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Stress responses in pigs postweaning: Effect of heavier hybrid and weaning intact litters

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ABSTRACT

Early and abrupt weaning of pigs is a stressful event. In addition, the use of hyperprolific sows with increasing numbers of live born piglets has decreased the average pig's birth weight, colostrum intake and weaning weight. The aim of this study was to investigate whether behavioural (tail posture, agonistic and abnormal behaviour), clinical (ear and tail lesions) and physiological (saliva cortisol) indicators of stress postweaning can be minimised by 1) using a pig hybrid that is heavier at birth and weaning, and 2) trough specific-stress-free housing where intact litters of pigs stay in the pen of birth after removing the sow at weaning. The study was a 2 × 2 factorial design, with pigs from Topigs Norsvin TN70 (**TN**) sows as a heavier alternative to the lighter pigs from DanBred LY sows (**DB**), and the specific-stress-free (**SSF**) weaning strategy as an alternative to the conventional weaning strategy (**CON**) where pigs are regrouped and moved to weaner pens. Fiftyseven litters, on average 26 days of age, were included. Behaviour recordings on the day (**d**) of weaning (d0), showed that the rate of fights and attacks were 23 and 42 times higher in CON compared to SSF, respectively. Furthermore, on d+1 pig in CON had a 9 (in DB) to 13 (in TN) times higher odds of having clinical ear lesions compared to SSF. Only on d+2, saliva cortisol was lower in TN-SSF than TN-CON, but not lower that DB-SSF. A higher rate of high tail posture was seen in CON d+1, which may reflect higher arousal due to mixing and moving. The rate of belly nosing was 5 times higher in the lighter hybrid (DB compared to TN), but increased from d+7 to d+14 in both hybrids. On d+7 the rate of ear directed behaviour tended to be higher in DB than TN, but the rate increased from d+7 to d+14 in TN to a similar rate as in DB. The rate of tail directed behaviour increased from d+7 to d+14 and tended to be higher in CON than SSF. In conclusion, specific-stress-free weaning reduced agonistic behaviour and ear lesions and tended to reduce tail directed behaviour postweaning. Using the heaver hybrid TN reduced belly nosing postweaning. However, since belly nosing and tail directed behaviour increased over time for both hybrids and weaning strategies additional measures are needed to reduce stress associated with early and abrupt weaning.

1. Introduction

At weaning, it is common practise to abruptly separate pigs from the sow, and to regroup and house the pigs in weaner pens until 30 kg of bodyweight (European Food Safty Authority, 2005). This practice causes stress in pigs due to the abrupt physiological, environmental and social changes (Campbell et al., 2013) which is evidenced by indicators such as low feed intake and reduced growth (Le Dividich and Sève, 2000), increased saliva and plasma cortisol (Ekkel et al., 1995; Escribano et al., 2019; Moscovice et al., 2022), agonistic behaviour and associated skin

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lesions (Turner et al., 2006; Escribano et al., 2019), and risk of development of abnormal behaviours (Bench and Gonyou, 2009) and post-weaning diarrhoea (Vente-Spreeuwenberg et al., 2003).

In Denmark the average number of total born piglets reached 19.8 piglets per litter in 2021 (Hansen, 2022). The use of hyper prolific sows and breeding towards larger litter sizes has reduced the average piglet birth weight and increased within litter variability (Quiniou et al., 2002). Higher birth weight, on the contrary, is associated with higher colostrum intake, higher blood plasma glucose and immunoglobulin G concentrations, better survival chances from birth until weaning, and a higher weight gain during the suckling period (Devillers et al., 2011). In addition, piglets of higher birth weight reach higher weaning and finishing weight (Declerck et al., 2016; Beaulieu et al., 2010). Hence, increasing pig birth weight and weaning weight may improve the pigs' ability to cope with stress postweaning.

The conventional weaning practice where moving and mixing of unacquainted pigs leads to agonistic behaviour and stress evidenced by increased saliva cortisol concentrations (Merlot et al., 2004), inhibited growth (Camp Montoro et al., 2021), and ear and body lesions (Turner et al., 2006; Escribano et al., 2019). Colson et al. (2012) found an additive increase in saliva cortisol concentration when pigs where both mixed with unfamiliar pigs and moved to a novel environment compared to staying in the farrowing pen at weaning. Ekkel et al. (1995) used the term Specific-stress-free (SSF) for housing pigs under optimal conditions where specific stressors such as moving and mixing are omitted. Housing pigs under SSF conditions reduced saliva cortisol and the number of haemorrhagic ear lesions and scratches on the day after weaning (Ekkel et al., 1995).

The present study is part of a larger experimental study on the effect of a heavier pig hybrid combined with an alternative SSF weaning strategy to improve the pig's resilience at weaning. Results on litter characteristics, postweaning growth and feeding behaviour (Winters et al., 2023), and play behaviour (Franchi et al., 2022, 2023) have previously been reported. The aim of the present study was to assess the effect of the heavier pig hybrid and SSF weaning in the pen of birth on stress and behavioural indicators. We used Topigs Norsvin TN70 sows as an alternative to the DanBred LY sow, as the TN70 previously gave birth to smaller litter sizes but heavier piglets (Schild et al., 2020; Kobek-Kjeldager et al., 2023). Modern litter sizes and the average number of functional teats (DanBred LY = 14 teats, Topigs Norsvin TN70 = 15 teats (Schild et al., 2020)) require larger space allowance for the sow and her litter. Therefore, farrowing pens for loose housed sows were used in the present study to enable housing of an intact litter from birth until four weeks postweaning. We hypothesised that the heavier hybrid TN, would have a less pronounced stress response postweaning than the lighter hybrid DB, and that pigs weaned under SSF conditions would experience less stress postweaning compared to pigs weaned in conventional weaner pens. Furthermore, we expected the heavier hybrid TN to show lower stress response compared to the lighter hybrid DB when weaned conventionally. Here we investigated the following stress indicators: saliva cortisol concentrations as a measure of the physiological stress (Escribano et al., 2019); agonistic behaviour and ear lesions; belly nosing, ear directed and tail directed behaviour as an indicator of abnormal behaviour (Bruni et al., 2008; Valros et al., 2021). In addition, a recent scientific focus on behaviour measures for affective states has associated tucked tail posture with tail lesions and biting (Larsen et al., 2018) and high or low tail posture with high or low arousal (Camerlink and Ursinus, 2020).

2. Material and methods

2.1. Study design

This study consisted of a 2 × 2 factorial design with the following sow hybrids and weaning strategies: The Topigs Norsvin TN70 sow (Topigs Norsvin L-line (Norsvin Landrace) × Topigs Norsvin Z-line (Large White)) (**TN**) as an alternative to the DanBred LY sow (DanBred Landrace × DanBred Yorkshire) (**DB**) in order to reduce litter size, but increase piglet birth and weaning weight, and the Specific-Stress-Free (**SSF**) weaning strategy where a intact litter stayed in the pen of birth as an alternative to conventional weaning where two litters were moved and mixed in a conventional weaner pen (**CON**). In total 24 DB and 33 TN litters were included over four batches and were randomly allocated to weaning strategy SSF or CON. The study was conducted from February to November 2020 at the pig research facilities at Aarhus University, AU Viborg, Denmark.

