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Investigating the effects of two weaning methods and two genetic hybrids on play behavior in weaner pigs (*Sus scrofa*)

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In conventional pig production, suckling pigs are typically weaned between 3 and 5 weeks of age. This involves separation from the sow, dietary change, and regrouping in a novel environment, which challenge the welfare of pigs. We investigated the effects of two weaning methods [conventional weaning: two litters mixed in a weaner pen of different size and design (MOVE) vs. litter staying in the farrowing pen after removing the sow (STAY)] and two genetic hybrids [DanBred Yorkshire × Landrace (approximately 21 total pigs born/litter; DB) vs. Topigs Norsvin TN70 Yorkshire × Landrace (approximately 16 total pigs born/litter with higher individual birth weight and weaning weight than DB; TN)] on play behavior across weaning. Both genetic hybrids were inseminated with semen of DanBred Duroc boars. Litters were reduced to the number of functional teats at birth. The durations of locomotor-rotational play (LOC) and social play (SOC) of 24 indoor-housed litters [pigs/litter: (average ± SD) 13 ± 2; age at the weaning day: 26 ± 2 days] were video-recorded continuously between 14:00 h and 22:00 h on days –1, 1, and 2 relative to weaning and statistically analyzed with mixed-effects modeling at the individual level. Before weaning, TN pigs performed LOC longer than DB pigs. On day 2 post-weaning, STAY pigs engaged in more SOC than MOVE pigs. Moreover, TN pigs and STAY pigs displayed a steeper increase in LOC from days 1 to 2 than DB pigs and MOVE pigs, respectively. We demonstrated that pigs belonging to the genetic hybrid with higher weight at birth and weaning spent more time playing on the day before weaning. Additionally, weaning pigs in the farrowing pen and, hence, avoiding social mixing and relocation to an unfamiliar environment had a positive effect on social play after weaning. Our study illustrates that weaning stress in pigs may be reduced by using a genetic hybrid featuring higher birth and weaning weight and by keeping litters intact in a familiar environment after weaning. This study also supports the use of play behavior as an animal welfare indicator.

KEYWORDS

locomotor-rotational play, social play, farrowing pen, genetic hybrid, video analysis, positive animal welfare

Introduction

In a natural setting, a sow seeks a secluded site to farrow and remains close to her pigs, who are nursed approximately 30 times daily for the first week after farrowing (Weary et al., 2008). Approximately 10 days post-partum (pp), nursing declines and pigs gradually become more nutritionally and socially independent, and by 4 to 5 months of age, pigs are fully habituated to solid food (Jensen and Stangel, 1992). Conversely, in modern pig production systems, pigs are abruptly weaned from their dam at approximately 3 to 5 weeks of age (Špinka, 2017). In addition to the abrupt separation from the sow, pigs typically face changes in the physical and social environments, as well as a shift from highly digestible sow milk to a less digestible dry solid feed (Dybkjær, 1992; Weary et al., 2008; Campbell et al., 2013). Hence, weaning is acknowledged to challenge the welfare of pigs as they are too physiologically and behaviorally immature to cope with these synchronous stressors. For instance, the abrupt dietary change can result in undernourishment, reduced growth, and increased susceptibility to illness such as diarrhea (Le Dividich and Sève, 2000; Leeb et al., 2014). Additionally, the early separation from the mother and subsequently the social mixing in a new environment can increase the frequency of aggression and occurrence of injuries (Pitts et al., 2000) and lead to abnormal behaviors such as belly nosing and re-directed suckling of ears or tails of other pigs (Fraser, 1975; Puppe et al., 1997). These practices also reduce play behavior. For instance, Donaldson et al. (2002) and Hötzel et al. (2010) found a reduced occurrence of play behavior in 3- to 4-week-old pigs on the first days following weaning. Additionally, Colson et al. (2012) reported reduced time spent playing in 26-day-old pigs socially mixed in a novel environment at weaning.

Indeed, one way of investigating the welfare consequences of weaning in pigs can be through play behavior. Pigs perform play behavior from the first day of life (Špinka, 2017), and this behavior is considered a relevant welfare indicator because it is typically performed when the animal's primary needs, such as food and safety, have been met (Newberry et al., 1988; Špinka et al., 2001; Held and Špinka, 2011), while suppression of play behavior may indicate the presence of physical, physiological, or psychological challenges. In addition to indicating the fulfillment of primary needs, and the fact that negative affective states are absent, the performance of play behavior by animals is associated with the presence of positive-valence affective states due to its correlation with increased activity of brain areas mediating hedonic properties of rewards (Burgdorf and Panksepp, 2007; Berridge and Kringelbach, 2008; Horback, 2014). Hence, play behavior is suggested to be a positive animal welfare indicator (Špinka et al., 2001; Boissy et al., 2007).

