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Early feeding experiences of piglets and their impact on novel environment behaviour and food neophobia

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A R T I C L E I N F O

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A B S T R A C T

We studied whether feeding experiences of piglets during lactation influence novel environment behaviour pre-weaning and food acceptance pre- and post-weaning. In a 2 × 2 arrangement, litters (n = 10/treatment) received creep feed as monotonous diet (MO) or four feed items as diverse diet (DD) and the feed was provided without substrate (CON) or partly hidden in substrate (SUB). Two groups of four piglets/litter were exposed to a novel environment test (NET) at d21, a familiar food test (FFT) with creep feed at d22 and a food neophobia test (FNT) at d24 (cheese and chocolate), which was repeated at d25. At weaning (d28), four piglets from the same treatment were grouped (n = 10 pens/treatment) and fed a weaner diet onwards. At d41 a FNT (dried apple and crisps) was performed. No differences were observed in the NET. In the FFT, DD-piglets took longer to sample ($P = 0.046$) and less DD- than MO-piglets sampled feed ($P = 0.02$). Feed consumption increased ($P < 0.001$) and vocalisations decreased ($P = 0.02$) from day 1–2 of the pre-weaning FNT. DD-piglets vocalised more during the pre-weaning FNT ($P = 0.03$), and, unlike MO, their latency to vocalise did not increase from day 1–2. DD-piglets explored ($P = 0.03$) and sampled feed less ($P = 0.007$) than MO-piglets, but percentages of piglets exploring and sampling feed did not differ between treatments. Feed presentation in substrate did not affect piglets' behaviour, except that the latency to sample feed did not decrease from day 1–2 for DD-CON, while it did for the other groups (interaction, $P = 0.047$). Post-weaning, DD-piglets took longer to explore the feed than MO-piglets ($P = 0.04$), and seemed more attracted to crisps, as they sampled it sooner ($P = 0.01$), and more DD-piglets sampled it ($P = 0.02$). MO-piglets, however, showed more exploration ($P = 0.006$) and sampling ($P = 0.01$) of apple, started sampling it sooner ($P = 0.01$) and a higher proportion of MO-piglets sampled apple ($P = 0.02$). Treatments did not affect the number and weight of the feed items consumed in the FNTs. Thus, early feeding experiences did not influence novel environment behaviour and feed intake in the FNTs, but pre-weaning dietary variety affected short- and long-term exploratory responses to novel feed. Unexpectedly, piglets with a diverse diet did not show signs of reduced food neophobia. This was possibly because of contrasts between the environment and the test conditions, resulting in MO-piglets being more motivated to explore in the tests than DD-piglets. Also the items selected as novel food may have played a role.

1. Introduction

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

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Provenza, 1993, 1994).

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

Several studies show that a diverse feeding experience in early life mitigates food neophobia. Breast-fed infants displayed lower neophobia towards new food than formula-fed infants (Sullivan and Birch, 1994; Maier et al., 2008) and breast feeding is a diverse feeding experience since the maternal diet affects the odour and flavour of the milk (Hausner et al., 2010). Moreover, prior exposure to unfamiliar foods reduced neophobia towards those food items, but also towards other unfamiliar foods in infants (Loewen and Pliner, 1999; Gerrish and Mennella, 2001; Maier et al., 2008) and lambs (Launchbach et al., 1997). It was also shown in lambs that a varied diet after weaning enhanced the acceptance rate of novel flavours and feeds that were given later in life compared to a monotonous mixture diet (Catanese et al., 2012; Villalba et al., 2012). How long the effects of early feeding experiences persist when dietary variety is not continued remain unknown, but these results suggest that dietary variety may be crucial in the development of cognitive abilities related to feeding behaviour (Catanese et al., 2012). In piglets, enriched housing during lactation by provision of edible substrates (straw, wood shavings, peat and branches) and extra space reduced neophobia towards novel feed items just before weaning as compared with barren housing (Oostindjer et al., 2011), which may have been the result of the diverse substrate experience. Dietary variety may not only be effective in reducing food neophobia, but may also reduce neophobia in general. For example, a varied versus monotonous diet given to lambs tended to result in a lower increase in rectal temperature after exposure to an open field test (Villalba et al., 2012).

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

2. Methods

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

2.1. Animals, housing and treatments

The animals, housing and treatments are described in more detail in Middelkoop et al. (2019). Briefly, forty litters were housed in farrowing pens (2.85 × 1.80 m), divided over two farrowing rooms, in three consecutive batches. During the study, litters were reared by multiparous sows, which were Topigs-20 and Norwegian Landrace x Topigs-20 sows inseminated by Tempo boar semen. From 4 days of age, piglets had *ad libitum* access to a concrete piglet feeding area (1.37 × 1.80 m), in front of the sow, including two feeders with four bowls/feeding spaces each (17.5 × 13.5 cm per bowl). Litters were assigned to one of four treatment combinations (10 litters/treatment) in a 2 × 2 arrangement, with dietary variety (DV) and feed presentation (FP) as experimental factors. In short, piglets received either one solid feed item, creep feed (3-mm pellet), as a monotonous diet (**MO**) or received four solid feed items simultaneously (creep feed, celery, cereal honey loops and peanuts in shell) as a diverse diet (**DD**), and the feed was either presented without substrate (**CON**) or hidden in substrate (**SUB**), which was sand, in one of the two feeders to stimulate natural foraging behaviour. Treatments were continued until weaning at 28.5 ± 0.2 days of age. Litter size was on average 12.9 ± 0.2 piglets/litter at weaning. At weaning, a subset of 160 piglets (n = 10 weaner pens/treatment with 4 piglets/pen), distributed over two consecutive batches, was relocated in two weaner rooms per batch until two weeks post-weaning. Piglets were mixed with conspecifics from the same pre-weaning treatment, and housed with two males and two females, which derived from three litters. Piglets were housed in pens of 2.76 × 1.20 m. All weaner pens were identical and all piglets were fed a commercially available nursery diet *ad libitum* (3-mm pellet). A metal chain with bolts was present as chew object for the piglets in the farrowing and weaner pens. No bedding material was provided.

To the aim of this study, eight piglets from each litter were selected on 21 days of age to study their behaviour in behavioural tests (i.e. 320 piglets in total). Piglets were selected based on the following criteria: 1) sex (equivalent male to female ratio) and 2) body weight at 19 days of age (close to the average weight of the litter). Piglets with a history of medication and leg/claw problems were excluded from selection. The eight selected piglets per litter were tested in two fixed groups of four piglets, resulting in 20 groups/treatment in the test before weaning. At weaning, piglets were selected based on sex and body weight (see Middelkoop et al. (2019) for details), and only piglets that were exposed to the pre-weaning behavioural tests were selected (160 out of 320 piglets), while again excluding piglets with health problems. The selected piglets originated from eight litters in MO-SUB and DD-CON and from seven litters in MO-CON and DD-SUB. Each weaner pen was tested as group of four piglets, resulting in 10 pens/treatment in the test after weaning (i.e. 160 piglets in total).