2.2. Animals and housing

All gilts were inseminated with DanBred Duroc semen (DanBred, DK) and farrowed in batch 1 and 2 whereafter they were re-inseminated and farrowed as second parity sows in batch 3 and 4. Not all sows became pregnant after insemination in batch 1 or 2, thus additional gilts were inseminated and farrowed as first parity sows in batch 3 and 4. The number of pens and litters included in the study are presented in Table 1. DB sows gave birth to larger litters than TN sows (DB = 19.0 liveborn; TN = 14.8 liveborn), but TN piglets had a higher birth weight (DB = 1.21 kg; TN = 1.35 kg). At weaning TN pigs were heavier (8.40 kg) than DB pigs (7.65 kg) although litter size on day seven before weaning did not differ between hybrids (DB = 12.3 piglets; TN = 12.2 piglets) (see Winters et al. 2023 for more details).

2.2.1. Lactation period

Gilts and sows farrowed in pens for loose-housed sows (FT30 pen, 3 × 2.2 m, SKIOLD, DK) as illustrated in Fig. 1 reproduced from Winters et al. (2023). The back pen wall had a metal bar and the wall opposite to the feeding trough was sloped. The feeding trough was 80 cm long made from polyconcrete (SKIOLD, DK) and constructed to allow piglets to eat from it. Four times daily, sows were fed a standardised lactation diet (Die Profil Lac, DLG, DK; 12.9 MJ metabolisable energy (**ME**) per kg, 15.6% crude protein). For the sow a drinking nipple was placed above the feed trough, while piglets had access to an Aqua-Level hinged water trough (31 × 17 × 11 cm, SKIOLD, DK). The sows were provided straw in a hay rack. The pen had a piglet creep area heated with an infrared heat lamp (Infrared lamp 150 W, InterHeat Inc, KR) and floor heating.

After ended farrowing, an analgesic (Metacam® 20 mg/mL, Boehringer Ingelheim, DK) was administered to each sow, and piglets were weighed, ear tagged, and had their umbilical cord cut. On each sow, the number of functional teats (teats with colostrum droplets) was counted and litters were equalised to a maximum of piglets equivalent to the number of functional teats. If sows had free available teats, surplus piglets were cross fostered within sow hybrid within the first 3 days postpartum (***pp***). If cross fostering of surplus piglets to functional teats was not possible, piglets were euthanised by blunt force trauma in the following order: 1) non-viable piglets (e.g., < 700 g) and 2) randomly selected piglets. Male piglets were castrated under local anaesthetic (injection of 0.5 mL per testis of Procamidor® vet. 20 mg/mL, Richter Pharma, AT) and received an analgesic (0.1 mL, Metacam® 5 mg/mL, Boehringer Ingelheim, DK). All piglets received an iron injection (1 mL SC, Viloferron®, 20%, Vilofarm, DK) on day 4 *pp*. Tails were kept intact. All piglets were vaccinated against Mycoplasma hyopneumoniae with

Table 1
Number of pens and litters (in parentheses) included in the study at weaning.

	Postweaning pens (litters)				
	DB-CON	DB-SSF	TN-CON	TN-SSF	
Batch					Sum
1	2 (4)	4 (4)	2 (4)	4 (4)	12 (16)
2	1 (2)	2 (2)	2 (4)	4 (4)	9 (12)
3	2 (4)	3 (3)	2 (4)	4 (4)	11 (15)
4	2 (4)	1 (1)	2 (4)	5 (5)	10 (14)
Total	7 (14)	10 (10)	8 (16)	17 (17)	42 (57)

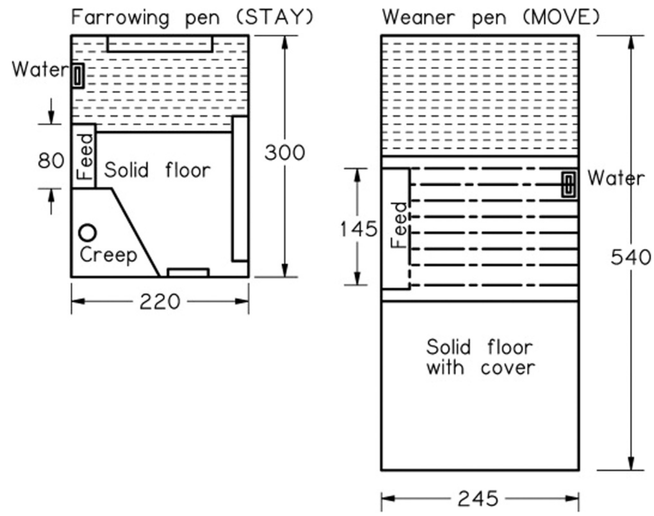

Fig. 1. The two pen types used in the study: a farrowing pen for loose-housed sows and a conventional two-climate weaner pen. At weaning (age [median, min-max]: 26, 22–30 days of age), litters allocated to weaning strategy SSF stayed in the farrowing pen after removing the sow. In weaning strategy CON two litters were mixed and moved to conventional weaner pens where they were housed until 28 days postweaning. Dimensions are given in cm. Reproduce from Winters et al. (2023).

Ingelvac MycoFLEX® (Boehringer Ingelheim, DK) on day 7 *pp* and immediately before weaning. Every morning, manure was manually removed from the pen floor, straw racks were filled, and approximately 130 g of fine chopped wheat straw and 400 g sawdust were provided on the floor and in the piglet creep area, respectively. The light was on from (HH:mm) 07:00–23:00, and the room temperature was decreased gradually from 21 °C at farrowing to 19 °C at weaning (automatic climate system, SKOV, DK). At farrowing, the floor temperature in the piglet creep area was approximately 35 °C, and floor heating and heath lamps were turned off as piglets started lying outside the creep area.

2.2.2. Postweaning

The two-climate weaner pens (used for litters in treatment CON), were 5.40 × 2.45 m with a roof (adjustable fibre panel positioned 85 cm above floor level, SKIOLD, DK) covering the solid floor equal to approximately 1/3 of the pen area. Pigs had access to a water trough similar to the one used in the farrowing pens. The weaner diet (Prime Midi Piller, DLG, DK; 14.8 MJ ME/kg, 19.3% crude protein, no medical zinc oxide) was provided in two hopper feeders (TR4, Rotecna, ES). Both the farrowing pens and the two-climate weaner pens provided a minimum of 0.3 m^2 open floor area per pig.

2.2.3. Weaning procedure

Litters were weaned after finished data collection (detailed below) around 14:00. In weaning strategy SSF, intact litters stayed in the farrowing pen after removing the sow, whereas in CON, two litters (within same hybrid) were mixed together and moved to a weaner pen in another section of the same building. Immediately after weaning, the pigs received a portion of chopped wheat straw (approximately 130 g per pen in SSF, double amount in CON) and sawdust (400 g per pen in SSF, double in CON) provided on the solid floor and covered area, respectively. The room temperature was increased to 24 °C at weaning and gradually decreased to 21 °C four weeks postweaning.

2.3. Data collection

Due to visible differences in weaning strategies and hybrids, blinding of observers were not possible during the clinical or behavioural recordings.