As reviewed by Weary et al. (2008), different management practices have been found to limit, at least partly, the negative effects of early weaning in pigs, for example, the use of housing systems that allow the dam and her offspring to voluntarily spend time apart, from day 11 pp at the earliest, to habituate the young to periods of separation and to encourage early consumption of solid food aiming to improve post-weaning performance (e.g., Damm et al., 2003; Hötzel et al., 2004). Alternatively, offering a diet with increased energy density prior to weaning reportedly led to increased food intake lasting for days after weaning (Pajor et al., 2002) and reduced post-weaning frequency of vocalizations (Weary et al., 1999). Furthermore, weaning pigs in the farrowing pen may avoid stressful handling and transportation as well as keep litters intact, thus limiting disruption of social organization and fighting following weaning (Puppe et al., 1997; Colson et al., 2012).

Selecting a genetic hybrid with an expected increased viability may contribute to increase the resilience of pigs to weaning. In Denmark, DanBred Landrace × Yorkshire hybrid sows are commonly used in commercial farms (Früh et al., 2014) and they are characterized by their high prolificity [in 2020, the average size litter 5 days pp was 19.6 pigs (Hansen, 2021)]. The high prolificity leads to economic and environmental benefits due to an increased number of weaned pigs per sow and consequent reduced carbon emissions per kilogram of slaughtered pig, respectively (Rutherford et al., 2013). However, the increased litter size is obtained at the expense of more live-born pigs with low birth weight (Pedersen et al., 2011) and low average rectal temperature within 24 h pp (Schild et al., 2020), leading to increased pig mortality and reduced viability. Another disadvantage is that sows' functional teats are being outnumbered by litter size (Moustsen and Nielsen, 2017), which leads to increased teat competition (Milligan et al., 2001). As total colostrum yield is reportedly independent of litter size (Foisnet et al., 2010), the consequences of this high prolificity are low pig survival chances and viability (Ocepek et al., 2017; Schild et al., 2020). An alternative breed, the Topigs Norsvin TN70 hybrid [Norsvin Landrace × Z-line (Yorkshire)], is characterized by a slightly reduced litter size (approximately 16 pigs 5 days pp; Schild et al., 2020), but live-born pigs with higher birth weight, which is associated with higher body weight on the expected day of weaning, higher average rectal temperature within 24 h pp (Quiniou et al., 2002; Kammersgaard et al., 2011), and higher growth from birth to weaning in outdoor organic systems compared with the DanBred genetic hybrid (Schild et al., 2020). Additionally, Schild et al. (2019) reported that Topigs Norsvin TN70 sows were more protective towards their pigs during pig handling (i.e., earmarking and castration) than DanBred sows. Increased sow attention to the whole litter, acquisition of passive immunity, and thermoregulation are crucial aspects for pigs' viability and performance during the suckling and post-weaning period (Baxter et al., 2008; Declerck et al., 2016).

Accordingly, the aims of the current study were to investigate the single and interactive effects of two weaning methods (conventional weaning: pigs moved to and mixed in a weaner pen vs. weaning in a farrowing pen for loose-housed sows) and two genetic hybrids (DanBred vs. Topigs Norsvin TN70) on play behavior in domestic pigs (*Sus scrofa*) during the days around weaning at 26 days of age. We hypothesized that, before weaning, Topigs Norsvin TN70 pigs would play more than DanBred pigs due to a higher liveweight and consequently improved thermoregulation and nutritional status. Additionally, we hypothesized that Topigs Norsvin TN70 pigs would be more resilient to weaning and consequently play more than DanBred pigs after weaning. Moreover, we hypothesized that the practice of keeping weaned pigs in the farrowing pen would represent a less stressful management practice and thus result in a higher performance of play compared with mixing litters in a weaner pen after separation from the dam.

Materials and methods

Experimental design

The present study was conducted from February to November 2020 at the Danish Pig Research Centre, Aarhus University, Foulum, Denmark. The animal experimental procedures and care of animal under study were carried out in accordance with the Ministry of Food, Agriculture and Fisheries, The Danish Veterinary and Food Administration under act 474 of 15 May 2014 and executive order 2028 of 14 December 2020, and under consideration of the ARRIVE Guidelines (Du Sert et al., 2020). The present study was part of a longer study examining the clinical, physiological, and behavioral effects of weaning intact litters from sows of different hybrids in farrowing pens for loose-housed sows (Winters et al., submitted).