2.2. Behavioural tests

Behavioural tests were all conducted in a 5.3 × 5.3 m arena, located in a different room, with wooden walls of 1.2 m and a concrete floor (**Supplementary Figure S1**). The test arena was surrounded by a corridor and the arena had one entrance with a vertical lift door. Piglets entered the arena from a start box. A 2 × 2 m square, painted on the floor in the centre of the arena, served as the feeding place when feed was provided in the tests. Piglets were individually marked using dark permanent hair dye to enable behavioural observations. Piglets were observed by well-trained observers through direct observation from the corridor adjacent to the test arena using Psion hand-held computers and tablets with the Pocket Observer 3.1 and 3.3 software package respectively (Noldus Information Technology, Wageningen, the Netherlands).

Transfer to the test arena was done in a transport cart. At the test arena piglets were put in the start box, the vertical lift door was opened, piglets were calmly guided into the arena, and the door was then closed. The tests started when all four piglets entered the arena and the total test time was 5 min per test. After the tests, piglets were returned to their home pen and faeces and urine were removed from the test arena. The order of testing was balanced for treatment and room. Temperature in the test room was kept the same as the temperature in the home pens.

2.2.1. Novel environment test

A novel environment test (NET) was conducted in the test arena at 21 days of age. Piglets encountered the test arena for the first time and their frequencies and latencies of vocalising, defecating, urinating and escape attempts (**Supplementary Table S1**) were scored live on group level by one observer that was blind to the treatment.

2.2.2. Familiar food test

A familiar food test (FFT) was conducted at 22 days of age. Creep feed, i.e. the same as provided in the home pen of the piglets, was distributed in 20 piles (5 piles/piglet) over the feeding place of the test arena (**Supplementary Figure S1**). In total, about 108 g of creep feed was provided. The latency to sample creep feed was recorded for each piglet. Sampling was defined as ‘taking a feed item in the mouth and chewing or eating it’. The intake of creep feed was also measured on group level by weighing the feed before and after testing.

2.2.3. Food neophobia tests

A food neophobia test (FNT) was conducted at 24, 25 (pre-weaning) and 41 days of age (post-weaning) in the test arena. In the pre-weaning FNT at 24 days of age, five cheese cubes (2.4 g per cheese cube sized 1.5 × 1.5 × 1.5 cm) and five differently coloured chocolate candies, i.e. button-shaped milk chocolates surrounded by a colourful candy shell (2.8 g per chocolate sized 1.3 × 1.3 × 0.7 cm), were provided per piglet as novel feed items in the test (based on Oostindjer et al., 2011). The feed items were evenly distributed over the feeding place of the arena (**Supplementary Figure S1**). The test was repeated with the same animals and same feed items (cheese and chocolate candy) at 25 days of age to study the acceptance rate of novel feed across time. In the post-weaning FNT at 41 days of age, five pieces of dried apple (12.2 g per apple piece sized 2.5 × 2 × 0.3 cm) and five curled paprika crisps (1.4 g per crisp piece sized 5 × 2.5 × 2.5 cm) were used per piglet.

Feed-related behaviours were observed live for each piglet, i.e. one observer per piglet, by continuous sampling for 5 min. Sniffing or touching the feed with snout were defined as ‘exploring feed’. Taking a feed item in the mouth, chewing or eating feed were defined as ‘sampling feed’, as previously described. The consumption of the feed items (in weight and in the number of items consumed) was determined on group level by weighing the two feed items separately before and after the test, and counting them after the test. Moreover, for each group of piglets, the frequencies and latencies of vocalising, defecating, urinating and escape attempts during the test were recorded (**Supplementary Table S1**) by the same observer as in the NET.

2.3. Statistical analyses

2.3.1. Data processing

The intake of creep feed in the FFT was negligible with on average 0.14 g/piglet and therefore excluded from analyses. One MO-SUB piglet laid down during the pre-weaning FNTs and was therefore excluded from analyses of feed-related behaviours. Data of one DD-CON piglet in the post-weaning FNT were excluded from analyses, because of a technical error with the hand-held computer. DD-SUB had two groups of three instead of four piglets tested in the pre- and post-weaning FNTs, as two piglets died in the period after the FFT. Urinating and escape attempts were seen very rarely in all tests and defecating was seen very rarely in the FNTs, and were therefore excluded from analyses. The percentage of piglets exploring the feed items was not analysed in the post-weaning FNT since all piglets explored the feed. The intake of feed (apple and crisps) in the post-weaning FNT could not be accurately determined in grams due to the presence of saliva. Therefore, only the number of feed items was analysed in the post-weaning FNT.

2.3.2. Data analyses

Data were analysed with the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Latencies (to vocalise, explore and sample), counts (the number of vocalisations and feed items) and feed intake were analysed in a linear mixed model (MIXED procedure). Data were transformed before analyses if model residuals were not normally distributed. No pieces of apple were consumed by DD-CON in the post-weaning FNT and the number of apple pieces eaten was therefore expressed as binary data (consuming apple pieces or not) and analysed in a Fisher’s exact test.

Time spent exploring and sampling feed were analysed in a generalised linear mixed model (GLIMMIX procedure) with a binomial distribution, logit link function and an additional multiplicative overdispersion parameter. In addition, the occurrence of defecating, exploring and sampling feed was expressed as a 0–1 variable (per group for defecating and per piglet for exploring and sampling) and analysed in a GLIMMIX procedure with a logit link function and binary distribution. If a sub-classification category showed no variation, i.e. all piglets or groups scoring 1, a Fisher’s exact test was used.

The (generalised) linear mixed models included the fixed effects of dietary variety (DD vs. MO), feed presentation (SUB vs. CON), their interactions, as well as batch (batch 1, 2 or 3 for the pre-weaning tests and batch 1 and 2 for the post-weaning test). Variables that were measured on piglet level (i.e. exploring and sampling feed in all tests including latencies, time spent on these behaviours and the percentage of piglets performing these behaviours) were analysed with a random group effect, i,e. the group of piglets subjected to the test together (nested within pen, treatments and batch). Pre-weaning variables that were measured on group level were analysed with a random pen effect (nested within treatments and batch). Day was used as fixed effect in the analyses of the pre-weaning FNT, which consisted of two days, with values of individual piglets (nested within group, pen, treatments and batch) or groups (nested within pen, treatments and batch) taken as repeated measurements, for piglet- and group-level data, respectively.

