where and how to eat (Galef and Giraldeau 2001; Nicol 2006). The mother can affect food neophobia and can provide information of particular use to her offspring, since piglets direct most of their attention to their mother. Furthermore, mother and offspring share a similar genetic and physiological background, and are thereby likely to have a similar physiological response to food items (Chesler 1969; Galloway et al. 2003; Provenza and Balph 1987; Thorhallsdottir et al. 1990). Using information from the mother on what food items to include in the diet may thus help the young animal to make the change from milk to a diet of solid food more easily.

Weaning the pig

Making the transition from milk to solid food easier is of particular importance for piglets in commercial pig husbandry. Piglets in commercial husbandry are generally weaned between 3 and 4 weeks of age for economic reasons. Piglets in the wild experience a more gradual weaning process that ends between week 14 and 17, resulting in piglets being fully adapted to eating and digesting solid food (Jensen and Stangel 1992). Piglets in husbandry conditions, in contrast, often have limited experience with solid food before weaning. As a result they often have a period of variable but generally low food intake immediately after weaning, accompanied by weight loss and a high occurrence of diarrhoea (Bolhuis et al. 2009; Brooks and Tsourgiannis 2003). The compromised growth and high occurrence of diarrhoea may lead to high costs for the farmer and high antibiotic use (Bruininx et al. 2001b).

To prevent the low postweaning food intake and high diarrhoea occurrence, piglets should start eating before weaning. Overall, there is a positive correlation between preweaning and postweaning food intake under commercial conditions (Berkeveld et al. 2007). Piglets classified as eaters preweaning have their first solid food intake postweaning faster than piglets classified as non-eaters, show higher food intakes postweaning and have higher body weights in the early postweaning period, and a higher absorption by the small intestine, which is negatively correlated with postweaning diarrhoea (Bruininx et al. 2002b; Kuller et al. 2007a; Kuller et al. 2007b).

The welfare of piglets is also compromised as they face many stressors in addition being malnourished and experiencing intestinal problems. The stressors around weaning under commercial conditions generally consist of maternal separation, relocation, a new physical environment and thus a new microbial environment. Piglets are typically mixed with unfamiliar piglets around weaning and thus also experience a new social environment, which often results in a period of some hours in which aggression is high (Blecha and Kelley 1981; D'Eath 2005; Weary et al. 2008). Besides high levels of aggression, newly weaned piglets also tend to show maladaptive behaviours such as belly nosing, mounting and manipulative behaviours such as chewing on another piglet's body. These behaviours are not only signs of high stress levels of the animal that performs the behaviour but can also compromise the welfare of the animal on which the behaviour is performed (Dudink et al. 2006; Dybkjær 1992).

High stress levels can also increase the incidence of diarrhoea (Rao et al. 1998; Taché et al. 1999). Weaning diarrhoea is a multi-factorial problem. High levels of stress may reduce the motivation of piglets to eat solid food after weaning, and this period of fasting can result in malabsorption and consequently diarrhoea when piglets start to eat again. The malabsorption is caused by a shortening of villi in the small intestine, thereby slowing the maturation of the gut, as well as reduced enzyme activity (Ball and Aherne 1982; Boudry et al. 2004; Hampson 1986; McCracken et al. 1999; Smith et al. 2010). Weaning can also result in inflammation and increased permeability of the gut, which in turn increases the chance of toxins and antigens entering other tissues (Moeser et al. 2007b; Spreeuwenberg et al. 2001). Another part of the problem is that the gut becomes more sensitive to bacteria (such as some strains of Escherichia Coli) which can also result in diarrhoea (Pluske et al. 1997). High stress levels can also directly affect the gastro-intestinal tract by changing motility and gastric emptying, again increasing the occurrence of diarrhoea (Rao et al. 1998). In order to prevent the physiological and behavioural problems associated with weaning and increase piglet health and welfare it is important to increase solid food intake both before and after weaning, as well as reduce stress around weaning.

Solutions for weaning problems?

Solutions to increase food intake of piglets before and after weaning have mostly focused on optimizing food and feeders for piglets. Feed companies provide piglet food that is optimized in texture, composition and taste, yet food intake around weaning is still variable and low, resulting in the above mentioned weaning-related problems. Some work has been done on optimizing feeder design for piglets, focusing on colour, shape and size, yet these adaptations did not significantly increase food intake, nor reduce variation in food intake (for example: Appleby et al. 1992; Edge et al. 2004; Edge et al. 2005; McLaughlin et al. 1983; Wattanakul et al. 2005).

Other strategies have focused on changing management of sow and piglets, leading to management strategies such as intermittent suckling, in which the sow is removed from the lactation pens for some hours per day to stimulate solid food intake of piglets. Piglets from intermittent suckling groups are not able to drink from the sow for 6 or 12 hours each day from day 14 of lactation until weaning (Berkeveld et al. 2007; Kuller et al. 2007b). Intermittent suckling piglets do show higher food intakes pre- and postweaning, as well as higher body weights postweaning, but the food intake is still variable. As the food intake is still variable and intermittent suckling requires a lot of labour from the farmer, intermittent suckling may not be the most optimal solution to reduce weaning-associated problems.

Multi-suckling and get-away systems socialize piglets already before weaning and result in higher growth rates pre- and postweaning, though still variable (Pajor et al. 1999; Weary et al. 2002). The positive effects of multi-suckling systems, however, are related to reduced stress and aggression around weaning and not to food intake per se (Hessel et al. 2006). Thus, intermittent suckling, multi-suckling and get-away systems decrease problems around weaning, but require a lot of effort and special adaptations to the farm, and still do not achieve an optimal food intake of all piglets in the pen.

Lessons from the wild

Piglets under (semi)-natural conditions are weaned much later than piglets in husbandry conditions, but there are other differences as well that could inspire solutions to optimize food intake before and after weaning of piglets in pig husbandry. Sows and piglets in the wild have a large home range up to 24 km2 and are able to use this home range completely, while sows and piglets under husbandry conditions often have a pen of around 6m2. Also, confining the sow in a farrowing crate throughout lactation is common practice in many countries (Saunders and McLeod 1999; Stolba and Wood-Gush 1989). The home range of wild pigs contains different substrates, such as grass, trees, dirt, branches, while pigs in husbandry conditions pigs are generally housed in barren pens without any substrates. Furthermore, piglets in the wild join their mother, as well as other members of the group and they forage together and eat the same food items, and piglets may already ingest solid items in the first week after birth (Jensen 1988; Jensen 1991). Sows and piglets in husbandry conditions eat different specialized feeds and eat at different places in the pen; the sow from a raised trough in the farrowing crate and the piglets from a feeder that is usually located in the back of the pen. Wild piglets and sows thus have more possibilities to interact with each other during the development of the piglets, while these possibilities are much more limited in pig husbandry. Enabling piglets to interact more with the sow and thus learn more from her before weaning may stimulate food intake before weaning and consequently increase food intake after weaning, thereby increasing performance, health and welfare of newly weaned piglets.

Learning from mom how to eat like a pig?

Piglets might be able to learn from the sow about how, what, when and where to eat, and could use different types of information for learning. Some of this information can be obtained by observation, using visual but also olfactory, gustatory and auditory cues. Observing what and where an experienced animal is eating increases preference for this type of food and location in several species, such as capuchin monkeys, cows, geese, red-winged blackbirds, Burmese fowl, canaries and rats (Cadieu et al. 1995; Fritz et al. 2000; Galef 1981a; Held et al. 2000; McQuoid and Galef 1993; Veissier 1993; Visalberghi and Addessi 2000a). Cues derived from observing may thus be important, but being able to participate in feeding may be even more effective in influencing food intake or preference (Bandura 1977). When visual observation is used, but no food is eaten immediately, there is a delay between the observation of the behaviour and performing the behaviour. Animals can learn with a delay, providing the delay is not too long and the eventual reward obtained by performing the behaviour contains calories (Black et al. 1985; Capaldi et al. 1987). In participation, however, there is no delay, as food is consumed during learning and the behaviour is thus rewarded immediately by nutrients and taste. Being able to directly link the cues (e.g. visual properties, taste and smell of food) with postingestive consequences (nutrients) is expected to result in a stronger learned association and could thus lead to a higher preference or intake of the food that the experienced demonstrator was eating (Sclafani 1997). This is the case in sheep, where being able to participate with the mother that is eating a food type unfamiliar to the lamb results in a higher intake of this food type 4 weeks later, while observation alone does not result in a higher food intake (Thorhallsdottir et al. 1990). Pigs can learn by observation of an experienced sibling (Nicol and Pope 1994b), but being able to participate may also be more important than being able to observe: piglets housed together with an experienced sibling show higher food intakes and weight gains after weaning than when the piglets are housed in adjacent pens with visual access to the experienced sibling (Morgan et al. 2001). Information from the mother is expected to be even more relevant than that of a sibling or unfamiliar animal, as young animals direct a lot of attention towards the mother. Additionally, the mother and offspring are likely to respond similarly to certain food items, more so than siblings (Chesler 1969; Provenza and Balph 1987). Indeed, learning from the mother proved more effective than learning from a familiar experienced animal in sheep and cats (Chesler 1969; Thorhallsdottir et al. 1990). Mothers of some species, fowl in particular, will even direct the offspring towards palatable and away from unpalatable food by use of vocalizations and food-directed behaviours (Nicol and Pope 1996; Sherry 1977). There are no indications that this also occurs in pigs, however. Yet, being able to learn by observing or participating with the sow before weaning is likely to have positive effects of food intake of piglets, both before and after weaning.

Learning where and what to eat

During observation of or participation with a sow that is eating, piglets may also obtain information on where to eat and what to eat, and may be more likely to eat at the same place as the sow and eat the same type of food as the sow. The presence of other animals or humans that are also eating is in general already enough to increase food intake, a process called social facilitation. In humans, for example, more food is consumed when there are more people present and eating at the dinner table, especially when these people are familiar to the person (De Castro and Brewer 1992; De Castro 1994). At this dinner table, two other processes are also likely to take place. Just because a person is already eating at the table makes it more likely for other people to come to that same location and also start eating, which is known as local enhancement (Hoppitt and Laland 2008a). This process is quite passive and is likely to work even if the demonstrator is not conscious (Galef 1981a). On the other hand, people at the dinner table may be more likely to eat the same food as the person that is already eating, which is known as stimulus enhancement. Stimulus enhancement has been shown to occur in canaries, rats and geese (Cadieu et al. 1995; Fritz et al. 2000; Heyes et al. 2000). There are indications that both local and stimulus enhancement are important in pigs, at least when the demonstrator is a sibling pig, (Nicol and Pope 1994b). Whether local and stimulus enhancement are also important in learning from the sow is not known.

Learning and enrichment of the environment

The amount and type of information that the young animal can learn from its mother or other conspecifics may greatly depend on the environment that the animals are in. If the environment is, for example, very barren, the mother may not be able to show the full behavioural repertoire, i.e., all the behaviours that the species will normally perform (Cox and Cooper 2001; Tuyttens 2005). The mother will also likely spend less time performing behaviours if the environment is very barren, and there is therefore less to learn for the young animals in a barren environment. The same goes for space allowance, but also for example for providing animal with only a short period of time to perform the behaviour, e.g. feeding sows only two meals per day.

Providing piglets and sow with an enriched and larger environment may increase the amount of information that piglets can acquire from the sow, allowing them to adapt better in challenging situations. Enrichment itself may also have beneficial effects on behavioural development and welfare of piglets. Enrichment is known to increase behavioural flexibility, reduce reactivity to stressful situations and decrease fear of novel objects and environments in rodents and similar results have been found in some pig studies (Beattie et al. 2000; Chapillon et al. 1999; De Jong et al. 2000; Leggio et al. 2005; Schrijver et al. 2002). An increased behavioural flexibility and reduced fear of novel objects may also result in a decreased reluctance to try novel foods. Providing various substrates such as straw may furthermore increase the development of feeding-related behaviours such as chewing, which is an important precursor of eating, independently of sow demonstration and make piglets more adapted to consuming solid food items (Cox and Cooper 2001; Herring 1985; Johnson et al. 2001; Miller et al. 2009). It can be hypothesised that providing substrate before weaning makes piglets better adapted to eating solid food and reacting to the stressors around weaning, but providing substrate after weaning may also be beneficial.

Providing enrichment later in life can also reduce the response of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system to stressor in rodents (Moncek et al. 2004). In pigs, enrichment may increase cortisol levels due to increased activity and not stress per se (Beattie et al. 2000; Koolhaas et al. 2011), indicating that providing enrichment after weaning may improve welfare of newly weaned by providing a distraction. It may also motivate piglets to explore instead of perform excessive stress-related behaviours (Bolhuis et al. 2005b; Fraser et al. 1991). Substrates used as enrichment, such as straw, can also be ingested by the piglets, and even if piglets are not eating solid food, eating substrate may stimulate intestinal functioning and promote maturation of the gut, thereby reducing weaning-related diarrhoea (McCracken et al. 1999; Pluske et al. 2007). Thus, providing enrichment before and after weaning has the potential to increase the ability of piglets to adapt to being weaned.

Learning from flavours in the maternal diet

Olfactory cues can also be used to obtain information about food and feeding from experienced conspecifics. The assumption is that the mother knows what type of food is good to eat, and the offspring should prefer to eat the same items that are eaten by the mother in large quantities. Rabbits, for example, develop a strong preference for juniper berry flavours experienced in faeces, in milk or already in utero (Bilko et al. 1994). Flavour cues from the maternal diet in utero can reach the fetus through the amniotic fluid and/or the placental blood stream and this exposure to cues generally results in a preference for these flavours later in life and consequently can positively affect the acceptance of foods with a similar flavour before and after weaning (Hepper 1988; Mennella et al. 2001; Schaal et al. 2000; Simitzis et al. 2008). This flavour preference can be strengthened even more when the flavour is also present in the maternal milk (Galef and Henderson 1972; Galef and Sherry 1973; Mennella 1995), whereby the continuity of flavour exposure is a likely factor for success, as exposure through milk alone does not increase flavour preference in all species (Hepper and Wells 2006).

When the flavour is present in the food after weaning, it is expected that animals will eat this food more quickly and in larger quantities, as they should have a preference for the flavour. Flavour learning during gestation and lactation (perinatal flavour learning) could potentially be used as a tool to help piglets to overcome neophobia for the solid food before and after weaning: the food in the weaning pen should be preferred and not be a novel food, as animals have experienced the flavour before birth and during lactation. The presence of a familiar flavour can also reduce stress levels in novel environments such as a test room or the weaning pen by making the novel environment less novel (Jones et al. 2002). It can thus be hypothesised that providing piglets with a flavour through which they were exposed perinatally increases postweaning food intake and reduces problems arising in the postweaning period.

AIM OF THE THESIS

The aim of this thesis was to explore whether providing piglets with more opportunities to learn from their mother about what, how and where to eat can increase food intake before and after weaning and consequently can reduce health and welfare problems after weaning. Currently the opportunities to learn from the sow are very limited as the sow is generally confined throughout lactation, fed a different feed than the piglets in a raised trough and sows and piglets are generally housed in barren pens. Three pathways of learning that could be improved were investigated: 1) direct learning from the mother through cues derived from observation and eating together, 2) learning in an enriched environment and 3) learning from flavour cues in the maternal diet. These pathways will be integrated in the discussion of the results in the general discussion at the end of this thesis.

02

MATERNAL PRESENCE AND ENVIRONMENTAL ENRICHMENT AFFECT FOOD NEOPHOBIA OF PIGLETS

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ABSTRACT

Young omnivores show food neophobia in order to avoid the potential harmful effects of ingesting unfamiliar food items. We investigated whether the presence of the mother and an enriched rearing environment would reduce food neophobia in piglets. A mother may provide information on suitable food types to include in the diet, whereas an enriched environment may stimulate behavioural development and reduce reactivity towards novel stimuli (including food). Five barren-reared or enriched-reared piglets per litter were exposed to two novel food items in the presence, and the other five per litter in the absence, of the mother in a 7 min test. Maternal presence reduced food neophobia profoundly as reflected in a reduced latency to touching the food, a higher proportion of piglets sampling the two different food items and a higher intake. Latency to touch the food, however, was affected by maternal presence more strongly for barren-reared piglets than for enriched-reared piglets, and in the absence of the sow, consumption of one novel food type and time spent in the feeding area were higher for enriched-reared piglets. Environmental enrichment does have the potential to reduce food neophobia, but the presence of the mother during the encounter with novel food seems more efficient in decreasing food neophobia of piglets.

Keywords:
food neophobia
piglets
maternal presence
environmental enrichment

INTRODUCTION

Omnivores have a broad range of potential food items that can be included in their diet. To avoid potential harmful effects of ingesting unfamiliar food, most young omnivores show food neophobia, i.e. an initial reluctance to eat novel food items (Rozin and Vollmecke 1986). Apart from dietary selection through a trial-and-error process of individual learning, young animals rely on older, experienced conspecifics for information on what to include in their diet (Nicol 2006). When learning about novel food, young animals may direct more attention towards their mother, who is more genetically related to her offspring and spends more time with them than most other adult conspecifics (Galef and Giraldeau 2001; Thorhallsdottir et al. 1990). Social learning regarding food intake may be more efficient when the animals are provided with stimulus- and substrate-rich environments, in which the mother can show the full range of foraging behaviours (Jensen 1988). Enrichment of the rearing environment also has the potential to reduce food neophobia independent of social learning. Environmental enrichment results in a higher behavioural flexibility and a lower reactivity towards unfamiliar stimuli (Leggio et al. 2005; Würbel 2001). As novel food can be seen as an unfamiliar stimulus, it can be hypothesised that enriched-reared animals would be less neophobic towards unfamiliar food than barren-reared animals.

The aim of this experiment was to investigate neophobia of barren- and enriched-reared piglets towards unfamiliar food items presented in the presence or absence of their mother. Piglets in pig husbandry generally show a reluctance to ingest solid food after weaning, resulting in health and welfare problems (Bolhuis et al. 2009). This makes piglets interesting subjects for research regarding food neophobia. We predicted that, in addition to maternal presence, an enriched rearing environment would reduce food neophobia in piglets. This effect might be observed particularly in piglets tested in the absence of the mother.

METHODS

The experiment was set up in a 2 x 2 factorial arrangement, with rearing environment (barren or enriched) and maternal presence during testing (sow present or absent) as factors, and was carried out in two successive batches using 16 multiparous sows and their offspring.

From one week before parturition, sows were either housed in barren (B; 9.2 m2, n = 8) or in enriched farrowing pens (E; 18.4 m2, n = 8). Enrichment consisted of wood shavings, straw (added to the pen daily), peat and branches (replaced once and twice weekly, respectively; see electronic supplementary material for further details). Sows were confined in a farrowing crate between days 0 and 4 after parturition. Litter sizes were set at 10 piglets per litter on day 4 (1 : 1 sex ratio, see electronic supplementary material). Sows were fed twice a day, while piglets had food available continuously. Both sows and piglets received standard food for lactating sows.

The behavioural test was conducted on day 25 after parturition in a 7.7 x 7.7 m arena, located in a different room, with walls of 1 m height and a concrete floor. A 2 x 2 m square, drawn on the floor in the centre of the arena, served as a feeding place. Sows and piglets were habituated to the arena and food types (sows only) before the test; see electronic supplementary material for procedures.

Five piglets from each litter were tested with the sow present in the arena (SP), and the other five piglets were tested in her absence (SA; see electronic supplementary material for a video). Five pieces of cheese and five differently coloured pieces of equally sized, chocolate-covered peanuts per piglet and sow were evenly distributed over the feeding place of the arena before the start of the test. Before testing, the sow was brought to another, empty pen. SP piglets were brought to the sow and sow and piglets were guided to the arena together. During SA trials the sow stayed in the other pen.

The test started when all the animals were present in the arena. Several behaviours were scored (see Electronic Supplement), and the number and weight of pieces of both food types still present in the arena at the end of the test were determined. Test time was 7 min.

Data were averaged per half litter. One SP group from a barren pen was excluded from the analysis because the sow was in oestrus. Data (see the electronic supplementary material) were analysed with generalized linear mixed models (SAS 9.0, SAS Institute Inc.), with sow presence, enrichment, their interaction and batch as fixed effects and pen as random effect, using the appropriate distributions and link functions.

RESULTS

Sow presence reduced latency to touch the food (F1,13 = 714, P < 0.0001, *[figure 1A]*) and the number of vocalizations (F1,13 = 1665, P < 0.0001, *[table 1]*) and increased percentage of piglets sampling cheese (F1,13 = 8.3, P = 0.01, *[table 1]*) and both food types (F1,13 = 8.2, P = 0.01, *[figure 1B]*), though not chocolate peanuts (F1,13 = 2.4, P = 0.14, *[table 1]*). SP piglets also ate more chocolate peanuts (F1,13 = 5.0 pieces, P = 0.04, *[figure 1D]*) and more pieces of both food types (F1,13 = 5.6, P = 0.03, *[table 1]*), though not cheese (F1,13 = 2.7, P = 0.12, *[figure 1C]*), and tended to spend more time in the feeding area than SA piglets (F1,13 = 4.6, P = 0.05), though not when corrected for latency to enter the feeding area (F1,13 = 0.42, P = 0.55, *[table 1]*). Food consumption by the sow was high (barren sows 12.7 and 16.0 enriched sows 12.8 and 14.0 pieces of cheese and chocolate peanuts on average, respectively), and there was a negative correlation between total pieces of food eaten by sow and by the piglets (r = -0.84, P < 0.001). Sows were not observed pushing the piglets towards the food, nor were special vocalizations observed.

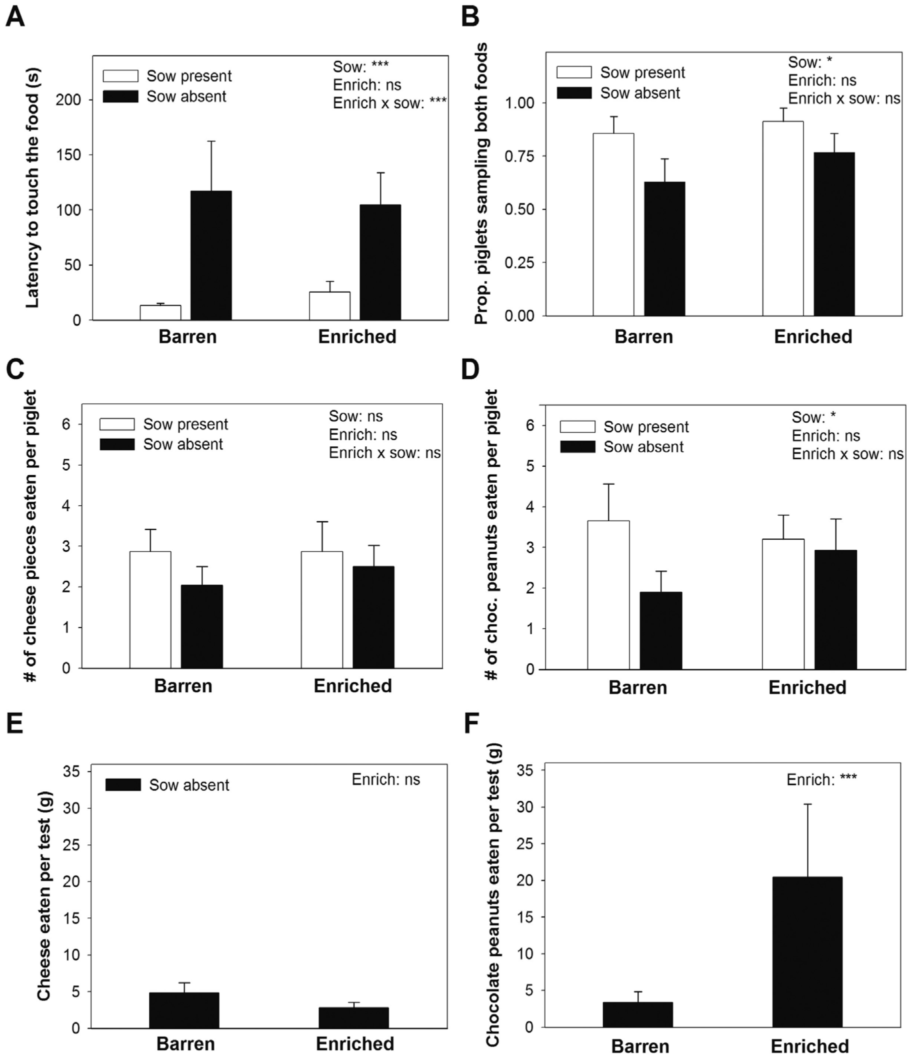
There were, however, differences between barren and enriched piglets in the potential of maternal presence to reduce food neophobia: maternal presence reduced latency to touch the food (sow x enrichment interaction, F1,13 = 32.0, P < 0.0001, *[figure 1A]*) and the number of vocalizations (sow x enrichment interaction, F1,13 = 4.3, P = 0.06, *[table 1]*) more strongly in barren-reared than in enriched-reared piglets. Environmental enrichment also affected some of the neophobia-indicators measured directly: enriched-reared piglets consumed (in grams), more chocolate peanuts than barren-reared piglets (SA only: F1,12 = 68.4, P < 0.0001, *[figure 1F]*), though not cheese (F1,12 = 3.6, P = 0.08, *[figure 1E]*) and spent more time in the feeding area than barren pigs (F1,13 = 4.0, P = 0.07), also when corrected for the latency time to enter the feeding area (F1,13 = 6.8, P = 0.02, *[table 1]*). The number of pieces of both food types eaten, vocalizations, latency to touch the food and the proportion of piglets sampling both food types, however, were not affected by environmental enrichment (all P values >0.1, *[figure and table 1]*).

table 1.

	barren		enriched		P-value		
	SP	SA	SP	SA	sow	enrich	enrich x sow
vocalizations (number)	28 ± 6	276 ± 45	24 ± 11	193 ± 61	**<0.001**	0.33	**0.06**
food items eaten (number per piglet)	6.5 ± 1.4	3.9 ± 0.9	6.1 ± 1.2	5.4 ± 1.2	**0.03**	0.44	0.18
piglets sampling chocolate peanuts (proportion)	0.94 ± 0.06	0.77 ± 0.12	0.94 ± 0.04	0.89 ± 0.06	0.14	0.34	0.41
piglets sampling cheese (proportion)	0.91 ± 0.04	0.80 ± 0.04	0.94 ± 0.04	0.83 ± 0.08	**0.01**	0.61	0.95
time spent in feeding area (percentage)	33.8 ± 4.0	22.9 ± 2.3	41.3 ± 4.9	35.2 ± 5.4	**0.05**	**0.07**	0.55
time spent in feeding area corrected for latency (percentage)	18.1 ± 5.5	21.2 ± 2.5	31.0 ± 3.2	32.8 ± 4.8	0.53	**0.02**	0.87

Food ingestion and exploration of piglets reared in a barren or enriched environment, tested in the presence (SP) or absence (SA) of their mother. Data are depicted as averages ± s.e.m. Bold values are values with $P < 0.1$.

figure 1.



Latency to touch the food (average per half litter, A), proportion of piglets sampling both food types (B), number of cheese pieces eaten per piglet (C), number of chocolate peanuts eaten per piglet (D), grams of cheese eaten per test (E) and grams of chocolate peanuts eaten per test (F) for barren-reared and enriched-reared piglets, tested with the sow present (white bars) or absent (grey bars). *** P<0.001, ** P<0.01, * P<0.05, # P<0.1, ns not significant.

DISCUSSION

This study demonstrates that the presence of the mother during exposure to novel food items reduces neophobia in piglets. Moreover, enriched-reared piglets appeared less neophobic in this experiment, irrespective of maternal presence. Enriched-reared piglets consumed more chocolate in the absence of the sow and spent more time in the feeding area of the arena than barren-reared piglets. The presence of the sow seemed, furthermore, to reduce neophobia more in barren piglets. This is, to our knowledge, the first demonstration of an effect of environmental enrichment on food neophobia. The effect could have been caused by differences in activity and thus energy requirements of barren and enriched piglets, however, piglets still regularly suckle their mother's milk and no differences in suckling frequency or duration were observed (data not shown). It is therefore more likely that the enrichment instead may have resulted in a lower responsiveness to the test situation, which is supported by the shorter time spent attending the food by barren piglets and by the finding that maternal presence tended to reduce the number of vocalizations more strongly in barren piglets. Enrichment may also have reduced food neophobia owing to enhanced experience with sampling unfamiliar items in the environment (Launchbaugh et al. 1997; Schrijver et al. 2002), possibly leading to improved foraging skills and a higher eagerness to explore (Jensen 1988; Rosenzweig and Bennett 1996).

The presence of the mother during exposure to novel food items, however, seemed to result in a stronger reduction of food neophobia of piglets than environmental enrichment, though the effect may be modulated by environmental enrichment, as the effect of maternal presence appeared more profound in barren-reared than in enriched-reared animals. Although all animals were thoroughly habituated to the test arena, we cannot rule out the possibility that the presence of the sow reduced stress of the piglets during the test. This is supported by the lower number of vocalizations given by piglets in presence of their mother, which could be indicative of lower stress levels (Weary et al. 1997). Increased stress levels in barren piglets may, in turn, lead to increased food neophobia, as has been shown in humans (Pliner et al. 1995) and sheep (Burritt and Provenza 1997). However, the time spent in the area close to the food did not differ between piglets tested with or without the sow. This suggests that piglets of both treatments spent the same time attending to the food but differed in consumption, pointing to a specific effect on food neophobia. The mother was previously familiarized with the food, and testing piglets with an unfamiliarized mother or with a mother who has experienced negative consequences of ingesting the food may give information on the learning processes involved. Social facilitation and local enhancement seem likely candidates as no active behaviours were made by the sow towards the piglets (Nicol 2006).

In conclusion, environmental enrichment does have the potential to reduce food neophobia, but the presence of the mother during the encounter with the novel food seems to overrule the possible effects of enrichment. The reduction of food neophobia by maternal presence and by environmental enrichment may be important to stimulate food intake of piglets in pig husbandry. Piglets often have their first experience with solid food without the sow present, whereas the current study suggests that the presence of the mother during contact with novel food items could stimulate food intake before weaning. Providing piglets with an enriched rearing environment may further decrease neophobia and increase adaptability of piglets to novel stimuli. Combining increased foraging opportunities together with the sow in an enriched environment may therefore reduce food neophobia, thereby improving health and welfare of newly weaned piglets.

Animal care and experimental procedures performed were approved by the Animal Care and Use Committee (DEC) of Wageningen University.

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03

EFFECTS OF ENVIRONMENTAL ENRICHMENT AND LOOSE HOUSING OF LACTATING SOWS ON PIGLET PERFORMANCE BEFORE AND AFTER WEANING

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ABSTRACT

We investigated effects of loose housing of the sow during lactation and enrichment of the pen pre- and postweaning on performance of newly weaned piglets. Before weaning, piglets (n = 320) were housed in an enriched (straw, wood shavings, peat and branches) or barren pen with a confined or loose-housed sow (n = 32). Loose-housed sows and their piglets could eat together from a family feeder, whereas confined sows and piglets had separate feeding troughs. Piglets (n = 256) were mixed postweaning, and 4 piglets from each litter were relocated to a barren pen (n = 32 pens) and 4 other piglets were housed in an enriched pen (n = 32 pens). Growth from d 15 until weaning was greater for piglets from enriched pens (4.38 ± 0.29 vs. 4.71 ± 0.21 kg/pig, $P < 0.05$) and tended to be greater for piglets with a loose-housed sow compared with piglets with a confined sow (4.41 ± 0.26 vs. 4.67 ± 0.24 kg/pig, $P = 0.10$). Preweaning feed intake was not affected by preweaning conditions ($P > 0.25$). Piglets from enriched preweaning pens ate more in the first 2 d postweaning than piglets from barren pens (first 48 h, barren 0.45 ± 0.05 kg/pig, enriched 0.53 ± 0.04 kg/pig, $P < 0.05$). Piglets relocated to an enriched pen after weaning showed a greater growth in the 2 wk after weaning (barren 5.5 ± 0.2 kg/pig, enriched 6.2 ± 0.2 kg/pig, $P < 0.0001$), had a profoundly reduced diarrhoea prevalence than piglets housed in barren pens after weaning (barren 2.4 ± 0.4 d, enriched 1.0 ± 0.3 d, $P < 0.0001$), and had a greater feed efficiency (barren 0.81 ± 0.03, enriched 0.85 ± 0.02, $P < 0.05$). Enrichment of the preweaning environment likely stimulates development of feeding behaviours and consequently increases feed intake immediately after weaning. Providing piglets with an enriched environment after weaning positively affected postweaning growth, feed efficiency, and incidence of diarrhoea, which may be caused by decreased stress levels or increased gut health. Enrichment of the pre- and postweaning environment thus seems important in improving performance and health of newly weaned pigs.

Keywords:

environmental enrichment
learning
piglets
sow housing
weaning

INTRODUCTION

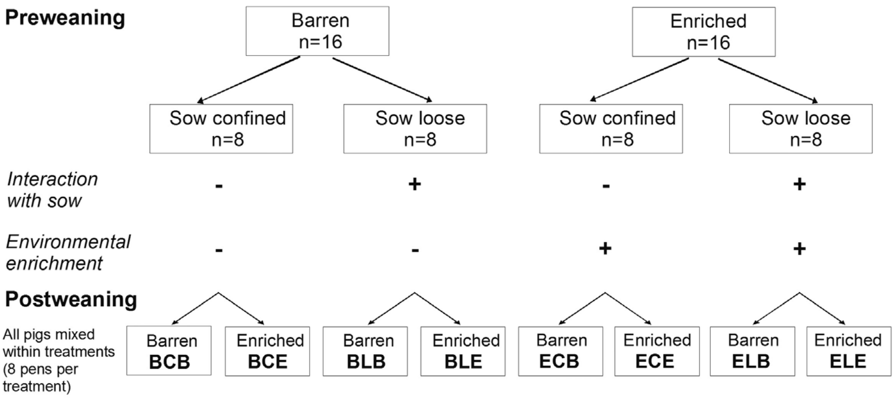
Timely and sufficient nutrient intake during the immediate postweaning period is crucial for the growth, health, and welfare of piglets (e.g., Bolhuis et al. 2009; Bruininx et al. 2002b). Despite several management strategies applied to encourage piglets to eat, most suckling and newly weaned piglets are still very reluctant to ingest solid feed (Berkeveld et al. 2007; Edge et al. 2005; Wattanakul et al. 2005).

Many young mammals rely on older, more experienced conspecifics, particularly their mother, for information when making the change from milk to solid feed (Galef and Giraldeau 2001). We recently demonstrated beneficial effects of prenatal exposure to flavours from the maternal diet on postweaning feed intake, growth, health and welfare of piglets provided with similarly flavoured feed after weaning (Oostindjer et al. 2010c). Feeding behaviour of piglets may also be stimulated by other forms of social learning, such as observation of the sow. In current housing systems, however, sows cannot demonstrate the full range of explorative and feeding-related behaviours as they are usually confined in a farrowing crate in a barren pen lacking rooting substrates throughout lactation. Provisioning rooting substrates during the preweaning period may also, regardless of sow behaviour, stimulate the development of feeding-related behaviours in piglets by stimulating explorative behaviour and reducing the behavioural and stress responses to unfamiliar stimuli such as feed (Jensen 1991; Würbel 2001). The aim of this study was to test whether an enriched preweaning environment or increased opportunities (or both) for piglets to interact with the sow improves performance of piglets housed in barren or enriched pens postweaning. Loose housing of sows and provisioning rooting substrates may increase the information transfer from sow to piglets, possibly resulting in an increased feed intake and growth postweaning.

METHODS

The Animal Care and Use Committee of Wageningen University approved the experiment. The experimental design was a 2 x 2 x 2 factorial arrangement of treatments, with preweaning environment (barren or enriched), confinement of the sow preweaning (sow confined in crate or loose-housed), and the environment postweaning (barren or enriched) as the factors *[figure 1]*, and was carried out in 2 successive batches.

figure 1.



Experimental setup. BCB = barren preweaning environment, confined sow, barren postweaning environment; BCE = barren preweaning environment, confined sow, enriched postweaning environment; BLB = barren preweaning environment, loose-housed sow, barren postweaning environment; BLE = barren preweaning environment, loose-housed sow, enriched postweaning environment; ECB = enriched preweaning environment, confined sow, barren postweaning environment; ECE = enriched preweaning environment, confined sow, enriched postweaning environment; ELB = enriched preweaning environment, loose-housed sow, barren postweaning environment; ELE = enriched preweaning environment, loose-housed sow, enriched postweaning environment.

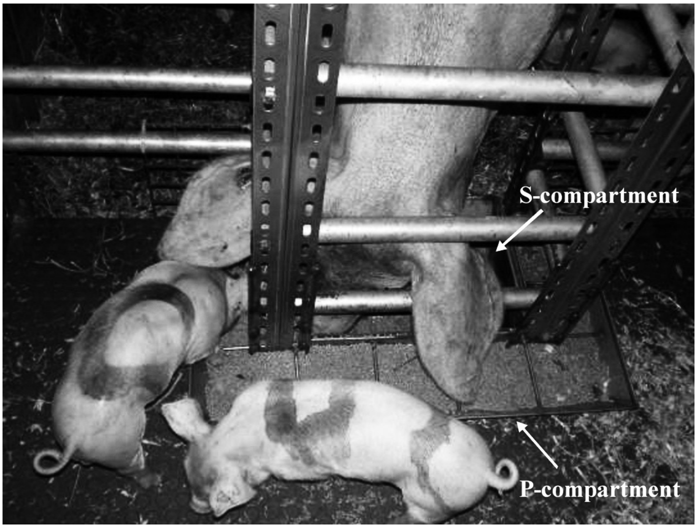
Animals and housing pre-weaning

A total of 32 multiparous sows and their piglets (Tempo x Topigs 30) were used. Sows were individually housed in barren (n = 16) or enriched (n = 16) farrowing pens from 1 wk before farrowing. Sample sizes were based on previous experiments in our department and on other studies investigating performance of newly weaned pigs (e.g., Bruininx et al. 2002b; Oostindjer et al. 2010c). Barren pens (9.2 m2) had partly solid (65%) and partly slatted (35%) floors. Slats were covered with mats the first 4 d after farrowing. No substrate was present in the barren pens, with the exception of a small amount of sawdust (approximately 5 L) on the first day after farrowing. Enriched pens (18.4 m2) were provided with wood shavings (550 L/pen, covering 65% of floor) and peat (400 L/pen, covering 35% of floor. Straw (1 kg/pen) and branches (3 large or 5 small branches per pen, none larger than 2 m long) were presented on top of the other substrates. Fresh wood shavings (70 L) and straw (0.5 kg) were added to the enriched pens every day, and peat (40 L) was added to the pen once per week. Branches were replaced 2 times per week. The increased pen size was also part of the enrichment treatment becaise it increases the expression of exploratory and locomotory behaviours (Bolhuis et al. 2005a). Parity was balanced between groups (average parity 5.03, SD 1.44). Sows were weighed and backfat was measured 1 wk before farrowing and immediately after weaning (prefarrowing: average BW 278 ± 20 kg, average backfat 17.4 ± 4.3, postweaning: BW 245 ± 23 kg, backfat 13.6 ± 3.7).

All sows were confined in a farrowing crate between d 0 and d 4 after farrowing. The farrowing crate was removed in one-half of the barren and enriched pens on d 4 (loose-housed sows, n = 16) and remained in place in the other one-half of the pens (confined sows, n = 16). Space allowance for confined sows was 1.68 m2, 7.15 m2 for barren-loose housed sows and 16.38 m2 for enriched-loose housed sows. Each pen contained a barred platform that was reachable for piglets but not for the sow to prevent crushing. A heating lamp was placed above the platform, and around farrowing a heating lamp was placed at the back of the sow.

A family feeder (Verbakel B.V., Sint Oedenrode, the Netherlands, *[figure 2]*), consisting of a compartment for the sow and a compartment for the piglets, was placed in the platform in all pens so that the sow compartment was outside of the heated piglet area and the piglet compartment was integrated into the piglet area. The piglet compartment of the feeder was shallow yet levelled with the top of the sow compartment to enable observation of the feeding behaviour of sows by piglets in the loose-housed groups. Mesh wire was placed on both sides of the feeder to minimize sows eating feed from the piglet compartment. The loose-housed sows were fed twice per day in the family feeder, and confined sows were fed twice a day in a standard feeder in the farrowing crate, which was raised and screened off for piglets. At the start of lactation, feed provisioning to sows was 1 kg, which quickly increased to 4 kg in the first week of lactation and gradually increased to a maximum of 6.5 kg at d 18 of lactation. Piglets of both confined and loose-housed sows had food available all day from d 4 onward in the family feeder. Sows and piglets both received standard feed for lactating sows (9.51 MJ of NE/kg, 14.9% CP, 0.68% ileal digestible Lys) to maximize information transfer from sow to piglet by allowing for learning through stimulus enhancement (Nicol 2006). This feed was mixed with piglet feed (10.91 MJ of NE/kg, 17.9% CP, 1.12% ileal digestible Lys) during the last 6 d before weaning for both sows and piglets to adapt piglets to the postweaning feed and thus minimize a poor feed intake caused by a sudden change in diet. The amount of piglet feed in the diet increased by 16.7% per day, resulting in 100% piglet feed being provided to sows and piglets on the day before weaning. Both feed types were designed to meet nutrient requirements according to Centraal Veevoeder Bureau (CVB) guidelines (CVB 2008). All pens had standard drinking nipples available for sows and piglets: 1 for confined sows and 2 for loose-housed sows, 2 for piglets at the back of the pen. No drinking nipples were available immediately next to the family feeder to avoid spilling of water on the piglet feed. Temperature was maintained at a minimum of 26°C around farrowing and thereafter gradually decreased to 22°C by 1°C every 4 d. *[figure 3]* shows the a schematic overview of the barren and enriched pens before removal of the farrowing crate.

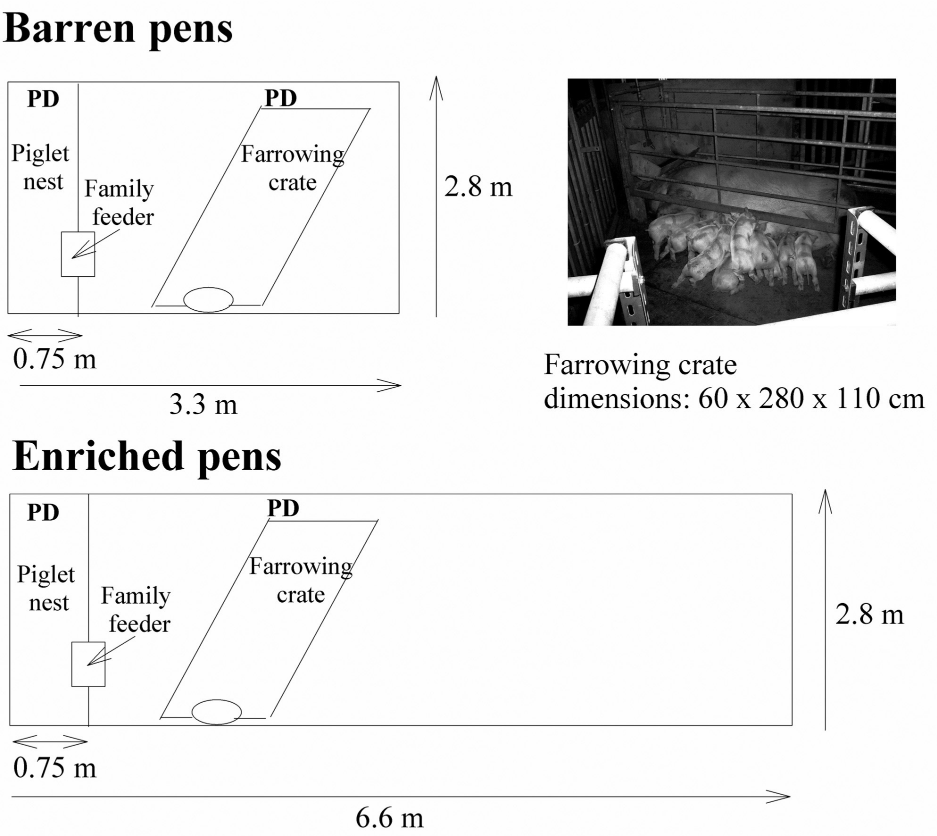
figure 2.



Family feeder used in the experiment in all treatments. Loose-housed sows were fed in the S-compartment of the feeder. Piglets in all treatments had feed available in the P-compartment.

Litter sizes were standardized to 10 piglets per litter with minimum use of cross-fostering, on d 4, and with a 1:1 sex ratio when possible (only failed in 5 out of 32 pens). Lights were on between 0700 and 1900 h and dimmed during the night. Pens were divided within batch into two observation groups, based on the moment of farrowing: an early and late group (difference of 3 d). Each group contained 2 pens from each preweaning treatment combination. The preweaning measurements that are described below were done on the average age of the respective observation group. Four piglets died preweaning, after d 3 of lactation, in the enriched-loose housed treatment (from 3 litters), 1 in the enriched-confined treatment and 1 in the barren-confined treatment.

figure 3.



Schematic overview of the barren and enriched pens before weaning, before the removal of the farrowing crates for loose-housed sows. Photo: farrowing crate and sow feeder for confined sows (barren environment). PD: piglet drink nipple.