2.3.1. Clinical recordings

Clinical recordings were collected on all pigs on the day (**d**) of weaning (d0) and on d+1, d+2, d+7, and d+14 postweaning. On these days, pigs were weighed, marked on their back for individual identification on video, and had their ears and tail assessed for lesions. Each pig had both ears assessed and categorised into one of five categories: 0) no ear lesions; 1) red/blush; 2) less than two fresh, red scratches without scab; 3) more than two fresh, red scratches without scab; or 4) minimum one fresh, red wound (min size 3 × 3 mm) without scab. In addition, the tail of each pig was assessed visually and by palpation and categorised into one of four categories: 0) no damage, 1) red and/or swollen, 2) bite marks, or 3) open wound (modified from Wallgren et al., 2019).

2.3.2. Saliva cortisol

Four piglets per litter were selected for saliva cortisol sampling. Two piglets were randomly selected amongst the 25% lightest pigs and two others amongst the 25% heaviest pigs in the litter based on their weight two days prior to weaning. Three pairs of technicians collected the saliva samples, where one of the two persons gently lifted and held the pig while the other person entered the Salivette® sampling swab (Sarstedt, DE) into the mouth of the focal pig for approximately 30 s. Saliva samples serving as a pre-weaning baseline were collected on d0 in batch 1, and on d-1 in batches 2, 3 and 4. Postweaning, saliva was collected on d+1 and d+2. On all three days, saliva samples were collected from 08:30–12:00. All saliva samples were stored on dry ice until centrifugation at 5 °C at 2000 × g for 10 min. From each sampling tube, 150 μL was transferred to an Eppendorf Tube® (Eppendorf, DE) and shipped on dry ice to the University of Murcia, Spain, for cortisol analysis by use chemiluminescent enzyme immunoassay validated in pigs (Escribano et al., 2012) with an intra- and inter-assay imprecision below 15% that was linear after serial sample dilutions.

2.3.3. Behaviour analyses

Digital cameras (Model DS-2CD2145FWD-I, 2.8 mm lens, HIKVISION, CN) were placed 2.3 m above floor level in the farrowing pens and at 2.8 m in the weaner pens. Video was recorded using Blue Iris v5 software (https://blueirissoftware.com) (1270 × 720 pixels, 15 fps) and video files were stored on external hard drives and analysed in The Observer® XT 15 (Noldus®) and Windows Media Player® (Microsoft®). The order of video observations was randomised by hybrid, weaning strategy and day.

2.3.3.1. Agonistic behaviour. Agonistic behaviour (fights and attacks) were recorded on d0, d+1, d+2 and d+7 using all occurrence sampling (Bateson and Martin, 2021). On d0, events and duration were recorded from the time of weaning (i.e., in SSF: when the sow was removed from the pen and in CON: when the pigs entered the weaner pen) and one hour forward and again from 20:00 until 21:00. On d+1, d+2 and d+7 events of agonistic behaviour were counted in the two time periods 13:00–13:30 and 20:00–20:30. A fight was defined as a reciprocal interaction between two pigs where both pigs use their head to attack (push, knock or bite) the other pig's head region with high intensity. An attack was defined as a non-reciprocal interaction where the performer pig uses its head to attack (push, knock or bite) the head region of the receiver pig. For both fights and attacks the event began at the first physical contact between two pigs and ended when one of the pigs withdrew and ended the physical contact. The minimum duration between two bouts of fights or attacks was 5 s. Agonistic behaviour was analysed by two observers (observer A observed batch 1 and 3, observer B observed batch 2 and 4). Due to technical problems, video from the following days were missing: d0 from 20:00–21:00 for all pens from batch 2 and 4 (seven CON and twelve SSF pens); d+7 for four pens from batch 1 (one SSF and three CON pens).

2.3.3.2. Tail posture. The tail posture of individual pigs was assessed on d-1 and d+1 using 15 min interval instantaneous scan sampling (Bateson and Martin, 2021) from 07:00 until 22:00 (60 scans per day). Tail posture was categorised as high, low or tucked (modified from Camerlink and Ursinus, 2020). High tail posture denoted a pig having the tip of the tail in or above the zero-position (zero-position: extension of the tail from the spinal cord in a slightly downward pointing direction following the natural curve of the spine). Low tail posture denoted a pig having the tail below the zero-position, but the tip of the tail pointing outwards (i.e., not between the hind legs). Tucked tail posture denoted a pig with the tip of the tail pointing downwards or even inwards between the pig's hind legs. Tail posture was only scored on those pigs that were standing on all legs at each instantaneous moment. Tail posture was not assessed if the pig or tail was out view. Tail posture was analysed by two observers (observer A observed batch 1 and 3, and observer B observed batch 2 and 4).

2.3.3.3. Belly nosing, ear directed and tail directed behaviour. Belly nosing, ear directed behaviour and tail directed behaviour were observed using all occurrence sampling (Bateson and Martin, 2021) on d+7 and d+14 from 20:30–21:30 where the performer and duration was recorded. Definition of belly nosing (modified from Main et al., 2005): The performer pig performs a rhythmic up-and-down massage movement with its snout directed towards the receiver pig's umbilical or inguinal region for at least 10 s. Definition of ear directed and tail directed behaviour (modified from Hakansson and Bolhuis, 2021): The performer pig has the receiver pig's ear or tail in its mouth or the nose touches the receiver pig's ear or tail region (a 10 cm radius from the base of the ear or tail) in an up, down, sideways or circling movement (nosing) for at least 5 s. Both pigs can be standing or lying. In batch 1, video was missing for one SSF and three CON pens on d+7. In batch 4, video was malfunctioning for four CON pens on d+14 and video from d+15 was observed instead.

2.4. Statistical analysis

All statistical analyses were performed in R version 4.2.1. P-values < 0.05 were considered significant and < 0.1 as tendencies.

2.4.1. Ear lesions

The five-level ear lesion score was made into a dichotomous variable with intact skin (category 0 and 1) vs skin not intact (category 2, 3 and 4), and analysed in a binomial generalised linear mixed effects model (**GLMM**) with logit link function (mixed effects logistic regression). The model corresponded to having the following fixed effects: hybrid, weaning strategy, day (d0, d+1, d+2, d+7 and d+14), their two-way and three-way interactions (×) but with weaning strategy only being included on d+1, d+2, d+7, and d+14, weaning age, technician pair (three paris) and a four-levelled categorical variable indicating each pig's weaning weight relative to its littermates (base on the quartiles (Q1-Q4) of weaning weight within litter). In practice, however, the model was reparameterised and analysed by defining and using one variable with the resulting 18 combinations of hybrid, weaning strategy and day. Batch, pen, litter and pig were initially included as random effects, however, batch was removed again due to convergence problems. Results from the statistical model are presented as probability estimates of ear lesions in Fig. 2**A** with the exact values and odds ratios (**OR**) with 95% confidence interval specified in Supplementary Table S1. Relevant pairwise comparisons among the eighteen hybrid × weaning strategy × day combinations were adjustment for multiple testing (56 tests) with Bonferroni's method and the adjusted *P*-values are referred to as P_{adj}.