Significant fixed effects were further analysed using *post-hoc* pairwise comparisons of least squares means using Tukey’s adjustment for three-way interactions. Data are presented as (untransformed) means ± SEM based on pen averages. Differences at $P < 0.05$ were considered statistically significant. In figures, only fixed effects at $P < 0.05$ are presented.

3. Results

3.1. Novel environment test

The number of vocalisations per piglet during the NET was not affected by dietary variety (DD vs. MO) or feed presentation (SUB vs. CON) before weaning, or their interaction (Table 1). The treatments did also not affect the latency of groups to vocalise and the percentage of groups that defecated during the NET (Table 1).

Table 1
Vocalising and defecating behaviour of a group of four 21-day-old suckling piglets in the novel environment test. In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages.

Behaviour	DD		MO		Significance		
	CON	SUB	CON	SUB	DV	FP	DV x FP
Vocalisations / piglet	99.0 ± 8.8	111.4 ± 3.9	95.4 ± 5.0	108.9 ± 12.7	0.82	0.14	0.99
Low-pitched vocalisations / piglet	89.3 ± 6.5	106.1 ± 3.7	90.4 ± 4.5	99.5 ± 11.2	0.81	0.08	0.55
High-pitched vocalisations / piglet	9.7 ± 3.1	5.3 ± 1.1	5.0 ± 1.3	9.4 ± 2.7	0.86	0.91	0.21
Latency to vocalise (s)	5.9 ± 0.8	6.0 ± 0.6	8.4 ± 1.5	6.5 ± 0.9	0.13	0.39	0.57
Percentage of groups defecating (%)	35	10	10	10	0.96	0.96	0.47

3.2. Familiar food test

MO-piglets (154.2 ± 13.9 s) had a shorter latency to sample the creep feed in the FFT than DD-piglets (194.7 ± 13.5 s, $P = 0.046$) and also more MO- (70.6 %) than DD-piglets (50.9 %) were sampling it ($P = 0.02$). Feed presentation or its interaction with dietary variety did not affect behaviour towards the creep feed (Table 2).

3.3. Food neophobia tests

3.3.1. Pre-weaning food neophobia test

3.3.1.1. Vocalisations. The latency to vocalise was affected by dietary variety ($P = 0.009$) and dietary variety x day ($P = 0.04$). The latency to vocalise of MO-piglets was higher on day 2 than on day 1 of the FNT, and also higher than that of DD-piglets on both days (Fig. 1A). Irrespective of treatments, the total number of vocalisations (Fig. 1B) was lower on the second (60.5 ± 4.3) compared to the first day of the FNT (68.2 ± 4.3, $P = 0.02$), which held both for low-pitched (day 1: 64.4 ± 3.8 vs. day 2: 57.4 ± 4.0, $P = 0.03$) and high-pitched vocalisations (day 1: 3.7 ± 0.8 vs. day 2: 3.0 ± 0.8, $P = 0.03$). DD-piglets (73.7 ± 3.7) vocalised more than MO-piglets (55.0 ± 4.5) during the FNT ($P = 0.03$), due to a higher number of low-pitched vocalisations (69.3 ± 3.2 ± vs. 52.5 ± 4.1, $P = 0.03$), of which the number of long grunts was higher (6.7 ± 0.7 vs. 3.8 ± 0.4, $P = 0.003$). No effect of feed presentation or its interaction with dietary variety was found.

Table 2
Behaviour of 22-day-old suckling piglets towards abundant creep feed in the familiar food test. In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV, dietary variety; FP, feed presentation. Data are expressed as means ± SEM based on pen averages. Significant *P*-values are presented in bold.

Behaviour towards creep feed	DD		MO		Significance		
	CON	SUB	CON	SUB	DV	FP	DV x FP
Latency to sample (s)	187.4 ± 22.1	202.8 ± 15.5	149.8 ± 19.7	158.1 ± 20.5	**0.046**	0.57	0.66
Percentage of piglets sampling (%)	50	51.9	67.5	73.8	**0.02**	0.63	0.71

3.3.1.2. Feed-related behaviour and feed intake. The latency to explore the feed (cheese plus chocolate) was affected by day ($P < 0.0001$) and the dietary variety x feed presentation x day interaction ($P = 0.02$). The latency to explore the feed decreased in all groups from the first to the second day of the pre-weaning FNT, but decreased in DD-CON the least (Fig. 2A). The latency to sample the feed was affected by day ($P < 0.0001$), dietary variety x day ($P = 0.003$) and the dietary variety x feed presentation x day interaction ($P = 0.047$). The latter interaction showed that the latency to sample the feed decreased from the first to the second day for DD-SUB, MO-CON and MO-SUB, but not for DD-CON (Fig. 2B).

Time spent exploring the feed was lower on the second (9.7 ± 0.5 %) than on the first day of the FNT (11.1 ± 0.7 %, $P = 0.002$, Fig. 2C), while time spent sampling the feed was higher on the second (32.0 ± 3.1 %) than on the first day of the FNT (25.7 ± 0.3 %, $P < 0.0001$, Fig. 2D). Time spent exploring (DD: 9.2 ± 0.6 vs. MO: 11.5 ± 0.6 %, $P = 0.03$) and sampling the feed (DD: 23.1 ± 2.7 vs. MO: 34.6 ± 2.8 %, $P = 0.007$) were both lower in DD- than in MO-piglets. No interactions between treatments and day were found.

The percentage of piglets exploring the feed was not affected by day, the treatments or their interactions (Fig. 2E). The percentage of piglets sampling the feed was higher on the second (87.7 %) than on the first day (83.9 %, $P = 0.04$), but was not affected by the treatments or their interactions (Fig. 2F).

The number of feed items eaten during the pre-weaning FNT was higher on the second (1.70 ± 0.23 feed items) compared to the first day (1.05 ± 0.17 feed items, $P = 0.0007$, **Supplementary Figure S2A**). Also the intake of feed in grams was higher on the second (2.12 ± 0.27 g) than on the first day (1.14 ± 0.15 g, $P < 0.0001$, **Supplementary Figure S2B**). No effect of treatments or interactions between treatments and day were observed for these parameters.