Animals and housing postweaning

Piglets were weaned at 29.2 (SD 2.7) d of age between 0900 and 1200 h. Four piglets per litter were allocated to enriched postweaning pens and 4 to barren postweaning pens. Allocation to barren and enriched pens was balanced for sex and BW (see *[table 1]* for weaning weights). Piglets were mixed at weaning with other piglets from the same preweaning treatment, resulting in 4 piglets per pen that were unfamiliar to each other. Barren and enriched pens were evenly distributed within the room. Temperature was maintained at a minimum of 25°C until d 4 after weaning and thereafter gradually decreased by 1°C every 2 d to 22°C.

table 1.

	preweaning barren				preweaning enriched				effects[3]						
	confined sow		loose-housed sow		confined sow		loose-housed sow								
	BCB	BCE	BLB	BLE	ECB	ECE	ELB	ELE	P	S	E	PS	PE	SE	PSE
number of pens	8	8	8	8	8	8	8	8							
weaning weight, kg	8.94 ± 0.57	8.97 ± 0.61	9.32 ± 0.49	9.28 ± 0.59	9.48 ± 0.60	9.49 ± 0.62	9.60 ± 0.52	9.54 ± 0.47	ns	ns	ns	ns	ns	ns	ns
growth d 0 – 14, kg	5.45 ± 0.22^{a}	5.92 ± 0.32^{b}	5.52 ± 0.15^{a}	6.49 ± 0.11^{b}	5.52 ± 0.26^{a}	6.08 ± 0.25^{b}	5.47 ± 0.13^{a}	6.30 ± 0.21^{b}	ns	ns	***	ns	ns	ns	ns
growth d 0 – 4, kg	0.81 ± 0.09^{a}	0.92 ± 0.09^{b}	0.81 ± 0.08^{a}	1.02 ± 0.06^{b}	0.80 ± 0.08^{a}	1.04 ± 0.08^{b}	0.82 ± 0.08^{a}	1.00 ± 0.03^{b}	ns	ns	***	ns	ns	ns	ns
growth d 4 – 7, kg	0.68 ± 0.10	0.49 ± 0.11	0.66 ± 0.14	0.83 ± 0.08	0.65 ± 0.16	0.68 ± 0.11	0.64 ± 0.07	0.70 ± 0.09	ns	ns	ns	ns	ns	ns	ns
growth d 7 – 14, kg	3.96 ± 0.16^{a}	4.53 ± 0.21^{b}	4.05 ± 0.12^{a}	4.64 ± 0.13^{b}	4.08 ± 0.21^{a}	4.37 ± 0.22^{b}	4.00 ± 0.13^{a}	4.6 ± 0.15^{b}	ns	ns	***	ns	ns	ns	ns
feed intake 2 wk, kg/pig	6.84 ± 0.28	6.82 ± 0.36	6.88 ± 0.26	7.41 ± 0.13	7.06 ± 0.34	7.16 ± 0.28	7.01 ± 0.21	7.45 ± 0.20	ns	ns	ns	ns	ns	ns	ns
feed intake first 4 h, g/pig	36 ± 6^{a}	11 ± 4^{a}	31 ± 11^{a}	23 ± 7^{a}	48 ± 9^{b}	46 ± 8^{b}	24 ± 8^{a}	31 ± 5^{a}	*	ns	ns	**	#	ns	ns
feed intake first 24 h, g/pig	195 ± 11^{a}	134 ± 16^{a}	182 ± 27^{a}	159 ± 30^{a}	288 ± 44^{b}	259 ± 39^{b}	171 ± 31^{a}	183 ± 15^{a}	*	ns	ns	**	ns	ns	ns
feed intake first 48 h, g/pig	493 ± 29^{a}	428 ± 23^{a}	423 ± 35^{a}	471 ± 42^{a}	546 ± 30^{b}	605 ± 66^{b}	494 ± 101^{b}	488 ± 46^{b}	*	ns	ns	ns	ns	ns	ns
feed efficiency d 0 – 14	0.79 ± 0.04^{a}	0.88 ± 0.02^{b}	0.80 ± 0.03^{a}	0.87 ± 0.02^{b}	0.83 ± 0.04^{a}	0.80 ± 0.03^{b}	0.81 ± 0.03^{a}	0.85 ± 0.02^{b}	ns	ns	*	ns	#	ns	ns
piglets ≥ 1 day diarrhoea	23^{a}	6^{b}	17^{a}	12^{a}	20^{a}	9^{b}	20^{a}	8^{b}	ns	ns	*	ns	ns	ns	#

1 Data are presented as averages ± S.E.M. Within a row, means with different superscript letters differ ($P < 0.05$).

2 Treatments: BCB = barren preweaning environment, confined sow, barren postweaning environment; BCE = barren preweaning environment, confined sow, enriched postweaning environment; BLB = barren preweaning environment, loose-housed sow, barren postweaning environment; BLE = barren preweaning environment, loose-housed sow, enriched postweaning environment; ECB = enriched preweaning environment, confined sow, barren postweaning environment; ECE = enriched preweaning environment, confined sow, enriched postweaning environment; ELB = enriched preweaning environment, loose-housed sow, barren postweaning environment; ELE = enriched preweaning environment, loose-housed sow, enriched postweaning environment.

3 Significance of treatment effects: PSC = preweaning enrichment (PE) × sow confinement (SC) interaction, SCE = sow confinement × postweaning enrichment (E) interaction, PPE = preweaning enrichment × postweaning enrichment interaction, PSCE = preweaning enrichment × sow confinement × postweaning enrichment interaction. * $P<0.05$, ** $P<0.01$, *** $P<0.0001$, # $P<0.1$, ns not significant.

Postweaning barren pens (3.2 m2) had a 65% solid and 35% slatted floor and no substrate. Enriched pens (6.4 m2) were provided with straw (0.5 kg), wood shavings (185 L), peat (135 L), and branches (3). Fresh wood shavings (70 L) and straw (0.5 kg) were added to the enriched pens every day, and peat (40 L) was added to the pen once per week. Branches were replaced 2 times per week.

Feed (the same as in the last days of lactation) was provided in 2 feeders per pen with 2 and 3 feeding spaces, respectively. Two drinking nipples were available per pen. Lights were fully lit between 0700 and 1900 h and dimmed during the night.

Performance and prevalence of diarrhoea

Preweaning feed intake of piglets could only be accurately measured over the last days before weaning due to spillage by piglets, consumption of piglet feed by the sow, and an accumulation of dust in the feed by the peat provided to the enriched pens. Therefore, feed intake was determined between d 23 of lactation and weaning (on average d 29) by removing all feed from the feeder with a vacuum cleaner and subtracting the leftover feed from the amount added. All piglets were weighed at birth, d 15 (average age, piglets within 1 observation group were weighed on the same day), and the day of weaning for each observation group. The measurement on d 15 was included because piglets were expected to start eating around that time.

Body weight was measured on d 0, 4, 7, and 14 postweaning. Feed intake was measured at 4 h, 24 h, 48 h and d 3, 7, 11, and 14 postweaning by weighing the feeder and subtracting the weight from the empty feeder weight plus the added feed. Prevalence of diarrhoea was examined daily by visual inspection of the area around the anus of all the piglets. Any presence of yellow, grey or watery brown faeces around the anus was taken as an indication of intestinal problems. The total number of days that a piglet suffered from diarrhoea within the first 2 wk after weaning was calculated and used for analysis.

Statistical analyses

Data were averaged per pen before analysis. Effects of pre- and postweaning environmental enrichment and opportunities to interact with the sow were analysed with mixed linear models in SAS (SAS Inst. Inc., Cary, NC).

Effects of preweaning enrichment, sow confinement, and their interaction on birth weight were analysed with a mixed model that also contained batch (first or second). Effects of preweaning enrichment, sow confinement, and their interaction on growth of piglets from birth to weaning were analysed with a mixed model that also included batch, birth weight, and age at weaning. Growth from the measurement around d 15 until weaning was analysed with the same model but also included observation group to correct for differences in time interval between these 2 measurements because piglets from both observation groups were weaned at the same day. Preweaning feed intake per piglet per day was analysed with a mixed model including preweaning enrichment, sow confinement, and their interaction, as well as batch and age at weaning. Total feed intake over the postweaning period was analysed using a mixed model with preweaning enrichment, sow confinement, postweaning enrichment, and their interactions, as well as batch as fixed effects. Feed intakes over the first 4, 24, and 48 h postweaning were analysed with the same model, as the first few days are the most critical period after weaning. The same model was also used for the analyses of growth postweaning, feed efficiency over the 2 wk postweaning and the number of diarrhoea days postweaning. Data are presented as means ± SEM based on pen averages.

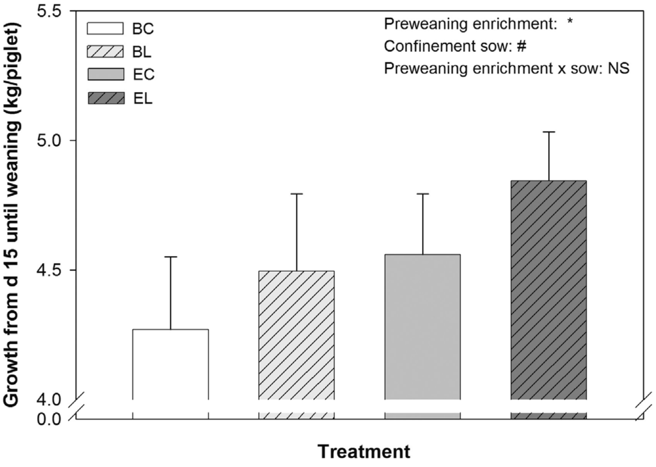
RESULTS

Preweaning performance

Piglets that were reared with a loose-housed sow after standardization had lighter birth weights than piglets with a confined sow, even though all sows were still confined until d 4 after farrowing (P < 0.05). Piglets of barren and enriched pens did not differ in birth weights, nor was there an interaction with sow confinement (barren-confined 1.46 ± 0.04, barren-loose 1.31 ± 0.07, enriched-confined 1.46 ± 0.06, enriched-loose 1.34 ± 0.04; P > 0.8). Growth from birth to weaning was not affected by preweaning enrichment or sow confinement (P > 0.1). However, piglets in an enriched preweaning environment had greater growth in the period from d 15 until weaning (4.38 ± 0.29 vs. 4.71 ± 0.21 kg/pig, P < 0.05, *[figure 4]*). Loose housing of sows tended to increase growth in this period as well (4.41 ± 0.26 vs. 4.67 ± 0.24 kg/pig; P = 0.10). No interaction was found between preweaning enrichment and confinement of the sow (P = 0.82).

Feed intake between d 23 and weaning was not affected by any of the treatments (barren-confined: 45 ± 11 , barren-loose: 58 ± 13, enriched-confined: 64 ± 10, enriched-loose: 64 ± 9 g·pig-1·d-1, all P > 0.55).

figure 4.



Growth between d 15 after birth until weaning for piglets housed in a barren or enriched environment, with a confined sow or loose-housed sow. BC = barren environment, confined sow; BL = barren environment, loose-housed sow; EC = enriched environment, confined sow; EL = enriched environment, loose-housed sow. Error bars: averages are given with the SEM.

Postweaning performance

There was no treatment effect on the total feed intake over the 2 wk postweaning *[table 1]*. Analysis of the feed consumption in the first 4 h after weaning, however, showed that piglets from barren preweaning pens that were placed in an enriched environment postweaning tended to have less feed intake than the other piglets (17 ± 6 g/pig vs. 36 ± 15 g/pig, preweaning enrichment x postweaning enrichment, $P < 0.10$, Table 1). Piglets housed preweaning in enriched pens consumed more feed in both the first 4 h and 24 h postweaning than piglets housed in barren pens preweaning (4 h: barren 25 ± 7 g/pig, enriched 37 ± 7 kg/pig; 24 h: barren 170 ± 20 g/pig, enriched 220 ± 30 g/pig, preweaning enrichment, $P < 0.05$). Preweaning enriched piglets housed with a confined sow, however, showed an even greater feed intake in the first 4 h and 24 h after weaning (4 h: 47 ± 8 g/pig vs. 26 ± 7 g/pig; 24 h: 270 ± 40 g/pig versus 170 ± 20 g/pig for enriched and barren preweaning housing, respectively, preweaning enrichment x sow confinement interaction, $P < 0.01$). The feed intake over the first 48 h postweaning was only affected by preweaning enrichment, with enriched-housed piglets consuming more feed than barren-housed piglets (barren 450 ± 50 g/pig, enriched 530 ± 40 g/pig, $P < 0.05$).

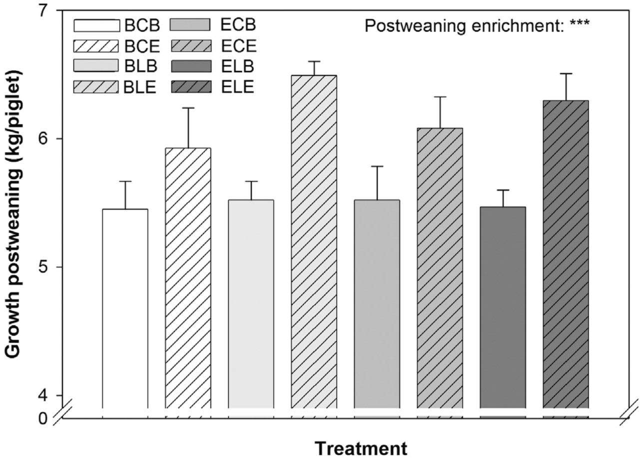
Growth of piglets postweaning was profoundly influenced by environmental enrichment postweaning: enriched-housed piglets showed a greater growth than barren-housed piglets over the 2 wk postweaning, independent of their preweaning treatments (barren 5.5 ± 0.2 kg/pig, enriched 6.2 ± 0.2 kg/pig, $P < 0.0001$, *[figure 5]*). Postweaning enrichment significantly increased growth in the first 4 d ($P < 0.001$). Postweaning enrichment did not significantly affect growth between d 4 and 7, yet increased growth from d 7 to 14 ($P < 0.001$, *[table 1]*). No effects of preweaning enrichment or sow confinement or their interactions were found on growth in the first 4 d, 7 d, or 2 wk postweaning ($P > 0.15$, *[table 1]*).

Feed efficiencies were greater for piglets housed in enriched postweaning pens (barren 0.81 ± 0.03, enriched 0.85 ± 0.02, $P < 0.05$). The reducing effect of postweaning enrichment on feed efficiency, however, tended to be stronger for piglets housed in barren pens preweaning (preweaning x postweaning, $P < 0.10$, *[table 1]*).

Piglets that were housed in an enriched environment postweaning had diarrhoea for fewer days after weaning than piglets housed in barren environments (barren 2.4 ± 0.4 d, enriched 1.0 ± 0.3 d, $P < 0.0001$, Figure 6). The reduction in diarrhoea by postweaning enrichment was less strong in barren-loose housed and enriched-confined housed piglets (preweaning enrichment x sow confinement x postweaning enrichment, $P < 0.05$, *[figure 6]*). There were more piglets in barren environments that had diarrhoea for 1 d or more than in enriched environments (generalized linear model with binary distribution, postweaning enrichment

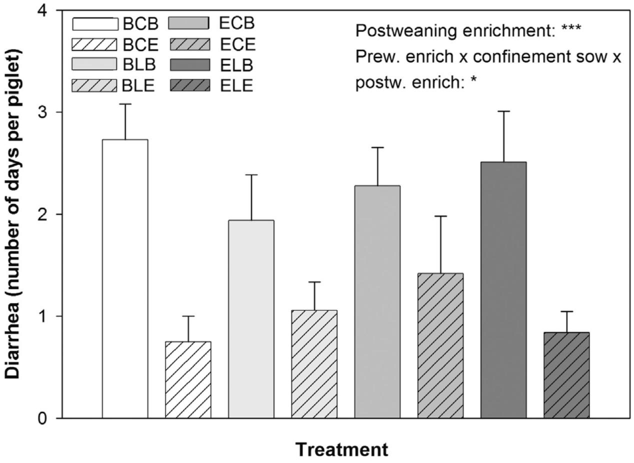
$P < 0.0001$), but this effect tended to be absent for piglets that were housed in barren environments with a loose-housed sow before weaning (preweaning enrichment x sow confinement x postweaning enrichment, $P < 0.06$, *[table 1]*).

figure 5.



Growth of piglets in the 2 wk postweaning for piglets preweaning housed in a barren or enriched environment, with a confined sow or loose-housed sow, and postweaning housed in a barren (clear bars) or enriched (striped bars) environment. BCB = barren preweaning environment, confined sow, barren postweaning environment; BCE = barren preweaning environment, confined sow, enriched postweaning environment; BLB = barren preweaning environment, loose-housed sow, barren postweaning environment; BLE = barren preweaning environment, loose-housed sow, enriched postweaning environment; ECB = enriched preweaning environment, confined sow, barren postweaning environment; ECE = enriched preweaning environment, confined sow, enriched postweaning environment; ELB = enriched preweaning environment, loose-housed sow, barren postweaning environment; ELE = enriched preweaning environment, loose-housed sow, enriched postweaning environment. Error bars: averages are given with the SEM.

figure 6.



Total days of diarrhea in the 2 wk postweaning for piglets preweaning housed in a barren or enriched environment, with a confined sow or loose-housed sow, and postweaning housed in a barren (clear bars) or enriched (striped bars) environment. BCB = barren preweaning environment, confined sow, barren postweaning environment; BCE = barren preweaning environment, confined sow, enriched postweaning environment; BLB = barren preweaning environment, loose-housed sow, barren postweaning environment; BLE = barren preweaning environment, loose-housed sow, enriched postweaning environment; ECB = enriched preweaning environment, confined sow, barren postweaning environment; ECE = enriched preweaning environment, confined sow, enriched postweaning environment; ELB = enriched preweaning environment, loose-housed sow, barren postweaning environment; ELE = enriched preweaning environment, loose-housed sow, enriched postweaning environment. Error bars: averages are given with the SEM.

DISCUSSION

This study investigated effects of environmental enrichment during farrowing and lactation, loose housing of the sow during lactation, and an enriched postweaning environment on performance and health of newly weaned piglets. We found, however, effects of preweaning environmental enrichment and sow housing on performance before weaning. An enriched preweaning environment and loose housing of the sow resulted in greater growth between d 15 and weaning, which is the period when piglets potentially start ingesting solid feed (Berkeveld et al. 2007). We cannot fully exclude the possibility that preweaning conditions may have affected growth by influencing milk production. It should be noted, though, that preweaning treatments did not differ in number and durations of sucklings or in BW and backfat loss of the sow (data not shown). Therefore, it is most likely that preweaning enrichment and loose housing of the sow enhanced growth between d 15 and weaning by stimulating feed intake even though, apart from the last preweaning days, intake could not be accurately measured.

Our findings are in agreement with studies on piglets housed in outdoor paddocks with a loose-housed sow and with increased environmental stimuli compared with indoor piglets. Outdoor piglets more often ingest solid feed preweaning compared with piglets reared indoors (Cox and Cooper 2001; Miller et al. 2007). Piglets reared outdoors have a broader explorative, social and feeding-related behavioural repertoire than indoor reared piglets (Johnson et al. 2001). The stimulus-rich outdoor environment may also stimulate the development of chewing, which is an important precursor of solid feed intake (Petersen 1994; Petersen et al. 1995), whereas participation with the sow during exploration and eating may also be beneficial for the development of feeding related behaviours through social learning processes, such as local enhancement and social facilitation (Galef and Giraldeau 2001).

The idea that the enriched rearing environment may have positively affected the development of feeding behaviour of piglets is supported by our finding that piglets housed in enriched pens preweaning showed greater feed intake in the period immediately after weaning (i.e., 48 h). The greater feed intakes postweaning may have been caused by an enhanced development of feeding-related behaviours, but the preweaning enrichment may also have increased adaptability of piglets to stressful processes such as weaning. Providing many stimuli early in life may result in reduced reactivity to unfamiliar stimuli later in life and decreased feed neophobia (Würbel 2001), thereby possibly increasing intake feed intake immediately after weaning.

Although feed intake in the first days after weaning was positively affected by preweaning environment, it was the enrichment in the postweaning pen that predominantly increased growth in the 2 wk after weaning. Moreover, postweaning enrichment reduced the occurrence of diarrhoea and increased feed efficiency. Feed intake in the first days postweaning was not greater for piglets housed in enriched postweaning pens, so the positive effects of postweaning enrichment may have been the result of properties of the enrichment that are unrelated to feed intake per se (e.g., content of the substrates provided and stimulation of the gut, stress, microclimate, and pen size). We can only speculate how these properties may have stimulated postweaning performance. The intake of straw as an energy source is an unlikely cause of the large difference in growth between barren and enriched housed pigs. Energy provided from straw intake seems to be insignificant, as demonstrated in pigs fed milled straw (Schrama and Bakker 1999). An effect of straw intake on the utilization of other dietary components, however, cannot be excluded (Staals et al. 2007). Furthermore, properties of the enrichment substrates, such as fibres, composition of microbiota, and an interaction between fibres and microbiota (Pluske et al. 2007), could have positively stimulated saliva production, the turnover of intestinal cells, and the maturation of the intestine of the growing pig, and thus gut health, leading to a reduced prevalence of diarrhoea, a greater nutrient absorption, and an increased growth (Jin et al. 1994; McCracken et al. 1999; Pluske et al. 1997; Popowics and Herring 2006).

Another important pathway by which environmental enrichment could potentially increase postweaning performance is through a reduction of stress. The weaning process consists of multiple stressors, such as a change in diet, a change in pen and social environment, and the separation from the sow (Weary et al. 2008). Stress negatively affects feed intake, and also impairs intestinal functioning, by inhibiting digestion and increasing intestinal permeability, which enhances the risk of intestinal inflammation and diarrhoea (Collins 2001; Moeser et al. 2007a; Soderholm and Perdue 2001). Enrichment of the pen is known to increase behavioural diversity in pigs of all ages, as it provides substrate for behaviours that could otherwise not be performed (Tuyttens 2005). The postweaning enrichment may have served as a distraction, thereby preventing stress-related behaviours and leading to decreased aggression and restlessness in the pen (Bolhuis et al. 2005a; Fraser et al. 1991). Piglets in the enriched postweaning pens could have created a more preferred microclimate due to the presence of straw, further reducing postweaning stress (Fraser 1985), though all piglets in the current experiment were housed at temperatures well within their thermo-neutral zone. The reduction of stress may thus have been important in reducing diarrhoea prevalence and thereby increasing postweaning growth, but it is very likely that this effect was strengthened by the potential positive effects of enrichment on gut functioning.

The effect of postweaning enrichment on growth may also have been caused by the differences in floor space allowance for the pigs. Several studies show that an increased density tends to leads to a reduction in performance in growing pigs, though the results are quite ambiguous because the studies differ in the number and the type of performance measurements that are affected (Brumm et al. 2004; Randolph et al. 1981; Turner et al. 2000). Also, it must be noted that the maximum space allowance provided in two of these studies is very close to or even less than the space allowance given to the barren piglets in this study postweaning (Brumm et al.: 0.8 m2 as maximum allowance vs. 0.8 m2 minimum allowance in the current study; Turner et al.: 32 kg/m2 as maximum allowance vs. 19 kg/m2 as minimum allowance at the end of the 2 wk period in the current study). Given the density ranges used in the present study, and the sufficient amount of feeding places provided, it is unlikely that performance was restrained by the smaller pen size in barren piglets compared with enriched piglets.

Few effects were found of housing condition of the sow during lactation in this study. Loose housing of the sow tended to increase preweaning growth, but did not have any beneficial effects on performance after weaning. The postweaning performance was, unexpectedly, predominantly affected by postweaning enrichment of the pen, independent of preweaning treatments, possibly overshadowing any effects of sow housing during lactation. Previous studies that used loose-housed sows found beneficial effects on performance and behaviour pre- and postweaning, but in general those studies used larger contrasts, such as indoor and outdoor rearing, of which increased opportunities to interact with the sow was just one of the many factors of which the treatments consisted (Cox and Cooper 2001; Gentry et al. 2002; Hötzel et al. 2004a; Johnson et al. 2001). Also, it must be noted that this study only describes measures of production performance and diarrhoea prevalence pre- and postweaning. It is very well possible that loose housing of the sow, and thus enabling piglets to learn more from their mother, has beneficial effects on behaviour and welfare of the piglets, as well as on sow welfare (Hötzel et al. 2004a; Verhovsek et al. 2007). Therefore, more research is needed before loose housing of the sow can be discarded as a valuable tool in increasing postweaning performance of piglets.

In conclusion, this study showed beneficial effects of an enriched preweaning environment, and to a lesser extent, of loose housing of the sow, on growth before weaning and feed intake after weaning of piglets, possibly mediated by preweaning feed intake. It seems, however, that especially enrichment of the pen postweaning is of great importance in improving performance and health of newly weaned piglets, which may be mediated by preserving gut functioning, either through intake of substrates or through stress reduction.

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04

EFFECTS OF ENVIRONMENTAL ENRICHMENT AND LOOSE HOUSING OF LACTATING SOWS ON PIGLET BEHAVIOUR BEFORE AND AFTER WEANING

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ABSTRACT

Weaning of piglets in commercial husbandry systems is earlier and more abrupt than would happen under natural conditions, resulting in low feed intakes and health and welfare problems in the immediate postweaning period. Piglets in commercial systems generally have limited opportunities to learn how to behave and eat like a pig. Interacting more with the sow, as well as providing enrichment, may stimulate the development of social and foraging related behaviours, and make piglets better able to adapt to the postweaning situation. Piglets were housed in a barren or enriched pen with a confined or loose-housed sow preweaning, and at weaning at d 29 were relocated to a barren or enriched pen postweaning (2x2x2 factorial arrangement, eight treatments, eight pens per treatments, four pigs per pen). Enrichment consisted of increased space allowance, straw, wood shavings, peat and branches. Behaviour was recorded on four days pre- (weekly) and postweaning (d 1, 5, 9 and 12). The development of social behaviours and foraging related behaviours was positively affected by enrichment of the lactation pen, but less by sow housing. Postweaning behaviour was most strongly affected by postweaning enrichment, with enriched housed piglets showing higher levels of exploratory behaviours (27 versus 10% of observations), lower levels of belly nosing (0.1 versus 1.0%) and manipulative behaviours (0.2 versus 1.5%), but also less time spent eating (8 versus 9%) than piglets in barren postweaning pens. Piglets from loose-housed sows showed lower levels of belly nosing (0.3 versus 0.7%) and manipulative behaviour (0.8 versus 0.9 %), as well as more chewing (6.1 versus 5.5 %), food exploration (1.0 versus 0.8 %) and play behaviour (0.9 versus 0.7%) postweaning than piglets from confined sows. Enrichment of the lactation pen in itself had few effects on postweaning behaviour, but interacted strongly with postweaning enrichment and sow housing, with piglets from a loose-housed sow switching

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weaning
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social learning

from a barren to enriched environment showing high levels of play behaviour while piglets from a confined sow switching from an enriched to a barren environment showed high levels of belly nosing and low levels of play (barren-loose-enriched: 1.8% of observations, enriched-confined-barren: 0.1% of observations).

Enrichment pre- and postweaning, as well as providing more possibilities to interact with the sow, can thus positively affect how piglet behaviour develops before weaning and how they adapt after weaning. It is important to realize that the preweaning environment, including sow housing, affects how piglets value their postweaning environment and may thus greatly affect piglet welfare around weaning.

INTRODUCTION

Weaning of piglets in commercial husbandry systems is, in contrast to that of wild piglets, quite abrupt and at an early age (Jensen and Stangel 1992). Many piglets have not yet consumed solid feed before weaning, and consequently often show a low feed intake in the immediate postweaning period (see Bolhuis et al. 2009 for review). The low feed intake leads to a reduced growth or even weight loss in the first days after weaning, as well as a high occurrence of diarrhoea (Pluske et al. 1997). The weaning process furthermore leads to a high incidence of maladaptive behaviours such as belly nosing and damaging of ears, tails and limbs of pen mates (Dudink et al. 2006; Torrey and Widowski 2006; Widowski et al. 2008). What causes the development of these typical behaviours is not fully understood, but getting piglets to eat solid food already at a young age may make piglets better able to adapt to the postweaning environment and food source, thereby reducing postweaning stress.

Several methods have been used to try to get piglets to eat more before weaning. Most studies involve optimization of the composition and palatability of piglet feed and presentation of the feed, which does result in a higher feed intake. These methods are not sufficient, however, to increase intake in all piglets (Appleby et al. 1992; Edge et al. 2004; Edge et al. 2005; Wattanakul et al. 2005). Management strategies such as intermittent suckling, multi-suckling and get-away systems have also been tried and were found in part to be successful too, but still not all piglets in the pen eat before weaning (Berkeveld et al. 2007; Svendsen and Svendsen 1997; Weary et al. 2002).

Learning from another animal what food types are safe to eat seems more efficient and less dangerous than trial-and-error learning (Nicol 2006) and may help piglets to increase their feed intake before weaning. The mother may be of particular interest for the young animal to obtain information from, as most of the young's attention is directed to the mother and mother and offspring are strongly genetically related (Galef and Giraldeau 2001; Thorhallsdottir et al. 1990). Currently, there are not many possibilities for social learning from the mother in standard lactation pens, as sows are generally confined to a farrowing crate, the sow trough is often inaccessible for piglets and sow and piglets eat different types of food at different locations in the pen. Providing piglets with the opportunity to eat together with their sow when she is loose housed in the lactation pen resulted in a higher preweaning growth, most likely due to a higher solid feed intake (Oostindjer et al. 2010b). Being able to sample novel foods together with the sow reduced food neophobia and increased intake of these novel foods compared to when the sow was absent (Oostindjer et al. 2011). The beneficial effects of providing possibilities for social learning on food intake and neophobia may be even larger when sow and piglets are also housed in an enriched environment, as providing substrate will broaden the behavioural repertoire of the sow (Tuyttens 2005). This enables piglets to obtain more information from the sow than when sow and piglets are housed in a barren environment.

Providing piglets with an enriched environment before weaning may, independent of learning from the sow, increase the development of foraging-related behaviours and can thereby positively affect feed intake preweaning. Chewing, for example, is necessary to properly process solid feed, and providing substrate on which piglets can chew, may stimulate the development of muscles important for chewing, as well as the chewing behaviour itself, which allows piglets to better adapt to ingesting solid feed (Herring 1985; Popowics and Herring 2006). Also, providing enrichment in the lactation pen, and thus increasing the number of stimuli the animals experience, may enhance behavioural flexibility of piglets and reduce fear of novelty, which could positively affect how piglets deal with the weaning stress (Chaloupkova et al. 2007; Oostindjer et al. 2011; Petersen et al. 1995). Similarly, providing enrichment after weaning can potentially make the weaning process easier for piglets as it can provide distraction and has the potential to blunt the cortisol response to weaning (Beattie et al. 2000; Dudink et al. 2006; Fraser et al. 1991; Moncek et al. 2004). Providing substrate results in less damaging behaviours later in life as it provides pigs with material to perform certain behaviours on that might otherwise be performed on pen mates, such as chewing on tails and ears (Bolhuis et al. 2005a).

The aim of this study was to investigate how sow housing (confined or loose), environment in the lactation pen (barren or enriched) and postweaning environment (barren or enriched) affect behavioural development before and after weaning. The focus was two-fold: focus on social behaviours, as maladaptive behaviours reflect reduced welfare of piglets after weaning, and focus on foraging-related behaviours, as it is important that those behaviours are developed properly to successfully make the transition from milk to solid feed. Our hypothesis was that having a loose-housed sow and an enriched pen will have positive effects on both foraging-related and social behaviours, but that the combination of preweaning enrichment, loose housing of the sow and postweaning enrichment will be optimal for behavioural development of piglets, resulting in more adaptive behaviours in the early postweaning period.

MATERIALS AND METHODS

The Animal Care and Use committee of Wageningen University approved the experiment. The experiment was set up in a 2x2x2 factorial arrangement regarding pen treatments, with preweaning enrichment (barren or enriched), sow housing (sow confined in farrowing crate or loose-housed sow with more sow-piglet interactions) and postweaning enrichment (barren or enriched) as factors. In total there were 64 postweaning pens, eight per treatment, with each pen containing four piglets that were unfamiliar to each other. The experiment was carried out in two successive batches.

Animals and housing preweaning

A total of 32 multiparous sows and their piglets (Tempo x Topigs) were used in the experiment. These sows were housed in barren pens in all gestations and lactations before the experiment, but received approximately 50 grams of straw daily while pregnant of the litters used in this experiment. Sows were housed individually in either a barren (B, N=16) or enriched (E, N=16) farrowing pen one week before farrowing. Barren pens (9.2 m2) consisted partly of solid (65%) and party of slatted (35%) floors and contained no substrate, except for a small amount of sawdust (approximately 5L) in the first 24 h after farrowing. The slats were covered with mats the first four days after farrowing. Enriched pens (18.4 m2) contained wood shavings (550 L per pen, covering 65% of the floor) and peat (400 L per pen, covering 35% of the floor). Branches (3 large or 5 small ones per pen, none longer than 2 metres) and straw (1 kg/pen) were provided on top of the other substrates. Fresh straw (0.5 kg) and wood shavings (70 L) were provided to enriched pens daily, peat (40 L) was added to the pen once a week, and branches were replaced twice per week. Enriched pens were larger than barren pens as part of the enrichment since increased space allowance can increase the expression of exploratory behaviour and locomotion (Bolhuis et al. 2005a).

All sows were confined in a farrowing crate between d 0 and 4 after farrowing. The farrowing crate was removed in half of the B and E pens on d 4 (loose-housed sows, L, N=16) and remained in place in the other half of the pens (confined sows, C, N=16). Each pen contained a barred platform with a heating lamp placed above it. The platform was reachable for piglets but not for the sow in order to prevent crushing of piglets. Assignment of sows to either barren or enriched pens or to loose-housing or confinement was balanced for parity.

The platform in each pen contained a family feeder (Verbakel B.V., Sint Oedenrode, The Netherlands, see Oostindjer et al. 2010b for photo), with a compartment for the sow and a compartment for the piglets. The sow compartment was on the outside of the barred piglet platform and the piglet compartment was on the inside to prevent sows from eating from the piglet compartment, while piglets could access both compartments. The piglet compartment of the feeder was shallow but levelled with the top of the sow compartment to allow piglets to observe the sows in the L group while eating. L-sows were fed twice a day in the family feeder and C-sows were fed twice a day in a standard trough in the farrowing crate, which was raised and screened-off from piglets to avoid piglets eating together with the sow. When feed was given to the sows, a small amount of feed was also given in the piglet compartment in all treatments. Piglets in all treatments had feed available continuously in the piglet compartment of the family feeder from d 4 onwards. Sows and piglets both were fed standard feed for lactating sows to maximize the transfer of information from sow to piglet. The feed was mixed in the last 6 d before weaning with a standard piglet feed which was fed to the piglets after weaning (Oostindjer et al. 2010b). All pens had standard drinking nipples available for sows and piglets and water was continuously available.

Litter sizes were standardized to 10 piglets per litter on d 4 with minimum use of cross fostering and with a 1:1 sex ratio when possible. Four piglets died in the EL treatment, one in the EC treatment and one in the BC treatment after d 4 of lactation (four due to crushing). Pens were divided into two observation groups within each batch to correct for differences in farrowing time: an early and late group, with a difference of 3 d. Each group contained 2 pens from each preweaning treatment combination. The preweaning observations described below were done on the average age of the respective observation group. A personality test (Bolhuis et al. 2004) was performed on day 10 of age to classify piglets as High resisters (active coping in response to stressors) or Low resisters (passive coping), which may predict the response to weaning. Lights were on between 07.00 and 19.00 h and were dimmed during the night. Room temperature during lactation was maintained at a minimum of 26 °C around farrowing and thereafter gradually decreased to 22 °C.

Animals and housing postweaning

Piglets were weaned at 29.2 (SD=2.7) d of age, between 09.00 and 12.00 h. Four piglets from each litter were allocated to enriched postweaning pens and four to barren postweaning pens, with allocation to B and E pens balanced for sex, backtest classification and weaning weight (see Oostindjer et al. 2010b). Piglets with a birth weight less than 0.9 kg, weighing less than 5.5 kg two days before weaning and piglets with a history of leg problems were excluded from selection. Average weaning weight of piglets allocated to barren and enriched pens postweaning was equal within preweaning treatments. Average within-pen variation in weight was equal for all treatments. Piglets were mixed at weaning with piglets from other litters from the same preweaning treatment.

Postweaning barren pens (3.2 m2) had a 65% solid and 35% slatted floor and contained no substrate. Enriched pens (6.4 m2) were provided with straw (0.5 kg), wood shavings (185 L), peat (135 L) and three branches. Fresh wood shavings (70 L) and straw (0.5 kg) were added to enriched pens daily, and peat

(40 L) was added to the pen once a week. Branches were replaced two times per week. Barren and enriched pens were evenly distributed within the room.

All pens contained two feeders with two and three feeding spaces, respectively. Feeders contained the same feed as provided in the last days of lactation. Every pen had two drinking nipples available and water was continuously available. Room temperature was maintained at a minimum of 25 °C in the first four days postweaning and thereafter gradually decreased by 1 °C every two days to 22 °C. Lights were fully lit between 07.00 h and 19.00 h and dimmed during the night.

Behavioural observations

Behaviour in the lactation pen was scored using live 2-min instantaneous scan sampling for 6h per day during six 1-hour sessions on d 7, 14, 21 and 28 of lactation. Observation hours started at 8.00, 9.15, 10.30, 14.00, 15.15 and 16.30 h. Behaviour in the postweaning pen was collected on d 1, 5, 8 and 12 postweaning. Data were collected using the Psion Workabout MX with the Observer 5.0 (Noldus Information Technology B.V., Wageningen, The Netherlands) installed on it. The behaviours of interest are listed in *[table 1]*. Activity was determined as all behaviours showing an active posture, thus excluding sitting, lying or sleeping.

table 1.

behaviour	description
Social behaviour	
Nosing pen mate	Touching or sniffing any part of the head or body of a pen mate
Aggressive behaviour	Ramming or pushing pen mate, with or without biting
Mounting	Standing on back of pen mate with front legs
Belly nosing	Rubbing belly of a pen mate with up and down movements of the snout
Manipulative behaviour	Nibbling, sucking or chewing part of the body of a pen mate
Foraging-related behaviour	
Exploring floor	Sniffing, touching, scraping the leg or rooting (substrate on) floor
Exploring fixtures pen	Sniffing, touching, chewing or rooting part of the pen above floor level
Chewing air/substrate	Chewing on straw or wood shavings or air
Exploring food	Sniffing, touching, rooting or chewing any food without sampling
Eating food	Eating or chewing any food
Drinking	Drinking from water nipple
Other behaviour	
Play	Shaking head while having substrate in mouth, rolling, pivoting, gambolling
Activity	All behaviour minus sleeping, lying and sitting.
Suckling	Massaging the udder and suckling (preweaning only)

Behaviours of interest, both pre- and postweaning.

Statistical analyses

All data were analysed using mixed linear models in SAS (SAS 9.0, SAS Institute Inc.). Behaviours were analysed as relative frequencies and arcsine-square root transformed if residuals were not normally distributed. Effects of enrichment and sow housing on preweaning behaviours were analysed with a model including lactation enrichment, sow housing and their interaction as main factors, as well as batch. The litter (i.e. the sow with which the piglets were reared) was included as a random factor. Behaviours postweaning and their development over time were analysed with a mixed linear model including lactation enrichment, sow housing, postweaning enrichment, week and their interactions, as well as batch. Postweaning pen was included as a random effect. Post-hoc pair wise comparisons were made using the least-square means, corrected for multiple comparisons with a Tukey adjustment. Data from the mixed linear models are presented as (untransformed) mean ± SEM based on pen averages.

RESULTS

Preweaning behaviour

Means and significances of preweaning enrichment and sow housing effects on preweaning behaviour are presented in *[table 2]*. Most social behaviours were unaffected by enrichment, with the exception of belly nosing and manipulative behaviour, both of which were lower in enriched pens than in barren pens (belly nosing: F1,27 = 12.6, P=0.002; manipulative behaviour: F1,27 = 67.6, P < 0.001). No effects of enrichment or sow housing were found on nosing pen mates, mounting or aggression (all P-values > 0.1).

Almost all foraging-related behaviours were affected by enrichment. Enriched piglets showed more chewing (F1,27 = 210.8, P < 0.001) and explored the floor more (F1,27 = 26.2, P < 0.0001), while barren piglets explored fixtures in the pen more (F1,27 = 5.1, P = 0.03) and were seen eating food more often (F1,27 = 8.0, P = 0.009) than enriched-housed piglets. Barren-housed piglets explored food more often than enriched piglets (F1,27 = 22.2, P <0.001), as did piglets from a loose-housed sow (F1,27 = 4.4, P = 0.05), mainly due to high food exploration of barren piglets with a loose-housed sow (enrichment x sow housing, F1,27 = 6.2, P = 0.02). Piglets housed with a loose-housed sow tended to explore fixtures in the pen less than piglets with a confined sow (F1,27 = 3.2, P = 0.09) and tended to explore the floor less (F1,27 = 3.5, P = 0.07), but did not differ in chewing on substrate from piglets housed with a confined sow (P = 0.34). Sow housing did not affect eating (P = 0.79). Drinking water was unaffected by both enrichment and sow housing (P > 0.21).

Play behaviour was higher in enriched housed piglets than in barren housed piglets (F1,27 = 17.6, P <0.001) but unaffected by sow housing. Activity was also higher in enriched piglets (F1,27 = 6.2, P = 0.02) and in piglets with a confined sow (F1,27 = 3.7, P = 0.04). Suckling was unaffected by enrichment or sow housing (all P > 0.37).

table 2.

	treatment				significance[1]		
behaviour (% of observations)	BC	BL	EC	EL	enrichment	sow housing	enrich x sow
social							
nosing pen mate	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.2 ± 0.1	ns	ns	ns
aggression	0.7 ± 0.05	0.8 ± 0.05	0.8 ± 0.05	0.5 ± 0.04	ns	ns	ns
mounting	0.2 ± 0.03	0.3 ± 0.03	0.3 ± 0.03	0.2 ± 0.03	ns	ns	ns
belly nosing	0.04 ± 0.008	0.06 ± 0.010	0.01 ± 0.005	0.01 ± 0.003	**	ns	ns
manipulation	0.4 ± 0.04	0.5 ± 0.04	0.2 ± 0.02	0.1 ± 0.02	***	ns	ns
foraging-related							
exploration floor	8.1 ± 0.3	7.6 ± 0.3	14.8 ± 0.4	10.9 ± 0.3	***	#	ns
exploration pen	2.5 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	1.4 ± 0.1	*	#	ns
chewing	0.7 ± 0.05	0.7 ± 0.04	2.9 ± 0.12	2.6 ± 0.11	***	ns	ns
exploration food	0.6 ± 0.04	0.9 ± 0.05	0.4 ± 0.04	0.4 ± 0.03	***	*	*
eating food	2.1 ± 0.1	2.2 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	***	ns	ns
drinking	0.1 ± 0.01	0.1 ± 0.1	0.2 ± 0.04	0.1 ± 0.02	ns	ns	ns
other							
play	1.1 ± 0.1	1.1 ± 0.1	2.1 ± 0.1	1.6 ± 0.1	***	ns	ns
activity	40.0 ± 0.7	38.1 ± 0.6	48.0 ± 0.7	40.4 ± 0.6	*	*	ns
suckling	15.2 ± 0.3	14.2 ± 0.3	15.4 ± 0.4	14.3 ± 0.3	ns	ns	ns

Social behaviours, foraging related behaviours and other behaviours in the first 4 weeks after birth for piglets in a barren pen with confined sow (BC), barren pen with loose-housed sow (BL), enriched pen with confined sow (EC) and enriched pen with loose-housed sow (EL).