2.4.2. Tail lesions

Due to a low number of pigs bitemarks or open wounds, the four level tail lesion score was made into a dichotomous variable with no tail lesions (category 0) vs tail lesions (categories 1, 2 and 3). In addition, d+2 was removed from the dataset due to no tail lesions in DB-SSF. The response variable tail lesions was analysed in a binomial GLMM with logit link function including hybrid, weaning strategy, day (d0, d+1, d+7 and d+14), their two-way and three-way interactions (×) but with weaning strategy only being included on d+1, d+7, and d+14, weaning age, technician pair (three pairs) and a four-levelled categorical variable indicating each pig's weaning weight relative to its littermates (Q1-Q4). The model was reparameterised and analysed by defining and using one variable with the resulting 14 combinations of hybrid, weaning strategy and day. Batch, pen, litter and pig were initially included as random effects, however, batch was removed again due to convergence problems. Results from the statistical model are presented as probability estimates of tail lesions in Fig. 2**B** with the exact values and OR specified in Supplementary Table S2. Relevant pairwise comparisons among the fourteen hybrid × weaning strategy × day combinations were adjustment for multiple testing (36 tests) with Bonferroni's method and the adjusted P-values are referred to as P_{adj}.

2.4.3. Saliva cortisol

Saliva cortisol concentrations per pig were log-transformed and analysed in a linear mixed effects model (**LMM**) including following categorical fixed effects: hybrid, weaning strategy, day (d-1, d+1, d+2), their two-way interactions and the three-way interaction but with weaning strategy only included d+1 and d+2. In addition, weaning age,

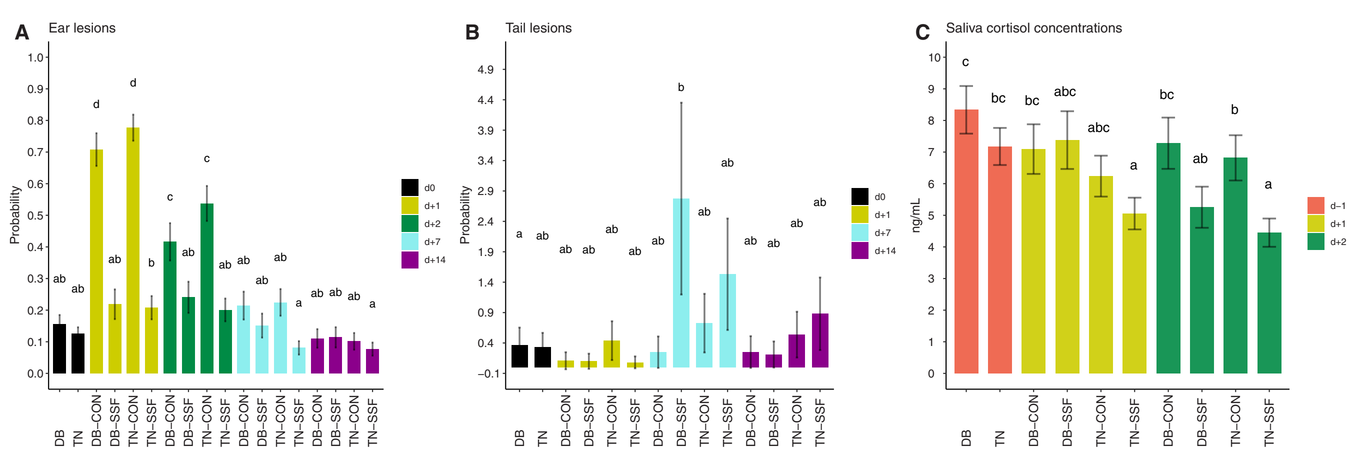

Fig. 2. A) The probability of ear lesions (± SE), (B) tail lesions (± SE) and (C) mean saliva cortisol concentration (± SE) in the hybrid DB and TN allocated to weaning strategy SSF or CON on given days (d) relative to the day of weaning (d0). Different letters indicate significant (*P* < 0.05) pairwise comparisons among hybrid, weaning strategy, day combinations within each panel.

pig weight category (pig weight d-2 among the 25% lightest or 25% heaviest in the litter), the identity of the technician pair (three pairs), and the duration (continuous) from the technicians entered the pen until saliva sampling on the individual pig were also included in the model. As for the analysis of ear lesions, the model was reparameterised by using a factor having the resulting 10 combinations of hybrid, weaning strategy and day. Batch was included as a random effect. Litter and pen were also included as random effects in a partially nested structure, as the random effects of litter and pen were identical for pigs in weaning strategy SSF. Furthermore, a continuous-time first order autoregressive covariance structure (corCAR1) was used to account for the correlation among repeated measurements from the same pig. The assumption of normality was checked by visual inspection of residual qq-plots. The estimated marginal means (EM-means ± SE) are illustrated in Fig. 2C and specified in Supplementary Table S3. *P*-values of pairwise comparisons among the ten hybrid × weaning strategy × day combinations were adjusted for multiple testing (21 tests) with Bonferroni's method.

2.4.4. Agonistic behaviour

Since fights were defined as a reciprocal action, each fight event was assigned to both pigs involved in the fight. For attacks, the behaviour was only assigned to the aggressor. The number of fights and attacks were summed per performer pig(s) on d0, d+1, d+2 and d+7. Fights and attacks were analysed in two separate GLMMs using a zero-inflated Poisson model with log link function to account for the large number that did not perform any fights or attacks. Hybrid, weaning strategy, day, their two-way and three-way interactions were included as categorical fixed effects together with weaning age, a four-levelled categorical variable indicating each pig's weaning weight relative to its littermates (base on the quartiles of weaning weight within litter) and observer (A or B). However, the tree-way interaction was removed in both models due to large standard errors because combination DB-SSF, d+2 and d+7 had no fights. In the model of fights, the zero-inflated part allowed differences among the four different hybrid × weaning strategies, whereas the model of attacks included one common zero-inflation parameter. Batch, pen, litter and pig were included as random effects, but batch was removed again in the model of attacks due to convergence problems. Total observation time varied among pens due to disturbance during data collection. Therefore, the logarithm of the total observation time in each pen was included as offset in the model. Results are presented as incidence rate ratios (**IRR**) with 95% CI Model estimates of the rate ± SE of fights and attacks (per pig per h) are illustrated in Fig. 3 and specified in Supplementary Table S4. P-values of pairwise comparisons among the eight hybrid × day combinations and eight weaning strategy × day combinations were adjusted for multiple testing (28 tests) with Tukey's method.

2.4.5. Belly nosing, ear directed and tail directed behaviour

The number of events of belly nosing, ear directed and tail directed behaviour were summed for each performer pig on d+7 and d+14 and analysed in three separate GLMMs using zero-inflated Poisson models with log link function. The following categorical fixed effects were included: hybrid, weaning strategy, day, their two-way and tree-way interactions, weaning age, and a four-levelled categorical variable indicating each pig's weaning weight category (quartile) relative to its littermates. The zero-inflated part allowed differences among the four groups of hybrid × weaning strategies. Batch, pen, litter and pig were included as random effects. However, in the model of belly nosing, the random effect of pig was removed again due to convergence problems. Results are presented as IRRs with 95% CI. Model estimates of the rate ± SE of belly nosing, ear directed and tail directed behaviour are presented in Fig. 4 and specified in Supplementary Table S5. For ear directed behaviour *P*-values of pairwise comparisons among the four hybrid × day combinations were adjusted for multiple testing (6 tests) with Tukey's method.