3.3.1.3. Feed-related behaviour towards cheese and intake of cheese. The latency to explore cheese was affected by day ($P < 0.0001$) and the dietary variety x feed presentation x day interaction ($P = 0.03$). MO-CON, MO-SUB and DD-SUB had a shorter latency to explore cheese on the second compared to the first day of the FNT, which was not the case for DD-CON (Fig. 3A). The latency to sample cheese was shorter on day 2 (128.2 ± 13.1 s) than on day 1 of the FNT (144.1 ± 13.6 s, $P = 0.03$) and shorter for MO- (116.4 ± 11.2 s) versus DD-piglets (156.0 ± 14.6 s, $P = 0.046$, Fig. 3B). No other fixed effects were found for the latency to sample cheese.

Time spent exploring cheese was lower on the second (5.2 ± 0.3 %) compared to the first day of the FNT (6.1 ± 0.4 %, $P = 0.0007$), and was lower for DD- (5.1 ± 0.4 %) than for MO-piglets (6.2 ± 0.3 %, $P = 0.048$, Fig. 3C**)**. Time spent sampling cheese was higher on the second (15.4 ± 2.2 %) compared to the first day of the FNT (12.2 ± 1.7 %, $P = 0.003$) and lower for DD- (9.2 ± 1.4 %) than for MO-piglets (18.4 ± 2.1 %, $P = 0.0003$, Fig. 3D). No effect of feed presentation or its interaction with dietary variety was found for time spent exploring and sampling cheese.

The percentage of piglets exploring cheese did not differ between treatments on the first day of the FNT. On the second day of the FNT, however, MO-SUB had a higher percentage of piglets exploring cheese than DD-SUB (Fisher's exact test, DV effect within SUB, $P = 0.03$, Fig. 3E). The percentage of piglets sampling cheese increased over time ($P = 0.02$), with more piglets sampling cheese on the second (71.6 %) than on the first day of the pre-weaning FNT (64.7 %, Fig. 3F).

The number of cheese cubes eaten was not affected by treatments or their interactions, but affected by day ($P = 0.02$) and increased from the first (0.18 ± 0.05 cheese cubes) to the second day (0.32 ± 0.07 cheese cubes, **Supplementary Figure S3A**). The intake of cheese in grams also increased from the first (0.41 ± 0.07 g) to the second day (0.74 ± 0.13 g, $P = 0.002$, **Supplementary Figure S3B**).

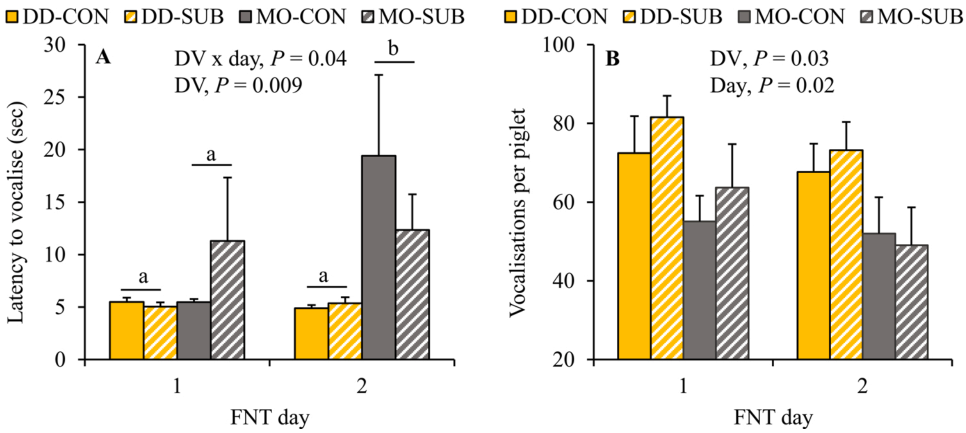

Fig. 1. The latency to vocalise (**A**) and the number of vocalisations (**B**) of piglets in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). In their home pen, piglets were provided with creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse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

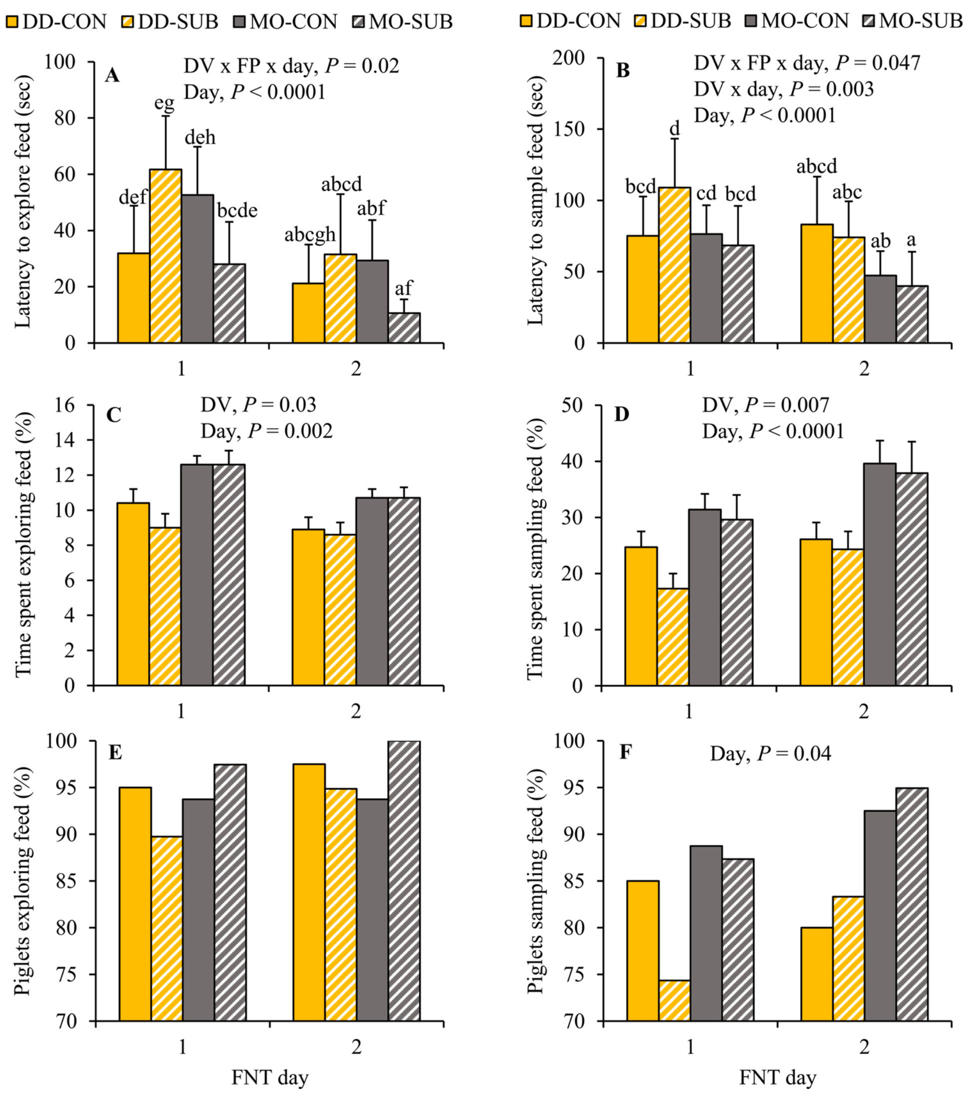

Fig. 2. Feed-related behaviour of piglets towards novel feed, i.e. cheese and chocolate, in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). The latency to explore (**A**), the latency to sample (**B**), time spent exploring (**C**), time spent sampling (**D**), the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