[1] Effects of enrichment of the lactation pen, sow housing and their interaction are indicated: *** P<0.001, ** P<0.01, *P<0.05, # P<0.1, ns=not significant

postweaning behaviour

Overall effects

The effects of preweaning enrichment, sow housing and postweaning enrichments on postweaning behaviour as average over all observation days are presented in *[table 3]*.

table 3.

	treatment								significance						
behaviour (% of observations)	BCB	BCE	BLB	BLE	ECB	ECE	ELB	ELE	LE	SH	PE	LExSH	LExPE	SHxPE	LExSHxPE
social															
nosing pen mate	2.4 ± 0.1	1.3 ± 0.1	2.4 ± 0.2	1.3 ± 0.1	2.3 ± 0.2	1.8 ± 0.1	2.2 ± 0.1	1.2 ± 0.1	ns	ns	***	*	*	ns	#
aggression	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	ns	ns	ns	*	ns	ns	ns
mounting	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	*	ns	ns	ns	ns	ns	ns
belly nosing	1.0 ± 0.2	0.1 ± 0.03	0.9 ± 0.2	0.03 ± 0.01	1.6 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	0.07 ± 0.02	ns	*	***	#	ns	ns	ns
manipulation	1.8 ± 0.2	0.2 ± 0.03	1.4 ± 0.1	0.2 ± 0.04	1.4 ± 0.1	0.2 ± 0.04	1.2 ± 0.1	0.2 ± 0.03	ns	#	***	ns	#	ns	ns
foraging-related															
exploration floor	9.4 ± 0.4	25.3 ± 0.9	7.7 ± 0.3	25.8 ± 0.7	6.8 ± 0.4	20.6 ± 0.8	6.2 ± 0.3	21.2 ± 0.6	***	ns	***	ns	ns	ns	ns
exploration pen	3.0 ± 0.2	3.7 ± 0.2	3.3 ± 0.2	3.8 ± 0.2	2.0 ± 0.2	3.4 ± 0.2	2.7 ± 0.2	3.4 ± 0.2	*	ns	***	ns	ns	ns	ns
chewing	1.9 ± 0.1	10.6 ± 0.4	1.6 ± 0.1	10.9 ± 0.4	1.3 ± 0.1	8.3 ± 0.4	2.0 ± 0.1	9.8 ± 0.4	*	#	***	*	#	ns	ns
exploration food	1.0 ± 0.1	0.7 ± 0.1	1.4 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	ns	*	***	ns	ns	#	ns
eating food	8.9 ± 0.3	8.8 ± 0.3	9.1 ± 0.4	7.1 ± 0.3	8.5 ± 0.4	7.9 ± 0.3	9.3 ± 0.1	8.4 ± 0.1	ns	ns	#	ns	ns	ns	ns
drinking	1.3 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	2.3 ± 0.2	1.4 ± 0.1	ns	ns	ns	ns	ns	*	ns
other															
play	0.5 ± 0.1	1.2 ± 0.1	0.4 ± 0.1	1.8 ± 0.1	0.1 ± 0.03	1.0 ± 0.1	0.3 ± 0.04	1.2 ± 0.1	***	*	***	ns	ns	#	*
activity	41.4 ± 0.9	61.3 ± 1.3	38.7 ± 0.9	61.8 ± 0.8	35.0 ± 0.9	55.2 ± 1.2	35.3 ± 1.0	54.7 ± 1.0	***	ns	***	ns	ns	ns	ns

Effects of enrichment of the lactation pen (LE), sow housing (SH), enrichment of the postweaning pen (PE) and their interactions on percentage of time spent on social behaviours, foraging related behaviours and other behaviours, expressed as % of observations, in the first 2 weeks after weaning for piglets from a barren lactation pen with confined sow in a postweaning barren pen (BCB), barren lactation pen with confined sow in a postweaning enriched pen (BCE), barren lactation pen with loose-housed sow in a postweaning barren pen (BLB), barren lactation pen with loose-housed sow in a postweaning enriched pen (BLE), enriched lactation pen with confined sow in a postweaning barren pen (ECB), enriched lactation pen with confined sow in a postweaning enriched pen (ECE), enriched lactation pen with loose-housed sow in a postweaning barren pen (ELB), enriched lactation pen with loose-housed sow in a postweaning enriched pen (ELE). *** P<0.001, *P<0.05, # P<0.1, ns=not significant

Social behaviour. Mounting was higher for piglets coming from a barren lactation pen than from an enriched lactation pen (lactation enrichment, F1,55 = 4.7, P = 0.03). Belly nosing and manipulative behaviour were both higher for piglets from a confined sow than from a loose-housed sow (belly nosing: F1,55 = 4.6, P = 0.04; manipulation: F1,55 = 4.0, P = 0.05). Piglets in the EC preweaning treatment spent more time nosing pen mates than piglets in EL preweaning treatment (BC: 1.9 ± 0.1, BL: 1.9 ± 0.2, EC: 2.1 ± 0.2, EL: 1.7 ± 0.1, lactation enrichment x sow housing: F1,55 = 4.8, P = 0.03). EC piglets also spent more time fighting than BC piglets (BC: 0.4 ± 0.1, BL: 0.6 ± 0.1, EC: 0.7 ± 0.1, EL: 0.5 ± 0.1, lactation enrichment x sow housing, F1,55 = 6.5, P = 0.01), and tended to spend more time belly nosing than EL piglets (BC: 0.6 ± 0.1, BL: 0.5 ± 0.1, EC: 0.9 ± 0.1, EL: 0.2 ± 0.1, lactation enrichment x sow housing: F1,55 = 2.8, P = 0.1).

Piglets in barren postweaning pens spent more time nosing pen mates than piglets in enriched pens (postweaning enrichment: F1,55 = 70.7, P < 0.001). Belly nosing and manipulative behaviour were higher for piglets housed in barren pens compared to piglets housed in enriched pens postweaning (belly nosing: F1,55= 28.8, P < 0.001; manipulation: F1,55 = 299.2, P < 0.001). Piglets that moved from a barren preweaning pen to an enriched pen postweaning showed low levels of nosing pen mates (BE: 1.3 ± 0.1), while piglets moving from an enriched preweaning pen to a barren postweaning pen and piglets staying in a barren environment showed particularly high levels of nosing pen mates (BB: 2.4 ± 0.1, EB: 2.3 ± 0.1), with levels of piglets that were housed in enriched environments both pre- and postweaning in between (EE: 1.5 ± 0.1, lactation enrichment x postweaning enrichment: F1,55 = 4.3, P = 0.04). The effect of postweaning enrichment on manipulative behaviour tended to be smaller for piglets coming from an enriched lactation pen than for piglets from a barren lactation pen (BB: 1.3 ± 0.1, BE: 0.1 ± 0.02, EB: 1.0 ± 0.1, EE: 0.2 ± 0.03, lactation enrichment x postweaning enrichment: F1,55 = 3.4, P = 0.07).

Foraging-related behaviour. Piglets from a barren lactation pen spent more time exploring the floor and the pen fixtures of the postweaning pen than piglets from an enriched lactation pen (floor: lactation enrichment, F1,55 = 18.7, P < 0.001, pen fixtures: lactation enrichment, F1,55 = 5.9, P = 0.02). Piglets from a barren lactation pen also chewed more than piglets from an enriched lactation pen (F1,55 = 5.9, P = 0.02). Piglets from a loose-housed sow tended to chew more than piglets from a confined sow (F1,55 = 3.3, P = 0.07) and spent more time exploring food (F1,55 = 4.8, P = 0.03). Piglets from the EC preweaning treatment chewed less than piglets from the other preweaning treatments (BC: 6.3 ± 0.3, BL: 6.3 ± 0.3, EC: 4.8 ± 0.3, EL: 5.9 ± 0.3, lactation enrichment x sow housing, F1,55 = 4.6, P = 0.04).

Piglets in an enriched postweaning pen spent more time exploring the floor and pen fixtures than piglets in a barren postweaning pen (floor: postweaning enrichment, F1,55 = 407.6, P < 0.001, pen fixtures: postweaning enrichment, F1,55 = 13.3, P < 0.001) and spent more time chewing (F1,55 = 679.1, P < 0.001). Food exploration levels were higher for piglets in a postweaning enriched pen than for piglets in a postweaning barren pen (F1,55 = 21.9, P < 0.001), but time spent eating tended to be higher in postweaning barren pens than in postweaning enriched pens (F1,55 = 3.5, P = 0.07). Levels of chewing tended to be especially high when the postweaning enriched piglets came from a barren lactation pen (BB: 1.8 ± 0.2, BE: 10.8 ± 0.5, EB: 1.6 ± 0.1, EE: 9.0 ± 0.5, lactation enrichment x postweaning enrichment, F1,55 = 3.4, P = 0.07). Food exploration tended to be particularly high in piglets from a loose-housed sow in a postweaning barren pen (CB: 0.9 ± 0.1, CE: 0.6 ± 0.1, LB: 1.3 ± 0.1, LE: 0.7 ± 0.1, sow housing x postweaning enrichment, F1,55 = 2.9, P = 0.09), as was time spent drinking (CB: 1.6 ± 0.1, CE: 1.7 ± 0.1, LB: 2.2 ± 0.2, LE: 1.6 ± 0.1, sow housing x postweaning enrichment, F1,55 = 4.8, P = 0.03).

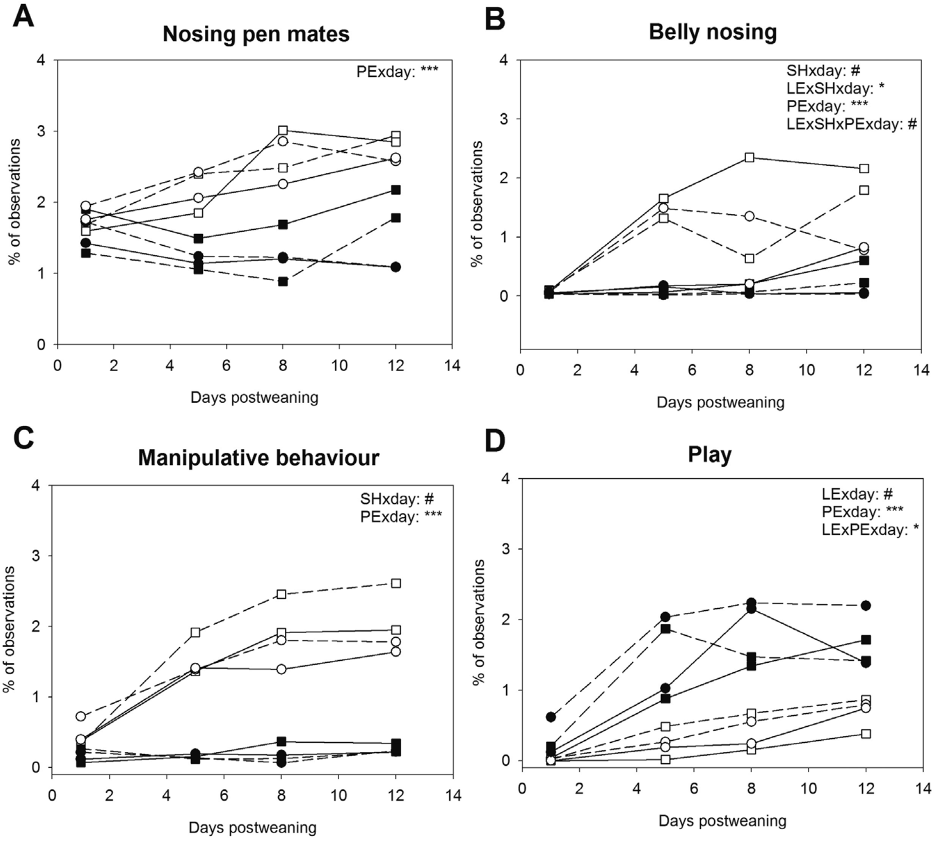
Other behaviour. Piglets from a barren lactation pen played more after weaning than piglets from an enriched lactation pen (F1,55 = 24.1, P < 0.001), and were more active after weaning (F1,55 = 12.8, P < 0.001). Play was also higher for piglets from a loose-housed sow compared to piglets from a confined sow (F1,55 = 5.5, P = 0.02) and piglets in an enriched postweaning pen compared to piglets in a barren postweaning pen (F1,55 = 160.8, P < 0.001). Activity was also higher for piglets housed in an enriched pen after weaning (F1,55 = 157.8, P < 0.001). Play behaviour was higher for piglets from a loose-housed sow that were housed in an enriched pen than for piglets from a loose-housed sow that were housed in a barren postweaning pen (CB: 0.3 ± 0.05, CE: 1.1 ± 0.1, LB: 0.4 ± 0.1, LE: 1.5 ± 0.1, sow housing x postweaning enrichment, F1,55 = 3.3, P = 0.08), and was particularly high in BLE and particularly low in ECB piglets (BCB: 0.5 ± 0.1, BCE: 1.2 ± 0.1, BLB: 0.4 ± 0.1, BLE: 1.8 ± 0.1, ECB: 0.1 ± 0.03, ECE: 1.0 ± 0.1, ELB: 0.3 ± 0.04, ELE: 1.2 ± 0.1, lactation enrichment x sow housing x postweaning enrichment, F1,55 = 4.3, P = 0.04).

Behavioural postweaning patterns over time

The temporal patterns of social behaviours and play behaviour in the first two weeks postweaning are shown in *[figure 1]*. Nosing pen mates was higher in postweaning barren pens but only from d 5 onwards (postweaning enrichment x day: F3,168 = 6.3, P < 0.001, *[figure 1A]*). Aggression was higher in barren postweaning pens on day 1 postweaning (barren: 1.15 ± 0.22, enriched: 0.61 ± 0.15% of observations) and higher in enriched postweaning pens on d 5 postweaning (barren: 0.23 ± 0.07, enriched: 0.40 ± 0.10% of observations, postweaning enrichment x day, F3,168 = 6.1, P < 0.001). Mounting tended to be higher for piglets in enriched pens on d 1 postweaning (barren: 0.83 ± 0.22, enriched: 1.31 ± 0.27 % of observations), but not on other days (postweaning enrichment x day, F3,168 = 2.5, P = 0.06). Belly nosing increased over time for postweaning barren piglets, with postweaning barren piglets showing higher levels on d 5, 8 and 12 postweaning (postweaning enrichment x day, F3,168 = 8.9, P < 0.001, *[figure 1B]*), with the exception of the ELB piglets (lactation enrichment x sow housing x postweaning enrichment x day, F3,168 = 2.2, P = 0.09). Belly nosing tended to be lower for piglets housed with a loose-housed sow preweaning on d 8 and 12 (sow housing x day, F3,168 = 2.4, P = 0.07), especially for piglets housed in preweaning enriched pens (lactation enrichment x sow housing x day, F3,168 = 2.9, P = 0.04). Manipulative behaviour increased only in barren postweaning pens and was practically absent on all observation days in enriched postweaning pens (postweaning enrichment x day, F3,168 = 23.6, P < 0.001, *[figure 1C]*), and tended to be lower for piglets from a loose-housed sow on d 8 postweaning (sow housing x day, F3,168 = 2.5, P = 0.06). Play behaviour increased more strongly in postweaning enriched pens over time (postweaning enrichment x day, F3,168 = 9.3, P < 0.001). Preweaning barren piglets tended to show more play behaviour on d 1, 5 and 8 postweaning (lactation enrichment x day, F3,168 = 2.1, P = 0.1, *[figure 1D]*), particularly those preweaning barren piglets that were housed in a postweaning enriched pen, while preweaning enriched piglets housed in postweaning barren pens showed the least play behaviour, with low levels on all days (lactation enrichment x postweaning enrichment x day, F3,168 = 2.8, P = 0.04).

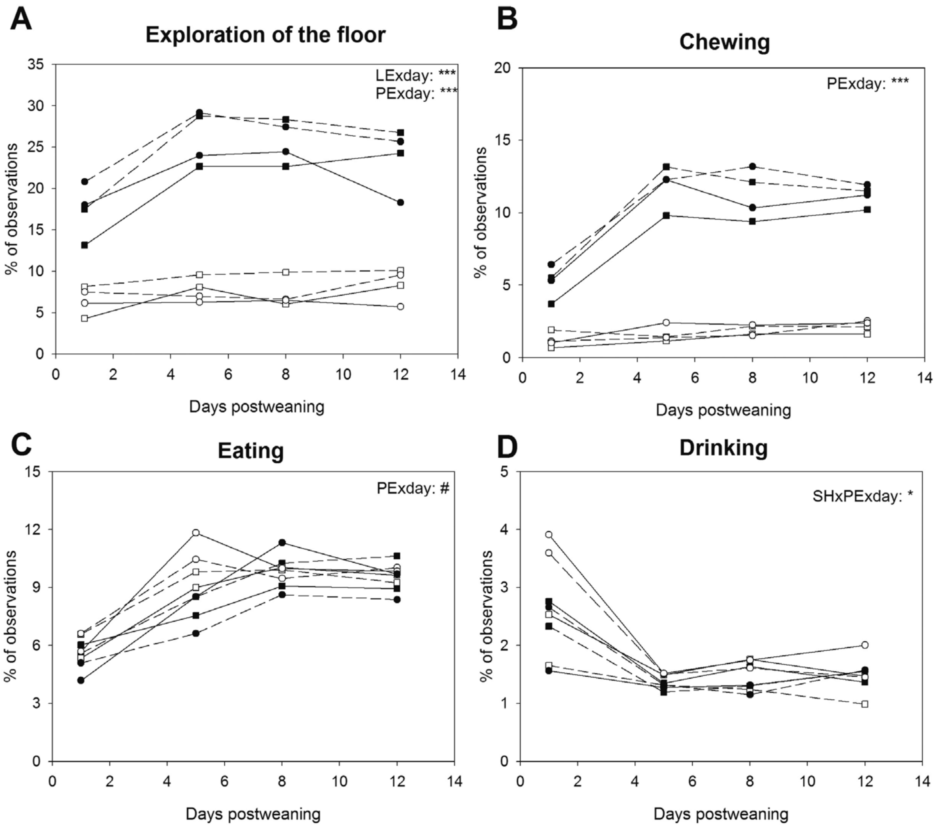
The temporal patterns of foraging related behaviours are shown in *[figure 2]*. Exploration of the floor of the pen was higher for piglets from preweaning enriched pens and remained higher compared to piglets in barren postweaning pens (lactation enrichment x day, F3,168 = 18.7, P < 0.001, *[figure 2A]*). Exploration of the floor was also higher in postweaning enriched pens, and increased between d 1 and 5 for enriched pens only (postweaning enrichment x day, F3,168 = 407.6, P < 0.001). Chewing increased for postweaning enriched piglets only (postweaning enrichment x day, F3,168 = 20.2, P < 0.001, *[figure 2B]*). Eating tended to be higher for piglets in barren postweaning pens on d 5 postweaning (postweaning enrichment x day, F3,168 = 2.6, P = 0.06, *[figure 2C]*), while drinking tended to be higher for piglets from a loose-housed sow in barren postweaning pens than for piglets from a confined sow in a barren postweaning pen on d 1 postweaning (sow housing x postweaning enrichment x day, F3,168 = 2.7, P = 0.05, *[figure 2D]*). Exploration of the pen and exploration of food were unaffected by any treatment x day interactions. Activity of piglets increased over time, especially after d 1, for postweaning enriched piglets while activity slightly decreased for postweaning barren piglets (postweaning enrichment x day, F3,168 = 8.6, P < 0.001, barren: 40.6, 37.3, 34.4, 38.0 % of observations, enriched: 52.0, 61.2, 59.8, 60.0 % of observations on d 1, 5, 8 and 12, respectively).

figure 1.



Effects of enrichment of the lactation pen (LE), sow housing (SH), enrichment of the postweaning pen (PE) and their interactions with observation day on percentage of time spent on social behaviours and play behaviour expressed as % of observations, in the first 2 weeks after weaning for piglets from a barren lactation pen with confined sow in a postweaning barren pen (BCB, open square, dotted line), barren lactation pen with confined sow in a postweaning enriched pen (BCE, closed square, dotted line), barren lactation pen with loose-housed sow in a postweaning barren pen (BLB, open circle, dotted line), barren lactation pen with loose-housed sow in a postweaning enriched pen (BLE, closed circle, dotted line), enriched lactation pen with confined sow in a postweaning barren pen (ECB, open square, solid line), enriched lactation pen with confined sow in a postweaning enriched pen (ECE, closed square, solid line), enriched lactation pen with loose-housed sow in a postweaning barren pen (ELB, open circle, solid line), enriched lactation pen with loose-housed sow in a postweaning enriched pen (ELE, closed circle, solid line). *** P<0.001, *P<0.05, # P<0.1.

figure 2.



Effects of enrichment of the lactation pen (LE), sow housing (SH), enrichment of the postweaning pen (PE) and their interactions with observation day on percentage of time spent on foraging related behaviours, expressed as % of observations, in the first 2 weeks after weaning for piglets from a barren lactation pen with confined sow in a postweaning barren pen (BCB, open square, dotted line), barren lactation pen with confined sow in a postweaning enriched pen (BCE, closed square, dotted line), barren lactation pen with loose-housed sow in a postweaning barren pen (BLB, open circle, dotted line), barren lactation pen with loose-housed sow in a postweaning enriched pen (BLE, closed circle, dotted line), enriched lactation pen with confined sow in a postweaning barren pen (ECB, open square, solid line), enriched lactation pen with confined sow in a postweaning enriched pen (ECE, closed square, solid line), enriched lactation pen with loose-housed sow in a postweaning barren pen (ELB, open circle, solid line), enriched lactation pen with loose-housed sow in a postweaning enriched pen (ELE, closed circle, solid line). *** P<0.001, *P<0.05, # P<0.1.

DISCUSSION

This study investigated the effects of pre- and postweaning enrichment and loose housing of the sow on the development of social and foraging-related behaviours of piglets before and after weaning. The results show that all three factors affect both social and foraging-related behaviours, with preweaning treatments still affecting behaviour postweaning.

Preweaning behaviour

Enrichment of the lactation pen reduced belly nosing and manipulative behaviour before weaning and increased exploration, chewing, play behaviour and activity. It also decreased time spent exploring feed and eating, but growth before weaning was higher in enriched-housed piglets (Oostindjer et al. 2010b), suggesting that piglets in barren pens may have used the feed to perform exploratory behaviours or that these piglets may have been less efficient in processing feed or had a different intake rate than piglets from enriched pens. The effects of enrichment on behaviour are not unexpected, as enrichment, when provided as substrate, increases the behavioural repertoire and gives animals the opportunity to perform some behaviours, such as chewing, better than when there is no substrate (Fraser et al. 1991; Petersen 1994; Petersen et al. 1995; Wilson and Green 2009). The novelty of substrates, as they were provided to the pen regularly, could have increased arousal, leading to higher levels of play behaviour, exploration and overall activity (Dudink et al. 2006; Wood-Gush and Vestergaard 1991).

Loose housing of sows reduced overall activity but increased food exploration preweaning. It is likely that the loose-housed sows' behaviour directed piglet behaviour towards the feeder and food. Social facilitation and stimulus enhancement are likely important learning mechanisms (De Castro 1994; Galef and Giraldeau 2001; Oostindjer et al. In press) and may positively affect behavioural development by stimulating behaviours towards proper stimuli at the proper time, making behaviour more targeted. Loose housing of sows also may have had positive effects on bonding between mother and piglets, and on synchronization of behaviour, which in turn can positively affect the development of adaptive social skills such as knowing when to stop a fight or responding to unwanted behaviours (Feldman 2007; Pettit and Harrist 1993). Providing piglets and sow with more possibilities to interact would thus have been likely to positively affect piglet behavioural development. Evidence for this can be found postweaning. Piglets from loose-housed sows showed lower and less sharply increasing levels of belly nosing and manipulation, more chewing, food exploration and play behaviour postweaning, suggesting that the sow had profound effects on the development of both foraging-related and social behaviours.

There were few interactions between sow housing and environmental enrichment on preweaning behaviour. Studies that compared outdoor-housed sows and their litters with indoor-housed sows should be in part comparable to the piglets with a barren-confined sow and piglets with an enriched-loose sow in the current study, yet the results are different. Piglets housed outdoors with their sow spend more time eating, exploring their environment and playing and seem to perform less damaging behaviours before weaning (Cox and Cooper 2001; Hötzel et al. 2004b; Johnson et al. 2001). Uncontrollable environmental stimuli such as temperature, moisture, enclosure size and also differences in sow behaviour can all affect behaviour, however (see for example Lopez et al. 1991; Lynch 1977), making it very hard to compare outdoor rearing with piglets reared indoors in an environmentally enriched pen with a loose-housed sow. Either way, it was expected that sows housed outside a crate in an enriched environment would be able to demonstrate the largest set of behaviours out of all treatments, yet we found very few interactions between preweaning enrichment and sow housing effects on behaviour before weaning. Sow behaviour, such as maternal care or even bonding between piglets and sows (Herskin et al. 1998), was likely affected by the enrichment and loose housing and could have affected piglet behaviour as well (Thodberg et al. 1999). It is thus not unlikely that, besides independent stimulation by enrichment and increased opportunities to interact with the mother, the enrichment may have facilitated better maternal care or bonding, thus leading to an improved behavioural development of enriched-housed piglets with a loose-housed sow. These effects do not become visible, however, until piglets are weaned, indicating that these sow housing effects are more related to the “behavioural baggage” that piglets obtain before weaning, which then may affect how the piglets deal with the stressors around weaning. Indeed, we found some lactation pen enrichment and sow housing interaction effects after weaning, with a marked difference between animals from an enriched pen with confined and enriched pen with loose-housed sow in both social and foraging-related behaviours.

Postweaning behaviour

These interaction effects of lactation pen enrichment and sow housing on postweaning behaviour are largely affected, however, by the presence or absence of postweaning enrichment. Postweaning enrichment reduced fighting and mounting on day 1, reduced nosing of pen mates, belly nosing and manipulative behaviours after day 1, reduced food exploration but increased explorative behaviours, play, activity and growth (Oostindjer et al. 2010b). The postweaning environment thus greatly affected the behaviours that piglets showed after weaning, either by a direct effect on the type of behaviours and the extent to which these behaviours can be performed by providing materials to perform the behaviours on, thereby increasing the behavioural repertoire. Postweaning enrichment can also affect behaviour by more indirect ways such as a reduction of stress or by providing distraction. The weaning process consists of multiple stressors (Weary et al. 2008), and the postweaning enrichment may have served as a distraction (Bolhuis et al. 2005a; Day et al. 2002; Fraser et al. 1991) by providing piglets with different motivations or different 'objects' to which they can direct their behaviours (pen mate to substrate). Piglets may have been less motivated to show aggression to establish a rank order directly after weaning if the enrichment is interesting enough, resulting in lower levels and perhaps different types of aggression (Melotti et al. accepted). Rooting behaviour may be directed at the enrichment, yet when this is absent, it may be directed at pen mates instead, leading to high levels of manipulative behaviours (Beattie et al. 1995). Enrichment can also have direct effects on stress, resulting in lower corticosterone responses, as shown in rats, to unpleasant situations such as handling when enrichment is present in the immediate environment (Moncek et al. 2004; Morley-Fletcher et al. 2003). Enrichment thus may have reduced the immediate stress response of piglets to weaning, which could have resulted in a differential pattern of behaviours in the first two weeks postweaning.

Most of the effects of preweaning enrichment on postweaning behaviour seem to be the result of large reductions or increases in behaviours for piglets switching from a barren to enriched or from an enriched to a barren pen. This is in part in concordance with a study done on effects of moderate enrichment and loss of enrichment on pig behaviour (Munsterhjelm et al. 2009). Piglets in our study that switched from a barren to enriched environment showed high levels of exploration and play behaviour, while piglets switching from an enriched to a barren environment showed high levels of belly nosing and low levels of play. The positive effects that enrichment can have on dealing with stressful situations may be higher when the enrichment is new and unexpected, leading to a higher motivation to explore the new pen than when piglets have always been in an enriched environment. In contrast, unexpectedly removing a positive thing (a reward) leads to frustration, stress and in some cases stereotypic behaviours (Latham and Mason 2010; Papini and Dudley 1997). The weaning process, independent of housing, may already be an example of unexpected reward loss, as maternal separation and the loss of milk as a food source can also be considered to be a unexpected loss of reward (Papini 2000). The loss of enrichment on top of the weaning stress may result in even higher levels of frustration and distress, resulting in high levels of stress-induced behaviours such as belly nosing and low levels of play behaviour as found in the current study, indicating a strongly reduced welfare. It is argued that animals might be better off when they never have experienced enrichment than when they have been in an enriched environment and were switched to a barren environment (Latham and Mason 2010).

Interestingly, there were some interactions between switching from environment and sow housing. The high levels of exploration and play in piglets switching from barren to enriched were especially high in piglets from a loose-housed sow, while the negative effects of switching from enriched to barren were more profound in piglets from a confined sow. It seems thus that sow housing preweaning has an effect on how piglets value their postweaning environment, depending on their preweaning environment. Due to differences in social interactions with the mother between housing systems, piglets from a confined sow may be in a more negative emotional state after weaning (see Mendl et al. 2009 for a discussion on positive and negative emotional states in animals), thereby responding more strongly to the loss of enrichment postweaning. Having a loose-housed sow during the preweaning phase thus seems to buffer the detrimental effects of the unexpected loss of reward, perhaps due to differences in maternal care, which is likely better in loose-housed sows, which can result in long-term effects on social behaviour and emotionality and possibly affects resilience to weaning stress (see Newberry and Swanson 2008). The results of the current study also indicate that having an enriched environment throughout the first six weeks of life, as well as a loose-housed sow, might be optimal.

In conclusion, enrichment pre- and postweaning, as well as sow housing, affect how piglet behaviour develops both before and after weaning. Piglets experiencing enrichment before weaning, as well as a loose-housed sow, may be better able to deal with the stressors around weaning. Providing enrichment postweaning has strong positive effects on piglet behaviour and welfare, and both preweaning and postweaning enrichment can be used to increase the development of social and foraging related behaviours that are important for a good adaptation to the postweaning environment. It is important however to realize that the preweaning environment affects how piglets value their environment postweaning and may thus greatly affect piglet welfare around weaning.

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05

LEARNING HOW TO EAT LIKE A PIG: EFFECTIVENESS OF MECHANISMS FOR VERTICAL SOCIAL LEARNING IN PIGLETS

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ABSTRACT

This study investigated which mechanisms of learning from the mother about foraging are important in piglets. The first experiment compared observation of the sow versus participation during eating. Piglet pairs could observe (observation piglets) or participate (participation piglets) with the sow while she was eating a flavoured feed in a test room for 10 minutes/day during five days. Piglet pairs that could eat food without cues from the sow and control piglets that had neither cues nor food were also exposed to the test room with their sow present but unable to eat. Piglets were tested during three days for 1 ½ hour/day and could choose between the sow's food and another flavoured food. Observation and participation piglets showed shorter latencies to eat and higher consumption and preference for the flavour eaten by the sow than control and eat without cues piglets. The second experiment compared local versus stimulus enhancement. Piglets observed the sow eating a flavoured feed from one of two feeders on different sides of the room for 10 minutes/day during five days. During the test phase there was a match or mismatch between location and the flavoured food eaten by the sow. Match piglets showed more behaviour towards and a higher consumption from the feeder where the sow was eating, while this was not true for mismatch piglets, suggesting a role of both local and stimulus enhancement. Observation, participation, local and stimulus enhancement thus seem important for piglets to learn from the sow.

Keywords:

social learning
piglets
participation
local enhancement
stimulus enhancement
weaning
mother

INTRODUCTION

Young animals face a challenge around weaning when they have to start selecting appropriate food types to include in their diet. Starting to eat every food type that an animal encounters is time consuming and potentially lethal, as the presence of toxins may result in the animal's first meal also being its last. Instead of this trial-and-error learning, it would be to the advantage of the young animal to obtain information from more experienced conspecifics on what, when and how to eat (see Galef and Giraldeau 2001 for review). Learning from a sibling or another conspecific of similar social rank and age could be beneficial when the demonstrator is more experienced (e.g. Held et al. 2000; Nicol and Pope 1994b). Adults are likely to have more experience with different food types and could provide the young animal with more information with a higher accuracy than a sibling. Young mammals generally direct more of their attention towards their mother than towards other conspecifics, as well as share a common genetic and physiological background and may thus respond similarly to different food types (Chesler 1969; Provenza and Balph 1987). It is therefore expected that learning from the mother would be especially important for the young animal to optimize its foraging strategy, although, depending on the species, other adults, such as the father or other more dominant group members, may also provide information.

The young animal can obtain this information from its conspecifics in different ways. Although it has been shown in several species that both olfactory and auditory cues can be important (see for example Bilko et al. 1994; Galef and Stein 1985; Nicol and Pope 1996; Woodcock et al. 2004), the main focus of most studies has been visual cues, often using a demonstrator and an observer. Lambs, for example, learn by observation of both dry ewes and their own mothers to ingest more healthy food and fewer pellets containing lithium-chloride (Thorhallsdottir et al. 1990). The largest effect, however, was obtained when lambs were also able to participate, e.g. interacting directly with the demonstrator and with the food on which the demonstrator is feeding, especially when the mother was the demonstrator. Participation might be more effective than observation because the time between observing the behaviour and performing the behaviour is very short (Bandura 1977). The exposure during participation furthermore includes both observational cues and positive post-ingestive consequences, e.g. nutritional reward, and thus a direct reinforcement (Myers et al. 2005; Sclafani 1997). Animals are able to learn with delayed reinforcement, assuming the reinforcement contains calories (Capaldi et al. 1987; Holman 1975), but the learned association of stimulus and reward can be stronger when the reward is provided quickly (Black et al. 1985; Richards 1981). On the other hand, participation may simply be following a knowledgeable conspecific to a food source, and the higher food intake in participation may simply be due to social facilitation by the knowledgeable individual (Visalberghi and Addessi 2000b). If this is the case, however, individuals that can participate are likely to obtain and retain less information about food (Beauchamp and Kacelnik 1991), which does not seem to be the case in for example lambs (Thorhallsdottir et al. 1990). Some of the experiments looking at the effectiveness of participation give animals the opportunity to scrounge, which can inhabit learning, leading to the conclusion that participation is less effective than observation. The individuals in these experiments instead learn to scrounge and do not learn the desired behaviour or food preference, by focusing on the demonstrator instead of on other stimuli (as discussed by Caldwell and Whiten 2003). Preventing scrounging as well as participation by separating the demonstrator from the observer may thus have positive effects on learning, making observation a potentially more efficient way of learning than "participation" (Giraldeau and Lefebvre 1987; Nicol and Pope 1994a). On the other hand, social contact and giving the opportunity to scrounge facilitates social learning and reduces food neophobia in marmosets (Caldwell and Whiten 2003; Voelkl et al. 2006), and participation with a free tutor resulted in successful foraging more often than observing a restricted tutor in chickens (Gajdon et al. 2001). Observation can thus be a useful way of learning, but participation with more experienced conspecifics is expected to give the young animal more information on which food to include in its diet.

Young animals can obtain information by observing or participating with adults and can use this information to learn different things. Amongst other things, they can learn where to eat and this can occur through, for example, local enhancement (Hoppitt and Laland 2008a). Local enhancement increases the possibility of the young animal performing a behaviour in the same location as an experienced individual and might result in the young animal obtaining information on where to eat. Local enhancement does not even require activity from the demonstrator, as seen in rats where the presence of an anaesthetized adult at a feeding site is sufficient to stimulate juveniles to approach and eat (Galef 1981b), and may thus even be caused by static visual and olfactory cues. Juvenile canaries also were shown to eat more from the same place as their parent, but also preferred the same food type as was consumed by the parent, suggesting that canaries use both the location and the type of food and may thus use stimulus enhancement and local enhancement to obtain information (Cadieu et al. 1995). Stimulus enhancement is used by some authors as a general term for the attraction of a conspecific to the site or object to which the demonstrator directed its behaviour and can thus overlap with the concept of local enhancement, and be caused by both olfactory and visual cues (see Hoppitt and Laland 2008b). Location and stimulus during demonstration can be disentangled, however, during testing of the observer and we will therefore consider them as two potentially different ways for animals to obtain information, with stimulus enhancement being specific for an object or food type. In this light, stimulus enhancement has also been demonstrated in rats (Heyes et al. 2000), geese (Fritz et al. 2000) and might even be involved in tool use learning in primates (see for example Ottoni and Mannu 2001). In most cases, however, local and stimulus enhancement will occur simultaneously, but disentangling the two ways to obtain information can give insight into what type of information has a higher priority for the young animal.

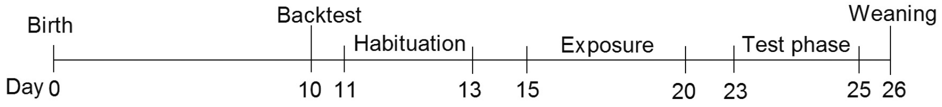
Pigs are interesting animals for studying how social learning affects the development of foraging behaviour, as pigs are very social and free-ranging piglets explore and forage together with their mother already from the first week after birth (Petersen 1994). Weaning occurs usually between week 13 and 18 (Jensen and Recén 1989; Jensen and Stangel 1992; Newberry and Wood-Gush 1985), giving piglets under (semi-)wild conditions a long period to learn from other individuals. Piglets spend this period exploring their environment, chewing on items, grazing, and slowly making the change from milk to solid food. Especially in the first four weeks, the piglets spend most of their time in close proximity of the sow, also when she is foraging (Jensen 1988), suggesting that social learning might be important in pigs.

The first experiment investigated whether learning is more effective when piglets can participate with the mother when she is eating (immediate reinforcement from consuming food), or whether it is enough to be able to observe the mother (and receive delayed reinforcement from food later), in addition to existing chemosensory cues that were present in all treatments. Previous work on learning from sibling pigs indicated that piglets can learn about food through observation (Held et al. 2000; Nicol and Pope 1994b), but that learning with direct reinforcement may be more effective (Morgan et al. 2001). Our hypothesis was thus that observation could be an effective way of learning by piglets, but participation might be an even more effective learning mechanism.

The first experiment investigated in what way piglets obtain information, but not what type of information piglets use. The second experiment therefore investigated which type of information is cues more important to piglets: the location where the demonstrator is performing the behaviour (local important) or the stimulus to which the demonstrator directs it behaviour (food, chemosensory cues more important). Some evidence of local enhancement in pigs has been found in a study by Nicol and Pope (1994b), but in another experiment in the same study, stimulus enhancement seemed to be more important, demonstrating that both these ways of obtaining information could be important for piglets.

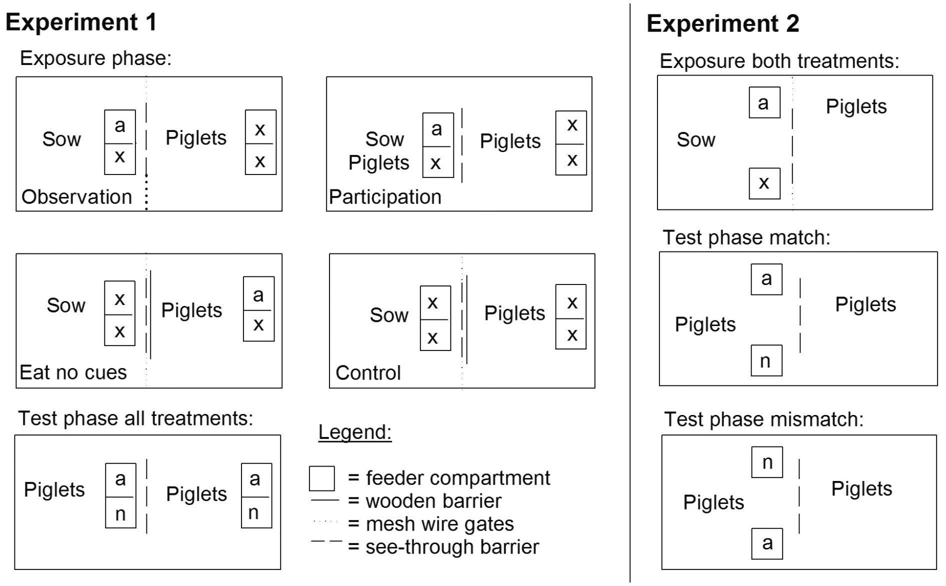
MATERIALS AND METHODS

The Animal Care and Use Committee of Wageningen University approved both experiments. The experiments were set up as a split-plot design, with sow as main plot and pairs of piglets assigned to different treatments as subplots to minimize the number of sows and piglets needed. Both experiments were carried out in multiple batches. A visual representation of procedures to which piglets from all treatments in both experiments were subjected is given in *[figure 1]*.



Timeline of procedures (backtest and weaning) and phases to which piglets from all treatments in both experiments were subjected

figure 2.



Layout of the test rooms during the exposure and test phases in experiment 1 and 2 for piglets of the observation, participation, eat without cues, control, match and mismatch treatments. X= covered food, a=food with flavour assigned to sow that is available, n= food that is not assigned to the sow and is available.

Test Room

Three similar test rooms were available during the experiment. The test rooms comprised a sow and a piglet compartment (each compartment 2.2 x 2.4 m,), which were separated by a metal see-through barrier (1.5 x 0.04 x 1.0 m), allowing nose and visual contact (see *[figure 2]*). In experiment 1, both compartments contained a feeder consisting of two adjacent sections (30 x 20 cm each). Each feeder section could be closed off with a wooden board, which contained four holes so that, when one section was closed off while the other was not, the odour of the feed in both sections could still be perceived *[figure 1]*.

In experiment 2, the test rooms contained two separate feeders of the exact same type and with the same dimensions as one feeder-section in experiment 1. The two feeders were placed near the barrier, but close to the two long walls, with a distance of 1.3 m from each other *[figure 2]*.

A wooden board, with the same dimensions as the barrier separating the two compartments, could be attached to the barrier in order to avoid visual information transfer. Two mesh wire gates were present on each side of the barrier and could be completely removed, closed, or opened to allow piglets, but not sows, to move from one compartment to the other. Nose and visual contact was possible through the gates but the feeders were not visible from the gates when the wooden barrier was in place. A drinking nipple for the sow was placed on both edges of the see-through barrier. Piglets could drink from drinking nipples in both compartments with the same placement as the sow nipples.

Feed During Testing

Each sow was randomly assigned to one of two flavoured feed types: strawberry or cherry-honey. Strawberry and cherry-honey were chosen because they both belong to the same odour family, fruity, (McLaughlin et al. 1983) yet are very different in their chemical composition (only 8 out of 30 and 60 notes in common) and both are accepted by pigs. The flavoured feed consisted of an unflavoured diet for newly weaned piglets (Nutreco Hendrix UTD, Boxmeer), with the addition of one of the flavours in powder form (Lucta S.V., Barcelona, Spain). Flavours and feed were mixed right before providing the animals with the feed. To ensure that the strength of the odour was similar for both the strawberry- and cherry-honey-flavoured diets, the flavours were mixed with the feed and presented to ten human panellists. At 1.5 g/kg for cherry-honey and 1.75 g/kg for strawberry, the flavours were judged to be equally strong. Both feed types (1 kg in each feeder section) were always present in the test rooms to avoid place-odour associations unrelated to sow feeding. One kilogram of food was chosen as it took most sows around 10 minutes to eat.

Habituation Procedures

Sows were habituated to the test room for three consecutive days starting nine days before the expected farrowing date. Sows were brought into each of the three test rooms twice, where they were presented with some feed scattered on the floor of the sow compartment. Time spent in the test room was gradually increased towards 10 minutes.

Sows and piglets were habituated on three consecutive days on day 11-13 after birth of the piglets. Each sow was randomly assigned to one of the three test rooms and sow and piglets were exposed and tested in this test room throughout the experiment. Sows were allowed to eat the assigned food in the test room twice per day, without piglets present. On day 11, the whole litter of piglets was habituated to the test room together with the sow twice, without food available. On day 12, pairs of piglets were habituated with the sow twice for 5 minutes. On day 13, pairs of piglets were habituated with the sow twice for 10 minutes, and without the sow for five minutes.

Data Analyses

The response variables that were analysed were as followed. Latency to start eating from the different feeders was included to get an idea of how long piglets took to their first meal and which feeder they visited first. Latency to start eating from any feeder containing the food eaten by the sow and latency to start eating from both feeders containing the food eaten by the sow were included to get an idea of whether the food eaten by the sow was in general more interesting to piglets from certain treatments, regardless of place, and whether piglets quickly sampled the food at both locations. In experiment 2, time spent at the feeder and frequency of visiting the feeder were included to get an idea of interest in the feeder and food, in addition to information on actual intake. The ratio of intake of feed from the sow's feeder to total food intake was analysed to see if piglets had developed a preference for the feeder where the sow had eaten. Intakes were analysed over the three test days as well as per day to look at generalisation effects. The initial choice might be more directed by the sow, but afterwards piglets might generalise more in feed type (both diets only different in flavour) or in location. Intakes were analysed from all feeders separately, as well as from sow and piglet feeders combined. The latter was done to look at whether learning from the sow can not only motivate piglets to start eating, but also to keep eating, leading to a higher intake of food before weaning.

Data from both experiments were analysed using generalized linear mixed models in SAS (SAS 9.0, SAS Institute Inc.). Data were averaged per pair of piglets. Consumption was analysed with a model including treatment, day and their interaction, as well as batch and the flavour assigned to the sow as main factors. Sow, nested within flavour and batch, and piglet pair, nested within sow, flavour, treatment and batch, were included as a random factors. Analyses by day and analyses of behaviours were analysed with the same model, excluding day and pair of piglets. The models in experiment 2 included a factor that mentioned whether the feeder where consumption or behaviour was measured was the feeder where the sow had previously eaten or not. This factor was tested in interaction with treatment or with treatment and day. Placement of the assigned flavour (left or right) in both experiments did not significantly contribute to the model and was therefore not included. A Gaussian distribution was used in the analysis when applicable; otherwise a lognormal distribution was used to obtain a good model fit. Post-hoc pair wise comparisons were made using the least-square means, corrected for multiple comparisons in Experiment 1 with a Tukey adjustment. Data are presented as means ± SEM based on pair averages.

EXPERIMENT 1

Animals and housing

Experiment 1 was carried out in four batches, using a total of twelve multiparous PIC (commercial synthetic sow line, includes Landrace and Large White breeds) sows obtained from a commercial farm and their piglets (Tempo (commercial boar line with Great York genetic background) x PIC) in total. Sows were individually housed in farrowing pens (2.2 x 2.4 m) from 10 days before farrowing onwards. All farrowing pens contained wood shavings and straw; both were accessible to both sow and piglets.

Sows were contained in farrowing crates from three days before farrowing until two days after parturition. Sows could access the space behind the farrowing crate from day two onwards until weaning, which provided an additional 2.2 x 1 m space. Sows farrowed within a four-day range in all four batches. Litters were standardized to 11 ± 1 piglets before day 3 by minimal use of cross-fostering. Lighting schedule was 12:12 with lights on at 7.00 h. Temperature was maintained at 25 °C around farrowing and gradually decreased to 21 °C at weaning. Heating lamps were provided for piglets in all pens.

Piglets did not have access to food in the farrowing pen. Sows were fed twice a day a standard commercial feed for lactating sows in an adjacent pen from day 4 after parturition onwards, to minimize learning about food by the piglets in the home pen. On post-farrowing habituation days and exposure days, sows received only a small portion of food in the morning (1 kg) to increase the motivation to eat in the test rooms. In the test room they were provided with 2 kg per day in two of the four exposure sessions (see below). Food availability in the test room was signalled with a specific sound to prevent frustration of sows. The portion in the afternoon on these days was the daily allowance minus the 1 kg fed in the morning and the 2 kg fed in the test room (daily allowance between 5.1 and 6.4 kg on day 11 and 20, respectively). Water was available ad libitum for sows and piglets. Piglets remained on the experimental farm after the experiment; sows were re-used in another behavioural experiment.

Treatments

Four pairs of piglets per sow (n=12) were exposed to one of the following treatments (each treatment n=12 pairs):

1) The possibility to observe (O): Piglets were able to observe the sow while she was eating in the test room.
2) The possibility to participate (P): Piglets were able to observe the sow and participate, thus eat the food with the sow, during the time she was eating in the test room.
3) The possibility to learn to eat without direct food-consumption cues from the sow (E): Piglets had access to food in the test room while the sow had not.
4) Control (C): Piglets were present in the test room without direct food-consumption cues from the sow nor the opportunity to eat by themselves.