2.4.6. Tail posture

The number of scans with tucked tails and high tails were analysed in two separate GLMMs. Due to a low number of scans where pigs had a tucked tail, this response variable was analysed using a zero-inflated Poisson model with log link function. The number of scans where pigs had a high tail posture was analysed correspondingly but without zero-

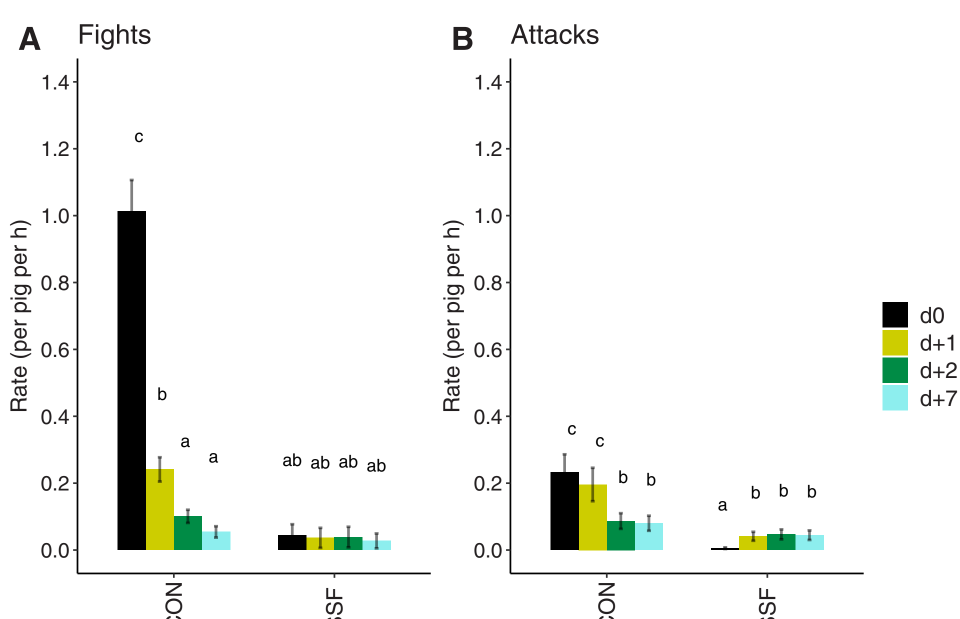

Fig. 3. The rate ± SE of agonistic behaviour per pig per day (d) on the day of weaning (d0) and d+1, d+2 and d+7 postweaning in pigs from the two hybrid DB and TN allocated to weaning strategy SSF or CON. Panels A and B show the interaction weaning strategy × day on the rate of fights and attacks, respectively. Panels C and D show the interaction hybrid × day on the rate of fight and attacks, respectively. The different letters indicate significant ($P < 0.05$) pairwise comparisons among the 8 combinations within each panel.

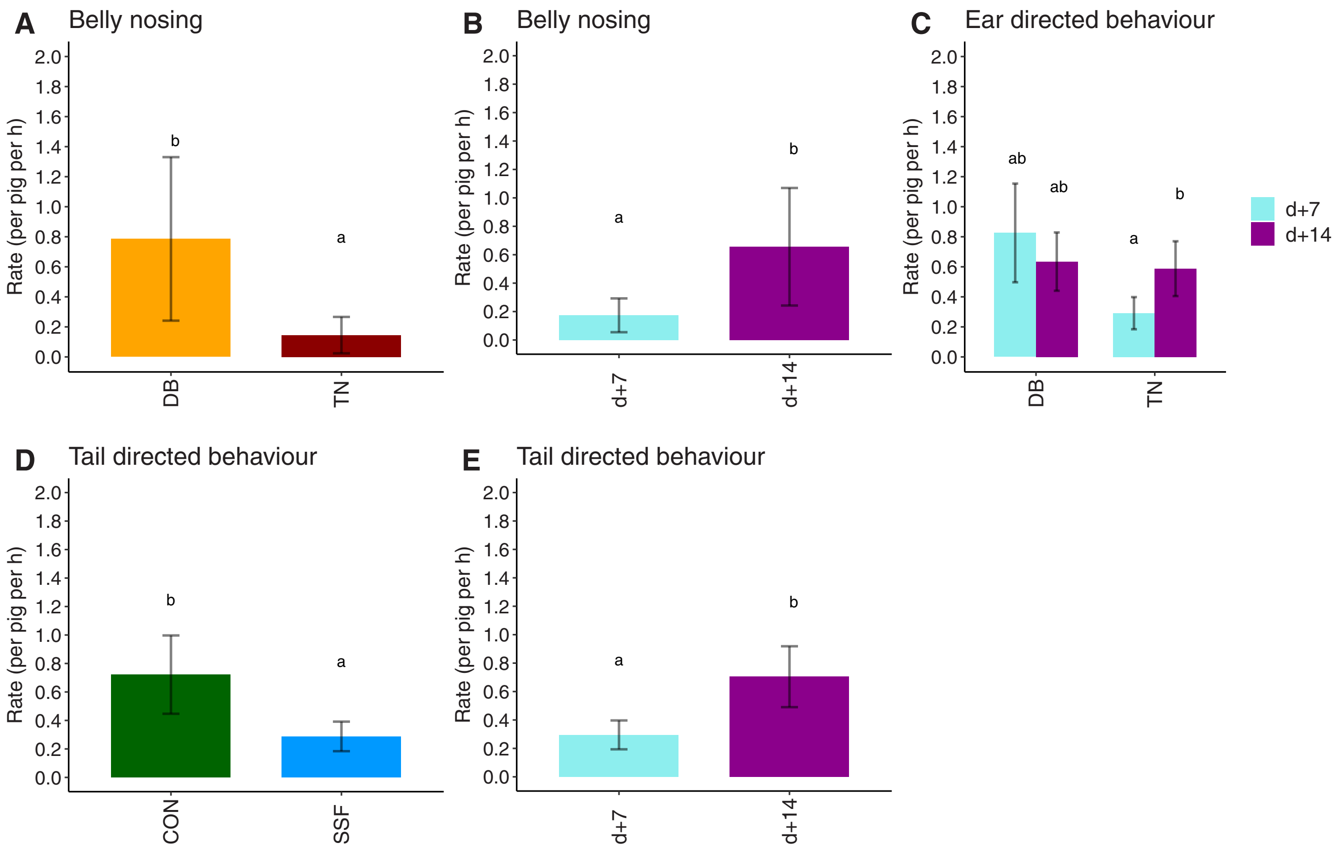

Fig. 4. The rate ± SE of belly nosing, ear directed and tail directed behaviour on day (d) +7 and d+14 postweaning in the two hybrids DB and TN allocated to weaning strategy SSF or CON. A) The main effect of hybrid on the rate of belly nosing, B) the main effect of day on the rate of belly nosing, C) the interaction hybrid × day on the rate of ear directed behaviour, D) the main effect of weaning strategy on the rate of tail directed behaviour, and E) the main effect of day on the rate of tail directed behaviour. The different letters indicate significant ($P < 0.05$) differences within each panel.

inflation. The logarithm of total number of tail posture scans per pig per day (min-max: 1–23 scans per pig per day) was included as offset in both models. The following categorical fixed effects were included in the models: hybrid, weaning strategy, day (d-1, d+1), their two-way and three-way interactions (with weaning strategy only being included on d+1) and observer (A and B). Again, the model was reparameterised by defining a factor containing the 6 combinations of hybrid, weaning strategy and day. The zero-inflated part allowed for differences between the 6 hybrid × weaning strategies × day combinations. Due to convergence problems only litter was included as random effect. Model estimates of the rate ± SE of tucked and high tails are presented in Fig. 5 and specified in Supplementary Table S6. *P*-values of pairwise comparisons among the six hybrid × weaning strategy × day combinations were adjusted for multiple testing (15 tests) with Tukey's method.