3.3.1.4. Feed-related behaviour towards chocolate and intake of chocolate. The latency to explore chocolate was shorter on the second (30.6 ± 6.4 s) than on the first day (69.2 ± 8.1 s, $P < 0.0001$), but was not affected by the treatments or their interactions (Fig. 4A). The latency to sample chocolate was not affected by day, the treatments or their interactions (Fig. 4B).

DD-piglets spent less time exploring chocolate than MO-piglets (DD: 4.1 ± 0.3 vs. MO: 5.3 ± 0.3 %, $P = 0.03$, Fig. 4C). Time spent sampling chocolate was affected by day ($P = 0.002$), showing that the time spent sampling chocolate was higher on the second (16.6 ± 1.6 %) compared to the first day of the FNT (13.5 ± 1.5 %, Fig. 4D). There were no other fixed effects observed for these parameters.

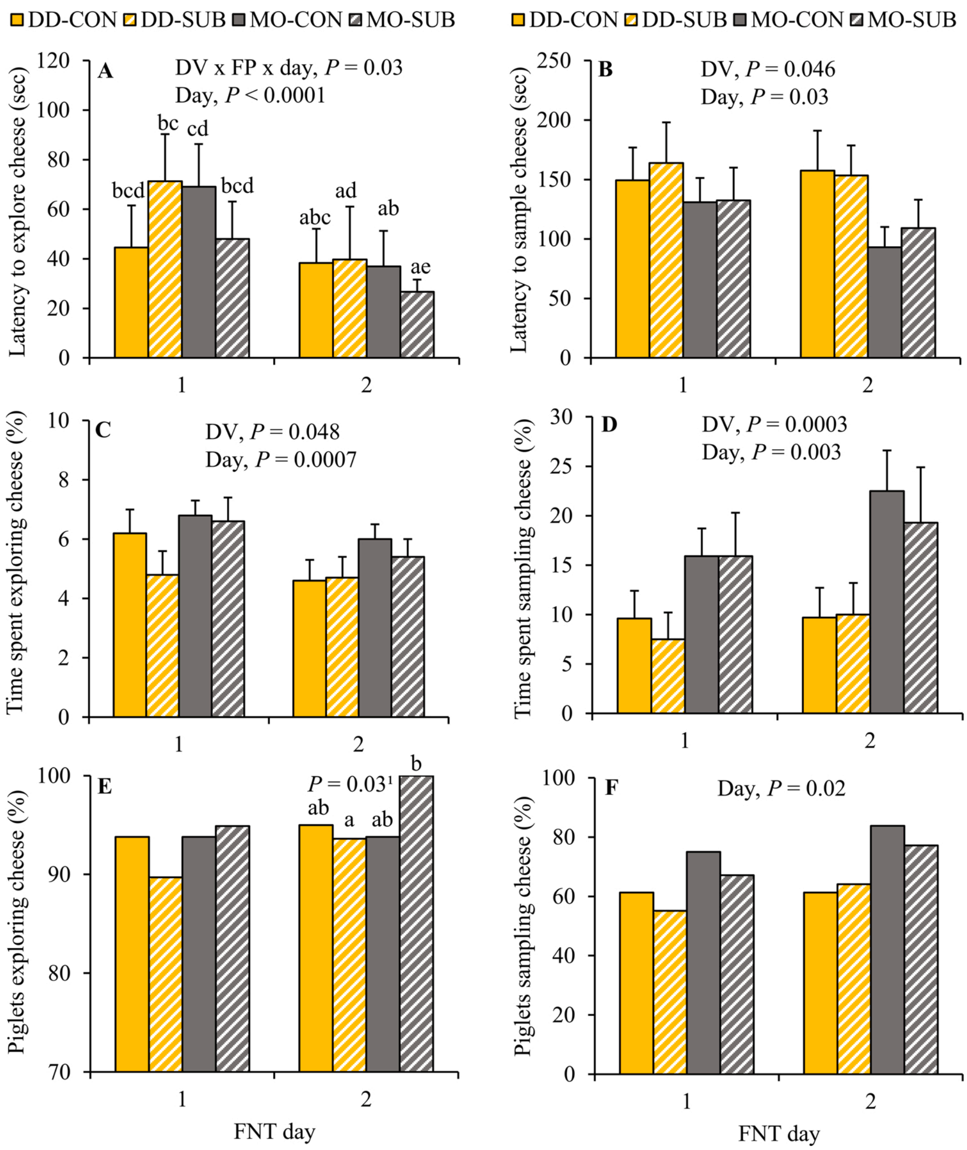

Fig. 3. Feed-related behaviour of piglets towards cheese in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). The latency to explore (**A**), the latency to sample (**B**), time spent exploring (**C**), time spent sampling (**D**), the percentage of piglets exploring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

The percentage of piglets exploring chocolate did not differ between treatments on the first day of the FNT. On the second day of the FNT, however, there was a higher percentage of MO-SUB versus MO-CON (Fisher's exact test, FP effect within MO, $P = 0.03$) and DD-SUB (Fisher's exact test, DV effect within SUB, $P = 0.03$) piglets that was exploring chocolate, with an intermediate percentage of DD-CON piglets (Fig. 4E). The percentage of piglets sampling chocolate was not affected by day, the treatments or their interactions (Fig. 4F).

Treatments did not affect the number of chocolates eaten (Supplementary Figure S4A) and the intake of chocolate in grams (Supplementary Figure S4B). Both the number of chocolates eaten (day 1: 0.87 ± 0.15 vs. day 2: 1.38 ± 0.21 chocolates, $P = 0.002$) and the intake of chocolate in grams (day 1: 0.73 ± 0.13 vs. day 2: 1.38 ± 0.21 g, $P < 0.0001$) were higher on the second compared to the first day of the FNT.