Piglets were assigned to pairs at postnatal day 10. A backtest was performed to classify piglets as either high resisters (more active coping response to stress) or low resisters (more passive coping response to stress, (Bolhuis et al. 2003; Hessing et al. 1993)). Each pair of piglets had an average weight near the average litter weight (on average 3.56 ± 0.2 kg) and consisted in general of a male and female and of a high and low resister. The number of males and females, as well as high and low resisters, was equal within and between treatments and non-cross fostered piglets were preferred for assigning piglets to treatments (two in O, three in E and three in C treatments). Cross-fostered piglets were only used when their body weight was not below 1 SD from the litter average.

Exposure phase

Piglet pairs were exposed to the test room with the sow during five days (day 16-20) for 10 min per day. During exposure trials of the O and P piglets, the wooden barrier was removed to allow piglets visual access to the sow compartment. The wooden barrier was present during exposure trials of the E and C piglet pairs. The gates between the sow and piglet compartment were open during exposure trials of P piglet pairs and remained closed in the other three treatments (see *[figure 2]* for overview). The sow had access to food during exposure of the O and P piglets only. Piglets in the E treatment had access to the section of the piglet feeder that contained the food type to which their sow was assigned. Placement of food types in sow and piglet feeders was the same (e.g. cherry left for sow and piglets).

Piglets were brought into the test room as a pair and guided into the piglet compartment. The piglet compartment was then closed off by the two gates separating sow and piglet compartment (except for the P treatment). In case of a trial during which the sow could eat, the appropriate section of the feeder was opened and the sow was brought into the test room. Two different sounds indicated trials with or without food access for the sow. Sow and piglets remained in the test room for 10 minutes, after which the sow was removed from the test room and placed in a small pen adjacent to it. The food, in case of a food trial, was covered and the piglets were guided back to the home pen. The test room was readied for the next trial and new piglets were brought in, and the process was repeated. Sow and piglets were brought back together after each second trial for the sow. Each pair of trials consisted of a food trial and a no-food trial for the sow. There was, on average, two hours between the first pair of exposure trials for one sow and the second pair of trials for the same sow. Faeces and urine were removed from the test room after each trial. All sows and piglets were exposed between 9.00 h and 14.00 h. Order of exposure was balanced over treatments and days.

Test phase

Piglets were tested for three consecutive days (day 23-25) without the sow present. Three pairs of piglets, each from different sows, were tested simultaneously in the three different test rooms. Order of testing was balanced between treatments and days. Barriers in the test rooms were removed and food was present and available in both sections of the piglet feeders and sow feeders, with the same placement of feed types as during the exposure phase. The piglets were guided into the test room and were able to explore and eat for 90 min, after which they were brought back to their home pen. Faeces and urine were removed after each trial. Test trials always started between 15 and 40 minutes after a suckling bout, thereby minimizing variation in feeding motivations.

Food was removed from the feeders after each trial and the leftover feed was weighed to determine consumption for both food types in both the piglet and sow feeder. A camera was present in each test room to record piglet behaviour. Latency to eat from each feeder and each food type was determined for each individual piglet.

EXPERIMENT 2

Experiment 2 was conducted in two batches, using a total of twelve sows and their litters (Tempo x PIC). Housing and standardization of litter sizes were the same as described for experiment 1, except sows were fed 2 kg in the morning and 1 kg in the test room.

Two pairs of piglets per sow were allowed to observe the sow while she was eating and were subjected to one of the following treatments (each n=12, see also *[figure 2]*):

1) A match of placement of food flavour types between exposure and test phase: control treatment. Both food types were in the same feeders during the exposure and test phase.
2) A mismatch of placement of food flavour types between exposure and test phase in order to distinguish between information use of location and stimulus properties.

RESULTS

Generally, sows paid little attention to piglets while the sow had the possibility to eat in the exposure phase. Interactions between sows and piglets typically consisted of nose contact at the mesh wire gates when the sow was not eating, though the time spent doing this was only a small proportion of the total test time, even when sows and piglets had no food available. Piglets in the observation treatment did show interest in the food the sow was eating, even though they could not reach it. The same goes for the participation piglets, yet we never observed that the sow pushed the piglets towards the food or anything similar.

EXPERIMENT 1

Latencies to ingest the food

Treatment during exposure affected latencies to the first ingestion of any food type from any feeder ($F_{3,33} = 4.74$, $P = 0.007$, data not shown) and post hoc tests showed that O (observation) piglets ate sooner than E (eat without cues from sow) piglets. Treatment similarly affected latency to sample food with the assigned flavour from both the sow and piglet feeder ($F_{3,33} = 4.85$, $P = 0.007$, *[table 1]*), with O piglets eating sooner than E piglets. Treatment also affected latency to the first ingestion of food with the assigned flavour in either sow or piglet feeder ($F_{3,33} = 5.96$, $P = 0.002$, *[table 1]*), with O piglets eating sooner than E piglets.

Treatment affected latency to eat from the food with the assigned flavour in the sow feeder ($F_{3,33} = 6.23$, $P = 0.002$, *[figure 3A]*), with O piglets eating sooner than control piglets (C) and E piglets. O piglets also tended to eat the feed with the non-assigned flavour in the sow feeder sooner than E piglets (treatment effect $F_{3,33} = 2.28$, $P = 0.097$, *[figure 3A]*). Treatment did not affect the latency to eat the assigned flavour food ($F_{3,33} = 1.10$, $P = 0.36$) or the non-assigned flavour ($F_{3,33} = 1.08$, $P = 0.37$, *[table 1]*) in the piglet feeder.

Food consumption during the test

Total consumption over all test days was not affected by treatment ($F_{3,33} = 1.08$, $P = 0.37$). Consumption of food with the assigned flavour over all test days was higher for O piglets than for control (C) piglets ($F_{3,33} = 3.20$, $P = 0.04$), while consumption of the food with the non-assigned flavour was unaffected by treatment ($F_{3,33} = 0.10$, $P = 0.96$). Consumption of food from the sow feeder with the assigned flavour was higher for O piglets than for C and E piglets ($F_{3,33} = 3.51$, $P = 0.02$), while consumption of food from the piglet feeder with the assigned flavour was unaffected by treatment ($F_{3,33} = 1.91$, $P = 0.15$). Consumption of food from the sow or piglet feeder with the non-assigned flavour was unaffected by treatment ($P > 0.68$). There were no significant interactions between treatment and day ($P > 0.1$). The consumption of assigned food over the three test days compared to total food consumption, and thus preference for the assigned flavour, was higher for O and participation (P) piglets than for E and

C piglets, but only when consumed from the sow feeders (sow feeders: F3,33 = 4.62, P = 0.008, piglet feeders: F3,33 = 0.59, P = 0.62, *[figure 3B]*).

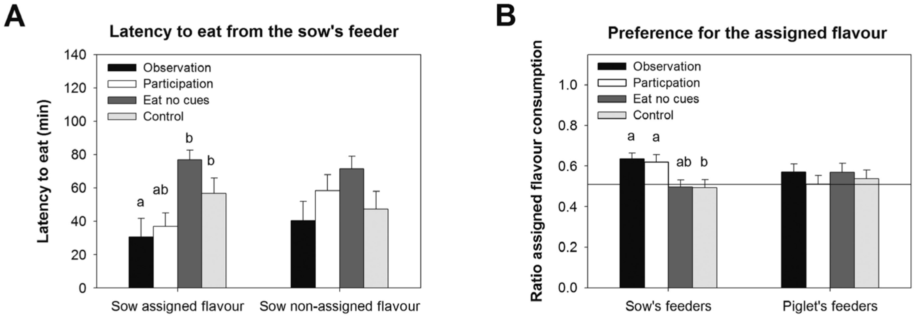
table 1.

	observation	participation	eat without cues	control	significance
latency to eat from sow or piglet assigned food (min)	29.5 ± 10.8^{a}	37.0 ± 8.0^{ab}	72.7 ± 5.5^{b}	47.9 ± 9.5^{ab}	P=0.002
latency to eat from both sow and piglet assigned food (min)	96.9 ± 16.4^{a}	116.8 ± 13.3^{ab}	160.1 ± 6.8^{b}	124.4 ± 14.1^{ab}	P=0.007
latency to eat assigned food in piglet feeder (min)	66.4 ± 7.4	79.8 ± 7.4	83.1 ± 3.4	67.7 ± 8.2	P=0.36
latency to eat non-assigned food piglet feeder (min)	66.2 ± 6.9	81.5 ± 5.7	77.1 ± 5.6	73.0 ± 5.2	P=0.37
consumption sow feeder assigned flavour d 1 (g/pig)	11.0 ± 2.9^{a}	9.1 ± 2.6^{a}	3.2 ± 1.2^{b}	2.3 ± 0.6^{b}	P<0.001
consumption sow feeder non-assigned flavour d 1 (g/pig)	5.1 ± 1.3	5.4 ± 1.6	4.3 ± 1.3	2.7 ± 0.5	P=0.18
consumption piglet feeder assigned flavour d 1 (g/pig)	4.7 ± 1.0	3.0 ± 0.8	3.2 ± 1.2	2.1 ± 0.3	P=0.12
consumption piglet feeder non-assigned flavour d 1 (g/pig)	3.2 ± 0.5	3.6 ± 0.8	2.5 ± 0.6	1.3 ± 0.3	P=0.10
consumption sow feeder assigned flavour d 2-3 (average, g/pig)	8.5 ± 2.7	8.6 ± 2.6	7.2 ± 2.5	3.6 ± 1.2	P=0.46
consumption sow feeder non-assigned flavour d 2+3 (average, g/pig)	3.5 ± 0.9	2.7 ± 0.6	4.9 ± 1.3	4.7 ± 1.4	P=0.88
consumption piglet feeder assigned flavour d 2+3 (average, g/pig)	2.8 ± 1.0	3.7 ± 1.0	3.0 ± 1.3	1.8 ± 0.5	P=0.25
consumption piglet feeder non-assigned flavour d 2+3 (average, g/pig)	1.4 ± 0.2	2.2 ± 0.4	2.2 ± 0.6	3.0 ± 1.1	P=0.78

Latency to eat from any feeder with the assigned flavour, both feeders with the assigned flavour, latency to eat from the piglet feeders and consumption from the sow and piglet feeders with the assigned and non-assigned flavoured foods on on day 1 and averaged over day 2 and 3 of testing for piglets that could observe the sow, participate with the sow, eat without cues from the sow and control piglets from experiment 1 [#]

[#] Treatments that were significantly different from each other are marked by different superscripts, within a row.

figure 3.



Behaviour and food intake of piglets during the test phase of experiment 1. Latency to feed from the sow feeder is presented in panel A and the ratio of assigned food consumption for sow and piglet feeders over total consumption from sow and piglet feeders over all three test days is presented in panel B. Treatments that were significantly different from each other are marked by different superscripts, within variable.

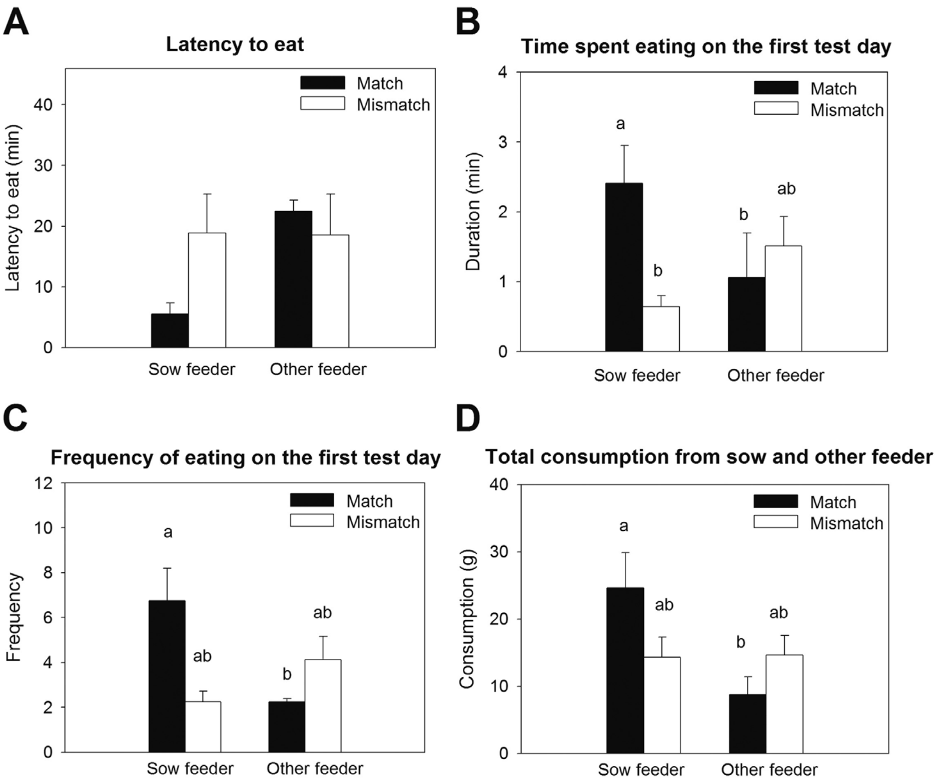
Most of the above mentioned effects were caused by the intakes on day 1 of testing. Table 1 shows the consumption from each feeder on day 1 and averaged for day 2 and 3. O and P piglets consumed more food from all feeders combined than E and C piglets on day 1, and E piglets consumed more than C piglets (treatment effect F3,33 = 8.29, P = 0.0003). Treatment also affected consumption of food with the assigned flavour on day 1 with O piglets eating most, then P, then E, and C piglets eating least (F3,33 = 7.76, P = 0.0005). O,P and E piglets consumed more food with the non-assigned flavour on day 1 than C piglets (F3,32 = 4.84, P = 0.007). Treatment affected consumption of the assigned flavoured food on day 1 (F3,33 = 11.45, P < 0.0001), but not of the non-assigned food (F3,32 = 3.32, P = 0.18) from the sow feeder, with O and P piglets consuming more assigned flavoured food than C and E piglets *[table 1]*. P piglets furthermore tended to consume more food with the non-assigned flavour from the piglet feeder on day 1 than C piglets on day 1 (F3,29 =2.32, P = 0.096). Treatment did not affect consumption from the piglet feeder with assigned flavour on day 1 (F3,30 =2.09, P = 0.12, *[table 1]*). Preference for the assigned flavour on day 1 was not affected by treatment (F3,33 = 2.09, P = 0.12), though O piglets did show a significant preference for consuming food which was assigned flavoured food (ratio: t-test with H0=0.5, t = 3.22, P = 0.008). Total consumption (F3,31 = 0.11, P = 0.95), consumption of assigned food (F3,31 = 1.46, P = 0.24), consumption of non-assigned food (F3,32 = 0.50, P = 0.69) and consumption from the separate feeders see *[table 1]* over days 2 and 3 were unaffected by treatment.

EXPERIMENT 2

Behaviour during the test phase

There were no effects of treatment or treatment x sow feeder interaction on latency to eat (P>0.16, *[figure 4A]*). Match piglets spent a longer time eating from the sow feeder than mismatch piglets on day 1 of testing and spent more time eating from the sow feeder than from the other feeder (treatment x feeder interaction, F1,27 = 9.56, P = 0.005, *[figure 4B]*). Match piglets also showed more frequent visits to the sow feeder than to the other feeder, while visits to the feeder by mismatch piglets did not differ between feeders (treatment x feeder interaction, F1,27 = 8.69, P = 0.007, *[figure 4C]*).

figure 4.



Behaviour and food intake of piglets during the test phase of experiment 2. Latency to feed from the sow feeder and the other feeder (A), duration of eating from the sow feeder and other feeder (B), frequency of eating from the sow feeder and other feeder (C) and consumption over the three test days from the sow feeder and other feeder (D) for piglets for which the feeder and flavour eaten by the sow matched with the observation period (match) or for which the flavour of the sow was not in the feeder of the sow (mismatch) from experiment 2. Treatments that were significantly different from each other are marked by different superscripts.

Consumption during the test

Piglets from the match treatment tended to consume more food over the three test days than piglets from the mismatch treatment (match: 58.4 ± 13 g, mismatch: 35.2 ± 7.5 g, F1,55 = 3.08, P = 0.09). Consumption of food from the sow feeder was higher than from the other feeder for match piglets but not for mismatch piglets across the three test days (treatment x feeder interaction, F1,91 = 6.86, P = 0.01, *[figure 4A]*). There were no differences between treatments in consumption from the sow feeder or the other feeder on the different days (P>0.17). Match piglets tended to consume a larger proportion of their food from the sow's feeder than mismatch piglets (match: 63, mismatch: 38 %, F1,49 = 4.03, P = 0.05).

DISCUSSION

This study investigated the effectiveness of observation versus participation and local versus stimulus enhancement on how piglets learn what and where to eat. Piglets can indeed learn by observing the sow, as indicated by the shorter latency to eat, higher total consumption and higher consumption of the sow's assigned food from the sow's feeder, of both observation and participation piglets compared to control piglets. Other work also indicates that piglets can use information obtained through observation of conspecifics' behaviour about where and what to eat (e.g. Held et al. 2000, Nicol & Pope 1994). What is quite surprising, however, is that participation of piglets was not more effective than observation alone in reducing latency to eat and increasing both total consumption and consumption from the feeder of the sow with the sow's assigned flavour, which is in contrast to previous similar studies in sheep (Thorhallsdottir et al. 1990) and pigs (Morgan et al. 2001). Direct reinforcement is generally thought to lead to stronger connections between stimulus (observing the sow) and reward (post-ingestive consequences) than delayed reinforcement (Black et al. 1985; Richards 1981) which, in this study, was a minimum of three days for observation piglets. Also, participation piglets had the opportunity to directly confirm that what they ate was the same as what was eaten by the sow, which could also be a type of reinforcement, although nose contact between sow and piglets was also possible in the observation treatment. One can wonder, however, how much visual information participation animals really did obtain. Social facilitation of food intake by the sow, e.g. the process whereby the presence of an eating individual increases food intake of another individual (De Castro 1994; Keeling and Hurnik 1996) could explain the higher intakes compared to piglets that ate without direct feeding cues from the sow. Visual information may not play an important role and instead of providing additional information to mere observation, participation could provide different, though useful information on where and what to eat. Animals of all treatments, however, had chemosensory information about what the sow was eating from her breath or possibly through the milk. This chemosensory information, however, did not lead to increased preference for the assigned flavour type of the sow in control and eat without cues from the sow piglets, again pointing out that observing the sow and interacting with the sow and the food is important in learning. Some sows also did not allow the participation piglets to eat on each exposure day (data not shown), so the actual reinforcement may in some cases have been relatively small and the reinforcement may thus have been more delayed. Participation piglets could, however, interact more with the sow, with the food and also observe the sow eating from every angle and thus obtain more information than observation piglets, though this did not result in a higher consumption and preference than observation piglets. The opportunity to scrounge may have been responsible for participation being less effective than expected (Giraldeau and Lefebvre 1987; Nicol and Pope 1994a), with animals learning more from the individual than from cues provided by food or location. If it were indeed the case that participation piglets learned less from the food or location, it would be expected that participation piglets would have performed worse than observation piglets three days after the exposure phase, which was not the case. What is clear is that suckling piglets that are inexperienced with ingesting solid food are able to learn about food and eating with a delayed reinforcement for at least three days.

This study furthermore shows that being able to learn from the sow is more effective than learning without cues from the sow, as seen in the higher consumption of observation and participation piglets in experiment 1 compared to eat without cues and control piglets, though consumption of piglets exposed to food in the test room without direct information from the sow was higher than that of control piglets. This effect is likely due to familiarization by repeated experience with the food during the exposure phase of piglets exposed to food in the test room without direct information from the sow (10 out of 12 pairs consumed food on three days or more during the exposure, data not shown), while this did not occur until the test phase for control piglets. Interestingly, some generalisation seemed to occur for all piglets. Intake of food with the assigned flavour was higher for piglets that could learn from the sow, but also intake of food with the non-assigned flavour was higher for piglets that could observe or participate, suggesting that these piglets generalised between food types that only differed in flavour (Birch 1999; Villalba and Provenza 2000a). On the other hand, piglets still preferred to eat food with the assigned flavour when looking over all three test days, suggesting that generalisation might reduce the reluctance to try novel foods, but experience with this novel food does not change the food preference established through social learning. Similarly, piglets did eat from both the piglet and sow feeders, suggesting generalisation of locations, but piglets that could learn from their mother still preferred the sow’s feeder.

Consumption was very similar between observation and participation piglets in experiment 1 and piglets from the match treatment that could learn by observation in experiment 2, again emphasizing that piglets learn by observing or interacting with the sow. The total consumption of piglets that experienced a mismatch between flavour and location in the test phase compared to the exposure phase, however, tended to be lower than that of the match piglets. The differences in consumption between match and mismatch piglets are likely due to mismatch piglets experiencing some difficulties with the change in food placement in the test phase. The ‘confusion’ of mismatch piglets suggests that they did remember where the sow was eating, though their intake from the sow feeder and the other feeder was similar, suggesting that location is used but not the only information source. In contrast, match piglets directed more behaviour towards the sow feeder, indicating that their behaviour was strongly affected by the behaviour of the sow during exposure. Mismatch piglets numerically visited the other feeder longer and more frequent, and it thus seems that piglets obtain or use information by stimulus enhancement as well, in this case through the chemosensory properties as both foods only differed in their odour. This is, in part, in accordance with a study by Nicol and Pope (1994b), who found in one experiment that pigs were more likely to direct behaviour towards an operant panel in the same colour and in the same direction as a demonstrator sibling pig, but in another location. In another experiment in the same study, however, pigs were more likely to eat from a feeder in which they had seen another pig forage than from another feeder, showing that local enhancement can also be important. Also in the current study, the confusion of mismatch piglets, as well as the preference of observation and participation piglets to eat from the sow feeder and not the piglet feeder, suggests that local enhancement is indeed used by piglets. One could argue, however, that the use of location in both the Nicol and Pope study and the current experiments has little relevance compared to the life of pigs in the wild, which often have a home range of multiple square kilometres, while the distances between locations in the experiments are in the range of meters. Piglets, however, stay close to their mother during foraging when they are still young and inexperienced and for the young pig it might be important to be able to distinguish between a food item that is eaten by the mother and a potentially toxic food item that is located only a meter away. Stimulus enhancement can be important for similar reasons, though as seen in experiment 2 whereby mismatch piglets had an overall lower intake of food, stimulus enhancement is not the only way that the piglet obtains information. Interestingly, stimulus enhancement seems to be used less in experiment 1 than in experiment 2, as true stimulus enhancement should have resulted in an increased consumption of food with the flavour consumed by the sow in both the sow and piglet feeder (Heyes et al. 2000). This was not the case: increased consumption was found in the sow feeder only. Observing the sow in the sow's compartment only may have motivated piglets to spend more time and eat more in the sow's compartment of the test room, as a general local enhancement of that part of the test room. Piglets in experiment 2 could only eat in the sow's compartment of the test room. This suggests that local enhancement is more important for the initial interest in a certain location (test room, location where mother is present) and initial investigation of the food, and when the approach is made, the stimulus itself becomes more important. Also the age of the piglets, the rank of the demonstrator (mother versus sibling) and previous experience with food and foraging may influence the balance between the use of local and stimulus enhancement and in inexperienced piglets that can learn from their mother before weaning, as in the present study, the balance might tip more towards stimulus enhancement.

Stimulus enhancement thus is important but does seem to require a more direct way of learning in piglets compared to rodents and rabbits, which show an increased preference after exposure to odours in the breath, on the skin and in faeces of experienced individuals (Bilko et al. 1994; Galef and Stein 1985; Galef 1989; McFadyen-Ketchum and Porter 1989; Valsecchi and Galef 1989). For rats the type of food that was eaten by the mother is very important in initial food selection, mainly caused by chemosensory cues in the milk (Galef and Henderson 1972), but previous studies in pigs showed little or no effects on preference of exposing piglets to flavours in the milk through the diet of the sow (Oostindjer et al. 2009; Oostindjer et al. 2010a). Pigs are more precocial than rodents and rabbits and can already use visual information at a young age and thus can make a more extensive assessment of the food, including colour, shape and also location of the food. Indirect learning from chemosensory cues could still be important in piglets (see Oostindjer et al. 2009; Oostindjer et al. 2010a) and all piglets from the sow in both experiments were exposed to the odour of the food that was eaten by the sow through the breath and skin of the sow when she returned to the home pen after the exposure trials. If olfactory cues were sufficient to establish a preference for the food that was eaten by the sow, the piglets that ate without cues from the sow and control piglets in experiment 1 would have been expected to eat more from the correct flavour than from the other flavoured food, yet they ate equal amounts from both food types, in contrast to observation and participation piglets. The coupling between olfactory cues of the food and visual and auditory (chewing) cues given by the sow eating the food, as well as properties of the food itself (smell, taste, appearance) seems important to influence the piglet's preference during its exposure to and interaction with the food, either directly or after a delay.

Being able to observe or participate with the sow, as well as learning about the type of food and to some extent the location where the sow eats, could be of great importance for piglets in commercial husbandry. These piglets are challenged in the period around weaning, as they are generally weaned abruptly and several weeks earlier than wild piglets (Bolhuis et al. 2009). The weaning is generally followed by low food consumption, with some animals showing a reluctance to ingest feed for up to three days or more (Brooks and Tsourgiannis 2003). The low food consumption is typically accompanied by low growth or even weight loss and a high prevalence of diarrhoea. Solutions for the problems arising around weaning mostly focus on increasing food consumption pre- and postweaning, as a high food consumption pre-weaning is associated with a high food consumption post-weaning (Berkeveld et al. 2007), and a reduction of the fasting period leads to improved gut health and less diarrhoea (McCracken et al. 1999). Most solutions, however, such as improving food palatability and feeder design, are not sufficient to increase food consumption (e.g. Edge et al. 2004; Edge et al. 2005). An alternative approach may be to increase opportunities for piglets to learn from their mother how, what and where to eat, as commercial husbandry situations currently provide limited possibilities for this. Typically, sow and piglets eat different types of food at different locations in the pen, whereby the sow eats from a raised through that is inaccessible for piglets. In a previous study we found that piglets benefit post-weaning from being able to learn from flavour cues in the maternal diet prenatally when these flavours are also present in the post-weaning environment (Oostindjer et al. 2010a). When looking at more direct forms of social learning, we discovered that providing piglets with enrichment and the opportunity to forage with their mother reduces food neophobia and increases pre-weaning growth and possibly food consumption as well (Oostindjer et al. 2010b; Oostindjer et al. 2011). The current study indicates that providing piglets with the opportunity to observe and interact with the sow while she eats and providing a match between sow and piglet food in smell, taste, appearance and also location, thereby optimizing learning through stimulus and local enhancement, could lead to higher preweaning consumption of food and consequently a better health and welfare of piglets after weaning.

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06

PRENATAL FLAVOUR EXPOSURE AFFECTS FLAVOUR RECOGNITION AND STRESS-RELATED BEHAVIOUR OF PIGLETS

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ABSTRACT

Exposure to flavours in the amniotic fluid and mother's milk derived from the maternal diet has been shown to modulate food preferences and neophobia of young animals of several species. Aim of the experiment was to study the effects of pre- and postnatal flavour exposure on behaviour of piglets during (re)exposure to this flavour. Furthermore, we investigated whether varying stress levels, caused by different test settings, affected behaviour of animals during (re-) exposure. Piglets were exposed to anisic flavour through the maternal diet during late gestation and/or during lactation, or never. Piglets that were prenatally exposed to the flavour through the maternal diet behaved differently compared to unexposed pigs during re-exposure to the flavour in several tests, suggesting recognition of the flavour. The differences between groups were more pronounced in tests with relatively high stress levels. This suggests that stress levels, caused by the design of the test, can affect the behaviour shown in the presence of the flavour. We conclude that prenatal flavour exposure affects behaviours of piglets that are indicative of recognition and that these behaviours are influenced by stress levels during (re)exposure.

Keywords:

chemosensory learning
preference
behavioural tests
neophobia
pigs

INTRODUCTION

Young animals face a major challenge around weaning. Instead of relying on their mother for the provisioning of healthy food, they need to start selecting healthy and nutritious food types by themselves. Olfactory cues that are transmitted from parents to offspring by means of skin, feathers or fur, faeces, diet or breath, may be used to select appropriate food sources and foraging sites (Bilko et al. 1994; Galef 1996; Morrow-Tesch and McGlone 1990).

Learning about nutritious food types may already take place before birth by transmission of chemosensory cues from the maternal diet to the offspring. Flavours can be transmitted to amniotic fluid, and may be perceived by the foetus during mouthing movements and ingestion of the fluid (El-Haddad et al. 2005; Mennella et al. 1995). In addition, flavours from the maternal diet can enter the foetal blood stream after crossing the placental barrier, and may be perceived through the foetal nasal capillaries (Schaal et al. 1995c). Schaal et al. (2000) showed that babies of mothers that ingested anise-flavoured foods during the last two gestational weeks showed a higher preference for anise compared to non-exposed infants in the first four days after birth. Similar effects of prenatal exposure to a particular flavour on olfactory preference have been shown in rats (Smotherman 1982b; Hepper, 1988) and dogs (Wells and Hepper 2006). Moreover, prenatal flavour exposure resulted in enhanced acceptance of similarly flavoured food around weaning in rabbits (Bilko et al. 1994) and sheep (Simitzis et al. 2008). Effects of prenatal flavour exposure on later preference might be strengthened if combined with flavour exposure through the maternal milk during lactation (Bilko et al. 1994; Désage et al. 1996; Galef and Henderson 1972; Hepper and Wells 2006; Mennella et al. 2001), although the relative effectiveness of prenatal as opposed to postnatal exposure may be dependent on the species under study. Prenatal exposure appears to affect preference in all species tested so far (see Bolhuis et al. 2009; Schaal and Orgeur 1992 for review), but it can be postulated that postnatal exposure without prenatal exposure may have the strongest effect in altricial species, in which the brain and the olfactory system are still relatively immature and plastic as compared with precocial species (Brunjes 1983). Precocial animals, on the other hand, generally start exploring and feeding relatively early in life, and therefore postnatal programming of preference, i.e. changing the structure and function of tissues such as the brain through early experiences (Seckl 2001), may be less beneficial for those species.

Early chemosensory learning may be of particular interest for piglets in animal husbandry, which are generally weaned abruptly and at a much younger age than would happen under (semi-)natural conditions (Jensen 1988). As a consequence, piglets often display a very low food intake and high stress levels in the first days postweaning (Bruininx et al. 2002b). Increasing the preference for a certain type of food may be helpful in motivating piglets to ingest solid food and thereby reduce welfare and health problems, such as diarrhoea and weight loss, that are associated with the low food intake around weaning (Jarvis et al. 2008).

Apart from influencing flavour preference, (early) exposure to flavours may also affect emotionality and stress-responsivity during re-exposure to these flavours. For instance, flavours associated with traumas increase alpha activity in the electroencephalogram of veterans suffering from post-traumatic stress disorder (McCaffrey et al. 1993). Flavours that are of personal significance affect emotionality through increased activity in the amygdala (Herz et al. 2004a). A neutral familiar flavour, such as vanilla, or a positive familiar flavour such as mother's milk, reduces crying and grimacing after administration of a heel stick and overall body movement during the heel stick procedure in babies (Rattaz et al. 2005). This means that during a test for flavour preference, which often takes place in a novel environment, the behaviour of previously exposed animals may be different from that of control animals due to the mere presence of the familiar flavour in the environment.

These potential stress-reducing effects of familiar flavours are often overlooked in experiments on the effect of early flavour exposure on preference. There is a large variation in the paradigms and observations used to assess flavour preference. For instance, in some paradigms the ingestion of the flavour (Hepper and Wells 2006) or feeding-related behaviours, such as pecking in flavoured substrate (Sneddon et al. 1998) are measured, whereas in others exploration and time spent in differentially flavoured locations in a Y-maze are assessed (Morrow-Tesch and McGlone, 1990). In the different paradigms used to assess flavour preference, stress levels induced by the test situation may vary, due to, for instance, novelty of the test environment or social isolation. The stressfulness of a test situation may in turn moderate the expressed flavour preference of animals that have pre- or postnatally been exposed to this flavour. For instance, in a relatively stressful test setting, exposed animals could be less inhibited to explore or ingest both the test flavour and control flavours than unexposed animals. So, on the one hand, relatively stressful test situations may increase the contrast in behaviour of exposed and unexposed animals. On the other hand, a stressful test situation may also mask flavour preferences if the mere presence of a familiar flavour in the test situation reduces neophobia and results in a lower reluctance of exposed animals to explore the unfamiliar control flavours. It is important to know whether the intrinsic stressfulness of a test situation interferes with the expressed preference within a paradigm, in order to facilitate interpretation of the response of the experimental animals. Comparing stress-related behaviours of previously exposed and unexposed animals within a test provides information on the changes in stress that are induced by the familiarity of the flavour. A comparison between different tests may provide information on different motivations of previously exposed compared to unexposed animals and can be used in the interpretation of flavour preference data.

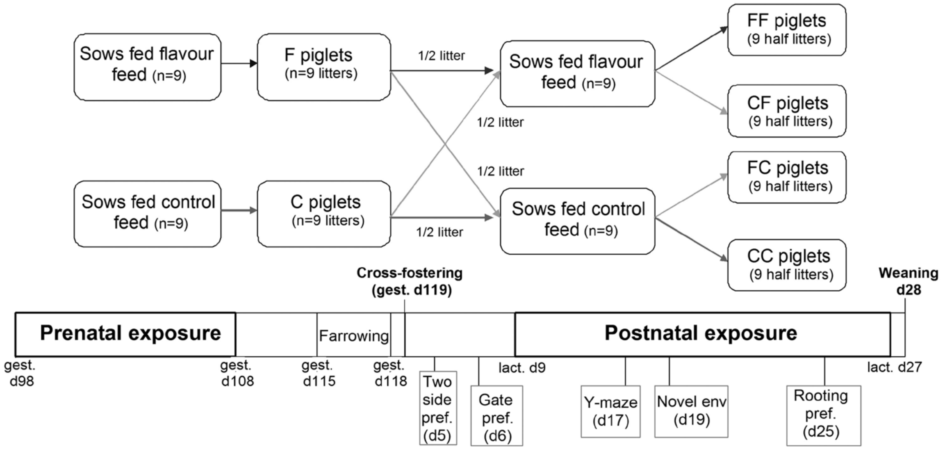
The aim of the current study was to test whether flavours experienced by piglets pre- and/or postnatally are determinants of flavour recognition before weaning. As the pig is a relatively precocial species that starts to explore and forage early in life, we hypothesised that prenatal flavour exposure and the combination of pre- and postnatal exposure would lead to behaviours indicating recognition, but postnatal exposure alone may not. We furthermore investigated whether the stressfulness of the flavour recognition tests used would affect behaviour of the animals during (re)exposure.

MATERIALS AND METHODS

Animals and housing

The experiment was approved by the Animal Care and Use Committee of Wageningen University. The experiment was set up in a 2 x 2 factorial arrangement and was carried out in two successive batches. A total of 18 multiparous gestating Great Yorkshire x Dutch Landrace sows were used. An overview of all experimental treatments and procedures is given in *[figure 1]*.

figure 1.



The experimental design and the timing of the different treatments, procedures and behavioural tests. Average days of age are given for each test; for exact age and variation per treatment group we refer to *[table 1]*..

Sows were either exposed to flavoured (F, n=9) or control (C, n=9) feed during days 98 to 108 of gestation (see below). During the last trimester of gestation, foetuses of several mammalian species appear to be able to detect and retain chemosensory information (see Schaal and Orgeur, 1992). Flavour treatment ceased on day 108, a week before farrowing, to prevent flavour exposure through mother’s faeces, milk or breath before the postnatal flavour treatment started (see below). All sows were provided with feed without any additions between day 109 of gestation and day 6 of lactation. In this period sows were gradually switched from gestation feed to feed for lactating sows. From farrowing onwards sows received only lactation feed.

From day 95 of gestation onwards, sows were individually housed in four different stables: two control stables and two flavour stables. On day 110 of gestation, sows were moved to four clean stables in which the test flavour had not been present before, and were placed individually in a farrowing pen of 3.54 m x 2.20 m. The sow was placed in a farrowing crate (2.16 m x 0.62 m) in the front-center of the pen. All sows farrowed within a four-day range (gestational day 115-118), and one day after the last sow farrowed, all piglets were cross-fostered to another sow. Piglets remained with their biological mother between birth and cross-fostering.

Each sow fostered ten piglets that she did not give birth to: five from an F-fed sow, and five from a C-fed sow. Both male and female piglets were used, and the distribution of piglets of different sexes between half litters was balanced for treatments. A half litter generally consisted of piglets originating from the same sow. In some cases of small litter sizes the half litter consisted of offspring from two different sows; piglets were always cross-fostered together with at least one sibling. Four additional sows participated in the experiment (one fed F and three fed C feed from days 98 to 108 of gestation), but were not included in this analysis. These sows only provided some piglets for the cross-fostering.
Half of the sows of each prenatal treatment were provided with F feed from day 9 to 27 ± 1.1 of age (mean± standard deviation) and the other half with C feed. Hence, piglets were exposed, through their (foster) mother's diet, to flavour from day 98 to 108 of gestation (FC), from day 6 to 24 of lactation (CF), during both gestation and lactation (FF) or not at all (CC, see *[figure 1]*.), n=9 half litters per treatment combination. See *[table 1]*. for an overview of the characteristics of the animals within the different treatments.

Piglets were weaned at 28 ± 1.1 days of age. No feed was provided to the piglets during the lactation period.

table 1.

	FF	FC	CF	CC
# of half litters	9	9	9	9
# of piglets	44	44	45	44
# of males	24	22	21	20
age at cross-fostering (days)	2.7 (0.9)	2.6 (0.9)	3.0 (1.1)	3.0 (1.3)
body weight at cross-fostering (kg)	1.7 (0.3)	1.7 (0.3)	1.7 (0.3)	1.8 (0.4)
body weight at weaning (kg)	8.7 (1.0)	8.7 (1.3)	8.6 (1.2)	8.5 (1.2)

Characteristics of animals exposed to anise pre- and postnatally (FF), only prenatally (FC), only postnatally (CF), or never (CC). Standard deviations are given within parenthesis.

Flavour exposure

Anise was chosen as the experimental flavour in this experiment. Anise is known to be accepted in the feed by pigs (Langendijk et al. 2007), and previous research in dogs and humans has shown that addition of this flavour to the maternal diet during gestation or lactation results in recognition by the offspring (Schaal et al., 2000; Wells and Hepper, 2006). All sows in the flavour groups received a daily dose of 350 mg trans-anethol (99%, Sigma-Aldrich), the molecule responsible for the anisic flavour (Karaali and Başoğlu 1995), given in two daily portions of 175 mg. This dose was based on a study done in humans (average anethol dose of 121.2 mg/day, Schaal et al., 2000), scaled for the average body weight of sows during late gestation. The portions of 175 mg anethol were dissolved into 20 mL soy oil and kept in 20 mL syringes in the dark. Syringes containing 20 mL of soy oil were prepared for the control sows, and were kept at a considerable distance from the anethol solutions to prevent any contamination.

The anethol solution (or plain oil) was sprayed on top of a portion of 300 g of food (standard commercial sow diets), which was between 4% and 10% of the total daily food intake depending on the gestational and lactational stage. Sows were fed this mixture in separate feeding troughs that could be placed into the trough of the home pen. Additional food was not given until the sow finished the treatment food, which resulted in an anethol uptake of 100% for sows in the experimental groups.

When the sow finished eating, the trough was removed from the pen and transported in closed plastic bags to a separate room for cleaning. A protocol was followed to prevent exposure of the control sows and piglets to the anethol. This protocol included feeding the control sows before the flavour sows; wearing gloves; keeping the exposure of anethol-contaminated objects, such as the troughs and syringes, to the air to a minimum by transporting them in plastic bags; and changing clothes between the morning and afternoon feeding period.

Behaviour during flavour exposure

The first two behavioural tests described below were carried out before the postnatal flavour treatment and thus only tested effects of prenatal exposure. The final three tests described were carried out during the postnatal exposure and thus tested both pre- and postnatal exposure effects. In most tests (see below), peppermint was used as a control scent. A small pilot study, performed before the current experiment, showed that piglets did not differ in preference for anisic and minty flavours. Peppermint was not used to flavour the food of the sows so that effects of flavour exposure on flavour preference, food intake, and growth could be examined after weaning (results will be reported elsewhere). Concentrations of the different flavour stimuli were determined before the experiment by a human panel, so that strengths of the scents were matched for anise and mint.

During the experiment, 36 half litters were used, 9 of each treatment. In each half litter, one piglet was kept naïve to the anisic flavour in the tests. The other four piglets all participated in both the two-side preference test (day 2) and the gate preference test (day 3 or 4). The naïve piglet was also handled in the two-side preference test and exposed to the gate preference test, but in the absence of the anisic or minty flavours. Subsequently, out of the four tested piglets, one was tested in the Y-maze test (day 14), two other piglets were tested in pairs in the rooting preference test (day 22 or 23, n = 20, only second batch) and the fourth piglet was tested in the novel environment test on day 16. The naïve piglet was used as the control in the novel environment test. The timing of the different behavioural tests is given in *[figure 1]*.

Each of the tests was classified as inducing low, moderate, or high stress levels before the experiment, based on whether the test was conducted alone or with siblings, in the home pen or elsewhere, and with or without habituation to the test environment and procedure. To validate this classification, the number of vocalizations given by the control animals (CC), as well as the percentage of control animals that defecated or urinated or tried to escape from the test environment were assessed to make a post-hoc classification of stress levels within a test. The Y-maze test and the rooting preference tests had higher post hoc stress levels than were previously assigned, perhaps due to less effective habituation than assumed. The number of vocalizations per piglet per minute, as well as the percentage of animals that defecated, urinated, or tried to escape are given in *[table 2]*.
The order of testing the half litters and placement of the experimental flavours within a behavioural test were always balanced for treatment.

table 2.

	mean vocal./ piglet/min	% animals defec./ urin.	% animals escape	pre-assigned stress level	post-hoc stress level
two side preference test	0 (0)	0%	0%	low	low
gate preference test	16.3 (14.6)	11%	0%	moderate	moderate
y-maze test	72.6 (21.9)	44%	0%	moderate	high
novel environment test (without anise)	65.3 (26.7)	55%	22%	high	high
rooting preference test	27.7 (5.2)	10%	0%	low	low-moderate

Mean vocalizations per piglet per minute, percentage of animals that urinated and/or defecated, percentage of animals that made an attempt to escape the test setup, and the preassigned and post hoc assigned stress levels for the five different behavioural tests. Standard deviations of the means are given within parenthesis.

Two-side preference test

The two-side preference test was carried out when piglets were 5.0 ± 1.1 days of age, and was done on a half litter basis. This test was based on the study by Hepper (1987), in which proximity to a scented object was considered a determinant of preference. The test was assumed to induce only low stress levels, because piglets were subjected to the test in their home pen together with siblings. The other half litter plus the 'naïve' piglet of the focal half litter remained behind a wooden board in the home pen during the test. The second half litter of the pen was tested immediately after the first half litter was tested.

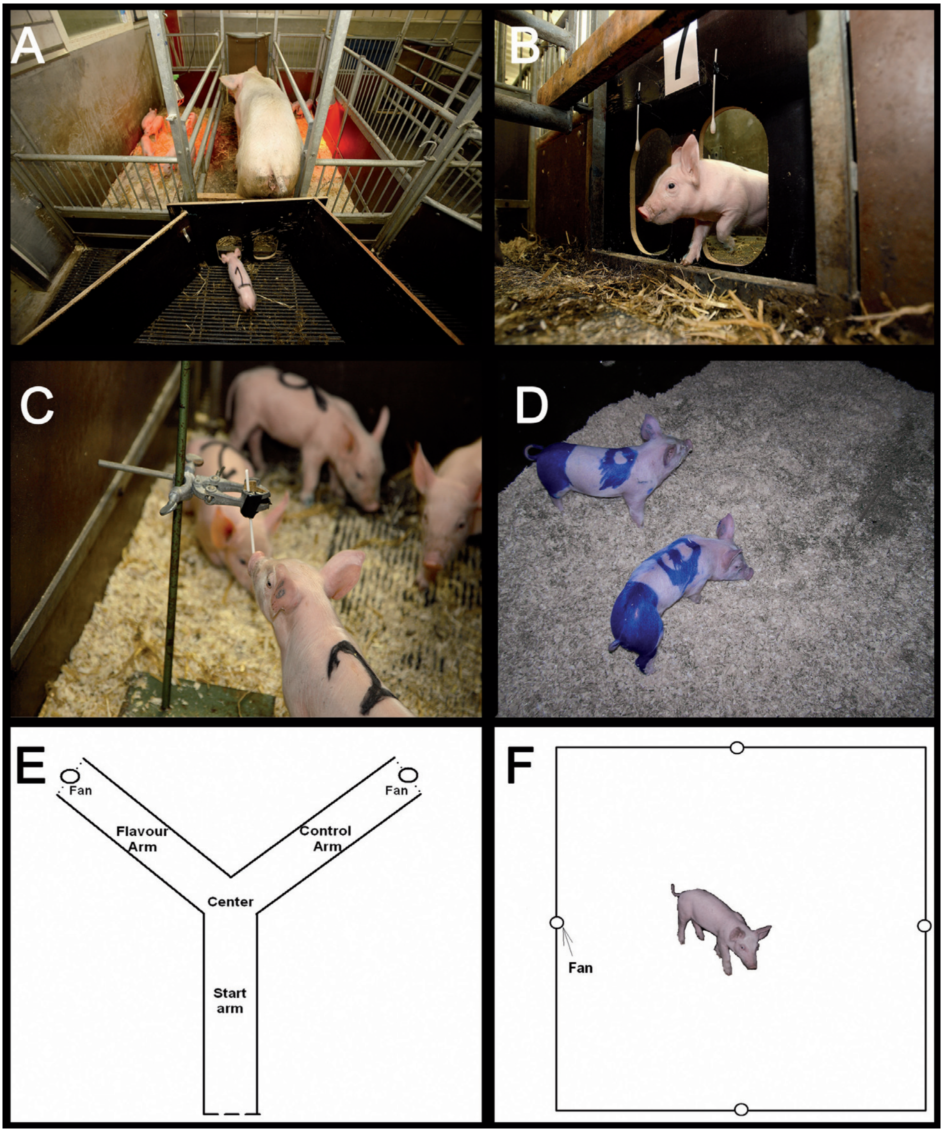
The focal piglets were presented with two test tube holders, each holding a cotton swab dipped in either anise seed infusion (33 g anise seed per litre), or peppermint infusion (8 g peppermint leaves per litre). Flavour infusions were used because of the close proximity of the animals to the stimuli. The test tube holders were placed against one wall of the pen with an equal distance between the test tube holders and the front and back of the pen. The test started at the moment the test tube holders were in place, and lasted 5 min. The behaviours of interest during this test were sniffing the swabs, chewing on the swabs, sniffing and/or chewing on the holders, and time spent on either the anise or control side of the test area (see *[figure 2C]* for an impression). Two people were positioned outside the pen against the wall in front of which the test tube holders were placed, one on each side of the area. Behaviour of one half litter could not be analysed due to technical problems with video recording.