3. Results

3.1. Ear lesions

The probability of a pig having ear lesions on a given day is illustrated in Fig. 2**A** (For specific values and odds ratios see Supplementary Table S1). On d0, just before weaning, the odds that a pig had ear lesions was similar in both hybrids. In weaning strategy CON, pigs from both hybrids had a higher odds of having ear lesions on d+1 compared to d0. In addition, the odds of having ear lesions was between 8.6 and 13.0 times higher on d+1 for CON compared to SSF ($P_{adj} < 0.001$). For both DB-CON and TN-CON, the odds of a pig having ear lesions decreased from d+1 to d+2, and stabilised on d+7 and d+14 to a level similar to before weaning. For weaning strategy SSF the odds of ear lesions did not increase after weaning irrespective of hybrid. In addition, the heaviest pigs within a litter (Q4) had a higher odds of having ear lesions than the lightest pigs within a litter (Q1) (See Fig. 6A and Supplementary Table S7). The factors technician pair and age at weaning were not significant.

3.2. Tail lesions

The probability of a pig having tail lesions (note: including all categories of lesions) is presented in Fig. 2B (Values and odds ratios are specified in Supplementary Table S2). The odds of tail lesions increased almost 8 times from d0 to d+7 In DB-SSF ($P_{adj} = 0.048$), whereas no increase in tail lesions was observed in the other hybrid × weaning strategy combinations. Pair of technicians scoring tail lesions was significant ($\chi^2_2 = 41.1$, $P < 0.001$), but weaning age and within litter weaning weight were not.

3.3. Saliva cortisol

Estimated marginal means of saliva cortisol concentrations are presented in Fig. 2**C** and Supplementary Table S3. On d-1, saliva cortisol concentrations were similar in the two hybrids. Saliva cortisol concentrations did not increase after weaning in any of the four hybrid × weaning strategy combinations. On the contrary, the saliva cortisol concentration decreased from d-1 to d+1 for TN-SSF, and remained lower on d+2 compared to before weaning. On d+1, no difference in saliva cortisol concentrations was found among the four hybrid ×

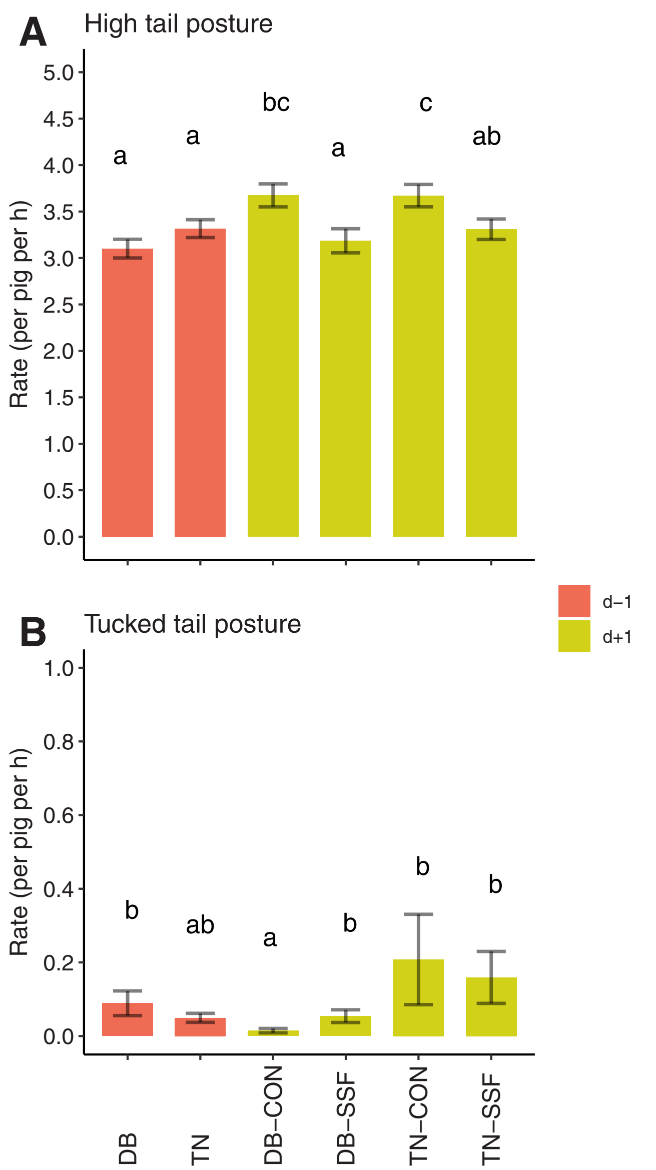

Fig. 5. The rate ± SE of A) high, and B) tucked tail posture on the day before (d-1) and after weaning (d+1) in pigs from the two hybrids DB and TN allocated to weaning strategy SSF or CON. The different letters indicate significant ($P < 0.05$) pairwise comparisons within each panel.

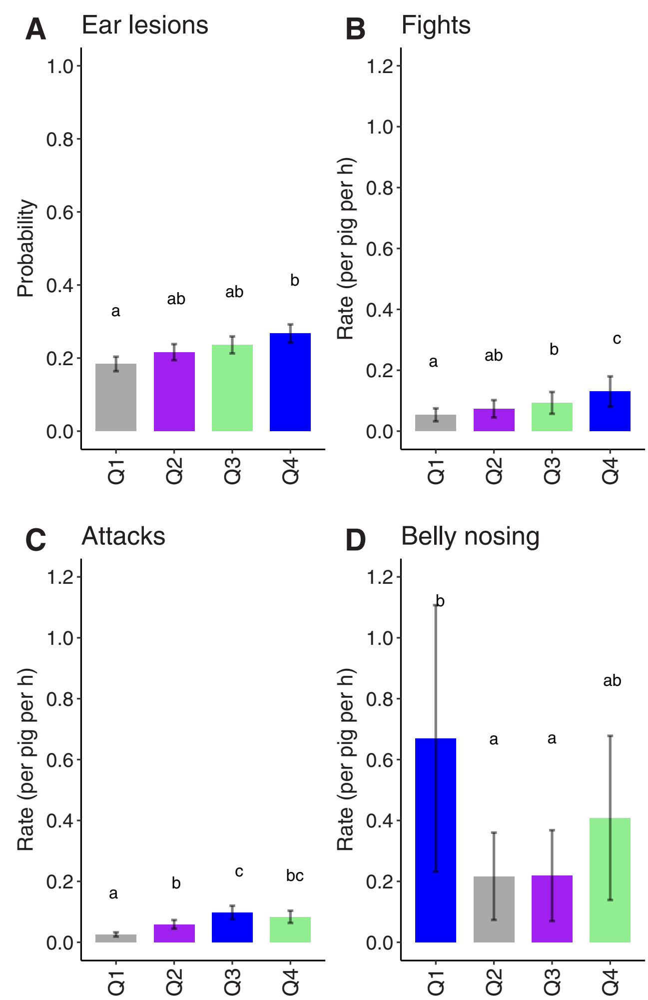

Fig. 6. The probability ± SE of ear lesions (A), and the rate ± SE of fights (B), attacks (C) and belly nosing (D) per pig weaning weight quartile (Q1-Q4) relative to its litter mates. The different letters indicate significant ($P < 0.05$) pairwise comparisons among the four weight quartiles within each panel.