3.3.2. Post-weaning food neophobia test

The latency to explore the feed presented in the post-weaning FNT (apples and crisps) was shorter for MO- (9.6 ± 0.9 s) than for DD-piglets (12.7 ± 1.3 s, $P = 0.04$). The latency to sample the feed, time spent on exploring and sampling the feed, the percentage of piglets sampling the feed and the number of feed items eaten were not affected by the treatments or their interactions (Table 3). The response of weaner piglets towards apple was affected by dietary variety, except for the proportion of groups that consumed at least some of the apple pieces (Table 3). Moreover, a dietary variety x feed presentation interaction ($P = 0.009$) was found for the latency to explore, showing that DD-CON had a longer latency to explore apple than the other three treatments. DD-piglets (147.1 ± 10.1 s) had a longer latency to sample apple than MO-piglets (98.2 ± 11.6 s, $P = 0.03$), and also spent less time exploring (DD: 4.0 ± 0.4 vs. MO: 5.4 ± 0.4 %, $P = 0.006$) and sampling apple (DD: 5.8 ± 0.6 vs. MO: 9.2 ± 0.9 %, $P = 0.01$) compared to MO-piglets. Lastly, there were less DD- (71.4 %) than MO-piglets (87.5 %) seen sampling apple ($P = 0.02$, Table 3).

The latency to sample crisps and the percentage of piglets sampling crisps were influenced by dietary variety (Table 3). DD-piglets (57.2 ± 9.5 s) had a shorter latency to sample crisps than MO-piglets (91.8 ± 12.2 s, $P = 0.01$) and a larger number of DD-piglets (96.1 %) were observed to sample crisps than MO-piglets (85 %, $P = 0.02$). No differences between treatment groups were observed in the latency to explore crisps, the time spent exploring and sampling crisps and the number of crisps eaten.

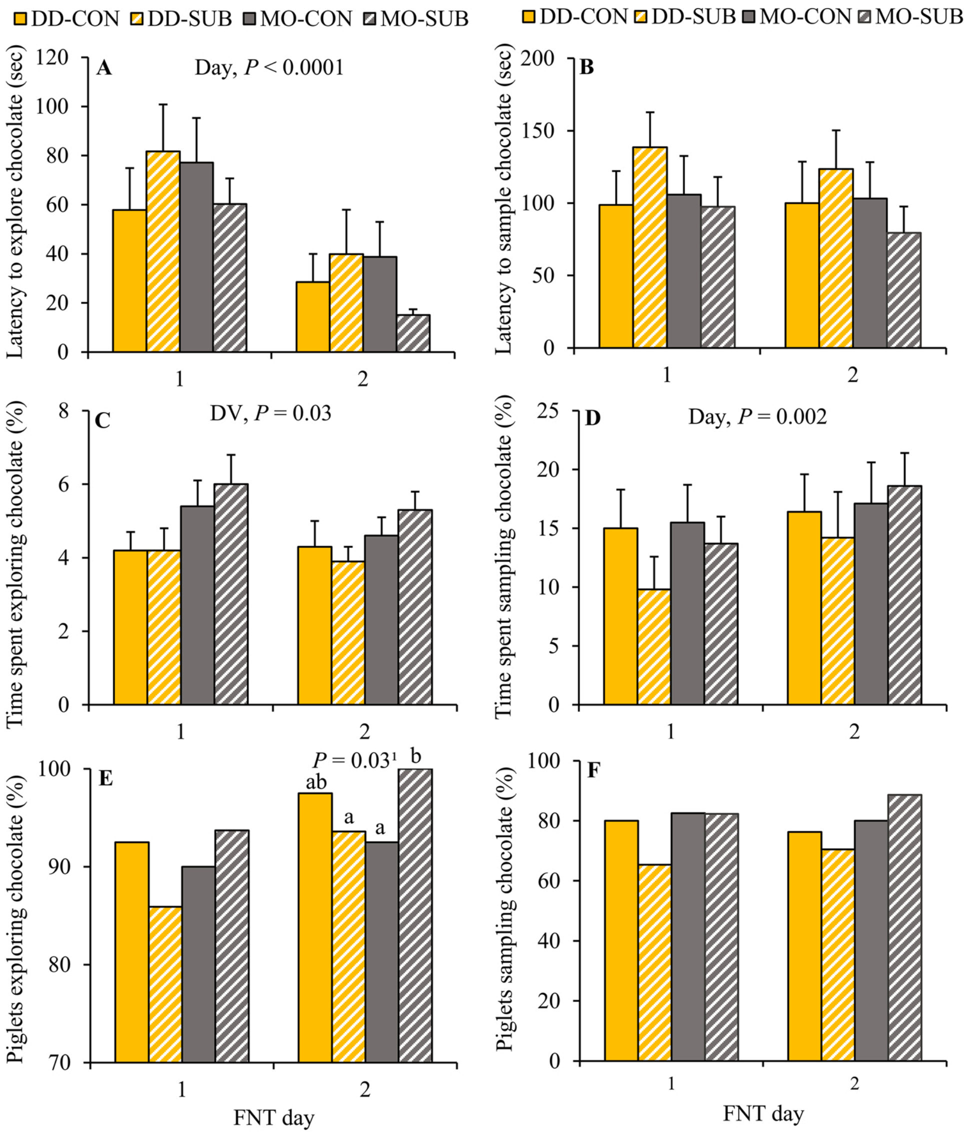

Fig. 4. Feed-related behaviour of piglets towards chocolate in the pre-weaning food neophobia test (FNT) at 24 (day 1) and 25 days of age (day 2). The latency to explore (**A**), the latency to sample (**B**), time spent exploring (**C**), time spent sampling (**D**), the percentage of piglets exploring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

4. Discussion

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

4.1. Effects of early feeding experiences in the pre-weaning tests

4.1.1. Novel environment test (NET)

We hypothesized that piglets exposed to dietary variety would have an attenuated fear response towards a novel environment. The latency to vocalise, the overall number of vocalisations, as well as low- and high-pitched vocalisations produced in the novel environment test, and the percentage of groups that defecated in the NET did not differ between the treatments however. Villalba et al. (2012) also reported no differences in vocalisations and escape attempts during an individual open field test between lambs exposed to a monotonous and diverse diet. However, they reported that lambs exposed to a diverse diet tended to have a lower stress-induced-hyperthermia after an individual open field test than lambs exposed to a monotonous diet.