Gate preference test

The gate preference test was carried out when piglets were 6 or 7±1.1 days of age. Piglets were separated from the sow and given the opportunity to return to the sow via either an anise-scented gate or a mint-scented gate. We expected piglets to have a preference for a familiar smelling gate to move from a nonsafe environment to a safe environment (the sow). The test was assumed to induce moderate stress levels due to a separation from the sow and siblings in a part of the home pen, which may not be favoured by piglets at that age.

The sow was confined to the farrowing crate and a wooden board of 0.8 x 0.6 x 0.03 m with two ellipse-shaped holes (gates) on each side of the centre (centre of the holes at 21.5 cm above the floor, hole diameter = 32.5 cm, distance between holes = 25 cm) was placed on the outside of the farrowing crate, behind the sow. A hook was present above each gate on the side of the board facing the sow to which a cotton swab dipped in anise or peppermint infusion (see two-side preference test for concentrations) could be attached. Placement of anise- and mint-flavoured swabs (left or right) was balanced for treatments. Two wooden boards (170 x 0.6 m) were used to close off the area behind the sow and were placed diagonally, leading to a trapezium-shaped arena of 60 cm near the sow and 258 cm on the far side of the sow *[see figure 2A]*. Two people were positioned

figure 2.



Photographs of three of the behavioural tests and general setup of the other three behavioural tests. Panel A shows the general setup of the gate preference test, with the piglets to be tested on the left side of the farrowing crate and the piglets that have been tested on the right. Panel B shows the two flavours above the different gates. Panel C shows chewing behaviour in the group preference test. Panel D gives an impression of the rooting preference test. Panel E shows the setup for the Y-maze test. Panel F shows the setup for the novel environment test.

on the outside of the trapezium, one on each side. The gate above which the anise was placed was kept the same within a pen to avoid mixture of scents. The two naïve piglets of the pen went through both gates, without the anise and mint flavour present, to provide scent marks from piglets on both gates. The naïve piglets remained behind wooden screens in the home pen during the test. Piglets that were already tested were kept separate behind a wooden screen from the piglets that still were to be tested to avoid contamination through body contact.

The test piglet was taken individually from the compartment left of the farrowing crate and was placed in the centre of the arena. It had to choose one of the gates (anise or mint scented) to return to the sow. The test ended when the piglet's entire body went through the gate, or when 5 minutes had passed *[figure 2B]*. The gates were cleaned with water after all piglets of the pen were tested. The test was videotaped with a camcorder and the following parameters were scored afterward: choice of gate, sniffing and chewing on the edge of the gate, as well as being on the left or right side of the test arena. Interest in the gates was calculated as time spent sniffing and chewing on the gate divided by the total test time.

Y-maze test

The Y-maze test was carried out when piglets were 17 ± 0.9 days of age and was done on an individual basis. The test was adapted from Morrow-Tesch and McGlone (1990), in which the time spent in the arm containing the test scent was a measure of preference. The test was assumed to induce somewhat higher stress levels than the previous tests due to temporary social isolation away from the home pen after habituation. A maze was built that consisted of 3 arms of 1.2 m x 25 cm, with walls of 1 m high. The maze was divided into four compartments: the start arm, the centre, the flavour arm and the control arm *[figure 2E]*. A wooden trapdoor was located at the end of the flavour and control arms, with a small ventilator placed in the middle of the door and with the centre of the fan at 31.5 cm height. The flavour and control arm were switched between batches, but not within a batch to minimize contamination risks. A petri-dish filled with four drops of anethol (99%) and peppermint oil was placed behind the flavour and control arm, respectively, in front of the ventilator that blew the scents slowly into the flavour and control arms (0.9 m3/min). Pure anethol and peppermint oil were used because the scent needed to be strong enough to carry throughout the respective arm of the Y-maze.

Piglets were habituated to the Y-maze on day 12 after cross-fostering without the flavours present, together with the other focal piglet from the same foster sow. During the actual test, the focal piglet was placed into the start arm of the maze and was left to explore the maze for 3 min. Vocalizations were scored live. The observer was positioned 5 m behind the start arm of the maze, outside of the visual range of the piglet. Time spent in each arm, locomotion and latency to enter arms were scored afterwards with video observations using focal sampling and continuous recording with the Observer 5.0 (Noldus B.V., Wageningen). The maze was cleaned between trials.

Novel environment test

The novel environment test was carried out when piglets were 19 ± 1 days of age. The test was assumed to induce, as compared to the other tests, relatively high stress levels due to isolation in a strange environment without habituation. A walled arena of 2.2 x 2.2 x 1.0 m was set up in a room unfamiliar to the piglets. All four walls contained a fan (see Y-maze). The naïve piglet of the half-litter was first subjected to the novel environment for 3 min in absence of any test flavour, but with the fans turned on. After the trials with the naïve piglets, the arena was scented with anethol (99%) through the fans in each wall of the arena *[figure 2F]*. Anethol was used to ensure the scent being present in the entire test arena. The same test procedure as described for the naïve piglets was followed for the test piglets. Number of vocalizations, escape attempts, defecation and urination were scored live from a video screen positioned outside the visual range of the focal piglet. The arena was cleaned after each trial with water and dried afterward.

Rooting preference test

The rooting preference test was carried out when piglets were 25 ± 0.4 days of age and done with sibling pairs on two consecutive days. The test was based on the “olfactory preference test” for chickens as described by Sneddon et al (1998) in which the amount of time spent on scented shavings was taken as a measurement of preference. This test was assumed to induce a relatively low level of stress due to the piglets being tested in pairs after habituation. A walled arena of 2.2 x 2.2 x 1.0 m was used to test the pairs of piglets. The piglets were habituated to the arena with unscented substrate on day 21 after cross-fostering for 5 min, together with the test pair of the other half litter from the same pen. During the actual test, half of the floor of the arena was covered with substrate scented with anisic flavour and the other half with peppermint-scented substrate (175 mg of 99% anethol dissolved in 20 mL oil and 20 mL peppermint oil, respectively, shaken in a bag with 18% of the substrate for the top layer). The two halves of the arena were divided by a low wooden beam (3.5 cm high). Piglets were introduced to the arena with the front legs on one scented half and the hind legs on the other half. One piglet faced the anise-scented half, and the other piglet faced the mint-scented half of the arena. Behaviour was scored live by two observers, using the Observer 3.0 installed on a Psion Organizer II LZ64, by continuous focal sampling. Two observers were positioned on the outside of the arena at both ends of the wooden beam that divided the two halves of the arena. Time spent on either side was scored, as well as exploration of the floor (rooting, sniffing and chewing substrate), standing alert (pricked ears and raised head), and contact behaviour (nosing head or body of the other piglet). The test time was 5 min after which the substrate was completely renewed. The side with the anise-flavoured substrate was constant between trials but reversed between days. *[see figure 2D]* for an impression.

Statistical analyses

Effects of pre- and postnatal flavour treatments were analysed with general linear models (GLM) in SAS (SAS 9.0, SAS Institute Inc). Data from tests in which more than one piglet from the same half litter performed the same test (two-side preference test, gate preference test and rooting preference test) were averaged per half litter. Latencies and the proportion of time spent on a behaviour were log- and arcsine square root transformed, respectively, when the residual variance was not normally distributed.

Behaviours in the two-side preference test and gate preference test were analysed using a model with prenatal treatment and batch as main effects. Preliminary analysis of the behavioural data from the gate preference test tended to show a choice bias for the left gate, irrespective of the flavour associated to the gate ($P = 0.08$, logistic regression), and therefore, the left and right gate were analysed separately. The choice for the anise or mint gate was analysed using a logistic regression model with prenatal exposure as class variable.

Behaviour in the Y-maze was analysed with a model including prenatal exposure, postnatal exposure, and their interaction, and batch as class variables. A logistic regression using the same factors was used to investigate differences between treatments in which arm of the Y-maze was entered first. Behaviour in the novel environment test was analysed in a GLM with prenatal treatment, postnatal treatment, and anise presence included, as well as their interactions and batch as class variables. The data in the rooting preference test were analysed with a model including prenatal treatment, postnatal treatment, and their interaction. Data are presented as mean ± standard error of the mean unless stated otherwise.

RESULTS

Two side preference test

In total, 79% of piglets tested showed any exploratory behaviour directed to the cotton swabs in the two side preference test. Out of these piglets, 84% explored the anise-flavoured swab, and 82% of the piglets explored the control-flavoured swab.

Time spent chewing or sniffing the anise-flavoured cotton swab or the holder of the swab did not differ between half litters exposed to anise prenatally and control half litters (chewing: 7.5 ± 2.4 s, sniffing: 4.7 ± 1.0 s, holder: 21 ± 3.9 s). There was also no effect of prenatal treatment on chewing and sniffing the control-flavoured swab, nor in exploring the holder of the control swab (chewing: 6.9 ± 2.2 s, sniffing: 3.7 ± 0.9 s, holder: 19.9 ± 3.8 s). Finally, no differences were observed between treatment groups in the time spent on the anise or control side of the pen (anise side: 154.8 ± 10.6 s, control side: 144.4 ± 10.7 s, all $P > 0.21$).

Gate preference test

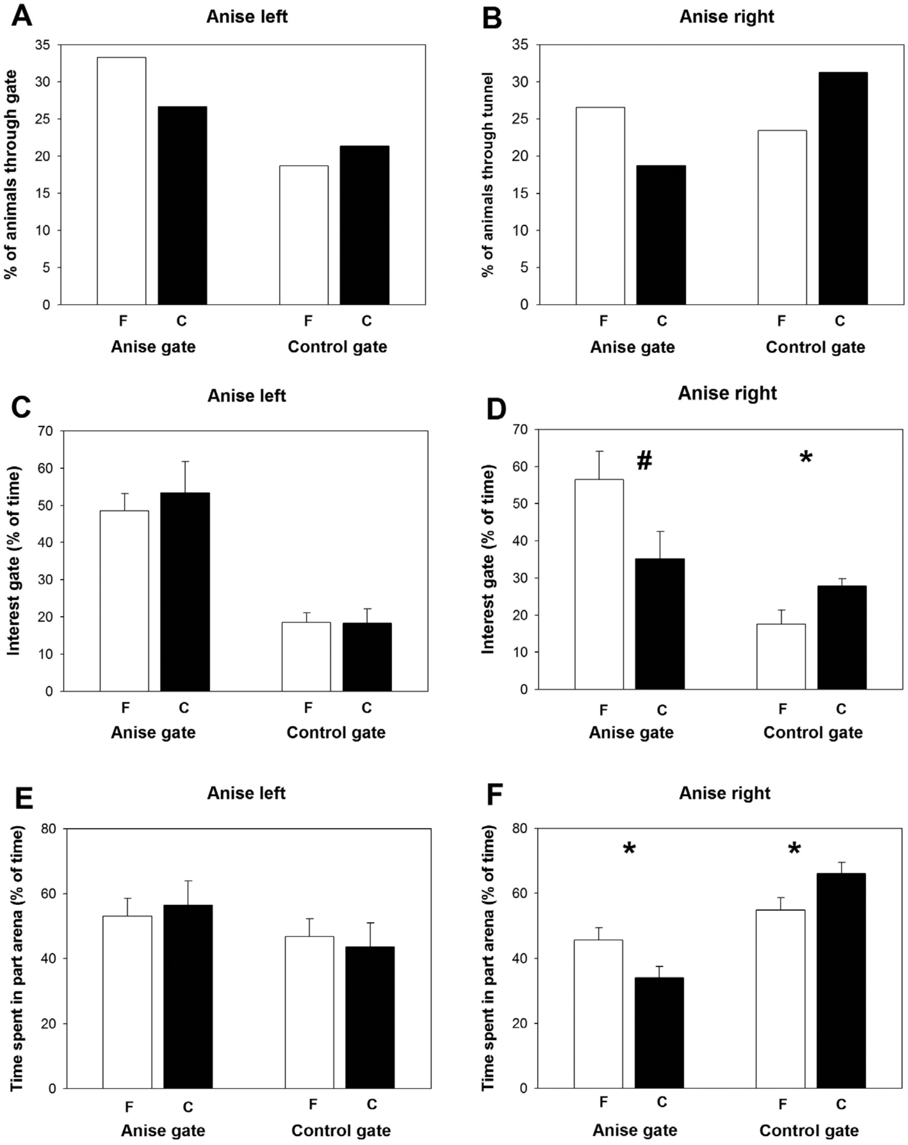
Three piglets did not choose any gate to return to the sow within the allotted time of 5 min in the gate preference test. The proportion of piglets choosing the anise gate with anise either above the left or right gate did not differ between piglets that were prenatally exposed to anise and controls ($P = 0.15$, *[see figure 3A, B]*).

Treatment did not affect interest in the anise and control gate or time spent on the left or right side of the test arena when anise was placed above the left gate (all $P > 0.53$, *[see figure 3C, E]*). When the anise was placed above the right gate, piglets exposed to anise prenatally tended to show a higher interest (i.e., sniffing and chewing) in the anise gate ($F_{1,15} = 4.1$, $P = 0.06$), and showed a lower interest in the control gate ($F_{1,15} = 5.1$, $P = 0.04$, *[see figure 3D]*) than control piglets. Piglets that were prenatally exposed to anise spent more time on the anise side of the test arena than control piglets, who spent more time on the control side of the arena when anise was above the right gate ($F_{1,15} = 5.2$, $P = 0.04$, *[figure 3F]*).

Y-maze test

Treatment did not affect the time spent in the flavour arm or the control arm of the Y-maze test nor the latency to enter the flavour arm (all $P > 0.26$). Piglets that were never exposed to anise through their mother's diet (CC) tended to enter the control arm sooner for the first time than piglets from the other three treatments (CC: 25.1 ± 4.8 s, FF: 57.2 ± 18.6 s, FC: 57.9 ± 14.3 s, CF: 53.6 ± 15.6 s, prenatal x postnatal interaction, $F_{1,35} = 3.14$, $P = 0.086$). Piglets exposed to anise prenatally only (FC) showed a longer latency to enter the centre of the Y-maze than piglets exposed to anise both pre- and postnatally (FC: 16.4 ± 3.1 s, FF: 6.4 ± 1.8 s, prenatal x postnatal interaction, $F_{1,35} = 4.40$, $P = 0.04$). There was no effect of treatment on time spent walking and standing ($P>0.23$), nor on vocalizations during the test ($P > 0.15$). No effects of the treatments on the percentage of piglets entering the anise arm first were found ($P > 0.56$).

figure 3.

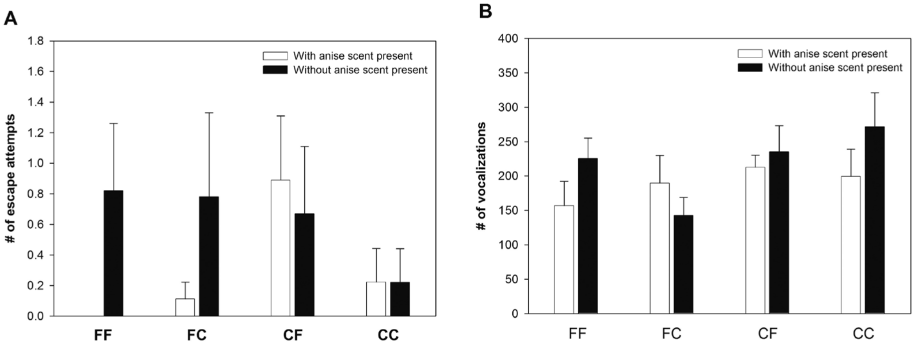


Percentage of animals choosing the anise-scented gate or the control-scented gate to return back to the sow for anise located above the left and right gate, respectively (A+B), interest in anise and control gates as percentage of time for anise above the left and right gates (C+D) and time spent on either the anise half or control half of the test arena for anise above the left and right gates (E+F) in the gate preference test for animals exposed to flavour prenatally (F) or control animals (C). * $P<0.05$, # $P<0.1$.

Novel environment test

Piglets that had prenatally been exposed to anise (FF/FC groups) showed fewer escape attempts in the novel environment test when anise was present in the test area compared with their siblings that were tested without the anise present (prenatal treatment x anise present interaction, F1,71 = 4.7, P = 0.04, *[figure 4A]*). No effect of anise presence on behaviour was found in the CF/CC groups. Piglets exposed to anise both pre- and postnatally (FF) had fewer vocalizations when anise was present than their siblings tested without anise in the test area (F1,71 = 5.2, P = 0.03, *[figure 4B]*). No effects of pre- or postnatal exposure were found on defecation and urination during the test (P > 0.23).

figure 4.

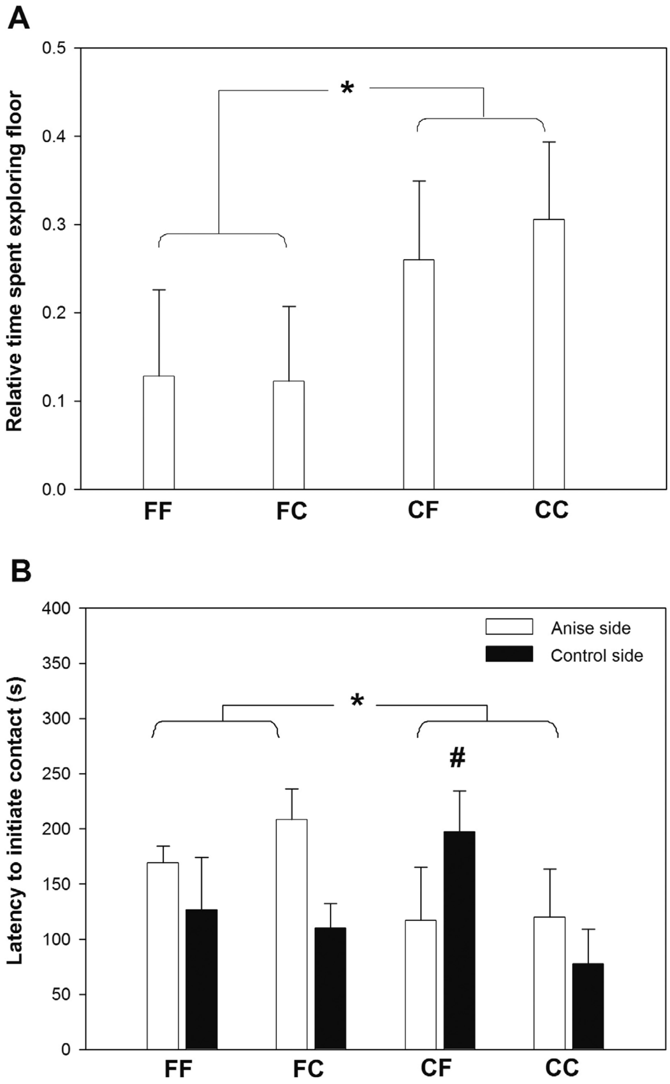


Number of escape attempts (A) and number of vocalizations (B) during the novel environment test with anise scent present (empty bars) or without the anise scent present (solid bars) in the environment for animals exposed to anise prenatally and postnatally (FF), only prenatally (FC), postnatally (CF), or never (CC). *P<0.05).

Rooting preference test

Pre- and postnatal treatment did not affect total time spent on either the anise or control side of the test arena nor total exploration (wall + floor) of either side in the rooting preference test (P > 0.19). Piglets that were prenatally exposed to anise (FF + FC) spent relatively less time exploring the floor on the anise side of the arena and more time exploring the floor on the mint side compared with CF and CC piglets (F1,19 = 4.8, P = 0.04, *[figure 5A]*). Treatment did not affect the time spent on contact behaviour (P > 0.1), but prenatal anise exposure (FF + FC) tended to increase the latency to initiate contact with the other piglet of the pair on the anise side (F1,19 = 3.8, P = 0.07, *[figure 5B]*). Piglets exposed to anise only postnatally (CF) tended to initiate contact later on the control side than piglets from the other treatment groups (F1,19 = 3.2, P = 0.09, Figure 5B). No effects of treatment on time spent standing alert were found (P > 0.47).

figure 5.



Relative time spent exploring the floor (A) and latency to initiate contact with the other piglet (B) on the anise (empty bars) and control (solid bars) side in the rooting preference test arena for animals exposed to anise prenatally and postnatally (FF), only prenatally (FC), postnatally (CF), or never (CC).
* P<0.05, # P<0.1.

DISCUSSION

Effects of prenatal and postnatal exposure on behaviour during re-exposure

This study demonstrates that piglets prenatally exposed to anisic flavour through the maternal diet differed in behaviour from non-exposed pigs during re-exposure to this flavour in various behavioural tests up to 23 days after birth, indicating recognition of the flavour. For instance, previously exposed piglets showed fewer vocalizations and escape attempts in the novel environment test and less exploration of the anise substrate as well as a longer latency to initiate contact with the other piglet on the anise half of the arena in the rooting preference test. In the gate preference, piglets showed increased exploration of the anise-scented gate after prenatal exposure, though only when the anise was present above the right gate, likely due to the piglets always being taken from the left side of the pen.

These results are in unison with those in other species, where prenatal exposure to a flavour led to recognition and a reduced aversion of this flavour later in life (Bilko et al. 1994; Hepper 1988; Schaal et al. 2000; Simitzis et al. 2008; Smotherman 1982a; Wells and Hepper 2006).

Providing flavour to piglets both prenatally and postnatally through the maternal diet had a larger effect on behaviour in the novel environment test than prenatal flavour exposure alone. Prenatal exposure alone may already organize the olfactory system to such an extent that receptor density, sensitivity, and reactivity to a flavour in the maternal diet are increased, as hypothesised by Hepper and Wells (2006). Indeed, it has been shown that prenatal exposure to juniper results in an increased sensitivity of the olfactory epithelium during re-exposure in rabbits (Semke et al. 1995). The continuity of flavour exposure after birth may be important to activate the changes in the olfactory system, as is seen in dogs, in which preference was shifted only after both prenatal and postnatal exposure (Hepper and Wells, 2006). Continuity may also be important in increasing flavour preference in piglets, but organization by prenatal exposure alone already significantly affected flavour preference in pigs.

Addition of the anisic flavour to the maternal diet during lactation alone, that is, without addition during gestation did not lead to behavioural changes in exposed piglets as compared with unexposed animals in the different tests. This is in contrast to studies in humans, rats, and rabbits, in which exposure through mother's milk alone led to a shift in preference (Bilko et al. 1994; Galef and Henderson 1972; Mennella et al. 2001). It is possible that the anethol ingested by the sows did not appear in their milk or in too low concentrations only. A study by Hausner et al. (2008) showed, however, that ingested anethol can be found in human breast milk after 2 h and is still present 8 h after ingestion. It is therefore unlikely that piglets were not exposed to anethol at all through their mother's milk. Another possibility is that all piglets, including the control piglets, have been postnatally exposed through the experience with anise in the group preference and gate preference tests, which would explain the lack of contrast between the non-exposed piglets and the postnatally exposed piglets. Experience with anise in these tests may have been associated with a positive environment (the home pen) and may therefore may have caused similar effects on stress and preference as postnatal exposure through the maternal diet. In line with this, infants that live with alcoholic parents show more behaviour directed at toys scented with ethanol, whereas infants whose mother often uses vanilla products directed more behaviours to vanilla-scented toys (Mennella and Beauchamp 1998). Mennella and Beauchamp (1991), however, suggest that the exposure to a flavour through mother's milk may have stronger effects on preference than mere olfactory exposure to the flavour because the suckling and chewing movements made during milk ingestion enhance retro-nasal stimulation. Furthermore, suckling is a highly rewarding experience for a young animal (Nowak et al. 1997) and the intake of the flavour in combination with milk may also result in satiety. The rewarding and satiating effects of milk intake may lead to a stronger association of the flavour to food, which is more relevant for the animal than mere exposure to flavours in the environment. The stronger association with food may induce a larger change in structures and functioning of the brain, resulting in a stronger preference for the flavour than when the flavour is only present in the environment. Also, in dogs, postnatal exposure to anise did not change the preference for this flavour (Hepper and Wells 2006). This suggests that, in some species, postnatal exposure alone may not be an important mechanism to modify the offspring's preference, whereas in others it is. The pig is a relatively precocial animal, and the brain of pigs, which has a perinatal growth spurt (Book and Bustad 1974), is probably less plastic in the period after birth than that of altricial species (Brunjes 1983), and therefore less sensitive to postnatal modification of flavour preference through milk. Furthermore, under (semi-)natural conditions piglets leave the nest to start exploring food items together with the sow starting a few days after birth (Jensen 1988). It may be adaptive to have a preference for healthy and available food types already before this period to avoid the intake of toxins, and this may be established mainly by prenatal experience. Humans, rats and rabbits start exploring food types later than piglets, whereas dogs are intermediates with their first exploration of solid food at 2 weeks of age (Scott and Fuller 1965). Consequently, there is more time available for postnatal programming of flavour preference before the first intake of non-milk food types in humans, rats and rabbits, and thus, postnatal flavour exposure could have, in contrast to pigs and dogs, additional benefits for programming.

Effects of pre- and postnatal exposure on stress during re-exposure

In this experiment we found several effects of prenatal exposure to a flavour on behaviours that are indicative of stress during re-exposure to the flavour. For instance, vocalizations and escape attempts were reduced in the novel environment test, and contact behaviour with the other piglet was reduced in the rooting preference test. This indicates that stress levels within the test setting were different for animals previously exposed to the flavour than for un-exposed animals. This is in line with studies in pigs, as well as in chickens, in which a scent to which animals were familiarized during rearing reduced stress-related behaviours in a novel environment (Jones et al. 2000; Jones 1985). The results of the current experiment suggest that investigating stress levels or stress-related behaviours during (re-)exposure in a stressful test setting can serve as a recognition test, irrespective of preference for the flavour, and may be a useful tool in research on prenatal and postnatal olfactory learning.

The reduced stress levels, caused by the familiarity of the flavour present in the test setting, may in turn have affected the behaviours measured as indicators of preference in different ways, as seen in the gate preference test and the rooting preference test. Animals that were prenatally exposed to anise directed more sniffing and chewing behaviour toward the anise gate in the gate preference test. A higher percentage of time spent exploring a flavour indicates a higher preference for the anise flavour for prenatally exposed animals compared to unexposed animals (Hepper, 1988; Schaal et al., 2000; Wells and Hepper, 2006). In the rooting preference test, however, animals prenatally exposed to anise showed less exploration of the anise-scented substrate and more exploration of the unfamiliar mint-scented substrate than unexposed animals, suggesting lower neophobia for the unfamiliar mint flavour. This finding may also point to recognition of the anise in previously exposed piglets. Pigs are neophilic in nature and explore familiar objects or individuals less than strange objects or individuals (Kristensen et al. 2001; Wood-Gush and Vestergaard 1991). Although both the rooting preference and gate preference tests did not induce high stress levels, there are several factors linked to the setup of the tests that can explain the differences in the expression of anise recognition found in both tests. On the one hand, the design of the test itself may have affected the response of the animals. Animals were forced to solve a task in the gate preference test: make a choice and escape the test situation, thereby returning to the sow. In the rooting preference test, animals were not forced to choose as the test was designed to induce explorative behaviour and the expression of their neophilic nature. This may have led to different motivations of the animals in both tests and thus a different expression of the preference resulting from prenatal exposure to the flavour.

On the other hand, the rooting preference test may have induced lower stress levels than the gate preference test, thereby inducing more neophilic behaviour for prenatally exposed animals. Animals were older in the rooting preference and thus less sensitive to stress induced by separation from the sow than piglets of 6 days old (Worobec et al. 1999), as well as more eager to explore relatively novel environments. Also, piglets were tested in pairs in the rooting preference test, whereas animals in the gate preference test were tested individually. Though the number of vocalizations per piglet was comparable for both tests, the motivation of the animals to vocalize may have differed greatly. Animals that are separated from sow and litter-mates, as in the gate preference test, may have vocalized more to reinstate contact with their pen mates and sow on the other side of the barrier, which may be indicative of stress. Animals in the rooting preference test may have had more social contact vocalizations, which are not indicators of stress. The lower stressfulness of the rooting preference test, further lowered by the familiarity of the anise flavour, likely induced the neophilic behaviour toward the mint flavour. In the gate preference test, however, the design of the test and the stress-reducing effect of the familiar flavour likely led to the observed increased expression of preference for anise by prenatally exposed animals. When designing a flavour preference test, it is thus important to consider the design of the test, the stress level it induces, the motivations of the animals within the test, as well as the effect the familiar flavour may have on the stress levels and the consequent expression of preference.

Conclusions and implications

In conclusion, prenatal flavour exposure through the maternal diet affected behaviour of piglets during re-exposure tests, indicating recognition of the flavour. Adding the flavour to the maternal diet after birth had an additive effect to prenatal exposure yet did not result in familiarization without prenatal exposure. Behavioural effects in preference and recognition tests may be modulated by the design of those tests, however. The stressfulness of the test, which may be lower for experienced animals because of the mere presence of a familiar flavour in the test environment, may interfere with the expression of preference. A next step will be to see whether prenatal exposure to a flavour also reduces stress and subsequently increases the acceptance and intake of flavoured food after weaning in piglets. If this proves true, the welfare and performance of piglets around weaning under the current husbandry conditions may be significantly improved.

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07

PRENATAL FLAVOUR EXPOSURE AFFECTS GROWTH, HEALTH AND BEHAVIOUR OF NEWLY WEANED PIGLETS

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ABSTRACT

Young animals can learn about flavours from the maternal diet that appear in the amniotic fluid and mother's milk, which may reduce neophobia for similarly flavoured food types at weaning. Flavour learning may be beneficial for piglets, which after the rather abrupt weaning in pig husbandry frequently show a period of anorexia, reduced health, and stress-induced behaviours. We investigated the effects of pre- and postnatal flavour exposure through the maternal diet on acceptance of a similarly flavoured food and subsequent growth, health and behaviour of newly weaned piglets.

Sows were offered anise-flavoured (F) or control (C) food during late gestation. Piglets were cross-fostered after birth, with each sow fostering 5 piglets from an F sow and 5 from a C sow. During lactation, sows were offered F or C food, resulting in FF, CF, FC and CC piglets. Piglets were weaned on day 25 and were given both control and flavoured food for two weeks using a double food choice approach.

The flavoured food was not preferred. Yet, prenatally exposed animals showed a higher food intake and a higher body weight in the first days after weaning, and a lower occurrence of diarrhoea than non-exposed piglets. Prenatal exposure also increased the latency to fight, and reduced oral manipulation of pen mates and mounting during the first two weeks after weaning.

Prenatal exposure, but not postnatal exposure alone, to anisic flavour through the maternal diet reduced weaning-associated problems in piglets and enhanced their health and welfare in the period after weaning.

INTRODUCTION

Young animals face a major challenge at weaning, when they have to make the transition from suckling mother's milk to the independent selection and ingestion of healthy and nutritious food items. Sampling potential food sources through a trial-and-error process of individual learning can be time-consuming and dangerous. Therefore young animals tend to rely on experienced conspecifics for obtaining information about appropriate food types and foraging behaviour, i.e. social learning (Galef and Giraldeau 2001). This social learning about food and feeding may be most effective when the information is provided by the mother, as mother and offspring generally share the same environment and thus encounter the same food sources (Sibly 1999; Thorhallsdottir et al. 1990). They furthermore are genetically related and are therefore likely to have a comparable physiological response to food items (Laland et al. 1993). The offspring may learn from the mother through mechanisms like imitation (Akins et al. 2002; Galef and Clark 1971; Miklosi 1999), local enhancement (Cadieu et al. 1995) or social facilitation (Keeling and Hurnik 1996; Visalberghi and Addessi 2000a). Social learning, however, may also take place more indirectly, through olfactory or gustatory cues from the mother's diet. These chemosensory cues may be transmitted from the mother to the offspring through fur, breath, or faeces (Bilko et al. 1994; Morrow-Tesch and McGlone 1990) and this information can be used to select appropriate food types.

Animals can already make use of chemosensory cues before birth, especially flavour cues from the maternal diet that reach the foetuses through the amniotic fluid and/or placental blood stream. Studies in several species, such as rats, humans and dogs have demonstrated that prenatal exposure to flavours from the maternal diet may lead to an olfactory preference for these flavours postnatally (Hepper 1988; Mennella et al. 2001; Schaal et al. 2000). Prenatal exposure to flavour has been demonstrated to lead, furthermore, to an increased acceptance of similarly flavoured food types after weaning in sheep (Simitzis et al. 2008) and rabbits (Bilko et al. 1994). The programmed preference for flavours that have been experienced prenatally may be strengthened when these flavours are also experienced postnatally through the maternal milk (Galef and Henderson 1972; Galef and Sherry 1973; Mennella 1995). Postnatal exposure through the milk alone, however, does not lead to increased preference in all animals: dogs did not prefer for the flavour to which they had been exposed only postnatally, although both pre- and postnatal exposures did lead to a higher preference (Hepper and Wells 2006). The plasticity of the brain after birth and the relative behavioural maturity of the offspring in the early postnatal phase may determine whether chemosensory learning through the mother's milk occurs, either independent of, or in addition to prenatal experience (Leon et al. 1984).

Early learning about healthy and nutritious food types may be of particular importance for piglets in commercial pig husbandry, which are generally weaned more abruptly and at an earlier age than occurs under (semi-)natural conditions (Jensen 1988). The weaning process consists of multiple stressors. Piglets face a change in diet, because most piglets, in contrast to their conspecifics in (semi-) natural conditions, are still fully dependent on sow's milk and have little or no experience with solid food, even though solid food is generally provided before weaning. Moreover, they are separated from the sow, relocated, and face a change in physical and hygienic and often also in social environment, as piglets are frequently regrouped at weaning. The confrontation with unfamiliar conspecifics results in vigorous fighting in the first hours after weaning (Fraser et al. 1998). In addition, the weaning stress can result in the occurrence of other harmful behaviours, such as 'belly nosing', a behaviour similar to massaging the udder of the sow except directed at pen mates, and the damaging of other body parts of pen mates by chewing and biting (Puppe et al. 1997; Torrey and Widowski 2006). Stress negatively affects food intake and intestinal functioning, both by the inhibition of the digestive system (Krahn et al. 1990; Smagin et al. 1999) and by increasing neophobia for the solid food, due to increased corticotropin-releasing hormone (CRH) levels (Britton et al. 1982; Job and Barnes 1995). As the food provided is unfamiliar to most piglets, the latency to first ingestion of food is long, sometimes up to 76 hours (Bruininx et al. 2002b), and many piglets have energy intakes below maintenance for the first four days after weaning (Le Dividich and Sève 2001). This period of anorexia, due to the stress of weaning and food neophobia, leads to changes in intestinal morphology, characterized by shortened villi and increased crypt depth (Bruininx et al. 2002a; Hampson 1986; Pluske et al. 1997). The changes in villous morphology lead to a reduced ability of the intestine to absorb nutrients, resulting in a loss of intestinal integrity, and reduced growth rates or even weight loss in the first days after weaning (Pluske et al. 1996). The period of anorexia is generally followed by a high incidence of compensatory feeding while the intestine cannot sufficiently absorb nutrients. The changes in gut functioning and weaning-related stress may lead to a high occurrence of diarrhoea, which also negatively influences growth rates (Barnett et al. 1989; Björk 1989). The ingestion of food shortly after weaning reverses most of the intestinal problems, suggesting that an immediate intake of nutrients after weaning is crucial to minimize health and welfare problems associated with poor intestinal functioning postweaning (McCracken et al. 1999). Thus far, however, most studies are unsuccessful in significantly increasing food intake of piglets after weaning (Widowski et al. 2008).

We hypothesised that providing piglets with flavoured food at weaning that matches the flavour in the maternal diet to which they have been exposed either pre- and/or postnatally, would reduce neophobia and increase preference for the flavour treated food. The reduced neophobia might in turn increase food intake in the first days after weaning, which will likely reduce the health and welfare problems associated with the weaning process. Piglets were either prenatally and/or postnatally exposed to flavour and offered both flavoured and unflavoured food at weaning. Postweaning food intake, growth and health status were evaluated. Behaviour of piglets was measured to get an indication of how well the animals cope with the stressors associated with the weaning process.

MATERIALS AND METHODS

Animals and housing preweaning

The Animal Care and Use Committee of Wageningen University approved the experiment. The experiment was set up in a 2x2 factorial arrangement and was carried out in two successive batches. A total of 23 multiparous gestating sows gave birth to the experimental piglets. Sows were exposed to control food (C, n = 11) or to identical food to which a flavour (see below) was added (F, n = 12) during days 98 to 108 of gestation. Mammalian foetuses of several species appear to be able to detect and retain chemosensory information during the last trimester of gestation and an exposure of less than 10 days was sufficient to induce postnatal recognition in rats and humans (Hepper 1988; Schaal et al. 2000). All sows were provided with unflavoured food between day 108 of gestation and day 6 postpartum. Flavour treatment ceased on day 108, a week before farrowing (gestation length in pigs is 115 days), to prevent flavour exposure through mother's faeces, milk or breath before the postnatal flavour treatment started (see below). Sows were housed individually in pens of 3.5 m x 2.2 m in four different stables from day 95 of gestation onwards: two control stables and two flavour stables. Sows were moved to four clean stables in which the test flavour had not previously been present on day 110 of gestation, in which they were housed individually in farrowing pens (3.5 x 2.2 m). All sows farrowed within a four-day range (gestational day 115-118). One day after the last sow farrowed, all piglets were cross-fostered to another sow. Each sow fostered ten piglets (Tempo x Topigs 30 line) from another sow: five piglets from a sow fed F-feed during gestation, and five from a sow fed C-feed during gestation. The distribution of piglets from both sexes was balanced for litters and treatments. A half litter generally consisted of piglets originating from the same sow. In some cases of small litter sizes the half litter consisted of offspring from two different sows; piglets were always cross-fostered with at least one sibling.

Half of the sows of each gestational dietary treatment were fed F food from days 6 to 24 of lactation, and the other half of the sows were fed C food. Hence, piglets were exposed to flavour through their (foster)mother's diet, either only prenatally, from days 98 to 108 of gestation (FC, n = 11 half litters), only postnatally from day 6 to 24 of lactation (CF, n = 11 half litters), both pre- and postnatally (FF, n = 12 half litters) or not at all (CC, n = 11 half litters). No food was provided to the piglets during the lactational period. Sows and piglets were provided with approximately 100 g of straw per day. Lights were switched on at 7:00 and off at 19:00.

Flavour exposure

Anise was chosen as the experimental flavour in this experiment. Previous research has shown that pre- and postnatal exposures to anise through the maternal diet results in recognition by the offspring in humans and dogs (Hepper and Wells 2006; Schaal et al. 2000). Anise is furthermore known to be accepted in food by pigs (Langendijk et al. 2007). All sows in the flavour (F) groups received a daily dose of 350 mg trans-anethol (Sigma-Aldrich), the molecule responsible for the anisic flavour (Karaali and Başoğlu 1995), provided in two daily portions of 175 mg. The dose was based on a study in humans (average anethol dose of 121.2 mg per day (Schaal et al. 2000)), scaled for the average body weight of sows during late gestation. The portions of 175 mg anethol were dissolved in 20 ml soy oil and were kept in 20 ml syringes in the dark. Syringes containing 20 ml soy oil without anethol were prepared for the control sows and were kept at another part of the experimental facility than the anethol solutions to prevent any contamination.

The anethol solution (or plain oil) was sprayed on top of a portion of 300 grams of food (standard commercial gestation or lactation sow diets), which was between 4% and 10% of the total daily amount of food provided, depending on the gestational and lactational stage. This food mixture was presented to the sows in separate feeding troughs which could be placed in the trough present in their home pen and removed immediately after the sow finished eating the anethol food, to minimize flavour exposure through the air. Additional food was given only when the sow completely finished the treated food portion, resulting in an anethol uptake of 100% for sows in the experimental groups. When the sow finished eating the treated food portion, the separate trough was removed from the pen and transported in closed plastic bags to a separate room for cleaning. A protocol was followed to prevent exposure of the control sows and piglets to the anisic flavour. This protocol consisted of feeding the control sows before the flavour sows, wearing gloves, keeping the exposure of anethol-contaminated objects (i.e. troughs and syringes) to the air to a minimum by transporting them in plastic bags and changing clothes between the morning and afternoon feeding period.

Animals and housing postweaning

Piglets were weaned at 28 days of age (average age ± standard deviation: FF 28 ± 1.1 days, FC 28 ± 1.0 days, CF 28 ± 1.2 days, CC 28 ± 1.1 days) and housed in pens of 2.1 m x 2.8 m containing five piglets from the same pre- and postnatal treatment. All piglets were mixed within treatments, resulting in pens containing animals that were generally unfamiliar to each other, with the exception of one (foster) sibling pair per pen. Ill-appearing piglets or piglets with a body weight lower than 5 kg were excluded from the experiment, resulting in ten FF pens, eight FC pens, eight CF pens and nine CC pens. Each pen contained either three males and two females, or two males and three females.

All pens were provided with a standard weaner diet (control food) on one side of the pen, and the identical food with 150 ppm of anethol added (flavoured food) on the other side of the pen. The control and flavoured food were each provided in two food troughs per pen with in total five feeding spaces for each flavour, in order to minimize competition for food. The powdered anethol (Lucta S.A., Spain) was mixed into the food at the moment of provisioning to ensure that the flavour would be present for some time after provisioning. Food was added when the trough appeared to be almost empty, and was renewed completely on the days when the food was weighed, from day 2 onwards (see below).

All piglets were housed on a bedding of wood-shavings. Each pen received approximately 60 grams of straw each day. Lights were switched on at 7.00 h and off at 19.00 h.

Performance and health measurements

Intake of both the control and flavoured food at pen level was determined at 6 h, 24 h, 48 h, 3 d, 5 d, 8 d, 12 d and 14 d after weaning by weighing the trough and subtracting trough weight. Body weight of piglets was measured at weaning and days 2, 5, 8 and 14 after weaning. The prevalence of diarrhoea for each individual piglet was examined daily by visual inspection of the area around the anus of the piglet. Any presence of yellow, grey or watery brown faeces around the anus was taken as an indication of intestinal problems. The total number of days that a piglet suffered from diarrhoea within the first two weeks after weaning was calculated and used for analysis.

Behavioural measurements

Video recordings of the first days after weaning were analysed using focal sampling, continuous recording with the Observer 5.0 (Noldus Information Technology B.V., Wageningen, The Netherlands). All piglets were individually marked by a back number to enable identification throughout the entire postweaning period. The behaviours scored were eating from the anise feeder, eating from the control feeder, nosing the anise feeder, and nosing the control feeder. Durations of these behaviours were recorded during the first 7 hours after weaning (i.e. from weaning until the onset of the dark period). Latencies of these behaviours were scored for each piglet using recordings from the first 48 h postweaning and averaged per pen for statistical analysis. Animals that had not eaten or nosed the feeders after 48 h were given the maximum time score of 48 h. In addition, aggression was scored in the first hour after weaning. Aggression consisted of biting, head knocking and pushing or ramming pen mates (Bolhuis et al. 2006).

Behaviour in the home pen was scored live using 2-min instantaneous scan sampling for 6 h per day on days 1, 5, 9 and 13 after weaning. Observation hours started at 8:00, 9:15, 10:30, 14:00, 15:15 and 16:30. Data were collected on the Psion Workabout MX with the Observer 5.0 (Noldus Information Technology B.V., Wageningen, The Netherlands) installed on it. The ethogram used is given in *[table 1]*. The observers were blind to the treatments of the different pens. They were trained before the experiment, and a high inter-observer reliability was achieved both within and between batches.

Statistical analyses

Data were averaged per pen before analysis. Effects of pre- and postnatal flavour treatments were analysed with mixed linear models in SAS (SAS 9.0, SAS Institute Inc.). Proportions of time, frequencies, durations and latencies were arcsine square root-, square root- and log-transformed, respectively, if the residual variance was not normally distributed.

Body weight, food intake and home pen behaviour were analysed with a repeated measures mixed model using an autoregressive covariance structure, including prenatal treatment, postnatal treatment, day and their interactions, as well as batch, as class variables. Food intake and body weight were also analysed for each day separately, to investigate effects in the first days after weaning, which is the most critical period. For this analysis a model was used which included prenatal treatment, postnatal treatment, their interaction and batch as class variables. The same model was used for the number of diarrhoea days and the behavioural video recording analyses. Data are presented as means ± SEM based on pen averages.

table 1.

behaviour	description
aggression	ramming, pushing or biting pen mate, including mutual fighting
mounting	placing both front hoofs on the back of a pen mate
manipulative behaviour	chewing on body part (e.g. ear, tail, flank, paw) of pen mate, or rubbing belly pen mate with force
social behaviour	touching head or body pen mate with nose
play behaviour	rolling, pivoting, shaking substrate, or running while barking
exploration	nosing, rooting or chewing substrate or objects in pen
inactivity	lying down with eyes open or closed
other	any other type of behaviour not mentioned above

Behavioural categories scored during live home pen observations.