weaning strategy combination. On d+2, TN-SSF had significant lower saliva cortisol concentrations than TN-CON, but not lower than DB-SSF. Saliva cortisol concentration was affected by the pair of technicians handling the pigs ($\chi^2_2 = 6.6$, $P = 0.035$), but not by weaning age, pig within litter weight category nor the duration from technicians entering the pen until saliva sampling of the individual pig (median = 2 min; min-max = 0–22 min).

3.4. Agonistic behaviour

3.4.1. Fights

The rate of fights per pig per hour are presented in Fig. 3A and the incidence rate ratios in Supplementary Table S4. There was a two-way interaction weaning strategy × day in that the rate of fights was 23 times higher on the day of weaning (d0) in weaning strategy CON compared to SSF (Fig. 3A). On the following days the rate of fight decreased in CON to a rate similar to that in SSF. For SSF, the rate of fights was low and did not differ among days. The interaction hybrid × day was significant, but only between non relevant pairwise comparisons. The interaction hybrid × weaning strategy and weaning age were not significant. Observer was significant ($\chi^2_1 = 8.2$, $P = 0.004$). The heaviest pigs in the litter (Q4) had a higher rate of fights than their smallest litter mates (Q1) (see Fig. 6B and Supplementary Table S7).

3.4.2. Attacks

The rate of attacks per pig per hour is presented in Fig. 3B and the incidence rate ratios in Supplementary Table S4. The significant two-way weaning strategy × day showed a 42 times higher rate of attacks on d0 in weaning strategy CON compared to SSF. Also on d+1 the rate of attacks was 5 times higher in CON than SSF, whereafter the rate of attacks reached a similar low level on d+2 and d+7. Again, the two-way interaction hybrid × day was significant, but only between non relevant pairwise comparisons. The two-way interaction hybrid × weaning strategy and factor weaning age were not significant. Observer was significant ($\chi^2_1 = 7.0$, $P = 0.008$). The rate of attacks increased stepwise with increasing pig weaning weight category i.e., heavier pigs within the litter attacked more often (Fig. 6**C** and Supplementary Table S7).

3.5. Abnormal behaviour

3.5.1. Belly nosing

The rate of belly nosing per pig per hour is shown in Fig. 4. The three-way interaction hybrid × weaning strategy × day and two-way interactions hybrid × weaning strategy and hybrid × day were not significant. The two-way interaction weaning strategy × day was significant ($P = 0.034$) but removed from the model, since pairwise testing showed only differences between non irrelevant comparisons. After removal of all interaction terms, there was a main effect of hybrid and day but not of weaning strategy (for details see Supplementary Table S4). Overall, the lighter hybrid DB had a 5 times higher rate of belly nosing compared to the heavier hybrid TN (Fig. 4A). In both hybrids, the rate of belly nosing increased almost 4 times from d+7 to d+14 (Fig. 4B). Weaning age was not significant. Pigs belonging to the lightest weight quartile within each litter (Q1) had a higher rate of belly nosing compared to pigs from Q2 and Q3, but not higher than the heaviest pigs (Q4) (Fig. 6D and Supplementary Table S7).

3.5.2. Ear directed behaviour

The three-way interaction hybrid × weaning strategy × day, and two-way interactions hybrid × weaning strategy and weaning strategy × day were not significant. The two-way interaction hybrid × day (Supplementary Table S4) was significant. On d+7, the rate of ear directed behaviour tended to be higher in the lighter hybrid DB than the heavier hybrid TN ($P_{adj} = 0.065$). However, the rate of ear directed behaviour doubled in from d+7 to d+14 in TN reaching the same rate as that in DB on d+14 (Fig. 4C). Weaning age was not significant. The rate of ear directed behaviour tended to be higher in the lightest pigs (Q1) compared to the heaviest pigs (Q4) within the litter ($P_{adj} = 0.10$) but there was no difference among the other quartiles (Supplementary Table S7).

3.5.3. Tail directed behaviour

The three- and two-way interactions between hybrid, weaning strategy and day were not significant. After removal of the interaction terms, there was a main effect of weaning strategy and day. The rate of tail directed behaviour has 2.5 times higher in CON than SSF, but increased in both weaning strategies from d+7 to d+14 (Figs. 4**D** and 4**E**). There was no effect of hybrid, weaning age nor within litter weaning weight quartile.

3.6. Tail posture

Due to varying amounts of active pigs in each observed image frame, the median (min-max) number of scans per pig per day was 9 (0−23) out of maximum 60 possible scans per day. The median (min-max) of scans on which a pig had a tucked tail posture was 0 (0−5) scans per pig per day, and a high tail posture 8 (0−22) scans per pig per day. Model estimates of rate of tucked and high tails per pig per h are shown in Fig. 5 and specified in Supplementary Table S6. In both hybrids, the rate of high tails increased from before (d-1) to after weaning (d+1) if pigs were allocated to weaning strategy CON. In addition, the rate of high tails was higher for CON compared to SSF irrespectively of hybrid (Fig. 5**A**). The rate of tucked tails was lower in DB-CON on the d+1 compared to before weaning (d-1), but no differences were seen in the other hybrid × weaning strategy combinations (Fig. 5**B**).

4. Discussion

4.1. Agonistic behaviour and ear lesions

As expected, and found in previous studies (Ekkel et al., 1995; Colson et al., 2012), SSF weaning in the pen of birth reduced agonistic behaviour and ear lesions compared to conventional weaning. In conventionally weaned pigs, the rate of fights and attacks decreased to a stable rate after the first two days postweaning indicating that the social hierarchy had been established (Meese and Ewbank, 1973). We found no difference in the rate of fights or attacks between the heavier and lighter hybrid. Irrespectively of hybrid, we found that the rate of fights, attacks and ear lesions increase with heavier within litter weight category, which supports the results of Prevolnik Povše et al. (2021) and Mesarec et al. (2021) that pigs initiating a fight in general are heavier than their opponent.