4.1.2. Familiar food test (FFT)

In the FFT, DD-piglets had a longer latency to sample creep feed and less DD- than MO-piglets were observed to sample it. As no differences were found in behaviour in the novel environment test preceding the FFT, it is unlikely that differences in the behavioural response to the test environment *per se* were responsible for this effect of dietary variety. Rather, differences in food neophobia and feed preferences towards the creep feed prior to the FFT may have played a role. DD-litters consumed more solid feed in general, but seemed to prefer creep feed the least of the four items that were provided to them in the farrowing pen (Middelkoop et al., 2019). As a result, DD-litters spent less time eating creep feed, tended to consume less creep feed and, within these litters, less piglets consumed creep feed compared to MO (Middelkoop et al., 2019).

4.1.3. Food neophobia test (FNT)

Irrespective of treatments, piglets produced a lower number of vocalisations, both low- and high-pitched, on the second as compared to the first day of the pre-weaning FNT. In other studies, a higher overall

Table 3
Behaviour of 41-day-old weaner piglets towards novel feed, i.e. dried apple and crisps, in the post-weaning food neophobia test. Piglets were exposed to the test in groups of pen mates. During lactation, piglets were fed creep feed as a monotonous diet (MO) or four feed items simultaneously as a diverse diet (DD) and the feed was presented without (CON) or with substrate (SUB) in one of two feeders. DV = dietary variety (DD vs. MO). FP = feed presentation (SUB vs. CON). Data are expressed as means ± SEM based on pen averages. Significant *P*-values are presented in bold. Superscripts without a common letter differ at *P* < 0.05.

Behaviour	DD		MO		Significance		
	CON	SUB	CON	SUB	DV	FP	DV x FP
Apple + crisps							
Latency to explore (s)	14.5 ± 2.1	10.9 ± 1.5	8.4 ± 1.0	10.8 ± 1.5	**0.04**	0.74	0.08
Latency to sample (s)	30.2 ± 7.0	35.9 ± 7.7	68.5 ± 20.9	39.4 ± 6.8	0.08	0.32	0.22
Time spent exploring (%)	14.8 ± 1.1	14.1 ± 1.0	15.9 ± 0.8	14.7 ± 1.1	0.34	0.30	0.71
Time spent sampling (%)	19.6 ± 3.0	21.0 ± 1.9	23.3 ± 4.2	25.5 ± 3.1	0.25	0.40	0.76
Percentage of piglets sampling	100	97.4	90	100	0.37	0.37	0.12[1]
No. eaten per piglet	0.39 ± 0.08	0.68 ± 0.18	0.58 ± 0.17	0.83 ± 0.31	0.68	0.21	0.80
Apple							
Latency to explore (s)	26.3 ± 2.6[a]	17.9 ± 1.7[b]	15.7 ± 1.7[b]	18.8 ± 2.3[b]	**0.03**	0.15	**0.009**
Latency to sample (s)	140.9 ± 16.9	153.3 ± 11.8	111.3 ± 19.4	85.2 ± 12.4	**0.01**	0.73	0.23
Time spent exploring (%)	4.0 ± 0.5	4.0 ± 0.5	5.5 ± 0.6	5.4 ± 0.5	**0.006**	0.92	0.90
Time spent sampling (%)	6.2 ± 1.0	5.5 ± 0.9	8.3 ± 1.4	10.1 ± 1.0	**0.01**	0.81	0.33
Percentage of piglets sampling	79.5	63.2	82.5	92.5	**0.02**	0.15	0.052
Percentage of groups that consumed items[2]	0	40	20	30	1.00	0.13	0.09
Crisps							
Latency to explore (s)	18.6 ± 3.0	16.2 ± 2.9	11.1 ± 1.6	17.5 ± 2.1	0.32	0.45	0.06
Latency to sample (s)	48.8 ± 11.1	65.5 ± 15.6	100.6 ± 18.8	82.9 ± 15.8	**0.01**	0.73	0.22
Time spent exploring (%)	10.7 ± 0.8	10.1 ± 0.7	10.5 ± 0.6	9.2 ± 0.8	0.47	0.20	0.66
Time spent sampling (%)	13.4 ± 2.4	15.5 ± 2.1	15.0 ± 3.0	15.3 ± 3.2	0.99	0.52	0.83
Percentage of piglets sampling	97.4	94.7	85	85	**0.02**	0.69	0.48
No. eaten per piglet	0.39 ± 0.08	0.52 ± 0.12	0.41 ± 0.09	0.50 ± 0.15	0.93	0.27	0.92

[1] Analysed in a Fisher's exact test. $P \geq 0.12$ for all comparisons.
[2] The no. of apple pieces eaten by DD-CON was 0. Data were therefore expressed as binary data (group consumed items yes or no) and analysed in a Fisher's exact test. DD-SUB tended to have a higher percentage of groups that consumed apple pieces than DD-CON.

number of vocalisations and a higher number of low- and high-pitched vocalisations were associated with novel situations that can elicit fear (Tallet et al., 2013; Leliveld et al., 2016). If results from these studies are also applicable to groups of piglets, these may suggest that piglets may have been less fearful on the second compared to the first day of the FNT.

Although piglets ingested the cheese and chocolate presented in the pre-weaning FNT with caution, as shown by low feed intake levels on both days, exposure to novel feed may not necessarily elicit fear, which could partly be related to the sensory properties of the feed items chosen (see below). Some groups of piglets displayed even object play with the feed items and locomotory play during the test, which may be indications of positive emotions and lack of fear. This seemed to occur more often in the post- than in the pre-weaning FNT though (personal observation by the observers). Instead of piglets being less fearful, on the other hand, piglets may have vocalised less as they spent more time sampling the feed on the second versus the first day of the FNT. Piglets became less neophobic towards the feed items presented in the test over time, as the time spent sampling the feed, the number of feed items eaten and the intake of feed in grams increased from the first to the second day of the test. Also the number of 'neophobic' piglets towards cheese decreased over time, as more piglets were sampling cheese over time, but this was not the case for chocolate. However, the percentage of piglets sampling chocolate was observed to be higher than the percentage of piglets sampling cheese on the first day of the FNT, suggesting the initial neophobic response towards cheese seemed to be stronger than towards chocolate. This may be explained by differences in feed ingredients (Solà-Oriol et al., 2011; chocolate: high in carbohydrates and cheese: high in dietary protein and fat) and sensory properties of the items, such as flavour profile (McLaughlin et al., 1983; Figueroa et al., 2019; chocolate: sweet (and contained vanilla aroma) and cheese: umami).