RESULTS

Growth and health measurements

Food intake

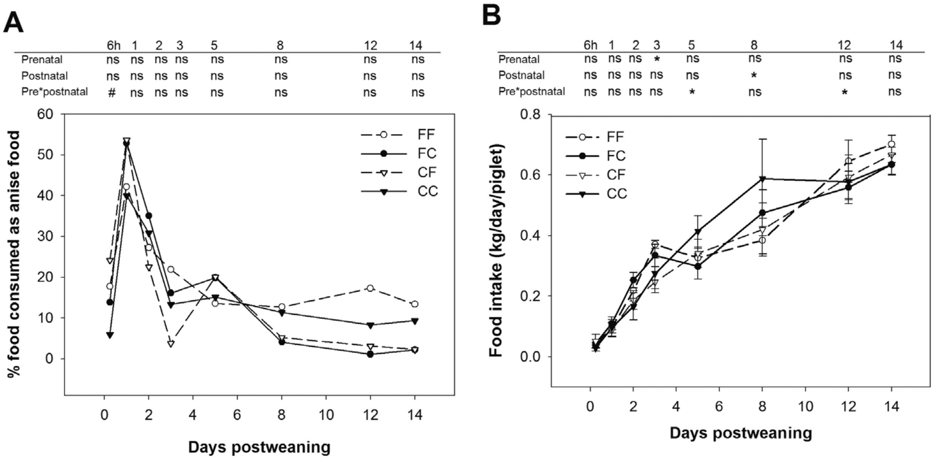
During the first two weeks after weaning, piglets consumed relatively less anise-flavoured food than control food *[figure 1A]*. Only during the first day postweaning, the proportion of anise-flavoured food approached 50% of total intake. The proportion of anise-flavoured food consumed over time differed between prenatally exposed and prenatally non-exposed piglets (prenatal x day interaction, F7,217 = 2.3, P < 0.05, *[figure 1A]*). Analysis per day revealed that, during the first 6 h after weaning, CF animals tended to eat more anise food compared to control food than CC animals (22.9 ± 11.4 versus 16.7 ± 6.8 %, pre x postnatal interaction, F1,29 = 4.0), with FF and FC animals showing intermediate levels. No other effects of prenatal exposure were found for separate days.

Prenatal and postnatal exposures to anise affected the course of total food intake (control + anise-flavoured food) during the first two weeks postweaning (prenatal x day interaction, F1,217 = 2.3, P < 0.05 and postnatal x day interaction, F1,217 = 1.8, P < 0.1, *[figure 1B]*). Prenatally exposed animals showed a higher food intake in the first days after weaning (day 3: 0.28 ± 0.02 versus 0.22 ± 0.02 kg/day/piglet, F1,30 = 7.3, P < 0.05, day 2: 0.18 ± 0.02 versus 0.14 ± 0.02 kg/day/piglet, F1,30 = 3.1, P < 0.1). After this period, animals that were not prenatally exposed showed a compensatory higher food intake (CC between days 3 and 5, 0.34 ± 0.04 versus 0.26 ± 0.03 kg/day/piglet for the other treatments, pre x postnatal interaction, F1,30 = 4.2, P < 0.05; FC and CC between days 5 and 8, 0.42 ± 0.06 versus 0.32 ± 0.04 kg/day/piglet for the other treatments, postnatal effect, F1,30 = 4.2, P < 0.05). FF animals showed a higher food intake between days 8 and 12 postweaning than animals of the other treatments (0.52 ± 0.06 versus 0.46 ± 0.04 kg/day/piglet, pre x postnatal interaction, F1,30 = 5.4, P < 0.05).

Body weight

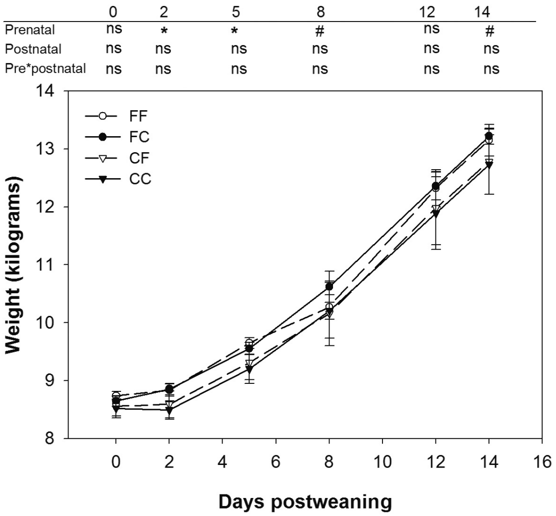
Birth weight or weight at weaning did not differ between the treatment groups (p > 0.2). Prenatally exposed piglets showed a higher body weight than prenatally unexposed piglets on day 2 after weaning (8.8 ± 0.1 versus 8.5 ± 0.1 kg, F1,30 = 5.1, P < 0.05) and day 5 after weaning (9.6 ± 0.1 versus 9.3 ± 0.2 kg, F1,30 = 5.3, P < 0.05, *[figure 2]*). Prenatally exposed animals still tended to have a higher body weight than prenatally unexposed animals on postweaning days 8 (10.4 ± 0.2 versus 10.2 ± 0.4kg, F1,30 = 3.4, P < 0.1) and 14 (13.2 ± 0.2 versus 12.8 ± 0.4 kg, F1,30 = 3.3, P < 0.1). There was no effect of pre- or postnatal treatment and their interactions with time on body weight over the entire postweaning period (prenatal: F1,30 = 2.7, P = 0.11; postnatal: F1,30 = 0.02, P = 0.89; pre x postnatal: F1,30 = 0.02, P = 0.88; prenatal x day: F5,154 = 0.35, P = 0.88; postnatal x day: F5,154 = 0.68, P = 0.64; pre x postnatal x day: F5,154 = 0.19, P = 0.97).

figure 1.



Percentage of food consumed in a double choice set up as anise food (A) and total food intake (B) during the first 14 days after weaning for piglets that were exposed to anise both pre- and postnatally (FF), prenatally (FC), postnatally (CF), or never (CC). * P<0.05, # P<0.1, ns not significant.

figure 2.

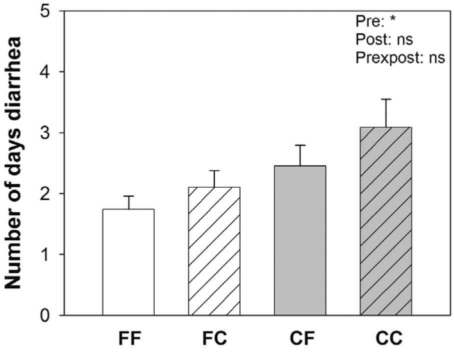


Body weight during the first 14 days postweaning for animals that had been exposed to anise both pre- and postnatally (FF), prenatally (FC), postnatally (CF), or never (CC). * P<0.05, # P<0.1, ns not significant.

Diarrhoea

Piglets that were prenatally exposed to anise suffered less days from diarrhoea after weaning than non-exposed animals (1.9 ± 0.2 versus 2.8 ± 0.3 days, $F1,30 = 4.8$, $P < 0.05$, *[figure 3]*). Postnatal exposure ($F1,30 = 2.1$, $P = 0.17$) or its interaction with prenatal exposure ($F1,30 = 0.06$, $P = 0.81$) did not affect number of diarrhoea days.

figure 3.



Percentage of food consumed in a double choice set up as anise food (A) and total food intake (B) during the first 14 days after weaning for piglets that were exposed to anise both pre- and postnatally (FF), prenatally (FC), postnatally (CF), or never (CC). * P<0.05, # P<0.1, ns not significant.

Behavioural observations

Video observations

Individual piglets varied largely in their latency to ingest food after weaning. The fastest piglets ingested their first meal at 0.5 min postweaning, whereas 11 piglets (6 FF, 1 FC, 1 CF and 3 CC) had not eaten at all during the first 48 h and were given the maximum time score. Latencies to start eating were strongly affected by pen (pen effect: GLM, $F30,131 = 5.59$, $P < 0.0001$). Pre- or postnatal exposure to anise through the maternal diet did not affect the time spent eating or nosing the feeders, nor the time spent on aggression in the first hour after weaning *[see table 2]*. Piglets that had been exposed to anise prenatally showed, however, a longer latency to initiate aggression than animals that had not been exposed prenatally (30.4 ± 4.2 versus 19.7 ± 2.1 min, $F1,30 = 5.8$, $P < 0.05$). Piglets that had been exposed to anise both pre- and postnatally showed a longer latency to explore the feeder containing anise food than piglets that had only been exposed prenatally or only postnatally (164 ± 68 versus 113 ± 107 min, pre x postnatal interaction, $F1,30 = 5.7$, $P < 0.05$). The CC treated piglets did not differ from any of the other groups in the latency to explore the anise feeder *[table 2]*.

table 2.

behaviour	FF	FC	CF	CC	Pre1	Post1	Pre* post[1]
durations (% of observed time)							
eating anise feeder	0.07 ± 0.02	0.19 ± 0.09	0.17 ± 0.09	0.07 ± 0.02	ns	ns	ns
eating control feeder	0.21 ± 0.07	0.21 ± 0.11	0.30 ± 0.21	0.30 ± 0.14	ns	ns	ns
nosing anise feeder	0.4 ± 0.09	0.6 ± 0.28	0.3 ± 0.14	0.3 ± 0.1	ns	ns	ns
nosing control feeder	0.5 ± 0.17	0.5 ± 0.17	0.3 ± 0.1	0.6 ± 0.3	ns	ns	ns
aggression	3.8 ± 0.67	3.7 ± 0.5	5.3 ± 1.5	4.8 ± 0.83	ns	ns	ns
latencies (min)							
eating anise feeder	568 ± 243	384 ± 208	502 ± 271	604 ± 209	ns	ns	ns
eating control feeder	822 ± 353	636 ± 254	431 ± 162	571 ± 245	ns	ns	ns
nosing anise feeder	164 ± 68	56 ± 54	57 ± 54	99 ± 45	ns	ns	*
nosing control feeder	99 ± 37	97 ± 62	43 ± 28	110 ± 56	ns	ns	ns
aggression	35 ± 7.7	25 ± 3.6	17 ± 1.8	22 ± 3.6	*	ns	ns

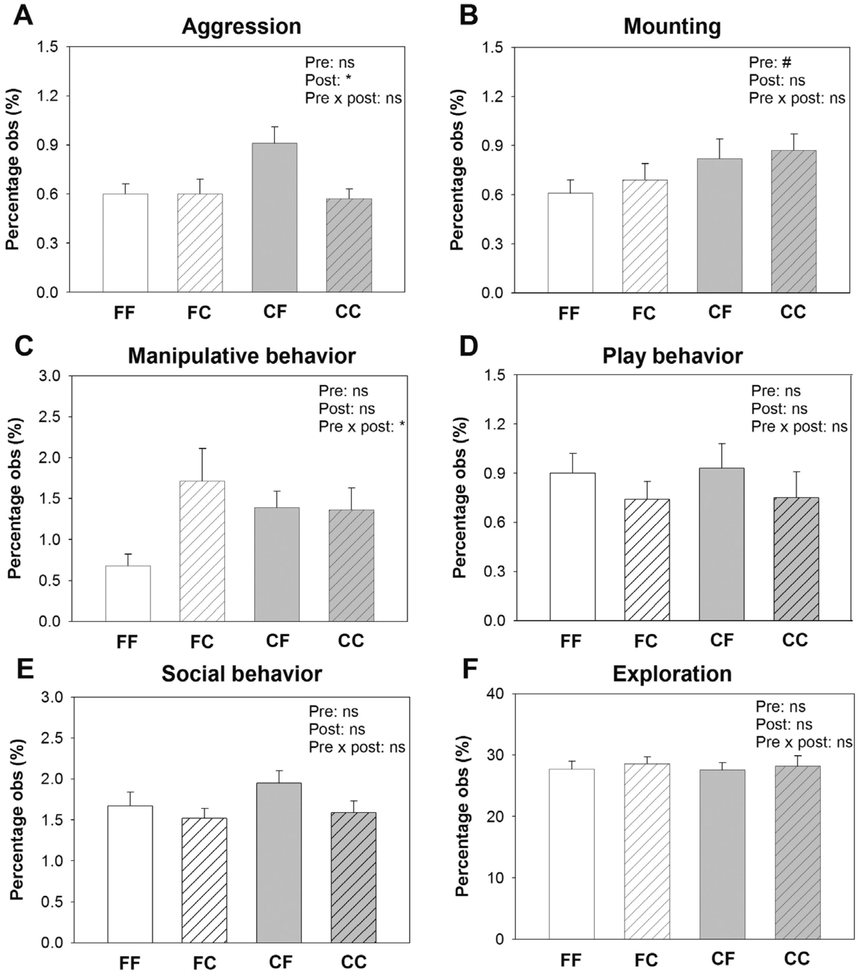
Behaviour (mean + S.E.M.) scored from video observations during the first hour (aggression), first seven hours (durations of other behaviours) and first 48 hours (latencies) postweaning.
FF = pre + postnatal exposure to anise, FC = prenatal exposure to anise, CF = postnatal exposure to anise, CC = no exposure to anise through maternal diet.
[1] Statistical significance
* Means differ significantly (P<0.05)

Home pen observations

Over the entire 2-week postweaning period, postnatally exposed piglets (FF + CF) spent more time on aggression than the other treatment groups (0.74 ± 0.06 versus 0.59 ± 0.05%, F1,30 = 4.5, P < 0.05, *[figure 4A]*), mainly caused by higher aggression levels in the CF group.

Prenatal exposure (FF + FC) tended to reduce mounting behaviour (0.64 ± 0.06 versus 0.84 ± 0.07 %, F1,30 = 4.0, P < 0.1, *[figure 4B]*). Piglets that had been exposed to anise both pre- and postnatally (FF) showed less oral manipulation of pen mates than piglets from the other treatments (0.68 ± 0.1 versus 1.49 ± 0.3 %, pre x postnatal interaction, F1,30 = 5.0, P < 0.05, *[figure 4C]*). No effects of pre- and/or postnatal treatment were found on play behaviour *[figure 4D]*, social behaviour *[figure 4E]*, exploration of the pen *[figure 4F]* and inactivity (FF: 52 ± 2.1%, FC: 49 ± 1.7%, CF: 50 ± 2.0%, CC: 49 ± 2.4%).

figure 4.



Aggression (A), mounting (B), manipulating pen mates (C), play behaviour (D), social behaviour (E) and exploration (F) during the 14-d postweaning period, in percentage of scans within the observations, for animals exposed to anise both pre- and postnatally (FF), prenatally (FC), postnatally (CF), or never (CC). * P<0.05, # P<0.1, ns not significant. Please note the differences in scaling.

DISCUSSION

In this experiment the effects of pre- and postnatal anise exposures, through the maternal diet, on preference and acceptance of similarly flavoured food at weaning were investigated, as well as the subsequent growth, behaviour and health in the postweaning period. We found that exposing piglets prenatally to anise flavour from the maternal diet resulted in a higher food intake in the first three days after weaning, a higher growth postweaning and a lower prevalence of stress-related behaviours and diarrhoea. These effects were, however, not caused by a high anise preference: anise-flavoured food intake in the postweaning period was generally low for all treatment groups. The longer latency of piglets exposed to anise both pre- and postnatally to explore the anise feeder suggests, however, that flavour exposure through the maternal diet does lead to recognition of the flavour as pigs tend to explore novel objects or conspecifics more than familiar objects or conspecifics (Kristensen et al. 2001; Wood-Gush and Vestergaard 1991).

There are different explanations for why prenatal exposure to a flavour leads to increased total food intake, without a preference for the flavoured food. Animals may have sampled both the anise food and the control food, and may have detected strong similarities between the two food types. The properties (texture, size, colour) of the anise and control food overlapped to a large extent, as both food types only differed in the anisic flavour added. Animals may have generalised their acceptance of the familiar anise food to the control food, which is a food type optimized in taste and smell for newly weaned piglets. The animals that were not prenatally exposed, on the other hand, might have taken longer to sample and develop a preference for either food source, as none of the food sources were familiar to them, resulting in a lower food intake in the first days after weaning. Generalisation of acceptance of food sources with overlapping properties has been reported to occur in pigs (Van Tien 2006), rabbits (Altbacker et al. 1995) and lambs (Villalba and Provenza 2000b) and seems an important mechanism in the reduction of neophobia for healthy, nutritious food types (Provenza and Villalba 2006). In contrast to other studies investigating preference after prenatal flavour exposure (Simitzis et al. 2008; Smotherman 1982b), there was a clear difference in preference between the anise and the control food irrespective of treatment. The control food is optimized for newly weaned piglets and it is thus likely that the addition of anise made the food less acceptable for piglets in general. Providing the piglets with just the anise-flavoured food may have led to even stronger effects of prenatal exposure, as piglets would not have been able to generalise their acceptance of the flavoured food to the unflavoured food. The provisioning of one type of food with the flavour used in exposure, preferably a preferred flavour, may be interesting for applications in pig husbandry.

Another mechanism through which prenatal exposure may have increased food intake after weaning, irrespective of preference, is through the stress-reducing effect a familiar flavour can have on the animals. Stress-reducing effects of chemosensory stimuli experienced in utero have been reported in humans (Varendi et al. 1998) and rodents. For instance, rats that were prenatally exposed to apple juice showed a preference for apple juice at 60 days of age, as well as lower corticosterone levels during (re-)exposure to the solution compared to control animals (Smotherman 1982b). In line with this, we found that stress-related behaviours in a novel environment, such as high-pitched vocalizations and escape attempts, were reduced when the flavour to which animals had been exposed prenatally was present (Oostindjer et al. 2009). Even though in the current experiment the anise-flavoured food was not consumed in large quantities, its odour was present in the pen, and the scent may have affected stress levels of the prenatally exposed animals, and consequently reduced neophobia, leading to a higher intake of the control food. This is in line with studies in humans and chickens, where familiar flavours from the rearing environment reduce stress-related behaviours (Jones 1985) and negative responses to pain (Rattaz et al. 2005) when present in an unfamiliar environment. In pigs however, stress in novel environments is only reduced by familiar flavours from the rearing environment in some cases, most likely depending on the properties of the odours used (Jones et al. 2000), as well as on the timing and context of the exposure: an odour which is relevant to the animal may have a stronger effect than a non-relevant odour. Continuity of flavour presence may be important here: a match between flavours in the prenatal, postnatal and postweaning period may lead to a stronger association of the flavour with a familiar, safe environment, thereby reducing stress levels (Hepper and Wells 2006; Oostindjer et al. 2009). However, a mere addition of flavour to the preweaning solid food of piglets may not increase intake after weaning (King 1979). This could be due to the timing of exposure, as prenatal exposure seems very important in increasing preference or reducing neophobia for the flavoured food, or the innate preference for the flavour used (Roura et al. 2008). On the other hand, cues from the maternal diet may lead to a much stronger effect of flavour exposure on subsequent food neophobia, due to the fact that they are continuously present, both pre- and postnatally, and animals are exposed to the flavours under reinforcing conditions such as suckling. Thus, to sufficiently prime the brain to the flavour, an association of the flavour with the maternal diet, especially prenatally, may be necessary to increase food intake and reduce stress after weaning, as well as an optimal timing of exposure.

The behavioural data in the current experiment support the idea that the familiarity of the flavour reduces stress after weaning. The longer latency to show aggression for prenatally exposed animals can be interpreted as a more immediate effect of the presence of the familiar flavour on stress or emotionality of the animals. Piglets generally show a short period of vigorous aggression after being introduced to a pen with unfamiliar piglets to establish a dominance ranking (Puppe et al. 1997). The familiar flavour may have altered priorities after weaning, possibly through altered anxiety or stress levels. The reduction in stress has long-term effects on the adaptability of piglets to the new (social) environment: mounting tended to be reduced in prenatally exposed animals, and pre- and postnatal exposures reduced oral manipulation of pen mates. Mounting behaviour can be a form of sexual play (Berry and Signoret 1984), yet is also displayed by adult females of high rank, suggesting that mounting may have a function in sustaining the dominance hierarchy (Arey and Edwards 1998). However, stressors such as weaning and mixing do increase mounting behaviour, indicating that it is induced by stress (Dudink et al. 2006), as is oral manipulation of pen mates (Dybkjær 1992). Both behaviours, which may result in skin lesions, infections and restlessness, indicate maladaptation and lead to impaired welfare for both the animal that performs the behaviour and for its victim.

The higher food intake of prenatally exposed animals resulted in a higher body weight for these animals in the first few days postweaning, yet the effect may also have been mediated by the lower prevalence of diarrhoea in this group. Diarrhoea after weaning is a multi-factorial problem. Part of it is caused by compensatory eating after a period of underfeeding, while the intestinal villi cannot absorb nutrients optimally (Ball and Aherne 1982). The compromised intestinal functioning, caused by low food intake directly after weaning, furthermore increases susceptibility to pathogens, which in turn can also cause diarrhoea (Pluske et al. 1997). The ingestion of small amounts of food directly after weaning may be sufficient to prevent problems leading to diarrhoea (McCracken et al. 1999). Diarrhoea prevalence may thus be directly correlated to food intake, yet stress levels of the animals may also affect intestinal integrity, diarrhoea prevalence and subsequently growth of the animals in the postweaning period. Stress responses, consisting of e.g. an activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system, inhibit digestion by delaying gastric emptying (Taché et al. 1999) and by stimulating colonic motility, resulting in a quick excretion of nutrient sources still present in the gut (Rao et al. 1998). Stress furthermore increases intestinal permeability, leading to an increased risk of intestinal inflammation and a further loss of intestinal integrity (Soderholm and Perdue 2001). If the presence of a familiar flavour reduces stress levels of animals that were prenatally exposed to this flavour, it is likely that gut functioning is less impaired compared to animals that were not prenatally exposed to the flavour. The reduced stress may furthermore have decreased neophobia for the solid food, resulting in a higher food intake and subsequently higher growth after weaning.

Postnatal exposure without prenatal exposure did not have an effect on most of the measured variables in this experiment, which is in contrast to effects of postnatal exposure in humans (Mennella 1995) and rats (Galef and Henderson 1972). As pigs are a semi-precocial species, and are thus relatively mature at birth compared to fully altricial species such as humans and rats, the brain may not be plastic enough for postnatal exposure to result in a strong familiarization with the flavour. Piglets furthermore start to explore their surroundings and possible food sources already in the first week after birth under (semi-)natural conditions (Jensen 1988). It may therefore be very adaptive if familiarization with flavours from the maternal diet already occurs before this period, to decrease the risk of piglets eating inappropriate or even toxic food types. In two small-scale studies in pigs, however, it was found that the addition of flavours different to the one used in the current experiment to both the lactating mother's diet and to the postweaning diet increased food intake and growth after weaning (Campbell 1976; King 1979). In contrast to the current study, the pigs only had access to flavoured food, suggesting that postnatal exposure may work when piglets are not provided with a choice between flavoured and control food. On the other hand, the properties of the flavour may be important for postnatal exposure to have a sufficient effect on postweaning food intake. For example, the use of highly preferred flavours combining pleasant odorants with positive tastants (umami, sweet or salty) enhances food appetence and lead to increases in food intake at weaning in unexposed pigs (Roura et al. 2007).

In conclusion, we found that prenatal exposure of piglets to anisic flavour through the maternal diet increased food intake and body weight and reduced diarrhoea and stress-induced behaviours in the first two weeks after weaning, suggesting improved health and welfare. Results indicate that the effects of prenatal exposure are likely to be established by either an increased acceptance of similar food types (generalisation) or a reduction in stress and neophobia by the mere presence of a flavour in the postweaning environment that has been experienced in utero. The potential stress-reducing effects of prenatally learned flavours merit further research.

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08

PERINATAL FLAVOUR LEARNING AND ADAPTATION TO BEING WEANED: ALL THE PIG NEEDS IS SMELL

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ABSTRACT

Perinatal flavour learning through the maternal diet is known to enhance flavour preference and acceptance of flavoured food in many species. Previously we found positive effects of perinatal flavour learning on food intake, growth and behaviour of piglets postweaning, but no increased preference for the flavour. This suggests that flavour learning in pigs works through a reduction of weaning stress by the presence of the familiar flavour instead. The aim of this study was to investigate whether perinatal flavour learning reduces stress at weaning, and whether the effect is stronger when the familiar flavour is present in the food. Sows were offered an anethol-flavoured diet (Flavour treatment) or control diet (Control treatment) during late gestation and lactation. Flavour and Control piglets were provided with anethol either in their food (Food treatment) or in the air (Air treatment) after weaning. Preweaning and postweaning treatments did not affect food intake, preference or growth in the first two weeks postweaning but flavour treatment reduced the latency to eat (24 versus 35 hours, $P = 0.02$) and within-pen variation in growth (SD within-pen: 0.7 versus 1.2 kg , $P<0.001$). Salivary cortisol levels tended to be lower four and seven hours postweaning for Flavour piglets compared to Control piglets (4 hours: 2.5 versus 3.0 ng/ml, $P=0.05$, 7 hours: 3.1 versus 3.4 ng/ml, $P=0.08$). Flavour piglets played more, tended to show less damaging behaviours and less vocalizations on day 1 postweaning than Control piglets, indicating that the familiar flavour reduced stress around weaning. Very few interaction effects were found between preweaning and postweaning treatment, and no effects of postweaning treatment. We conclude that in the newly weaned pig, perinatal flavour learning results in a reduction of stress when the familiar flavour is present, regardless of whether the flavour is provided in the food or in the air.

Keywords:

perinatal flavour learning
piglet
weaning
stress
development feeding behaviour
familiarity

INTRODUCTION

Young animals need to make important choices regarding what items to include in their diet around weaning. To make these choices, many animals use information from more experienced conspecifics, because trial-and-error learning is time consuming and potentially lethal (Galef and Giraldeau 2001). An important source of information is the mother, as young animals generally direct most of their attention to her. Due to their shared genetics, their responses to food types should be very similar and thus the information obtained from her is likely to be relevant (Chesler 1969; Provenza and Balph 1987).

Young animals can start learning which food types are healthy and nutritious from the mother before weaning, and even before birth. Flavours from the maternal diet can reach the foetus before birth through the amniotic fluid, where the flavours are perceived during mouthing movements or ingestion of the amniotic fluid (El-Haddad et al. 2005; Mennella et al. 1995). Flavours that cross the placental barrier may also reach the foetus via the foetal blood stream, where they can be perceived in the nasal capillaries (Schaal et al. 1995a). Studies on prenatal flavour learning have shown that the offspring of several species, such as humans, rats, dogs, rabbits, cats, sheep and even chickens and frogs, show a preference or reduced aversion to flavours to which they have been exposed before birth or hatching (Becques et al. 2010; Bilko et al. 1994; Hepper 1988; Hepper 1992; Hudson and Distel 1999; Marlier et al. 1998; Mennella et al. 2001; Nicolaïdis 2008; Schaal et al. 1995a; Schaal et al. 2000; Simitzis et al. 2008; Sneddon et al. 1998; Wells and Hepper 2006). Additionally, flavours may reach young mammals after birth through the mother's milk. This continued exposure may strengthen the preference for these flavours as seen in humans, rats, dogs and rabbits (Brake 1981; Coureaud et al. 2002; Galef and Henderson 1972; Hausner et al. 2008; Hepper and Wells 2006; Wuensch 1978). Thus, at weaning, young animals will more readily accept foods containing flavours to which they have been exposed through the maternal diet. This is due to an increases preference for these flavours (Forestell and Mennella 2007; Hausner et al. 2009).

In our earlier work we investigated whether perinatal flavour learning would increase preference and stimulate intake of flavoured foods in piglets (Oostindjer et al. 2009; Oostindjer et al. 2010a). We chose the pig as a model as perinatal flavour learning might potentially be used to improve piglet health and welfare in the immediate postweaning period. Weaning in husbandry occurs abruptly and at an earlier age than in the wild. As a result, many piglets are not yet adapted to eating solid food at weaning, making weaning a stressful event for piglets under husbandry conditions (Bolhuis et al. 2009). We found that piglets exposed to anise flavour (anethol) through the maternal diet, in particular prenatally, recognized anise flavour during lactation, but did not show a clear preference for it (Oostindjer et al. 2009). Moreover, in mildly challenging situations prenatally exposed piglets showed less signs of stress in the presence of the flavour than non-exposed piglets. In the early postweaning period perinatal flavour learning positively affected food intake and growth of piglets, but exposed piglets again showed no preference for the anise, and typically chose control food over the flavoured food that was provided (Oostindjer et al. 2010a). It is possible that piglets were initially attracted to the flavoured food but quickly generalised to the control food (Launchbaugh et al. 1997; Villalba and Provenza 2000b), resulting in an increased overall food intake. On the other hand, the mere presence of the familiar flavour in the postweaning environment may have reduced stress (Dudink et al. 2006; Oostindjer et al. 2009; Oostindjer et al. 2010a). Odours that are familiar to the piglet, for example through association with the mother or the safe and familiar home cage, can reduce stress-related behaviours and neophobia as demonstrated in humans and chickens and our previous study on pigs (Goubet et al. 2007a; Jones 1985; Oostindjer et al. 2009; Rattaz et al. 2005). These odours may have been responsible for reduced weaning stress, which would account for the positive effects of perinatal flavour learning in newly weaned piglets (Oostindjer et al. 2010a).

The aim of this study was to gain more insight in the mechanism of perinatal flavour learning by investigating whether perinatal flavour learning and consequent re-exposure to the flavour can reduce stress of piglets after weaning. We investigated if it is necessary to have the familiar flavour in the postweaning food, or if the same effects on stress and adaptation to weaning can be obtained by having the familiar flavour present in the air.

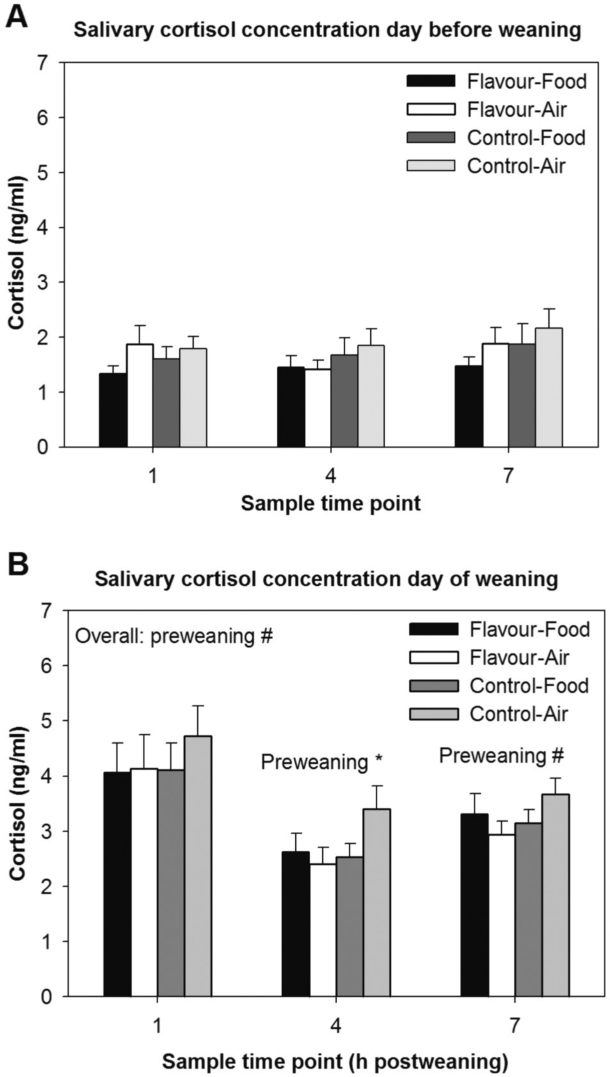
RESULTS

Cortisol levels on day of weaning

Preweaning treatment tended to affect cortisol levels over the three time points measured postweaning, with piglets in the Flavour treatment showing lower levels than Control piglets (F1,43 = 3.53, P = 0.07, *[figure 1]*). Flavour-exposed piglets showed lower cortisol levels four hours after weaning (F1,41 = 4.17, P = 0.05) and tended to have lower cortisol levels seven hours after weaning (F1,42 = 3.19, P = 0.08). Cortisol levels over the three time points measured postweaning were unaffected by postweaning treatment (P = 0.57), or by an interaction between pre- and postweaning treatment (P = 0.21).

When looking at the differences in cortisol levels between the day before weaning (basal samples) and the day of weaning, the Control-Air piglets showed the highest increase in cortisol concentration at the three time points (Control-Air: 2.29 ± 0.4 ng/ml, Flavour-Food: 2.12 ± 0.4 ng/ml, Flavour-Air: 1.66 ± 0.4 ng/ml, Control-Food: 1.58 ± 0.5 ng/ml preweaning x postweaning interaction, F1,43 = 4.52, P = 0.04). Control-Air piglets showed higher cortisol levels than Control-Food and Flavour-Air piglets four hours after weaning (Flavour-Food: 1.5 ± 0.3, Flavour-Air: 1.1 ± 0.3, Control-Food: 0.9 ± 0.3, Control-Air: 2.0 ± 0.4, preweaning x postweaning, F1,41 = 4.28, P = 0.04). A preweaning x postweaning interaction was found seven hours after weaning as well (F1,42 = 3.12, P = 0.09). Preweaning treatment (P = 0.63) and postweaning treatment (P = 0.62) did not affect the increase of cortisol concentrations.

figure 1.



Salivary cortisol levels on the day of before (A) and the day of weaning (B), sampled at the same time points on the day, for piglets exposed to Flavour through the maternal diet or Control piglets, housed in postweaning pens containing the flavour in the Food or in the Air. *: P<0.05, #: P<0.1

Latency to eat, food intake and growth

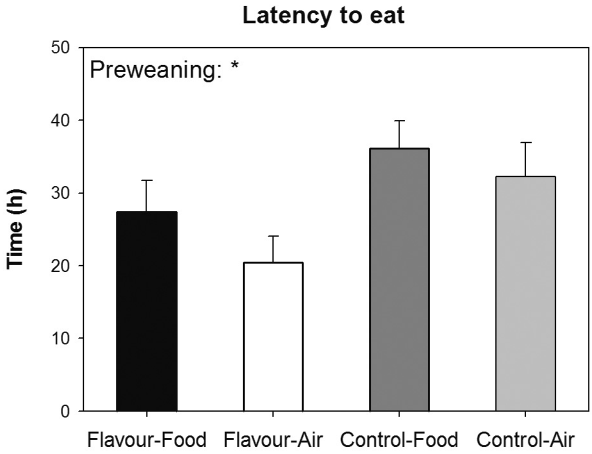
Latency to eat is presented in *[figure 2]*, and other measures of food intake and growth are presented in *[table 1]*. Food intake and body weight were unaffected by preweaning and postweaning treatments on any particular day and only presented over the total postweaning period.

Piglets that were exposed to anise flavour before weaning showed a shorter latency to their first meal than control piglets (preweaning treatment: F1,43 = 6.34, P = 0.02). Postweaning treatment (P = 0.18) and its interaction with preweaning treatment (P = 0.70) did not affect the latency to eat. Total food intake in the first two weeks postweaning was unaffected by preweaning treatment (P = 0.65), postweaning treatment (P = 0.73) and by the pre- and postweaning treatment interaction (P = 0.86). The amount of anise food consumed by the piglets that had anise flavour in the postweaning food was unaffected by preweaning treatment (P = 0.91). Total growth was unaffected by preweaning treatment (P = 0.33), postweaning treatment (P = 0.59) or the treatment interaction (P = 0.11), but the variation in postweaning growth within pens was smaller for piglets exposed to flavour before weaning (F1,43 = 19.2, P < 0.001). Within-pen variation in growth was unaffected by postweaning treatment (P = 0.42) and the interaction between pre- and postweaning treatment (P = 0.91). The percentage of piglets within pen that lost weight in the first three days was not affected by preweaning treatment (P = 0.13), postweaning treatment (P = 0.40) or the treatment interaction (P = 0.90). Numerically, however, fewer piglets in the preweaning Flavour group lost weight in the first three days postweaning, particularly the piglets in the Flavour-Air group.

Behaviour

Piglets that were exposed to flavour preweaning showed more play behaviour in the first two weeks postweaning (F1,43 = 7.60, P = 0.009, *[figure 3A]*). Play behaviour tended to be higher for Flavour-Air piglets than for piglets from other treatments (preweaning x postweaning interaction, F1,43 = 3.17, P = 0.08, *[figure 3A]*) and was unaffected by postweaning treatment (P = 0.16). Piglets that were exposed to flavour before weaning tended to manipulate pen mates less than control piglets (F1,43 = 3.22, P = 0.08, *[figure 3B]*). Manipulative behaviour was unaffected by postweaning treatment (P = 0.51) and its interaction with preweaning treatment (P = 0.54). The percentage of pigs that vocalized per pen tended to be lower for the Flavour piglets on day 1 after weaning than for Control piglets (F1,35 = 3.93, P = 0.06, *[figure 3C]*). Vocalizations were unaffected by postweaning treatment (P = 0.45) and its interaction with preweaning treatment (P = 0.47).

figure 2.



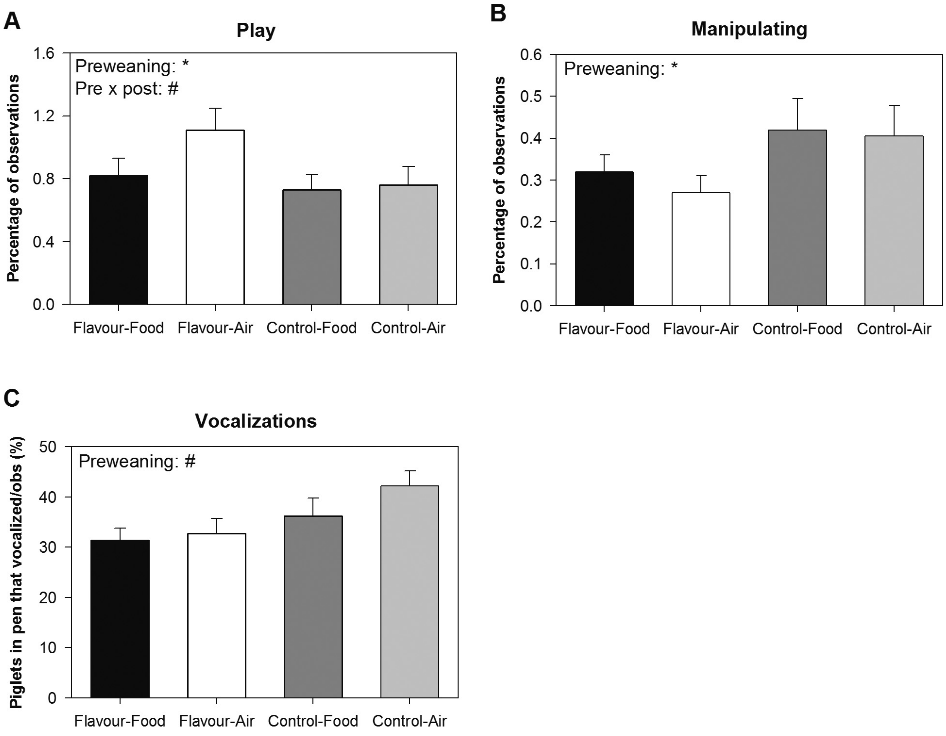
Latency to eat for piglets exposed to Flavour through the maternal diet or Control piglets, housed in postweaning pens containing the flavour in the Food or in the Air. *: P<0.05

table 1.

	treatment				p-values		
	flavour-food	flavour-air	control-food	control-air	pre	post	pre x post
total food intake postweaning (kg)	18.8 ± 1.3	18.9 ± 0.9	18.3 ± 1.4	18.7 ± 1.0	0.65	0.73	0.86
total anise intake postweaning (kg)	4.0 ± 1.3	-	3.7 ± 1.3	-	0.91	-	-
weaning weight (kg)	8.41 ± 0.25	8.54 ± 0.23	8.46 ± 0.25	8.58 ± 0.24	0.84	0.62	0.99
total growth postweaning (kg)	3.80 ± 0.27	3.95 ± 0.21	4.16 ± 0.29	3.86 ± 0.27	0.33	0.59	0.11
variation growth within pen (kg)	0.72 ± 0.08	0.66 ± 0.11	1.12 ± 0.12	1.04 ± 0.09	<0.001	0.42	0.91
pigs losing weight in the first 3 days (%)	16.7 ± 6.4	6.2 ± 3.3	25.0 ± 10.2	18.7 ± 8.2	0.13	0.40	0.90

Growth and food intake after weaning

figure 3.



Play behaviour (A), manipulative behaviour (B) and percentage of piglets per pen that vocalized on day 1 after weaning (C). Piglets were exposed to Flavour through the maternal diet or were Control piglets, housed in postweaning pens containing the flavour in the Food or in the Air. Please note the differences in scale. *: P<0.05, #: P<0.1

DISCUSSION

This study indicates that perinatal flavour learning followed by re-exposure to the familiar flavour at weaning reduces stress in piglets. Flavour-exposed piglets tended to show a faster decrease in salivary cortisol levels after weaning, indicating that the initial stress response was similar for all piglets but that the familiar flavour resulted in a faster recovery. The reduced stress levels were also reflected in the piglets' behaviour, with less vocalizations on the day after weaning and more play behaviour after weaning. Low levels of vocalizations and high levels of play after weaning indicate lower stress levels induced by weaning-related stressors such as maternal separation, relocation and general frustration (Chaloupkova et al. 2007; Dudink et al. 2006; Dybkjær 1992; Weary et al. 1997). There were also effects on the longer-term on variation in growth and behaviour that indicate that stress was reduced and piglets were better able to adapt to the postweaning situation (Botermans and Svendsen 2000; Chaloupkova et al. 2007; Dudink et al. 2006; Dybkjær 1992; Macrì et al. 2007; Weary et al. 1997). Animals are generally more reluctant to eat and try novel foods in unfamiliar environments (Burritt and Provenza 1997) and the shorter latency to ingest solid food of flavour-exposed piglets indicates that the familiar flavour either made the food less novel or the pen less unfamiliar. Time to eat the novel food, as well as cortisol, behaviour and growth, did not differ between piglets that received the flavour in the food or in the air. It is thus not necessary to provide the flavour in the food, which suggests that it is particularly the odour component of the familiar flavour that reduces stress.

Odours that are familiar and associated with a positive experience or environment can result in a strong positive memory recall and consequently affect mood, referred to as the Proust phenomenon (Chu and Downes 2000a, b; Herz 2002; Willander and Larsson 2006). The association of the odour with a positive context may be strongest when the context is related to the mother (Sevelinges et al. 2009; Sullivan and Toubas 1998), though it is not necessary to learn these associations in this particular positive context to affect emotionality and mood (Epple and Herz 1999; Herz 2002). It seems, however, that for hedonic or aversive valence of flavours, it is necessary to make the association with the odour with either a positive or negative experience or environment (Herz et al. 2004b). The amygdala plays an important role in making both positive and negative associations, as well as in creating the emotions during re-exposure to the odour and consequent memory evocation (Buchanan et al. 2003; McGaugh et al. 1993; Winston et al. 2005). Although most studies that looked at how familiar odours or flavours affect emotionality, memory evocation and mood have been performed in humans, there is also evidence from work on chickens and our earlier work in pigs that familiar flavours can also reduce stress and possibly influence emotionality in animals (Jones 1985; Jones et al. 2002; Oostindjer et al. 2009). In the current study, flavours in the amniotic, which is a very attractive and positive substrate for young animals (Arias and Chotro 2007; Mennella and

Beauchamp 1998; Schaal et al. 1995b; Varendi et al. 1998), may have resulted in an association of the flavour with a very positive context: the mother. Whether flavours in the prenatal or postnatal environment are more important for the learning of the association remains unclear from this study, but our previous work in pigs showed practically no effects of postnatal exposure only (from day 6 onwards) (Oostindjer et al. 2009; Oostindjer et al. 2010a). This suggests that the association is made prenatally or in the very early postnatal period, although continuity of flavour exposure might be important to strengthen the association (Coureaud et al. 2002; Guiraudie-Capraz et al. 2005). The association of the flavour with a familiar environment may have positively affected emotions of piglets at weaning when they were re-exposed to the flavour in a stressful situation, thereby reducing stress levels and possibly enhancing ‘mood’. Higher levels of play behaviour by the piglets that were previously exposed to the flavour support the view that their perception of the postweaning period was less negative than that of control animals. The more familiar odour of the pen, due to the presence of the flavour, may also have made the postweaning pen a less novel experience, thereby increasing adaptation to weaning and reducing the negative effects that are generally induced by weaning (Bolhuis et al. 2009).

The results also indicate that the positive valence of the flavour, or the odour component of the flavour, is not generalised to the taste component of the flavour in newly weaned piglets. Flavour-exposed piglets did not have a preference for the anise food, which is in contrast to most studies on perinatal flavour learning that do find a specific preference for and acceptance of foods that are flavoured with the familiar flavour (Becques et al. 2010; Bilko et al. 1994; Hepper and Wells 2006; Marlier et al. 1998; Mennella et al. 2001; Nicolaïdis 2008; Schaal et al. 2000; Simitzis et al. 2008; Todrank et al. 2011). The strong preference of the control food over the anise food by piglets in this study suggests that the palatability of the food was reduced by addition of the flavour, and the use of a highly preferred flavour would likely have positive effects on piglet behaviour when provided in the food. Perinatal flavour learning, however, can reduce the reluctance to accept foods with low palatability (Birch 1998; Hausner et al. 2009; Mennella and Beauchamp 1999; Mennella et al. 2009), but this was not observed in the current study, possibly due to the two-choice feeding protocol used. Despite this, it still seems that the effects of perinatal flavour learning are different in newly weaned piglets than in other species tested. In our view, the positive association with the mother may have overruled any other effects on preference due to the re-exposure to the flavour in a very stressful situation (weaning).