4.2. Saliva cortisol

Saliva cortisol concentration was used as stress biomarker reflecting hypothalamic-pituitary-adrenal activity as an response to stressors (separation from the sow, mixing of unfamiliar pig and moving to an novel environment) at weaning (Ekkel et al., 1995; Moscovice et al., 2022). Surprisingly, we did not see an increase in saliva cortisol from the day before to the day after weaning, nor any difference among the four hybrid × weaning strategy combinations on the day after weaning. We used a similar sampling method and timing as Colson et al. (2012), Yang et al. (2018) and Escribano et al. (2019) but were, in contrast to these studies, unable to detect an increase in saliva cortisol when pigs were conventionally weaned. However, in Colson et al. (2012) saliva cortisol did not increase postweaning in pigs that stayed in the farrowing pen. Surprisingly, we found lower saliva cortisol concentrations after weaning (from d+1 in TN and d+2 in DB) compared to before weaning when pigs stayed in the farrowing pen (SSF weaning). According to Jarvis et al. (2008), saliva cortisol was higher during the suckling period compared to one week postweaning. We therefore speculate that the lower saliva cortisol concentration postweaning may be a consequence of the absence of the hourly suckling periods in combination with prolonged periods of resting when weaned in the farrowing pen. On d+2, the SSF weaning strategy lowered saliva cortisol concentrations in the heavier hybrid TN, although it was not lower than in the lighter hybrid DB when weaned the same way. Hence, our results only partly supports Ekkel et al. (1995) and Colson et al. (2012) in that weaning intact litters in the pen of birth reduces saliva cortisol compared to conventional weaning.

4.3. Tail posture

In this study, we assessed the pig's tail posture on the day before and day after weaning and expected un-thriving pigs to have a higher rate of tucked tail posture as a potential indicator of negative affective states (Camerlink and Ursinus, 2020) caused by pain, sickness or incidences of tail biting in the pen (Larsen et al., 2018). However, we did not see an increase in the rate of tucked tails on the day postweaning in any of the four hybrid × weaning strategy combinations. On the contrary, the lighter hybrid DB had a lower rate of tucked tails when weaned conventionally. Our results on tail lesion scores support that tail biting was not present at the time of weaning, which may be reflected in the overall low rate of tucked tails. We found a higher rate of high tails in conventionally weaned pigs. High tail posture has been associated with neutral or positive affective state, but may also indicate high arousal irrespective of valence (Camerlink and Ursinus, 2020). In the present study, the increased rate of high tails may therefore indicate a state of high arousal as a consequence of being moved to a new environment with novel social stimuli. Nevertheless, we only recorded tail posture in pigs that were standing upright as recommended by Camerlink and Ursinus (2020). Thus, we cannot rule out that unthriving pigs that were lying down were missed using this recording method. More research is needed to understand the communicative value of the different tail postures in relation to the animal's activity budget and to optimise the recording method.

4.4. Abnormal behaviour

As expected, the lighter hybrid DB performed more belly nosing compared to the heavier hybrid. According to Colson et al. (2006) and Bench and Gonyou (2009), belly nosing increases in frequency over the first weeks postweaning, which was also the case in the present study, where the rate of belly nosing increased in both hybrid from day 7–14 postweaning. Hence, with the present weaning strategies and weaning age, development of belly nosing could be delayed, but not avoided, by using the heavier hybrid. Torrey and Widowski (2006) found that performers of belly nosing tended to be smaller at birth and weaning, and have a lower postweaning growth compared to their littermates. Therefore Torrey and Widowski (2006) suggested that belly nosing is related to the individual pig's nutritive status, as pigs that suckled the sow more frequently were less likely to develop belly nosing postweaning. In the lighter hybrid DB, factors such as lower birth weight and larger litter size may influence suckling success and nutritional status, which postweaning may trigger the development of abnormal behaviour. According to Valros et al. (2021), pigs with a higher growth rate during the suckling and postweaning period perform more pig directed nosing behaviour. Also ear directed behaviour tended to develop earlier in the lighter hybrid DB compared to the heavier hybrid TN. In our study, the number of piglets were reduced to the maximum number of functional teats for ethical reasons. Removing surplus piglets on day 3 *pp* may have reduced the negative effect of large litters especially in the hybrid DB on the measured stress and behaviour responses.

Irrespectively of hybrid, we found a higher rate of belly nosing in the lightest within litter weight category although the heaviest weight category was not significantly different from the lightest. The abrupt dietary change from sow milk to a grain based diet is a risk factor for developing abnormal behaviours postweaning (Valros, 2018; Prunier et al., 2020), and our previous study (Winters et al., 2023) showed that the heaviest pigs at weaning lost most weight and that heavier pigs had less feed trough visits.

Araújo et al. (2010) reduced pig directed nosing behaviour in newly weaning pigs by feeding highly digestible protein sources (spay-dried plasma protein and milk powder) opposed to feeding corn or soybean meal diets, and Vodolazska et al. (2022) increased pigs' postweaning feed intake by offering liquid feed during the suckling and postweaning period in combination with weaning at 35 days of age. Hence, using a heavier hybrid combined with initiatives that eases the transition from sow milk to solid feed (diet composition and/or later weaning) may alleviate stress associated with hunger during the early postweaning period and thereby reduce the risk of pigs developing abnormal behaviours.

Regarding weaning strategy, we expected that SSF pigs would to perform less abnormal behaviour, since novel social- and environmental interactions were omitted. However, no effect of weaning strategy was seen on belly nosing and ear directed behaviour. Colson et al. (2012) found no difference in the frequency of massaging and sucking behaviour between pigs housed with littermates in the farrowing pen or pigs that were mixed and moved postweaning. However, Colson et al. (2012) only observed during the first two days postweaning which may have been too early postweaning to detect these abnormal behaviours as the present results show. Gardner et al. (2001), found no association between higher stocking density and the frequency of belly nosing in early weaned pigs (12–14 days of age) during the first 21 days postweaning. Hence, other factors such as the abrupt dietary change, rather than social stress caused by mixing and moving may trigger the development of belly nosing in young pigs. On the contrary to belly nosing, social and environmental stress are potential risk factors for tail biting (Valros, 2018), which may explain higher rate of tail directed behaviour in our pigs that conventionally weaned. Our results on tail scores did not show problems with tail lesions during the first two weeks postweaning although redness/swelling and tail directed behaviour could be a precursor for tail biting later on in life (Ursinus et al., 2014).

5. Conclusion

Opposed to conventional weaning (CON), specific-stress-free (SSF) weaning in the pen of birth reduced agonistic behaviour, ear lesions and reduced tail directed behaviour. The heavier hybrid TN had a lower rate of belly nosing than the lighter hybrid DB. However, using the heavier hybrid did not reduce the motivation to perform abnormal behaviour, since the rate of belly nosing and ear directed behaviour increased over time. On d+2, the SSF weaning strategy reduced saliva cortisol in the heavier hybrid TN compared to CON, although saliva cortisol was not lower compared to the lighter hybrid DB housed the same way. In the conventional weaning strategy, no difference was found between the TN and DB pigs on stress and behaviour indicators. Although SSF weaning and using a heavier hybrid could reduce some measures of stress, additional initiatives that mitigate stress associated with the abrupt dietary change (e.g., increasing pre-weaning feed intake or weaning age) are needed.

Ethics approval

The procedures used in this study were in accordance with the Danish legislation on animal experimentation (Ministry of Food Agriculture and Fisheries of Denmark, 2014).

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CRediT authorship contribution statement

Jeanet Francisca Maria Winters: Conceptualisation, Methodology, Investigation, Formal analysis, Writing – original draft. **Cecilie Kobek-Kjeldager:** Writing review & editing, supervision. **Leslie Foldager:** Formal analysis, Writing – review and editing, supervision. **Fernando Tecles:** Formal analysis, review. **Lene Juul Pedersen:** Conceptualisation, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data are not deposited in an official repository.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.106106.

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