We expected the decline in fear-related behaviour and increase in sampling behaviour to be stronger for dietary diversity and feed hidden in substrate. However, the findings of the current study do not support this hypothesis, and, for some parameters the opposite seemed to be true for piglets with a diverse versus monotonous diet. Feed presentation in substrate did not affect the feeding behaviour and feed intake of piglets in the pre- and post-weaning FNT, which is in line with the absence of a feed presentation effect on feeding behaviour, feed intake and the percentage of eaters in the home pen (Middelkoop et al., 2019). DD-piglets spent less time on exploring and sampling feed than MO-piglets. Moreover, the latency to sample and explore the feed decreased from the first to the second day of the FNT, but this was not or less the case for DD-CON respectively. Piglets with a diverse diet did not differ in intake of the novel feed items from piglets with a monotonous diet. When we exposed the piglets to a novel feed at weaning that was provided *ad libitum* as described in Middelkoop et al. (2019), DD- and MO-piglets did also not differ in their feed intake in the first four hours after weaning, neither between d0–1, d1–2 and d2–5 post-weaning. Together with these previous reported findings, the current results suggest that DD- and MO-piglets did not differ in food neophobia, against our expectations. Potential explanations of these results will be discussed below.

Firstly, the feed items used in the pre-weaning FNT may not have been 'novel' enough, overruling a potential treatment effect on food neophobia, as pigs seem to have innate preferences for umami and sweet flavours (e.g. McLaughlin et al., 1983; Nofre et al., 2002; Guzmán-Pino et al., 2019), that are present in cheese and milk chocolate. It should be noted, though, that food neophobia towards cheese and chocolate still appeared to be influenced by environmental factors, i.e. presence of the sow during the test and environmental enrichment in the farrowing pen, in previous research (Oostindjer et al., 2011).

The novel feed items in the FNT may also have differed too largely in sensory properties (appearance, flavour, taste and texture) from the diverse feed items in the home pen of DD-piglets to enable stimulus generalisation and thereby to increase the intake of the novel feed items (Mennella et al., 2008). Besides, DD-piglets developed strong feeding preferences in the home pen (Middelkoop et al., 2019) and may therefore be more 'picky' in their eating behaviour (Dovey et al., 2008). Although not tested, DD-piglets may have preferred (some of) the feed items in their home pen over the feed items in the FNT, resulting in a negative contrast between the test situation and the home pen. In support of the latter, the latency to start vocalising did not become longer for DD-piglets from day 1 to day 2 of the FNT, while it did for MO-piglets. In addition, DD-piglets vocalised more than MO-piglets in the pre-weaning FNT, and a higher number of vocalisations has been associated with negative emotions (Leliveld et al., 2016). Conversely, the monotonous creep feed diet in the home pen of MO-piglets may have resulted in a positive contrast between the test situation with the choice of two feeds compared to the home pen with only one feed. The creep feed also appeared to be least preferred by DD-piglets that had the choice of four feed items during lactation (Middelkoop et al., 2019). As MO-piglets did not have access to a diverse range of solid feed items during lactation, they may have been more motivated to explore the novel feed items during testing than DD-piglets, as reflected by a larger time spent on exploring and sampling the feed. The increased motivation to perform a behaviour when it has been limited by certain housing conditions has been called a 'rebound effect' (Edwards-Callaway, 2015). Others have suggested previously that barren housing may result in such a rebound effect during testing in a novel object and human interaction test, and that barren-housed piglets were therefore more explorative and less anxious towards novelty compared to enriched-housed piglets in such tests (Olsson et al., 1999; Backus et al., 2017). However, no signs of such a rebound effect in barren- versus enriched-housed piglets were previously observed towards novel feed in a FNT (Oostindjer et al., 2011) and, similarly, in CON- versus SUB-piglets in the FNTs in the current study.

To conclude, the limited number of solid feed items, i.e. only one, to explore in the home pen of MO-piglets may have resulted in a higher motivation to explore the two novel feed items in the pre-weaning FNT compared to DD-piglets. Moreover, DD-piglets may potentially have preferred the feed items in their home pen over the feed items in the test room. Together, these may explain the higher feed exploration level and lower number of vocalisations of MO- versus DD-piglets in the pre-weaning FNT. Thus, the food items experienced in the home pen and how they relate to the novel food items likely play an important role in the exploration of novel food in pigs. Also, the innate preference of pigs for umami and sweet taste may have overruled potential treatment effects on food neophobia towards the feed items, i.e. cheese and milk chocolate, in the pre-weaning FNT.

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

Although piglets from all treatments were housed under the same conditions and received the same feed post-weaning, DD-piglets still differed in their behaviour towards novel feed from MO-piglets when tested two weeks after weaning. DD-piglets seemed more attracted to crisps, while MO-piglets seemed more interested in dried apple. Consequently, no major differences were reported in the overall response towards the novel feed items. DD-piglets may have been more attracted to crisps as result of their saltier diet in the home pen (Middelkoop et al., 2019). In human infants, the acceptance pattern of novel food appeared to be specific to the previous food profile they experienced (Mennella et al., 2008). This may also be the case in piglets, as the diet composition of the pre-weaning and post-weaning diet were found to interact on the amount of weaner feed ingested after weaning in other studies (Torrallardona et al., 2012; Heo et al., 2018). Alternatively, the fact that DD-piglets were less attracted to apple but instead preferred crisps may also relate to the sensory properties of the items. It can be argued that apples are perceived as less 'novel' to pigs, as sweet is a hedonic taste in pigs (e.g. McLaughlin et al., 1983; Nofre et al., 2002; Guzmán-Pino et al., 2019). The crisps may therefore represent a more novel item that the MO-piglets avoided more strongly than the DD-piglets. If so, these results could point to a (mildly) reduced food neophobia in DD-piglets that was not revealed in the pre-weaning test. Taken together, the findings of the current study suggest that early feeding experiences with a different diet can have long-lasting effects on feed preferences, at least up to two weeks after the treatments were not reinforced anymore. Our findings are consistent with previous studies, who also reported differences in short- (during dietary treatment) and long-term feeding preferences (after end of dietary treatments) in piglets exposed to different diets early in life (Figueroa et al., 2013; Blavi et al., 2016; Clouard et al., 2016).

5. Conclusions

Early feeding experiences, i.e. dietary variety and feed presentation in substrate, did not influence novel environment behaviour before weaning and feed intake in the food neophobia tests. Against expectations, piglets provided with a diverse diet showed lower levels of exploring and sampling of novel feed items than piglets provided with a monotonous diet before weaning. Whether these changes in exploratory behaviour towards the feed were accompanied by differences in food neophobia or motivation to explore remained inconclusive. Moreover, feed preferences as result of dietary variety were observed in the food neophobia test after weaning. Yet, this study showed that early experiences with a different diet can have short-term and long-lasting effects on the responsiveness to novel feed in young piglets. It is therefore important to consider the interaction between new and previous diets, when exposing pigs to dietary transitions.

Declaration of Competing Interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.applanim.2020.105142.

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