In conclusion, it seems that for the pig, perinatal flavour learning results in a reduction of stress when the familiar flavour is present in a challenging situation. Also, the smell of the familiar flavour alone is enough to obtain the stress-reducing effects, indicating that the positive postweaning effects of perinatal flavour learning do not necessarily involve an increased preference for the flavour.

MATERIALS AND METHODS

Ethics statement

This study was carried out in strict accordance with the recommendations in the European Guidelines for accommodation and care of animals. The protocol was approved by the Institutional Animal Care and Use Committee of the University of Wageningen (Protocol Number: 2010014c).

General setup

The experiment was set up in a 2x2 factorial design including postweaning pen treatments, with preweaning treatment (Flavour or Control) and postweaning treatment (flavour in Food or flavour in Air) as factors. The experiment was conducted in two successive batches.

Animals and housing preweaning

A total of 24 multiparous PIC Camborough sows (commercial synthetic sow line, includes Landrace and Large White breeds) and their offspring (Tempo (commercial synthetic boar line with Great York genetic background) x PIC Camborough) were used. Sows were assigned to the Flavour (n=12) or Control (n=12) treatment group and received anise flavoured or control food, respectively, between days 98 and 115 of gestation (sows generally give birth on day 115 of gestation), and from day 2 after giving birth until weaning the piglets on day 25. Sows were individually housed in four different rooms (2 flavour, 2 control) per batch from day 95 of gestation onwards, in farrowing pens of 3.5 x 2.2 m. Sows were placed in a farrowing crate (2.2 x 0.6 m) from day 115 of gestation until 3 days after birth of the piglets. All pens had a small layer of wood shavings and were provided with approximately 100 grams of straw daily. Litters of piglets were standardized to 10-12 piglets, when necessary, by cross-fostering within treatment groups before day 3 after birth. Both males and females were used. A personality test (backtest) was performed on day 10 to classify piglets as either high resisters (more active coping response to stress and novelty) or low resisters (more passive coping response to stress and novelty), (Bolhuis et al. 2003; Hessing et al. 1993), in order to make a balanced assignment of piglets to pens postweaning (see below). No food was provided to the piglets during the lactation period, but water from drinking nipples was available to all piglets. Lights were on between 7:00 and 19:00.

Flavour exposure protocol

Anise was chosen as the experimental flavour and provided to sows and piglets in the same dosage as reported previously (Oostindjer et al. 2009; Oostindjer et al. 2010a). Sows in the flavour group received a daily dose of 350 mg trans-anethol (99%, Sigma-Aldrich), which is the molecule responsible for the anisic flavour, given in two daily portions of 175 mg. The portions of 175 mg anethol were dissolved into 20 ml soy oil and kept in 20 ml syringes in the dark to prevent the anethol from disintegrating. Control sows received two daily portions of 20 ml soy oil. Control syringes were kept in a separate location in the experimental building to prevent contamination.

The anethol solution (or soy oil) was sprayed on top of a portion of 300 grams of food (standard commercial sow diets), which was between 4 and 10 % of the total daily food intake of sows, dependent upon the gestational and lactational stage. Additional food was not given until the sow finished the small portion of food. All sows in the flavour group consumed the anise and oil treated food. The protocol to prevent exposure of control sows and piglets to the anethol included feeding control sows before flavour sows, wearing gloves, transporting anethol-contaminated objects in plastic bags, and changing clothes after entering the flavour rooms.

Animals and housing postweaning

Piglets were weaned at 25 ± 2 days and housed in pens of 2.1 m x 2.8 m, with in total four unfamiliar piglets per pen from the same preweaning treatment. Only eight pigs per litter were used, of which four piglets were assigned to the Food treatment (flavour in food), and four to the Air treatment (flavour in air), resulting in 12 Flavour-Air pens, 12 Flavour-Food pens, 12 Control-Air pens and 12 Control-Food pens. Each pen contained two males and two females, with one of each sex being a high resister and one being a low resister, as defined by the backtest. Weaning weights of piglets were balanced between postweaning treatments (see Table 1 for weaning weights).

Each pen had two feeders, one on each side of the pen. Each pen also had an air permeable container that was placed in the back of the pen and could be touched but its contents could not be accessed by the piglets. The Food treatment pens had one feeder with control food (standard weaner diet, SpeenSelect, Rijnvallei) and one feeder with control food with 150 ppm of powdered anethol added. The permeable container in the Food treatment pens contained 1 kg of control food. The feeders in the Air group both contained control food, while the permeable container in these pens contained 1 kg of 150 ppm anethol-flavoured food, thus the flavour was only present in the air in these pens. For both postweaning treatment groups the powdered anethol (Lucta S.A., Spain) was mixed into the food for the feeders and containers as designated above at the moment of provisioning. Food in the feeders and containers was replaced on days 2, 5, 8 and 11 postweaning. Each pen was provided with a small layer of wood shavings as bedding and approximately 50 g of straw per day. Lights were on at 7:00 and off at 19:00. Water was available from two drink nipples in each pen.

Postweaning measurements

Piglets were weighed at day 0, 3, 7, 11 and 14 postweaning. Food intake was measured by weighing back the feeders at 5h, 24h, days 2, 3, 5, 8, 11 and 14 postweaning. Latency to eat was determined by video observations, in which the time between weaning and the first time the piglet spent more than three seconds with its head deep in the feeder was calculated for each individual piglet. If a piglet had not eaten after the third day, the maximum score was given for this piglet, which was the time from weaning until midnight on the third day (n=10, maximum scores between 61 and 63 hours).

Saliva samples for cortisol measurement were collected on the day of weaning at 1, 4 and 7 h postweaning from the two males piglets from each pen. Basal samples of these piglets were collected on the day before weaning at the same time points on the day. Piglets were presented with three cotton swabs on which they readily chewed after they had been habituated to the swabs three and two days before weaning. Swabs were collected into Salivettes® and stored on ice until all piglets for that time point were sampled. Salivettes were then centrifuged at 3000 RPM (870 RCF) for 10 minutes and stored at -20 °C until analysis. Salivary cortisol was determined using a solid-phase radioimmunoassay kit (Coat-a-Count Cortisol TKCO, Diagnostic Products Corporation, Apeldoorn, The Netherlands) modified for pig salivary cortisol as described in a previous study (Ruis et al. 1997).

Behaviour of piglets in their pen was scored on day 1, 5, 9 and 13 after weaning using 2-min instantaneous scan sampling for 6 h per day. Data were collected using the Psion Workabout MX with the Observer 5.0 (Noldus Information Technology B.V., Wageningen, The Netherlands) installed on it. Behaviours that indicate whether a piglet adapted to weaning well are play behaviour (running around the pen, pivoting, rolling, sliding, gambolling or substrate play) and manipulating pen mates (nibbling, sucking or chewing body parts of pen mates). Play was expected to be lower in piglets that experienced the weaning process as very stressful (Dudink et al. 2006; Dybkjær 1992), and manipulative behaviour was expected to be higher in piglets that experienced the weaning process as very stressful (Dudink et al. 2006; Dybkjær 1992). The percentage of piglets per pen that vocalized during behaviour sampling for that specific pen was also determined every two minutes on day 1 after weaning, as high vocalization rates postweaning indicate stress or frustration (Chaloupkova et al. 2007; Oostindjer et al. 2009; Weary et al. 1997).

Data analysis

All data were analysed using mixed linear models in SAS (SAS 9.1, SAS Institute Inc.). Behaviours (expressed as proportions of time) were arcsine-square root transformed when the residuals were not normally distributed. Effects of pre- and postweaning treatments on cortisol levels on the day of weaning and on the difference between basal levels and levels on the day of weaning were analysed with a repeated model. This model included preweaning treatment (Control or Flavour), postweaning treatment (Food or Air), day and their interaction, as well as batch as main factors, and postweaning pen and piglet as random factors. Growth, food intake, latency to eat, home pen behaviour, vocalizations and cortisol levels by time point were analysed with a model including pre- and postweaning treatments, their interactions and batch as main factors, and pen within treatment as random effect. Within-pen variation of growth (standard deviations) was also analysed with this model. Post-hoc pairwise comparisons were made using the least-square means, corrected for multiple comparisons with a Tukey adjustment. Data are presented as (untransformed) mean ± SEM based on pen averages.

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09

GENERAL DISCUSSION

Introduction

Piglets in commercial, intensive husbandry systems face several challenges in the period around weaning, as they are generally weaned more abruptly and earlier than in the wild. As a result, many piglets have no experience with solid food prior to weaning and show a low food intake and growth in the early postweaning period. Weaning is also associated with a high prevalence of diarrhoea and high levels of stress and maladaptive behaviours and is thus an event that compromises piglet performance, health and welfare (Bolhuis et al. 2009). It is important that piglets start to eat solid food early, preferably before weaning, to allow behavioural and physiological maturation, thereby reducing problems associated with weaning (Berkeveld et al. 2007; Bruininx et al. 2002b). The aim of this thesis was to explore whether providing piglets with more opportunities to learn from their mother about what, how and where to eat can increase food intake before and after weaning and, consequently, reduces health and welfare problems after weaning. Currently the opportunities for piglets to learn from the sow are very limited as the sow is mostly confined throughout lactation, fed a different feed than the piglets and fed in a trough that is often inaccessible for piglets. Sows and piglets are generally housed in barren, stimulus-poor pens, which limit the amount of (foraging-related) behaviours that sows and piglets may perform. The three previously described pathways through which piglets can learn more from the sow will be discussed here: 1) direct learning from the mother through cues derived from observation and eating together, 2) learning in an enriched environment and 3) learning from flavour cues in the maternal diet. The three pathways will be discussed together to draw conclusions on which pathways of learning are important to stimulate the development of piglet food intake and the development of the capacity to adapt to the stressors associated with weaning. It is not only important to get piglets to eat early and to enhance their adaptability to the postweaning situation, but it is also important that this occurs for all piglets in the pen and that variation in postweaning performance among piglets is low. Potential sources of between-individual variation will be discussed and effects of the three learning pathways on variation in performance will be shown. To conclude, recommendations are given to enhance piglet food intake, growth, health and welfare around weaning. Finally, the contribution of this thesis to knowledge on development of human feeding behaviour will be explored in a separate section at the end of the discussion.

Direct learning from the mother

An obvious way for most young mammals to learn from their mother is through direct learning by observation or participation (Chesler 1969; Galloway et al. 2003; Provenza and Balph 1987; Thorhallsdottir et al. 1990). The young animals can follow the mother to a food source and observe the behaviours she performs, or eat together with the mother and immediately use the cues that are provided by the mother to perform the behaviour by themselves (Galef and Giraldeau 2001). In Chapter 2, 3 and 4 we found positive effects of having the opportunity to eat together with the sow on food neophobia, intake, growth and foraging-related behaviour of piglets before weaning. The sow is therefore important in getting piglets to eat, though the effects of having more opportunities to interact with the mother are broader than just stimulating food intake. Piglets that were allowed to interact more with the sow showed more play behaviour and less damaging behaviour (belly nosing and manipulative behaviour) after weaning, which is likely due to changes in behavioural development before weaning, as seen in Chapter 4. The effect of interaction with the sow on belly nosing also suggests that more interactions with the sow during lactation increases the ability to cope with stress around weaning (Bolhuis et al. 2006).

The importance of the mother in the first experience with food can also be deducted from the results in Chapter 5. Piglets that were able to observe or could participate with their mother while she ate a flavoured food ate more and faster from this flavoured food than piglets that were able to sample food without information from the sow and piglets that had no opportunities to learn nor had previous experience with solid food. This was particularly the case on the first day of testing at 23 days of age. The mother may be of particular importance for the young animal in identifying which food types are healthy and nutritious and safe to eat, thereby reducing neophobia for those particular food items that the mother eats (Ammaniti et al. 2004; Dovey et al. 2008; Galloway et al. 2003). After the initial intake of food and a positive experience with this food, young animals tend to generalise towards similar food types and increase their exploration and intake of these food types as well (Birch 1999; Launchbaugh et al. 1997; McQuoid and Galef 1993; Villalba and Provenza 2000b). For the initial intake of food it is therefore important to have a match between food of mother and offspring. When eating behaviour has been established, generalisation will facilitate intake of similar food types that may be even more relevant to the offspring.

Directing attention to the same place where the mother is present and eating, local enhancement (Cadieu et al. 1995; Hoppitt and Laland 2008a), may be the first step for the young animal towards identifying healthy food items, before specific sensory properties of the food such as smell, colour and shape are known. The actual preference for a food item may be more determined by observation of the mother while she directs behaviour or attention towards a certain food item or stimulus, stimulus enhancement (Fritz et al. 2000; Heyes et al. 2000; Visalberghi and Addessi 2000a). Information from observation of the mother may then be coupled to the specific sensory properties of the food. Piglets use information from both the location and the food type that mother was eating, yet the results of Chapter 5 indicate that it is particularly information from the food type that the mother was eating that is used to establish preference and increase food intake.

The information about food type and location can be obtained both by observation of and participation with the experienced animal. However, the shorter the delay between observation and performing the behaviour, the more effective information use may be. Therefore, participation may be a more efficient way of learning than observation (Bandura 1977; Black et al. 1985; Sclafani 1997). As seen in Chapter 5, however, observation of the sow and participation with the sow were both effective in stimulating piglet food intake and preference. Previous studies on scrounging in other animals support the idea that often, young animals simply follow the mother to the feeder (local enhancement) and are stimulated to eat by her presence (social facilitation, Caldwell and Whiten 2003). These learning processes are rather passive, and the results indicate that participation is not additive to learning through observation in pigs. One must note, however, that in the experiment in Chapter 5, piglets that were able to participate were not always allowed by the sow to eat from the food, which may have limited information transfer from sow to piglet, making participation less effective than it otherwise would have been. This also indicates that, in contrast to for example hens (Wauters and Richard-Yris 2002), we have no indications that the sow has an active role in teaching the piglets to eat. In some cases, the motivation of the sow to eat might be so high that access to the food by other individuals, including piglets, will be prevented. When the price of eating the mother's food is high for the mother, such as missing an essential portion of her daily caloric intake, a conflict arises between the experienced anima and the animal that is learning. Experienced pigs may change their foraging strategy depending on the presence of other pigs that are likely to steal their food (Held et al. 2000; Held et al. 2010), and other species have developed tactics to deceive conspecifics to prevent scrounging by other animals that are using various ways to learn from them (Bugnyar and Kotrschal 2002, 2004; Fujita et al. 2002; Hauser 1992). In practice, sows are fed in two portions of concentrate food and their daily ration is close to ad lib feeding. The sow needs the calories that are provided to her by the food to produce enough milk and maintain body condition, and most sows quickly finish their meal once it is provided to them. The time in which sows are actually eating may thus constrain the amount of information that piglets can obtain from the sow during participation. Feeding the sow in more portions or feeding her feed with the addition of substrate or fibres may result in the sow eating for a longer period of time, thereby increasing the number and duration of learning possibilities for piglets. In Chapter 5, a total observation or participation time of only 5 x 10 minutes was enough, however, to significantly stimulate food intake of piglets. Further studies are necessary to investigate whether increasing duration and number of learning opportunities will further increase piglet food intake before weaning.

While the mother is an important and reliable source of information regarding what, where and how to eat (Chesler 1969; Galloway et al. 2003; Provenza and Balph 1987; Thorhallsdottir et al. 1990), young animals may also use information from siblings or other group members. Indeed, piglets can be stimulated to eat by being able to participate with an experienced piglet in the period after weaning (Morgan et al. 2001). Piglet behaviour within a litter or pen is strongly synchronized: when one piglet approaches the feeder, chances are high that another pig will join to eat as well (Keeling and Hurnik 1996). If the mother does not play a role in stimulating food intake of piglets, the first piglet in a litter that becomes interested in food needs to learn how, what and where to eat by trial-and-error learning, which is less efficient than learning from an experienced individual. After one piglet in the litter has learned the correct behaviour through trial-and-error learning, this piglet may become a model for the other piglets in the litter. The mother has an important role in overcoming food neophobia, and having the opportunity to learn more from the mother will make it more likely that piglets will start sampling food due to decreased food neophobia They are also likely to learn how, what and where to eat more efficiently due to the presence of an experienced animal. Then, once one piglet starts eating, other piglets in the litter may learn from that piglet as well. It is thus expected that the process of getting piglets to eat will occur faster and earlier when the sow can provide information compared to when piglets can only use information from other piglets. What should be noted is that when piglets lack information from the sow but still have the opportunity to eat preweaning, the food intake pre- and postweaning is still higher than that of piglets that have no previous experience with solid food (Chapter 5, Berkeveld et al. 2007), indicating the importance of an early intake of solid food.

Mere exposure to food increases the likelihood that young animals start eating and increases overall food intake (Birch 1998). This may be particularly true when the young animal is repeatedly exposed to a variety of foods: acceptance of a novel food is enhanced when there is previous experience with a number of different foods (Launchbaugh et al. 1997; Mennella et al. 2008). The young animal is, however, less likely to develop a resilient preference for a food type when the consumption of the food is unrelated to information coming from the mother (Cheslock et al. 2004). Instead the young animal can only use information from sensory properties and post-ingestive consequences of consuming the food to develop a preference. As a result, animals that learn through repeated exposure to different foods without information from experienced animals are more likely to easily generalise to similar food types (Launchbaugh et al. 1997; Villalba and Provenza 2000b). It might be that their preference is less fixed, but also because these animals may be more flexible in their food choice due to their earlier exposure with different food types and thus different stimuli (Mennella et al. 2008). This idea is supported by the results in Chapter 5. Piglets that could learn from their mother and choose from two flavoured food sources showed a preference for the flavoured food that was also consumed by the mother, while piglets that were exposed to one of the flavoured foods without opportunities to learn from the mother generalised to another flavoured food. They showed equal intakes of both flavoured foods, but in smaller quantities than the piglets that could learn from their mother. Both the importance of learning through stimulus enhancement, and the effect that the mother has on the development of food preference indicate that it is important to provide piglets and sow with food that matches, at least in flavour. Which exact properties (smell, taste, colour, texture, size, composition) should match remains unknown so far and may be vital knowledge for feed manufacturers to investigate to be able to give a feed optimal for sow and piglet performance that is also optimal for transfer of information from sow to piglets. In summary, the mother plays a critical role in overcoming food neophobia, stimulating overall food intake, and in establishing preference of piglets for certain foods.

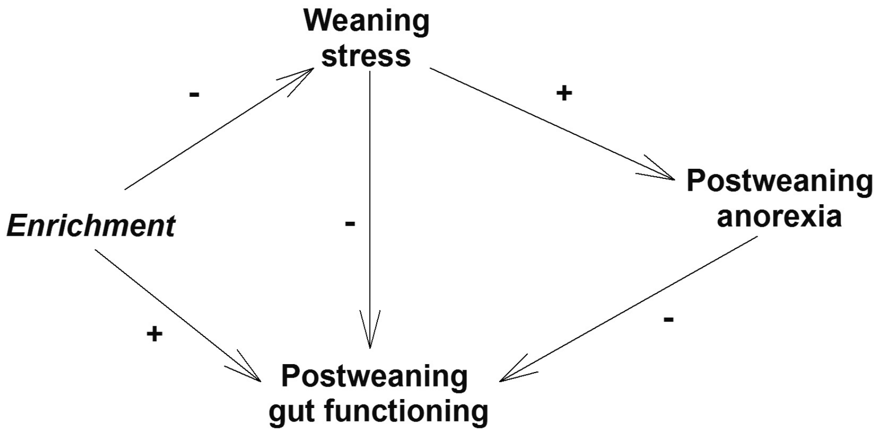
Learning in an enriched environment

The second learning pathway examined in this thesis was learning in an enriched environment. The questions were if the role of the mother can be further enhanced by providing sow and piglets with an enriched environment, and if an enriched environment in itself can also positively affect piglet performance and behaviour around weaning. The use of environmental enrichment in husbandry conditions was hypothesised to affect the amount and type of behaviours that the sow will show, particularly when the sow was also loose housed. Environmental enrichment was also thought to increase opportunities for piglets to practice more and different behaviours. Indeed, preweaning enrichment positively affected the development of piglet behaviour. Piglets that were housed in an enriched environment before weaning showed less damaging behaviours and more exploration and play behaviour than barren housed piglets, suggesting substantial effects of enrichment on behavioural development of piglets before weaning (Chapter 4). Enrichment also positively affected growth and there are strong indications that this effect was due to effects on preweaning intake of solid food. Solid food intake preweaning could not be accurately measured but preweaning enrichment reduced preweaning food neophobia (Chapter 2) and resulted in a higher postweaning food intake (Chapter 3). These positive effects of enrichment may in part have been the result of increased behavioural flexibility or lower stress-reactivity due to the increased number of stimuli in the early (enriched) environment (Beattie et al. 2000; De Jong et al. 2000; Leggio et al. 2005; O'Connell et al. 2005; Petersen et al. 1995; Schrijver et al. 2002). If animals are more exposed to novel things (objects, environment), they possibly also better able to cope with a novel food and a novel postweaning pen.

Although preweaning enrichment positively affected piglet food intake and behaviour, there were very few interactions between sow housing and enrichment. Most effects seemed to be additive instead of interactive. Nevertheless, it is likely that the sow was influenced by enrichment as well, either in her exploratory behaviour, or in her maternal care (Champagne et al. 2003; Herskin et al. 1998; Thodberg et al. 1999), which may in turn have affected piglet behaviour. Indeed, sow housing (loose or confined) interacted with preweaning enrichment in influencing postweaning behaviour (Chapters 3, 4), suggesting that an optimal combination of being able to interact with the sow and an enriched environment preweaning increases the ability of piglets to adapt to the postweaning situation.

Postweaning enrichment, however, seemed even more important in allowing piglets to better adapt to the postweaning situation. Providing postweaning enrichment resulted in a higher postweaning growth, a higher feed efficiency, a lower occurrence of diarrhoea, and resulted in less damaging behaviours and more exploratory and play behaviour (Chapters 3, 4). These effects are likely due to a reduction in weaning stress by providing distraction and a more positive environment postweaning (Bolhuis et al. 2005a, 2006; Day et al. 2002; Dudink et al. 2006; Fraser et al. 1991). Stress around weaning is caused by the changes in physical environment, social environment, maternal separation, food source and transportation. Increased levels of adrenaline and cortisol will help the animal to cope with the challenging situation (Koolhaas et al. 2011). There are downsides, however, to experiencing high stress levels, from a welfare and from a physiological perspective. Animals may be unable to adapt quickly to a new situation when the situation is very challenging, particularly when there is a loss of control of the situation, despite the proper physiological response. When animals are unable to cope and stress-levels remain high, the risk of developing behavioural problems and health issues that compromise welfare, such as maladaptive behaviours and gastro-intestinal problems, is increased (Moberg 2000; Wiepkema and Koolhaas 1993). Stress also reduces eating behaviour and the processing of food in the gastro-intestinal tract (Krahn et al. 1990; Rao et al. 1998; Taché et al. 1999), which may be particularly detrimental for newly weaned pigs that already are challenged by having to make the transition from milk to solid food abruptly. The period of low food intake that often occurs after weaning will result in a degradation of villi length and an increased crypt depth in the small intestine. The low food intake can also cause inflammation, which reduces the ability of the gut to properly absorb nutrients and results in diarrhoea (Ball and Aherne 1982; Boudry et al. 2004; Hampson 1986; McCracken et al. 1999; Moeser et al. 2007b; Pluske et al. 1997; Spreeuwenberg et al. 2001). The gut may be further compromised by high stress levels through increased secretion of corticotropin-releasing-hormone (CRH) which has a detrimental effect on the mucosal barrier of the intestine and increases the permeability of the gut, making the pig more susceptible for pathogens and toxins entering the blood stream (Van den Wijngaard et al. 2010). Piglets that already have a high intake of solid food before weaning are likely to eat more after weaning as well (Berkeveld et al. 2007). Therefore, stimulating early food intake is essential in minimizing the period of anorexia postweaning and thus reducing gastrointestinal dysfunction. When piglets do not eat solid food yet they may benefit from being able to ingest enrichment (materials such as straw). The fibres in the enrichment material may stimulate the gastro-intestinal tract, thereby reducing the detrimental effects of postweaning anorexia (Pluske et al. 1997; Pluske et al. 2003). Reducing stress levels is also crucial because high stress levels will reduce food intake (Krahn et al. 1990) and this reduction can likely be accomplished by providing enrichment materials. The relationship between weaning stress, anorexia, gut functioning and the hypothesised effect of postweaning environmental enrichment are presented in *[figure 1]*. The positive effects of postweaning enrichment on growth, health and behaviour found in Chapters 3 and 4 indicate that enrichment may have indeed reduced stress of piglets around weaning. This is supported by the effects of postweaning enrichment on piglets that were previously housed in a barren pen. These piglets showed a lower food intake than other piglets in the first few days postweaning, yet they gained more weight and had less diarrhoea than piglets that were housed in barren pens during the first two weeks postweaning. These measures are only indirect indicators of stress. Investigating physiology and morphology of the gut in the early postweaning period in relation to housing conditions may help to understand how enrichment increases postweaning performance, because it may show whether the previously described effects of enrichment on performance, health and behaviour are mediated by stress and reduced inflammation or gut permeability, or whether the ingestion of enrichment may also have positive effects on gastro-intestinal functioning.

figure 1.



Relation between weaning stress, postweaning anorexia and postweaning gut functioning and the hypothesised effect of postweaning enrichment. Arrows indicate one factor affecting another, + indicates an increase and – indicates a decrease.

What is crucial to realise is that it may be better for a piglet to not have experienced any enrichment than to have had an enriched environment and subsequently lose it. Piglets that were housed in enriched pens preweaning and housed in barren pens postweaning showed low levels of play behaviour and high levels of belly nosing compared to piglets were housed in barren environments both pre- and postweaning (Chapter 4), indicating a compromised welfare of piglets that lost environmental enrichment. The change in environment may be perceived as a loss of reward (Papini 2000), because the environment changes from positive (presence of the mother, presence of enrichment) to more negative (no presence of the mother nor of enrichment). This change in environment is larger than for piglets that are housed barren both pre-and postweaning (only loss of presence of the mother). Reward loss is associated with high stress levels and can eventually result in the development of stereotypic behaviours (Latham and Mason 2010; Papini and Dudley 1997). Gaining a reward may, on the other hand, increase welfare, at least in the period after the reward is received. Piglets that were housed in barren pens preweaning and housed in enriched pens postweaning showed high levels of play and exploratory behaviour postweaning, yet the higher preweaning levels of belly nosing and manipulative behaviour and lower levels of play suggests that welfare before weaning was lower than that of piglets housed in an enriched environment preweaning. Thus, preweaning and postweaning enrichment can greatly improve performance, health and welfare of piglets, but it is desired that, when enrichment is provided before weaning, it is also provided after weaning.

Learning through flavour cues in the maternal diet

Flavour cues coming from the maternal diet can reach the young animal in utero via the amniotic fluid, or via the milk during lactation (for review: Bolhuis et al. 2009; Schaal 2010). These flavour cues provide the young animal with information about the “feeding environment” in which it will have to choose what to include in the diet. Strong flavour cues are likely items that the mother eats in large quantities and these are the items that the young animal should also eat and will likely show a preference for (Bolhuis et al. 2009). Learning from flavour cues from the maternal diet was one of the pathways through which piglets could learn from the sow. Providing piglets with food postweaning that contains flavours that had been experienced via the maternal diet was hypothesised to increase postweaning food intake due to an increased preference for the flavour.

The results from this thesis show that flavour learning can indeed be used as a tool to increase postweaning food intake of piglets. Particularly prenatal, as opposed to postnatal, flavour learning resulted in a higher food intake, a higher growth and a reduced diarrhoea prevalence in the first two weeks postweaning. Prenatal flavour learning also resulted in less damaging behaviours postweaning, suggesting an overall improvement of welfare of newly weaned piglets (Chapter 7). Piglets that were able to learn from a flavour in the maternal diet did, however, not show a preference for this flavour in both the preweaning behavioural tests or in the postweaning double-choice feeding situation. Behaviours of piglets in behavioural tests preweaning were, however, modified in the presence of the familiar flavour, suggesting that piglets did recognize the flavour (Chapter 6). The modification of behaviour was strongest in behavioural tests that elicited relatively high stress levels, suggesting that the presence of the familiar flavour may have reduced stress. This may also have occurred at weaning and may have been, in part, responsible for the positive effects of flavour learning on performance and behaviour in Chapter 7. The stress-reducing effect of the presence of the familiar flavour at weaning was confirmed in the experiment described in Chapter 8. The presence of the familiar flavour alone in the postweaning environment was enough to reduce cortisol levels, reduce variation in growth, reduce the number of vocalizations and increase play behaviour after weaning. The possibility to learn from flavours from the maternal diet and providing these flavours in the postweaning environment can thus enhance performance, health and welfare of the newly weaned pig.

Developing a preference for a certain food is not simply a matter of repeated exposure to the food. The moment in development when the animal is exposed to the flavour, as well as the context in which the exposure occurs, are important in the development of a preference, or even an aversion for certain flavours. For example, human infants could learn to accept a protein hydrolysate formula, but only when they were younger than 3 ½ months of age (Mennella et al. 2011). In rats, the first intake of a food source after birth results in a strong preference for this food source, or an odour coupled to this food source and this preference is hard to modify later in life (Cheslock et al. 2004). The context in which the preference is learned may play a role in the resilience of the preference, as food types or flavours that are experienced in a relevant and positive context may result in a stronger preference than exposure in a neutral or negative context. Human infants, for example, prefer the odour of amniotic fluid over the odour of formula milk in the first days after birth (Marlier et al. 1998). They were exposed to the amniotic fluid for a longer time than to the formula milk, but it is likely that it is not just a matter of longer exposure but the higher preference is instead caused by a positive association with the mother. Being exposed to a flavour during a negative context, on the other hand, results in a strong aversion for the flavour (Capretta et al. 1973). Preference or aversion will be established when it is relevant for the animal to be attracted or avoid a certain odour. Cues that signal whether an animal should avoid or prefer may be provided by the positive or negative context in which the exposure occurs (Capretta et al. 1973; Jones et al. 2000). The emotional valence of the stimulus, provided in part by the context, will enhance memory of this flavour, and may elicit a strong evocation of memory during re-exposure that is coupled with the appropriate emotion (Chu and Downes 2000a; Herz 2002; Willander and Larsson 2006). This may also explain why children that experienced a certain odour during a frustrating task that ends in failure show a reduction in cognitive performance during another task in which the same odour as experienced before is present (Epple and Herz 1999). Odours or flavours associated with a positive context, such as the mother, are likely to elicit a positive emotion during re-exposure and may result in a strong preference. Piglets also prefer substrates that are linked to the mother such as skin secretions and faeces (McGlone and Anderson 2002; Morrow-Tesch and McGlone 1990). In this thesis, flavours that were associated with the mother due to their presence in the amniotic fluid or in the milk, did reduce stress during re-exposure at weaning, possibly due to mood-enhancing properties (Chapter 8). Preference for the flavour in the food compared to the control food was not observed, however. The flavour that was used, however, may have been less palatable than the flavour of the control food, so the absence of a preference for the flavour may have been a matter of relativity instead. Using a palatable flavour may be more efficient in providing piglets with a positive situation after weaning, as both the odour in the pen and the flavour of the food will be liked.

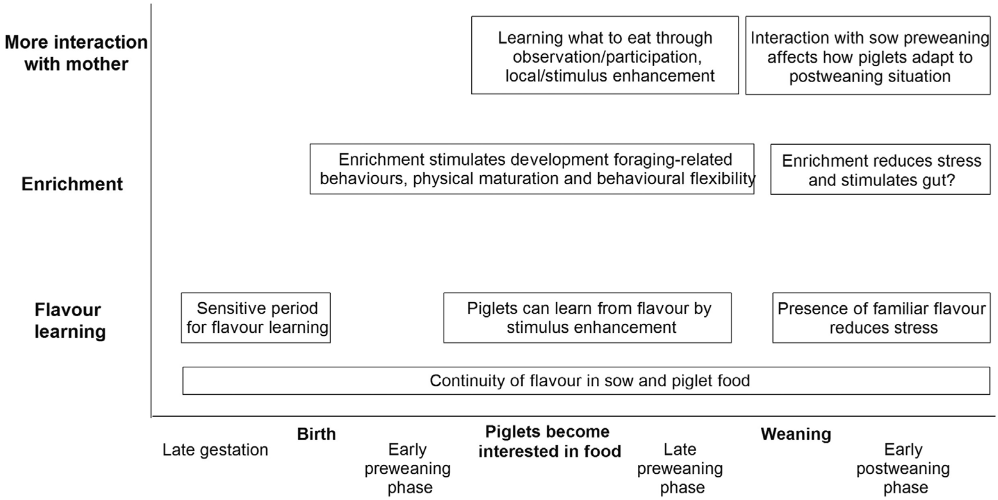
The results described in Chapters 6 and 7 are mostly related to effects of prenatal flavour learning, while Chapter 8 discussed effects of perinatal flavour learning. In the pig, exposure to a flavour prenatally may be essential for later recognition of the flavour and modification of behaviour. There is, however, also some evidence for positive effects of flavour learning through milk and mere exposure on food intake of piglets around weaning (Campbell 1976), though a reduction of stress by the familiar flavour may have played a role there as well. Nevertheless, the olfactory and gustatory system of the relatively precocial pig should be sufficiently developed in late gestation to perceive the flavours and develop a preference for them (Book and Bustad 1974; Brunjes 1983, Chapter 6, 7). The pig brain, however, may less plastic during lactation than the brain of rodents, and therefore learning postnatally through the milk may be less effective in pigs. It is likely, however, that a continuous exposure to the flavour will results in a more resilient learning of the flavour (Coureaud et al. 2002). The idea that flavours in the maternal diet predict the (feeding) environment later in life should include a certain continuity (Guiraudie-Capraz et al. 2005; Schaal 2010). When the flavour has been experienced before birth, but is not present in the environment during lactation, the effect that the familiar flavour has on postweaning behaviour is likely less strong than when the flavour is always present. Continued exposure to the flavour may not only lead to learning through amniotic fluid and milk, but may also enhance preference due to its presence in the mother's food. In utero, the flavours are exposed in the safe context of the maternal environment. During lactation, flavours are also experienced in the safe context of the maternal environment, but as the piglet matures and starts to explore the food, attention to odour cues may change and piglets may prioritize information coming from flavours experienced in the context of food. The association that the animal makes with the sensory cues of the food (smell, taste) are different in both situations: association with the mother (familiar flavour) or association with food (learning of association between flavour and post ingestive consequences). To establish the stress-reducing effects of the familiar flavour, the association with the mother may be most effective, while preference may be established by having the association with the food. This may be mediated by the behaviour of the mother: when the mother is seen eating the food, the cues from the food may be more likely to perceived in the context of food, and at the same time preference for the flavour cues of the food may be established by stimulus enhancement and social facilitation as seen in Chapter 5. Thus, for the most efficient flavour learning, (palatable) flavours should be present in the maternal diet before and after birth of the young animal and in the food that the young animal encounters on its first exploration of food. These flavours should match the flavours in the food that the mother is observed to eat, as well as in the postweaning food and environment. This creates an ultimate match between flavours predicted before birth and flavours that are actually present in the feeding environment.

Learning how to eat like a pig

Getting piglets to eat solid food early is an important to reduce postweaning problems (Bolhuis et al. 2009). In order to establish an early intake of solid food, a few conditions have to be met. Piglets need to be physically mature enough to ingest (e.g. teeth development, motor skills) and digest (gut maturation, enzymes, intestinal flora) solid food and piglets need to have the behavioural skills to locate, explore and eat the food (Herring 1985; Miller et al. 2007; Popowics and Herring 2006; Ross and Nijland 1998). Piglet also need to be motivated to explore and ingest solid food. When they are not stimulated by curiosity or the behaviour of the mother, food intake is likely to be low and variable among piglets at the age at which they are currently weaned. Under stressful conditions the motivation to eat is expected to be even lower: acute stress reduces food intake, at least partly mediated by high levels of CRH, and animals are less likely to consume novel foods when the environment in which they encounter the novel food is also novel (Burritt and Provenza 1997; Krahn et al. 1990; Vallès et al. 2000). Hunger is usually a good motivator to eat, and may be a reason for the smallest piglets in the litter to start eating solid food before weaning, in order to compensate for their low milk intake (Appleby et al. 1992; Pajor et al. 1991). After weaning piglets are likely to become hungry after several hours, but this is not enough to initiate food intake in all piglets, and some piglets will show a high intake of water to fill their stomach instead (Brooks and Tsourgiannis 2003; Dybkjær et al. 2006; Widowski et al. 2008). Therefore, the motivation to eat solid food after weaning that is generated by hunger signals may be overruled by other signals that reduce the motivation to eat, likely mediated by stress. As animals are typically not stressed before weaning, and piglet eating behaviour may be stimulated by the behaviour of the sow, the interest of piglets in solid food may be greater in the late preweaning phase in comparison to the early postweaning phase. The late preweaning phase may therefore be a good moment for piglets to start eating and may facilitate a better postweaning food intake as well (Berkeveld et al. 2007). Piglets would follow their mother to feeding sites and start sampling food in the first two weeks of life under (semi-)natural conditions, indicating that the piglet is indeed able to learn how to eat in the late preweaning phase (Meynhardt 1978; Newberry and Wood-Gush 1985).

In this thesis, food intake of piglets was stimulated by providing piglets with more possibilities to learn how to eat: learning from the mother, learning in an enriched environment and learning from flavour cues in the maternal diet. The results show that these three pathways of learning do increase food intake and growth of piglets around weaning, at least in part mediated by reduced stress levels at weaning. A proposed time line of the effects of being able to interact more with the mother, enrichment and flavour learning on development of the ability to adapt to the postweaning situation, based on the results from this thesis, is given in *[figure 2]*. Preweaning food intake and growth were improved when piglets had the opportunity to explore novel foods together with the sow, observe the sow and participate with her while eating and when piglets were reared in an enriched environment. Postweaning food intake and growth were improved by providing enrichment before and after weaning, though only effective when the postweaning environment was not made worse by providing enrichment before weaning but not after weaning. Interestingly, the negative effects of a loss of enrichment at weaning were reduced by increased opportunities to interact with the sow before weaning, indicating that the importance of learning from the sow goes beyond learning how to eat. The preweaning social and physical environment may therefore be important in how piglets evaluate the postweaning situation, and should be considered in management of piglets around weaning. The opportunity to learn from flavour cues in the maternal diet increased postweaning food intake and growth around weaning as well, possibly mediated by the stress reducing effects of the familiar flavour. It is possible that preweaning food intake could also be improved by flavour learning, though this was not investigated in this thesis. There are strong indications, however, that the flavour of the food is also important in learning from the mother by observation (Chapter 5). To facilitate optimal flavour learning and learning by observation, it may be important to provide a match between flavour of the sow's food both during gestation and lactation, and flavours in the piglet food during lactation and after weaning. It may be even better to provide sow and piglets with exactly the same food to motivate piglets to initiate eating solid food. After food intake has been established, a more properly nutritious food could be provided to piglets. When the flavour of this food matches that of the sow's food and the previous experienced food, it is likely that piglets will generalise to the new food, thus resulting in a smoother transition between foods. From a developmental perspective it may also be worthwhile to investigate the effects of providing a diverse range of foods (or flavours) to the sow and piglets during all stages of development. Providing a diverse range of foods or flavours may also be considered a possible way of enriching the environment. The large number of olfactory and taste stimuli then experienced may lead to a larger adaptability to novel stimuli, will provide piglets with the opportunity to learn about multiple food sources, and the diversity in olfactory stimuli may serve as a form of enrichment for the olfactory-driven pig.

figure 2.



Proposed time line of effects of being able to interact more with the mother, enrichment and flavour learning on development of the ability to adapt to the postweaning situation.

This thesis showed is that environmental enrichment, being able to learn from the sow by increased interaction opportunities and learning from flavour cues in the maternal diet will enhance the development of piglet behaviour, motivation of piglets to explore and motivation to ingest food. Whether combining the three learning pathways that were investigated in this thesis will be more effective than applying them separately is still unknown and should be investigated under commercial circumstances. The effects on performance per se are not very large (postweaning growth increase of max. 13%). Instead the positive effects of learning are likely due to a general enhancement of the ability to adapt to the postweaning situation. In other words, providing piglets with the opportunity to learn from the sow, providing enrichment and providing the opportunity for flavour learning is a form of 'health insurance' that will decrease the likelihood that a piglet will experience performance, health and welfare problems postweaning. Providing piglets with this 'health and welfare insurance' will likely allow a reduction in antibiotic use. Antibiotics use is very high in pigs up to 25 kg (on average 25 days of treatment in the first 74 days of life) and a large part of this antibiotic use occurs in the early postweaning period (MARAN 2009). Reducing antibiotic use is currently high on the political agenda in the Netherlands, and an important focus point of the pig husbandry sector. Increasing the ability of piglets to better adapt to eating solid food, to novel stimuli and to stressful situations by allowing piglets to learn more may be an important tool to reduce antibiotics use in pigs, in addition to increasing performance, health and welfare of newly weaned piglets.

Variation: not all pigs eat like pigs

Variation between animals instead of between treatments can be a source of frustration in research in general, but is also a source of frustration in reducing weaning problems in pigs. Latency to eat after weaning, for example, can vary between minutes after entering the pen up to more than 60 hours (Brooks et al. 2001, data from experiments in this thesis). Changes in feed, feed troughs and management strategies such as intermittent suckling may increase overall performance of piglets before and after weaning, but still there is a large variation between animals in how well they adapt to eating solid food and consequently adapt to being weaned. In this section some sources of variation will be discussed and the effects that learning more from the sow, an enriched environment and flavour learning have on variation between individual piglets will be discussed.

There are several sources of variation that affect how well piglets develop food intake and behaviour and how they adapt to the postweaning situation. Birth weight tends to be positively correlated with a higher preweaning food intake and weight gain (Milligan et al. 2002; Pajor et al. 1991), though other studies found that birth weight is negatively correlated with preweaning food intake, with smaller piglets compensating a low milk intake with eating of solid food (Appleby et al. 1992; Pajor et al. 1991). Birth weight, as well as preweaning weight gain, is typically thought to be positively correlated with postweaning weight gain (Pajor et al. 1991), though others have found a negative correlation (Appleby et al. 1992). Birth weight may also affect growth later in life, in particular the tendency to store fat (Poore and Fowden 2004). Birth weight can thus be a source of variation in the development of food intake and growth in the pig, but there are also other variables that influence behavioural and physiological development of the young pig.

Sex of the pig will not only influence the development of sexual behaviour, but will also influence growth and may affect the way piglets will adapt to the postweaning situation. Males and females differ in their susceptibility to preweaning mortality (Lay et al. 2002), and although growth before weaning is not necessarily linked to sex, males and female pigs do show marked differences in several endocrine factors related to growth and metabolism, such as growth hormone and thyroid hormones in the first 42 days of age (Carroll et al. 1998). Behavioural development before weaning also differs for males and females: males show more social behaviour, aggression and mounting than females, while females spend more time eating and tend to show more suckling behaviour than males (Kranendonk et al. 2006, Oostindjer et al. unpublished). After weaning females may eat and grow more (Bruininx et al. 2001a; Dybkjær et al. 2006). Females have a lower susceptibility to stress compared to males, and this difference is even larger when male piglets are castrated (rodents and pigs, Bowman et al. 2009; Lay et al. 2002). This suggests that male piglets may benefit more from management strategies to reduce weaning problems than females, as females seem to be better able to adapt to weaning. There may also be differences in how the management strategies influence behaviour of males and females. Indeed, male and female piglets in this thesis differed in how they responded to the three pathways through which they could learn more from the sow. Prenatal flavour learning, for example, increased postweaning growth in the first 14 days postweaning more in males than in females (Flavour-males: 4.6 kg, Control-males: 4.0 kg, Flavour-females: 4.4 kg, Control-females: 4.4 kg). On the other hand, being able to interact more with the sow tended to increase postweaning growth in females more than in males (Interact-males: 5.8 kg, Control-males: 5.9 kg, Interact-females: 6.1 kg, Control-females: 5.6 kg, unpublished data). Sex is an important source of between-individual variation and it is important to realize that some management strategies to reduce weaning-associated problems may be more effective in increasing performance of only males or females.

Another source of variation is the strategies than animals use to cope with challenging, stress-evoking situations and novelty. There are two strategies that can be used to cope with challenges: a proactive coping style and a reactive coping style (Koolhaas et al. 1999). Animals with a proactive coping style will actively deal with changes in their environment, will explore novel environments fast but superficially, show more impulsive aggression and are also more rigid in their behaviour. Animals with a reactive coping style will deal more passively with acute stress, will be reluctant to explore novel environment or objects, use more information from their environment, are less aggressive and more flexible in their behaviour. When piglets are weaned they are exposed to a novel environment, deal with unfamiliar pen mates and have to start eating soon after weaning, so piglets with a proactive coping style may be better able to deal with the postweaning situation. Indeed, proactively coping piglets from the experiment described in chapters 2, 3 and 4, classified by using a backtest (Bolhuis et al. 2003; Hessing et al. 1993), were observed to start eating sooner after weaning than reactive piglets and tended to show a higher growth in the first four days postweaning (Ooms et al. 2010). On the other hand, reactively coping piglets possess a larger behavioural flexibility than proactive coping piglets, and this behavioural flexibility may allow pigs to better adapt to the new situation (Geverink et al. 2004). Furthermore, as reactively coping animals make more use of information from their environment, it is likely that they will use more information from other animals in learning what, where and how to eat (Kurvers et al. 2010). Therefore, reactive piglets are hypothesised to use more information provided by the mother, and are hypothesised to use other cues from the environment, such as enrichment, which can positively affect their ability to cope with weaning. There were only subtle differences between coping styles in how the three pathways through which piglets could learn more from the sow affected the postweaning performance of piglets in this thesis. Being able to interact more with the sow reduced postweaning diarrhoea compared to control piglets more in proactive piglets than in reactive piglets (Interact-proactive: 1.3 days, Control-proactive: 2.1 days, Interact-reactive: 1.8 days, Control-reactive: 1.5 days). The proposed lower neophobia of proactive piglets may have stimulated an early intake of food, further enhanced by behaviour of the sow, and consequently could have resulted in a higher postweaning food intake and thus less diarrhoea. On the other hand, pre- and perinatal flavour learning tended to reduce postweaning diarrhoea more in reactive piglets than in proactive piglets (Flavour-proactive: 1.9 days, Control-proactive: 2.2 days, Flavour-reactive: 1.4 days, Control-reactive: 2.2 days). The stress-reducing effect of having the familiar flavour in the postweaning pen may have been more effective in reactive piglets as they use more information from the (flavoured) environment. Timing of effects may be of importance: reactive piglets may use more information from previous environments (the prenatal, maternal environment) compared to proactive piglets. Proactive piglets may take more information from the immediate environment than from previous environments (food intake established in late postweaning phase, continue eating), though reactive piglets may still use more information from the environment in general than proactive piglets (Bolhuis et al. 2005a). The timing-hypothesis is supported by earlier work on the interactions between coping style, early environment and current environment, in which the behaviour later in life was influenced by early enrichment predominantly in reactive piglets (Bolhuis et al. 2006).

In summary, sex and coping style interact with the possibilities to learn from the sow about what, where and how to eat, and can profoundly affect variation in the ability to cope with weaning. Low variation between individuals is desired by farmers and indicates that all animals adapt well to the postweaning situation. To investigate whether the three learning pathways decreased variation between piglets in postweaning performance, the coefficients of within-pen variation (standard deviation within pen divided by the mean of the pen) of postweaning growth were calculated. The effects of pre-, post- and perinatal flavour learning, eating together with the sow and pre- and postweaning enrichment on the coefficients of variation are given in *[table 1]*. Both postnatal and perinatal flavour learning reduced within-pen variation. The familiar flavour may reduce stress and reduce the novelty of food and the pen for all animals within the pen, resulting in a more similar food intake and growth. Being able to eat together with the sow tended to reduce variation in growth as well. This may have been established via two pathways: stimulation of early food intake and as a result a higher postweaning food intake, or by a better ability to adapt to novel environments (Chapter 4). Variation between individual piglets can be reduced by providing piglets with more opportunities to learn from the sow what and where to eat. Some sources of variation, however, such as sex and coping style, will influence how piglets are affected by being able to learn more, and will be a constraint in reducing variation between piglets in postweaning performance.

table 1.

	treatment	control	p-value
prenatal flavour learning	22 ± 2	25 ± 2	0.36
postnatal flavour learning	20 ± 2	28 ± 2	0.01
perinatal flavour learning	18 ± 2	29 ± 2	0.004
eating with the sow	15 ± 1	19 ± 2	0.08
preweaning enrichment	18 ± 1	17 ± 1	0.68
postweaning enrichment	19 ± 2	15 ± 2	0.10

Effects of opportunities to learn from the sow on between-individual variation in postweaning growth from day 0 - 14 postweaning, expressed as the coefficient of variation[1]

[1] Coefficient of variation=standard deviation within-pen / mean of pen

Recommendations for pig husbandry

This thesis demonstrated that information from the sow plays a critical role in the development of independent feeding and reducing weaning-related problems. To create optimal opportunities for piglets to learn from the sow, the first intake of solid food by piglets should be stimulated by providing the same food as is eaten by the sow during lactation. Ideally, piglets should have the opportunity to eat this food together with the sow, or at least observe the sow while she is eating. It is crucial to match the food of sow and piglets during gestation, lactation and weaning, at least in flavour but preferably in more sensory aspects, to elicit the maximum potential for flavour learning, as well as learning by stimulus enhancement and reduce stress after weaning. Enrichment could be provided both before and after weaning to stimulate the development of solid food intake and further reduce postweaning stress.

The above mentioned changes in food, management and feeder designs are not all easily implemented in existing systems. Providing sow and piglets with the same food with the same flavour was judged as an easy, viable solution for the short-term by stakeholders (see Appendix for results from the stakeholder workshop). Providing enrichment and having sow and piglets eat together would be easier to implement in new systems, butt some changes can be made on a shorter term to provide piglets with more opportunities to learn. The sow's food trough can be lowered for easier access for piglets, allowing piglets to observe the sow while she eats. Ingestion of enrichment or roughage may increase gut maturation and piglets may therefore benefit from food types that include a form of roughage, thus avoiding the current problems of enrichment blocking the waste removal system. In the future, larger pens with loose housed sows or even outdoor rearing of sows and piglets are desirable, whereby piglets have the opportunity to eat together with the sow and explore different substrates. This would require a complete renovation of existing stables, yet may be easier implementable in future designs of stables. These changes in stable and pen design, as well as management, might increase the cost price of meat. For a proper implementation of the results from this thesis there should therefore be balance between the costs and the benefits for both pigs and consumers, which is partly driven by the consumer's willingness to pay for meat from healthier pigs with a higher welfare.

HOW HUMANS LEARN TO EAT LIKE A PIG

The results in this thesis may also provide information that can be used to understand how human eating behaviour develops. Similarly to pigs, the first intake of solid food of human babies is often a specialized food for infants which looks, smells and tastes differently than the food that is consumed by the mother. Acceptance of healthy foods such as vegetables is typically considered a problem in children, and the results of this thesis, as well as previous research in humans, indicate that the mother plays an important part in motivating children to accept novel food items (Dovey et al. 2008; Forestell and Mennella 2007; Galloway et al. 2003; Pliner et al. 1995). It may even be desired for mother and infant to eat the same food, to allow the infant to learn from the mother by observing, participating and by stimulus enhancement. Care also has to be given not to eat unhealthy foods together with or in the presence of the infant, as bad eating habits may be transferred from mother to infant (Ammaniti et al. 2004). Piglets learn very rapidly to eat the same food as their mother (an exposure of 5 x 10 minutes was enough to significantly increase food intake compared to piglets that could not learn from the mother, Chapter 5), and the strength and speed with which infants learn from the mother may be underestimated in humans. Experiencing a large diversity of (dietary) stimuli, a form of environmental enrichment, may increase behavioural flexibility and the likelihood of acceptance of novel foods (Birch 1998; Dovey et al. 2008; Forestell and Mennella 2007; Mennella et al. 2008).

Allowing infants to eat the same food as the mother at the same time would also allow for optimal postnatal flavour learning, by matching flavours from the maternal diet with flavours in the infant diet. Not all infants get the opportunity for optimal postnatal flavour learning, however, as not all infants are breast fed and thus obtain only information from flavours in the formula and not the maternal diet. There are indications that the acceptance of novel foods is lower in formula-fed infants than in breast-fed infants (Gerrish and Mennella 2001; Marlier et al. 1998; Mennella and Beauchamp 2002). Prenatal flavour learning, however, occurs for all infants and affects food preference later in life. A diverse maternal diet that includes healthy food types such as vegetables and fruits is thus likely to positively affect food preference of the infant later in life, promoting acceptance of these foods. Flavours from the maternal diet, or from the maternal environment, may also be used in stressful situations such as administration of injections or the first visit to the day-care in order to reduce acute stress (Goubet et al. 2007b; Rattaz et al. 2005; Sullivan and Toubas 1998; Varendi et al. 1998).

Furthermore, there is a large individual variation in food preference and to what extent the individual wants and likes to eat. This thesis indicates that part of the variation may be due to sex differences and coping style of the individual. It may be possible to link the variation found in food preference and intake to sex or coping style differences. Knowing what the cause of the variation is may allow for specific dietary solutions or strategies to reduce food neophobia in children or reduce intake of unhealthy food types in adults.

10

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11

APPENDIX

IDENTIFYING VIABLE WAYS TO FACILITATE VERTICAL INFORMATION TRANSFER TO REDUCE WEANING PROBLEMS IN PIGS: RESULTS FROM A STAKEHOLDER WORKSHOP

Marije Oostindjer

INTRODUCTION

Many pigletablets show an insufficient feed intake in the immediate postweaning period and high levels of stress, resulting in a growth check or even weight loss, a high incidence of diarrhoea and maladaptive behaviours. Thus, weaning may compromise the welfare and health of piglets, but also results in high use of antibiotics and has negative financial consequences for pig farmers. This thesis contains descriptions of several experiments in which piglets were provided with opportunities to learn directly from the sow by eating together, with pen enrichment and with opportunities for flavour learning. To stimulate implementation of the results from this thesis in pig husbandry practice, a stakeholder workshop was organized. The aims of this workshop were to share the results of the project with stakeholders with a wide range of interests and to identify which ways of improving vertical information transfer used in the different experiments are viable for short-term and long-term implementation.

MATERIALS AND METHODS

The workshop was held on the 22nd of December 2010 in Wageningen, The Netherlands. The duration was approximately 6 hours including lunch and coffee breaks. The general program consisted of an introduction to the topic, a presentation of the main results from chapters 2-8 of this thesis, a session on short-term implementation and a session on long-term implementation.

Participants

Stakeholders that were invited to participate in the workshop had either a scientific interest in the topic, were interested in improving piglet health and welfare from a non-commercial view point, or had a potential financial interest in implementation of the results. The total number of participants was 36. Of these participants, 14 were linked to Wageningen University, knowledgeable on pig behaviour and welfare, pig nutrition, ethics or economics-related issues. Ten participants were from the pig food industry, one from a pig feeder production company, four were pig farmers or represented pig farmers, one from the animal protection society, two veterinarians, three were linked to the Ministry of economics, agriculture and innovation (EL&I) and one participant was representing the main funder of the project, STW. There were 23 male and 13 female participants and an independent chair person.

Short-term implementation

The short-term implementation session consisted of three rounds of brainstorming. This brainstorming was done from five different points of view. The points of view were: the pig, the farmer, the feed company, the pig stable designer and the consumer. Participants were divided into the five work groups for the first round to make heterogeneous groups, but after the first round the choice of view point was free. In the first round, participants tried to give as many thoughts and ideas as they could that were related to the subject. These thoughts were then grouped and prioritized. In the second round participants discussed the thoughts from the first round and gave their own ideas as well, resulting in most cases in some firm statements on the topic and implementation, clearly identifying key issues. The third round was used to again prioritize these thoughts and combine them into a coherent set of key issues and implementations that were deemed viable for the short term, and these key issues and ways to implement the results were presented by a representative of the work group and discussed in a plenary session.

Long-term implementation

The long-term implementation session was a plenary session in which the participants were asked to imagine a visit to a farm 20 years from now *[figure 1]*. They were then asked to mention all the things that they would see on this farm. This same question was asked again but then participants were asked to think outside of the box even further. Half the group was then asked to write the ideas coming from these two rounds of questioning and any other ideas on a post-it and put it in a cost-benefit matrix, putting it in a high or low costs section and in a high or low benefits section. The other half of the group was then asked to vote in favour of or against these ideas with a limited amount of green and red stickers. This would identify key issues for the long term.

table 1.

key issue	pig	pig farmer	feed company	stable designer	consumer
1. learning directly from sow by eating together	piglets need to learn how to eat, can learn by observing sow, lower feeder.	piglets can follow sow and eat with her if sow is loose housed.	feeder should be lowered and sow and piglets should be fed more often to increase learning possibilities.	piglets need to eat early. eat and explore together with sow. eat and drink from a long trough or family feeder, more times per day.	
2. enrichment in the form of straw or fibres in the diet to stimulate feeding-related behaviours	straw is good enrichment but difficult in management. give fibrous food instead.	providing enrichment to both sow and piglets to stimulate exploration. diversity in substrate.	fibres could be added to the feed of piglets, possibly as enrichment.	sow and piglets should be given a handful of straw, or an enrichment-carrousel to work for enrichment.	
3. matching maternal environment with piglet environment by odours or flavours	flavours could be added to the feed of sow and piglets.	match environment with that of the mother, keep the connection.	flavours could be added to the feed of sow and piglets, but which?	use odours in the weaning pen to match with environment of mother.	
4. look at behaviour of the pig to see what the pig needs	stay with the biology of the animal, take lessons from the wild. later weaning, no teeth clipping, chewing is good.	know the behaviour of your animals.		the pig is smart.	organic pig farming in the whole country is not realistic.
5. look at the sow and what she needs to facilitate learning by piglets	breed for sows that are social and provide piglets with learning opportunities.	sow should be able to build a nest and have diversity in behaviours. look at interactions piglet-sow .			
6. optimizing feed of piglets to stimulate eating		piglets should eat lactation-feed just like the sow.	lactation-feed is cheap piglet feed, make it large and flexible to maximize chewing. add fibres.	piglet feed should be diverse in feed structure, use of fibres.	
7. costs-benefits of welfare and performance of piglets	better welfare is worth the costs: enforced welfare by policy makers.	making money is important, as is pleasure in working. farmer needs pig, pig needs farmer.	costs need to be worth the benefits. flavours, yes. solutions need to be manageable too.		consumer wants to eat happy pigs but price is very important. retailer can force consumer to choose welfare-friendly meat.
8. attitude towards pigs	animal-human interactions might be important in facilitating weaning and learning.	look at the animal as an individual, less business-like.	need to work towards robust systems.		logo's like the "good farming star" and "good life stars" can make citizen and consumer more alike and aware.
9. choice of what meat to eat by consumer					meat needs to be cheap, healthy and taste good no antibiotics. pr could help consumers choose antibiotic-free and welfare friendly meat.

Characteristics of animals exposed to anise pre- and postnatally (FF), only prenatally (FC), only postnatally (CF), or never (CC). Standard deviations are given within parenthesis.

RESULTS AND DISCUSSION

Short-term implementation

As many viable ways to implement the results were mentioned in more than one work group, the ways to implement the results were grouped into key issues that are listed in *[table 1]* and discussed below.

- **Key issue 1:** Being able to learn from the sow by eating together. This was mentioned in four out of five groups and seems a very viable implementation. This can be achieved without de-intensifying farming. Lowering the feeder or providing a family feeder enables piglets to eat together with the sow and this way piglets can observe as well as practice the feeding behaviour, which may stimulate early feed intake. Loose-housing of sows may further increase what piglets can learn, and this is deemed positive as long as sows can be confined to a farrowing crate around farrowing.
- **Key issue 2:** Enrichment in the form of straw or fibres in the diet to stimulate feeding-related behaviours. This is important to give sows the opportunity to show a wide range of feeding-related behaviours, provide piglets with more possibilities to practice these behaviours, and perhaps increase behavioural flexibility. Currently, however, providing substrates on the floor is difficult to manage as they can block the manure-disposal system. Enrichment may instead be provided in a to-be-designed enrichment carousel or possibly in the feed as fibrous particles.
- **Key issue 3:** Matching maternal environment with piglet environment by odours or flavours. By providing flavour in the feed of sow and piglets, the feed postweaning and in the pen in which piglets are housed postweaning, the new pen and feed will be less unfamiliar, possibly resulting in reduced weaning stress and a higher intake of food postweaning.
- **Key issue 4:** Look at behaviour of the pig to see what it needs. In the wild piglets would forage together with their sow, eat the same type of food and encounter many different (food) stimuli. In order to successfully implement the results and increase postweaning performance, it is important to keep this in mind.
- **Key issue 5:** Look at the sow and what she needs to facilitate learning by piglets. Sows should be stimulated in such a way that they are willing and able to show more behaviours that stimulate early feed intake of piglets. This can be done by management strategies (loose housing, feeding more often) but making a more social sow that shows proper maternal behaviour may be also be a breeding goal.
- **Key issue 6:** Optimizing feed of piglets to stimulate eating. Currently piglets receive very small pellets of feed, which may be less efficient in enhancing development of chewing and feeding behaviour. A larger pellet that is more elastic and stimulates piglets to chew more might be more efficient in increasing preweaning feed intake and consequently increasing feed intake after weaning. Lactation feed might also stimulate feed intake of piglets, as piglets will be able to learn more about taste and appearance from their mother's diet if they eat the same feed.

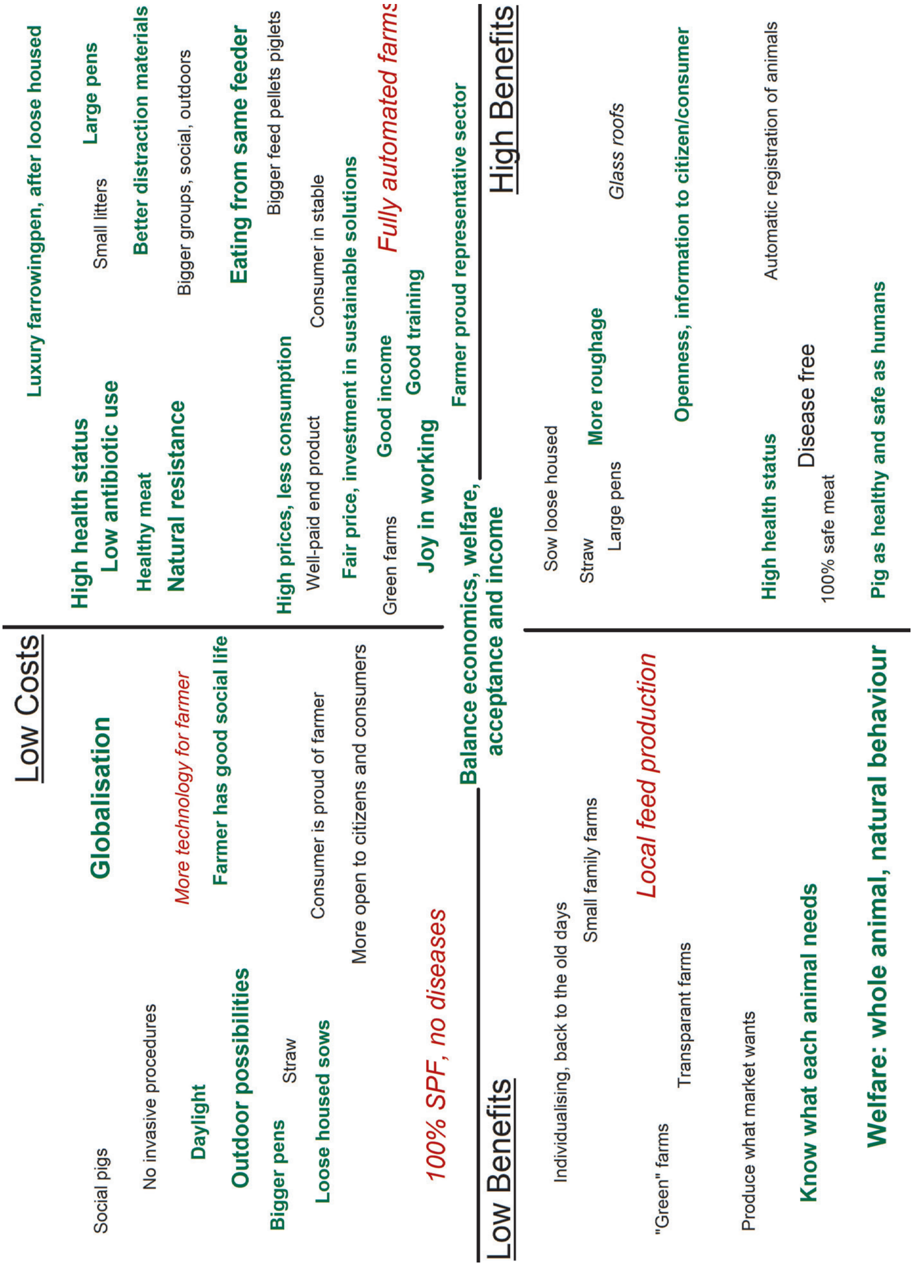
- **Key issue 7:** Costs-benefits of welfare and performance of piglets. The above mentioned key issues do have a certain cost, and it is important to show that the benefits for farmer and piglets outweigh the costs to facilitate implementation of the solutions. Policy makers can play a role by enforcing solutions to increase welfare of pigs, but this will increase prices unless there is a bonus for pig farmers who abide by the rules. Consumers, however, do want to eat "happy pigs", but are not eager to pay a high price for their meat. Retailers can force consumers to buy pig-friendly meat by not providing cheaper options in the store.
- **Key issue 8:** Attitude towards pigs. Making consumers more aware with certain logos, thus changing their attitude towards pigs and meat, may also make them more willing to buy the more expensive meat . Changing the attitude of farmers can also help. Farmers should see their animals more as individuals, and take into account that their own attitude towards their animals can affect stress levels and consequently affect piglet health and welfare around weaning.
- **Key issue 9:** Choice of what meat to eat by consumer. Always keep in mind what the consumer wants when specifying PR strategies and marketing. Focus should be on healthy meat that has been produced without antibiotics.

Long-term implementation

The long-term implementation session resulted in a number of thoughts, preconditions and ways to implement the results, of which many were placed in the low costs-high benefits section of the cost-benefit matrix. The cluster of ideas involving healthy and safe meat with low antibiotic use was deemed to be very important for future pig production, though going to specific-pathogen-free farming was not deemed desirable. Providing pigs with larger pens, outdoor facilities, distraction materials, bigger social groups and the possibility to learn more from the sow by eating together was also well-liked by the participants. When the farmer likes his/her work, has a good income, is trained and proud then production of pigs with good health and welfare may become more easy. This does not, however, mean that the farms should become fully automated: involvement of the farmer is important.

Interestingly, the cluster of thoughts related to social pigs in larger pens or groups with distraction and learning possibilities was also found in the high costs-high benefits section, in the low costs-low benefits section and also in the low benefits-high costs section. There were always participants in favour of these ideas, regardless of the section in which these ideas were found, suggesting that even when the costs are high there might be a market for meat produced in systems that are in between conventional and organic farming. Openness about farming, safety of the meat and welfare of the pigs were also often mentioned, suggesting that when consumers knows what they are paying for, they might be willing to pay more.

figure 1.



Representation of the issues raised in the long-term solutions session. Issues were categorized into low costs-high benefits, low costs-high benefits, high costs-high benefits and high costs-low benefits, and where then voted on. Issues presented in bold, green font were deemed positive by a majority of the voters. Issues in cursive, red font were deemed negative by a majority of voters. Issues in normal, black font were either not voted on, or there was an equal amount of voters in favour or against. Issues in the larger fonts were issues that were voted on: the larger the text, the more people voted on the issue.

CONCLUSIONS

For the short-term, a number of viable ways to facilitate vertical information transfer can be distilled from the key issues raised. Enabling sow and piglets to eat together seems a good solution when the trough of the sow can be lowered and feeding frequency of sows and piglets can be increased, especially when sows and piglets can both eat feed for lactating sows. Adding flavour to the feed of sow and piglets was deemed another viable implementation, but piglet feed itself may also be further optimized, for example by improving composition and pellet size. Providing roughage such as straw would be a good idea if the current systems could manage the straw. Improving consumer awareness and consumer and farmer's attitude towards pigs may make them more motivated to provide money to make these sometimes costly changes.

For the long-term, the focus should be on changing the design of pig stables, providing piglets and sows with larger pens, possibly outdoor runs in which they can interact with other pigs, distraction materials and the possibility to eat from the same feeder. This should be done while keeping in mind that the meat should remain healthy and safe, farmers health and work joy should not be compromised and antibiotic use should be minimal. These changes might be costly but consumer awareness may make it more likely that the income of the farmer remains sufficient and farmers remain motivated to produce pigs with good health and welfare.

Thus, the project has resulted in a number of viable ways to facilitate vertical information transfer to reduce problems around weaning, for both the short and long term, but for implementation of all the different solutions, there needs to be a good balance between costs, benefits and the consumer's willingness to pay more for welfare-friendly, safe and healthy meat.

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ENGLISH SUMMARY

Piglets in commercial husbandry are generally weaned between 3 and 4 weeks of age for economic reasons, which is earlier and more abrupt than would occur under (semi-)natural conditions. Many piglets have no experience with solid food before weaning, resulting in low food intake and growth after weaning. Newly weaned piglets also often show a high incidence of diarrhoea and maladaptive behaviours after weaning due to the low food intake and high stress levels at weaning and thus reduced health and welfare. The aim of this thesis was to explore whether providing piglets with more opportunities to learn from their mother about what, how and where to eat can increase food intake before and after weaning and consequently can reduce health and welfare problems after weaning. Young animals learn from experienced conspecifics what, where and how to eat. This social learning may also be important for piglets and when given the opportunity, piglets would follow the sow while she is searching for food and eating. In pig husbandry, however, the opportunities to learn from the sow are very limited as the sow is often confined throughout lactation, fed a different feed than the piglets in an inaccessible, raised trough. Sows and piglets are also mostly housed in rather barren, stimulus-poor pens. Three pathways of learning that could probably be improved in current pig husbandry were chosen: 1) direct learning from the sow through cues derived from observation and eating together, 2) learning in an enriched environment and 3) learning from flavour cues in the maternal diet.

Providing piglets with more opportunities to directly learn from the sow may stimulate the development of feeding related and social behaviours, may result in a higher food intake before weaning and consequently result in a higher food intake after weaning. Providing piglets and sow with an enriched environment may increase the amount of information that piglets can acquire from the sow because the sow can show more behaviours. Enrichment may also provide piglets with the opportunity to practice behaviours, and enrichment early in life may increase the ability to adapt to stressful situations, such as being weaned abruptly. The experiment described in Chapters 2, 3 and 4 investigated effects of increased opportunities to directly learn from the sow and an enriched environment. Piglets were reared with a confined or loose-housed sow in a barren or in an enriched (straw, wood shavings, peat and branches) environment. Piglets could eat together with the sow in a family feeder if the sow was loose-housed. After weaning half of each litter was housed in a barren pen and half in an enriched pen.

The barren and enriched housed piglets from the loose-housed sows were exposed to a novel food test either with their mother present or absent. Being able to eat novel foods together with the mother resulted in a lower reluctance to approach and eat the novel food and a higher intake. Piglets from an enriched environment were already less reluctant to eat the novel food, regardless of whether their mother was present or not, and also had a higher food intake (Chapter 2).

Being able to eat together with the sow and being reared in an enriched environment also had positive effects on feeding-related behaviours and growth in the home pen (Chapter 3, 4). Enrichment of the lactation pen also had positive effects on postweaning food intake, indicating that preweaning enrichment can help to stimulate early food intake in piglets (Chapter 3). Being reared with a loose-housed sow had positive effects on behaviour postweaning, suggesting that the sow is important in the development of adaptive behaviour (Chapter 4). Postweaning enrichment had profound positive effects after weaning: a higher growth in the two weeks after weaning, a profoundly lower diarrhoea prevalence and a higher feed efficiency (Chapter 3). Piglets also showed more play and less damaging behaviours postweaning when housed in an enriched pen (Chapter 4). These positive effects of enrichment may be due to decreased stress levels or increased gut health. Preweaning and postweaning enrichment did interact with each other: piglets from an enriched lactation pen housed in a barren pen postweaning showed a lower performance, more damaging behaviour and low levels of play in the first two weeks postweaning, so if enrichment is provided before weaning it is important to also provide it after weaning. Enrichment of the pre- and postweaning environment, and being able to eat together with the sow thus seems important in improving performance, health and welfare of newly weaned pigs.

There are many learning processes involved in eating together: observing what the mother does, participating, learning about the location of the food and learning about the type of food that the mother eats. Chapter 5 shows that learning from the sow is more effective than exposing piglets to the food without being able to learn from the sow. Observing or participating with the sow while she is eating were both effective in stimulating piglet food intake and in establishing a preference for the food eaten by the sow. Piglets use information about the location where the food was present, but prioritize information about the type of food that the sow eats. This indicates that it is important to let sow and piglets eat together, but particularly to provide sow and piglets with the same food.

The results from the experiments on flavour learning further stress the importance of matching sow and piglet food, at least in flavour. Young animals can learn from flavours in the maternal diet, which enhances recognition, preference and acceptance of foods containing these flavours around weaning. In the first experiment piglets were exposed to anise flavour through the maternal diet before birth, during lactation, both, or never, before weaning. Piglets that were exposed to the flavour before birth recognized the flavour after birth in several behavioural tests. The clearest effects on behaviour were found in tests eliciting a moderate stress response, suggesting that the mere presence of a familiar flavour may reduce stress (Chapter 6). Piglets were provided with control food and flavoured food after weaning, and piglets that were exposed to the flavour through the maternal diet did not show a preference for the flavoured food. Prenatally exposed piglets did, however, show a higher food intake, less diarrhoea and less damaging behaviours (Chapter 7). The lack of preference for the flavour, together with the results from the preweaning behavioural tests suggest that the effects were due to reduced stress at weaning, caused by the presence of the familiar flavour. This idea was confirmed in Chapter 8. Piglets that were exposed to anise flavour through the maternal diet both before and after birth, or never, were provided with the anise flavour in the air or in the food after weaning. Piglets that were exposed to the flavour showed lower levels of cortisol after weaning, less variation in growth, less damaging behaviours, more play behaviour and less vocalizations, all indicative of reduced stress. It did not matter whether the flavour was present in the food or only in the air, suggesting it is particularly the odour of the familiar flavour that is important in reducing weaning stress.

In conclusion, information from the sow plays a critical role in the development of independent feeding and reducing weaning-related problems. Currently, piglets do not have enough opportunities to learn from the sow, and lactation pens and food of sows and piglets could be improved to create possibilities for sow and piglets to eat together and to eat the same food during lactation. Enrichment may be provided both before and after weaning to further stimulate the development of solid food intake. Reducing stress through enrichment and providing a flavour associated with the sow's diet will further ensure an improved performance, health and welfare of newly weaned piglets.

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NEDERLANDSE SAMENVATTING

Biggen in de intensieve varkenshouderij worden meestal al op 3 tot 4 weken weggehaald bij hun moeder (spenen), waarna zij geen melk meer kunnen drinken en dus zelfstandig moeten eten. Dit spenen gebeurt abrupter en op een jongere leeftijd dan onder natuurlijke omstandigheden. Hierdoor hebben veel pas gespeende biggen nog geen ervaring met het zelfstandig eten van vast voer, waardoor zij in de eerste dagen na spenen vaak weinig eten en slecht groeien, of zelfs gewicht verliezen. Pas gespeende biggen hebben ook vaak last van diarree en vertonen beschadigende gedragingen, zoals oor-, poot-, staart- en flankbijten, wat aangeeft dat het spenen zorgt voor stress. Spenen resulteert daardoor vaak in slechte resultaten voor de varkenshouder, en een verminderde gezondheid en welzijn voor de big. Door de biggen al op vroege leeftijd aan het eten te krijgen zijn biggen beter aangepast op de situatie na spenen, waardoor speenproblemen verminderd worden. In dit proefschrift werd gekeken naar manieren om een vroege opname van vast voedsel te bevorderen. Dit werd gedaan door biggen meer mogelijkheden te geven om te leren van hun moeder over wat, hoe en waar ze moeten eten. Onder meer natuurlijke omstandigheden kunnen biggen al op jonge leeftijd mee eten met hun moeder, en ook van andere dieren is bekend dat het kunnen leren van de moeder belangrijk is voor een optimale ontwikkeling van eetgedrag. Onder commerciële omstandigheden zijn deze mogelijkheden om te leren van de moeder vaak beperkt, omdat de zeug weinig bewegingsvrijheid heeft, een ander voer krijgt dan de biggen en de biggen vaak niet mee kunnen kijken en eten met de zeug omdat de voerbak te hoog hangt. Zeugen en biggen zijn ook vaak gehuisvest in kale, stimulusarme hokken waar geen wroet- en kauwmateriaal, zoals stro, beschikbaar is waarop ze eetgedragingen kunnen oefenen. In dit proefschrift werden drie manieren onderzocht waarop biggen mogelijk meer kunnen leren wat, hoe en waar te eten. De eerste manier was leren tijdens het mee eten met de zeug, de tweede manier was het leren in een verrijkte omgeving, en de derde manier was leren van geur-/smaakstoffen (aroma's) in het voer van de zeug, al voor de geboorte.

In hoofdstuk 2, 3 en 4 werd onderzocht hoe samen kunnen eten met de zeug en een verrijkte omgeving bij kunnen dragen aan het verminderen van problemen na spenen. Samen kunnen eten en samen exploreren van voedsel geeft biggen de mogelijkheid om meer gedragingen te zien en te leren van de zeug, zoals voedsel zoeken, kauwen en eten. In een verrijkte omgeving kunnen biggen mogelijk meer gedragingen zien van de zeug, meer zelf oefenen en verrijking kan het aanpassingsvermogen aan onbekende situaties vergroten. In het experiment groeiden biggen op met een zeug met weinig bewegingsvrijheid of een loslopende zeug, in een kaal hok of een verrijkt hok. In de hokken was een familievoerbak geplaatst waaruit biggen samen met de zeug konden eten indien zij los liep.
Biggen werden een paar dagen voor het spenen in een testruimte blootgesteld aan onbekend voedsel (kaas en chocoladepinda's). Tijdens deze blootstelling was de moeder aanwezig of niet. Biggen die met hun moeder in de testruimte werden geplaatst namen sneller een hap van het onbekende voer en aten meer

tijdens de test dan biggen die blootgesteld waren zonder hun moeder. Opgroeien in een verrijkte omgeving zorgde ervoor dat biggen ook meer geneigd waren dit onbekende voedsel te eten, ongeacht of de moeder aanwezig was of niet (Hoofdstuk 2).

Het mee kunnen eten met de zeug en opgroeien in een verrijkt hok had ook een positief effect op groei en op eetgedrag in het hok voor spenen (Hoofdstuk 3, 4). Verrijking van het hok voor spenen zorgde er ook voor dat voeropname na spenen hoger was, wat aangeeft dat verrijking kan helpen om biggen al vroeg te laten wennen aan het eten van vast voer (Hoofdstuk 3). Het opgroeien met een loslopende zeug zorgde ervoor dat er meer positieve gedragingen (spelen) en minder beschadigende gedragingen te zien waren na spenen, wat aangeeft dat meer mogelijkheden voor interactie met de zeug door de zeug los te laten lopen en zeug en biggen samen te laten eten een positief effect heeft op welzijn van biggen na spenen (Hoofdstuk 4).

Het effect van verrijking na spenen werd onderzocht door na spenen de helft van de biggen in een kaal hok en de andere helft in een verrijkt hok te plaatsen. Verrijking na spenen had grote effecten: biggen in een verrijkt hok na spenen groeiden harder in de eerste twee weken na spenen, hadden minder last van diarree en hadden een hogere voerefficiëntie dan biggen in een kaal hok na spenen (Hoofdstuk 3). Biggen in een verrijkt hok lieten ook meer positief en minder beschadigend gedrag zien na spenen (Hoofdstuk 4). Deze positieve effecten van verrijking worden mogelijk veroorzaakt doordat verrijking stress na spenen kan verminderen, en darmgezondheid kan bevorderen wanneer wroetmaterialen opgegeten worden. De huisvesting van biggen tijdens de lactatieperiode beïnvloedde het effect van verrijking na spenen: als verrijking wel aanwezig was in het kraamhok maar niet na spenen deden biggen het slechter dan de andere biggen, wat aangeeft dat verrijking ook na spenen aanwezig moet zijn als het voor spenen aanwezig was. Verrijking van het hok voor en na spenen en meer kunnen leren van de zeug bevordert dus groei, voeropname, gezondheid en welzijn van biggen.

In hoofdstuk 5 werd in detail gekeken naar welke informatie belangrijk is voor de biggen. Uit deze experimenten bleek dat biggen zowel kunnen leren door het observeren van de moeder en door met haar mee te kunnen eten. Deze twee manieren van leren zijn allebei efficiënter dan biggen te laten eten zonder informatie van de zeug. De plaats waar de zeug eet is belangrijk, maar nog belangrijker is het type voer dat de zeug eet. Het is dus niet alleen belangrijk is om zeug en biggen samen te laten eten op dezelfde plaats, maar ook om ze hetzelfde voer aan te bieden.

Informatie over het type voer dat de zeug eet, met name aroma's, kan ook al voor de geboorte en tijdens lactatie via de melk door jonge dieren waargenomen worden, waarna zij makkelijker voertypen accepteren rondom spenen waarin dit aroma ook aanwezig is. In het eerste aroma experiment werden biggen blootgesteld aan anijsaroma via het zeugenvoer voor de geboorte, tijdens lactatie, beide of nooit. Uit verschillende gedragstesten bleek dat de biggen het anijs herkenden wanneer hun moeder voer met anijsvoer had gegeten tijdens de dracht. Dit werd vooral duidelijk in testen waarbij de biggen licht gestrest waren, wat aangeeft dat het bekende aroma mogelijk stress verlaagt (Hoofdstuk 6). Na spenen kregen biggen voer met anijsaroma en gewoon voer. Biggen die blootgesteld waren aan anijs via het zeugenvoer hadden geen voorkeur voor het anijsvoer. Wel lieten biggen die voor de geboorte blootgesteld waren aan anijs via het voer van hun moeder een grotere groei zien, een hogere voeropname, minder diarree en minder beschadigend gedrag na spenen (Hoofdstuk 7). De resultaten uit Hoofdstuk 6 en 7 suggereren dat een bekende geur in de omgeving voor verminderde stress rondom spenen zou kunnen zorgen, en dat de positieve effecten van het leren van aroma's voor de geboorte niet het gevolg zijn van een verhoogde voorkeur voor voer met een soortgelijk aroma. Dit werd bevestigd in Hoofdstuk 8. Biggen die al dan niet blootgesteld waren aan anijs via het voer van hun moeder tijdens dracht en lactatie kregen in het hok na spenen anijsaroma in het voer of alleen anijsgeur in de lucht. Biggen die konden leren van aroma's voor spenen hadden lagere cortisol niveaus na spenen, minder variatie in groei, minder beschadigend gedrag en vocalisaties, en meer spel, wat allemaal aangeeft dat stress lager was in deze biggen dan in biggen die nooit blootgesteld waren aan anijs voor spenen. Het maakte niet uit of het anijsaroma in het voer zat of alleen in de lucht. Dit geeft aan dat vooral de aanwezigheid van een bekende geur belangrijk is in het verminderen van stress rondom spenen.

De zeug speelt dus een belangrijke rol in het stimuleren van vroege opname van vast voer in biggen, het verminderen van stress rondom spenen en de ontwikkeling van aanpassingsvermogen aan de situatie na spenen. Momenteel zijn er meestal weinig mogelijkheden voor biggen om van de zeug te leren. Kraamhokken en voer van zeug en big zouden aangepast kunnen worden om biggen de mogelijkheid te geven mee te eten met de zeug van hetzelfde voer. Verrijking kan aangeboden worden zowel voor als na spenen om de ontwikkeling van zelfstandige voeropname te stimuleren. Stress rondom spenen kan verlaagd worden door het geven van verrijking en het geven van een aroma waaraan de biggen voor de geboorte zijn blootgesteld via het zeugenvoer. Het stimuleren van vroege voeropname en het verminderen van stress rondom spenen is belangrijk in het verhogen van groei, voeropname, gezondheid en welzijn van pas-gespeende biggen.

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> “Enjoy the unknown” – And One

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CURRICULUM VITAE

Marije Oostindjer was born on the 23rd of February 1985 in Zeist. She graduated from secondary school “CSG Vincent van Gogh” in Assen in 2002. In the same year she started her BSc in Biology at the Rijksuniversiteit of Groningen, with the specialization Ecology, and graduated in 2005. In the same year she was selected for the Research Master Behavioural and Cognitive Neurosciences at the Rijksuniversiteit Groningen and participated in the Animal and Human behaviour track. Her first master thesis investigated the relationship between individual characteristics and the ability to cope with dietary changes in rats. Her second thesis investigated the effects of testosterone on energy expenditure, immune status, behaviour and morphology of the Black-Headed Gull. She graduated in 2007 with honours, and started at Wageningen University as a PhD student in the same year. While working on her PhD thesis she also spent three months at the Centre European des Sciences du Gout as a visiting researcher, working on perinatal flavour learning and olfactory sensitivity in mice. Her PhD-thesis work was awarded with a Storm-van der Chijs travel stipend for promising female PhD students, the WIAS Science Day oral presentation prize and the WIAS publication price. After obtaining her PhD degree she aims to obtain a post-doc position to continue her career in research.

PUBLICATIONS

Refereed scientific journals and book chapters

- M. Oostindjer; J.E. Bolhuis; M. Mendl; S. Held; H. Van den Brand; B. Kemp (2011) Learning how to eat like a pig: effectiveness of mechanisms for vertical social learning in piglets. Animal Behaviour, in press.
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- H. Van den Brand; M. Oostindjer; A. Van Enckevort; J.E. Bolhuis. A large pellet diameter increases early feed intake of suckling piglets. Submitted to Journal of Animal Science.

CONFERENCE PROCEEDINGS AND ABSTRACTS

- M. Oostindjer; J.E. Bolhuis; M. Mendl; S. Held; H. Van den Brand; B. Kemp (2011) Learning how to eat like a pig: effectiveness of mechanisms for vertical social learning in piglets. In: Proceedings of the 45th Congress of the International Society for Applied Ethology, 31 July – 4 August, Indianapolis, USA.
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- Boerderij - ISSN 0006-5617
- M. Oostindjer; A.C. Bartels; M. Ooms; J.E. Bolhuis (2008) Kiest de big het poortje met de geur van zeugenvoer? Boerderij, 93 - ISSN 0006-5617 - p. 21.

Training and supervision plan

The basic package (3.0 ECTS)	
WIAS Introduction Course	2009
WIAS Course on Philosophy of Science and Ethics	2008

International conferences (5.6 ECTS)	
42nd Congress of the International Society for Applied Ethology, Dublin, Ireland	2008
Society for Behavioural Neuroscience 12th annual meeting, Groningen, The Netherlands	2008
15th Benelux Congress of Zoology, Liege, Belgium	2008
43rd Congress of the International Society for Applied Ethology, Cairns, Australia	2009
44th Congress of the International Society for Applied Ethology, Uppsala, Sweden	2010
AChemS 2011 Annual Meeting, St. Pete Beach, USA	2011

Seminars and workshops (2.9 ECTS)	
WIAS seminar ‘Personalities’	2007
Nederlandse Vereniging voor Gedragsbiologie meeting, Dalfsen/Soesterberg, the Netherlands	2007-2010
WIAS Science Day, Wageningen, the Netherlands	2008-2011
WIAS/Adaptation Physiology seminar ‘Lactation management’, Wageningen, the Netherlands	2009

Presentations (13.0 ECTS)	
Oral presentation at WIAS seminar ‘Personalities’, Wageningen, the Netherlands	2007
Oral presentation at 42nd Congress of the International Society for Applied Ethology, Dublin, Ireland	2008
Oral presentation at Nederlandse Vereniniging voor Gedragsbiologie meeting, Dalfsen, the Netherlands	2008
Poster presentation at the WIAS Science Day, Wageningen, the Netherlands	2009
Oral presentation at the 43rd Congress of the International Society for Applied Ethology, Cairns, Australia	2009
Oral presentation at the WIAS/Adaptation Physiology seminar ‘Lactation management’, Wageningen, the Netherlands	2009
Poster presentation at workshop Making the most of behavioural data, London, UK	2009
Oral presentation at the 44rd Congress of the International Society for Applied Ethology, Uppsala, Sweden	2010
Poster presentation at the 20th Conference of the European Chemoreception Research Organization, Avignon, France	2010
Poster presentation at the STW Jaarcongres, Nieuwegein, the Netherlands	2010
Oral presentation at the Nederlandse Vereniging voor Gedragsbiologie meeting, Soesterberg, the Netherlands	2010
Oral presentation at the WIAS Science Day, Wageningen, the Netherlands	2011
Poster presentation at the AChemS Annual Meeting, St. Pete Beach, USA	2011

In-depth courses (12 ECTS)	
Statistics for the Life Sciences	2008
Nutrition in the Omics Era	2008
Epigenisis-Epigenetics: Physiological consequences of prenatal programming	2008
Smell and Taste 01	2008
Interpretation of Animal Stress Responses	2009
Design of Animal Experiments	2009
Making the most of behavioural data: Rules, Relations and Robotics	2009
Orientation on Mathematical Modelling in Biology	2011

Professional Skills Support Courses (10 ECTS)	
Techniques for Writing and Presenting Scientific Papers	2008
PhD Competence Assessment	2008
Supervising MSc thesis work	2009
Interpersonal Communication	2009
Giving Lectures	2009
Talents & Topics program	2011

Research Skills Training (2 ECTS)	
External training of 3 months at the CESG, Dijon, France	2010

Didactic Skills (26 ETCS)	
Lecturing in BSc courses Adaptatiefysiologie-1 and Perinatology	2009-2010
Lecturing in MSc course Health, Welfare and Management	2011
Assisting practical Inleiding Dierwetenschappen	2009
Assisting coaching project group Adaptatiefysiologie-2	2009
Coaching group and grading papers Health, Welfare and Management	2009/2011
Coaching project group in Animal Experimentation	2009
Reviewing papers and judging research proposals of Research Master Cluster students	2008-2010
Supervising 13 BSc-MSc students	2007-2010

Management Skills Training (9 ECTS)	
Organization of WIAS Science Day	2009-2010
Member of the WAPS Council	2008-2010
Member of the Education Committee	2008-2009
Member of the Wageningen PhD Council	2010
WUR Representative for the Promovendi Network Netherlands	2009-2010

Education and Training total	82.0 ECTS

COLOPHON

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