The experimental design followed a 2 × 2 factorial arrangement with the following factors: weaning method [two litters grouped in a conventional two-climate weaner pen (MOVE) vs. intact litter that stayed in the farrowing pen at weaning after the sow was removed (STAY)] and sow genetic hybrid [DanBred Landrace × Yorkshire (approximately 21 total pigs born/litter; DB) vs. Topigs Norsvin TN-70 Yorkshire × Landrace (approximately 16 total pigs born/litter; TN)]. Both genetic hybrids were inseminated with semen of DanBred Duroc boars.

Animals, housing, and management

Lactation period

Twelve gilts (6 DB and 6 TN gilts) were included in one batch. After farrowing and lactation, these sows returned in a second batch as second-parity sows. In total, 306 pigs (50% castrated males, 50% females) from 24 litters [pigs/litter: (average ± SD) 13 ± 2] over two batches were included in this study. The farrowing unit had two sections, each containing eight 3.0 × 2.2 m farrowing pens for loose-housed sows (FT30, Jyden A/S, Denmark). In each pen, the floor was 41% slatted and 59% solid concrete, the sides of the pen were made of metal bar and equipped with sloped walls to reduce the risk of pig crushing by the sow. A hayrack was placed on the entrance next to the covered pig creep area (0.9 m^2). The solid floor surface was heated, and the creep area was equipped with a 150 W infrared heat lamp (InterHeat CO., South Korea) providing 35°C in the creep area at farrowing. The temperature was gradually decreased until the heater was turned off in week 3 pp. One week before the expected date of farrowing, three DB and three TN gilts/sows were allocated to each of the two rooms and, within room, sows were randomly allocated to farrowing pen. The farrowing pen was equipped with a low 80 × 28 cm polyconcrete feed trough (Jyden A/S, Denmark) allowing pigs to eat together with the sow. A water drinking nipple was placed 5 cm above the deepest point of the feed trough for the sow, permitting mixing of the lactation diet with water. This water drinking nipple was closed at weaning to omit water in the feed trough where the weaner diet was delivered post-weaning. A 31 × 17 × 11 cm pig water trough (Aqua-Level system with hinged trough, Jyden A/S, Denmark) positioned next to the feed trough on the slatted floor provided *ad libitum* access to water throughout the experimental period.

Sows received analgesic (Metacam®) and all pigs were weighted, ear tagged, and had their umbilical cord cut following farrowing, if it ended before 15:00 h, or on the next morning, if farrowing ended after 15:00 h. The number of own pigs nursed by the sows was adjusted to be equal to the number of functional teats. Surplus pigs within sow hybrid were cross fostered within the first 3 days pp to sows that had fewer pigs than functional teats. If no sows were available for cross-fostering, surplus pigs were euthanized by blunt force trauma. Non-viable pigs and pigs below 700 g were euthanized first followed by randomly selected pigs. On the day before weaning, the numbers of pigs per pen in the DB and TN genetic hybrids were 12 ± 2 and 13 ± 2, respectively. Pigs received 1 ml of subcutaneous iron injection (Viloferron®, 20%) on day 4 pp and male pigs were castrated after receiving a local anaesthetic (0.5 ml of Procamidor®Vet 20 mg/ml per testis) and analgesic (0.2 ml of Melovem® 20 mg/ml i.m.). Pigs were vaccinated with Ingelvac MycoFLEX® on day 7 pp and again at weaning. No pigs were tail-docked or tooth-clipped.

The artificial light was off from 22:00 h to 07:00 h. Every morning, feed troughs were emptied before first feeding, and manure was removed. Straw racks were filled with wheat straw daily and approximately 130 g of fine chopped wheat straw were provided on the solid floor daily. Approximately 400 g of sawdust was provided in the pig creep area daily. At farrowing, the room temperature was set at 21°C and gradually decreased to 19°C at weaning.

Sows were fed a pelleted standardized lactation diet (Die Profil Lac, DLG, Frederica, Denmark, metabolizable energy: 12.9

MJ/kg, 15.6% crude protein) following the Danish recommended feeding curve (Tybirk et al., 2018) delivered 4 times daily at 08:30 h, 11:00 h, 15:30 h, and 22:00 h. If the feed trough was empty in the morning, from day 14 pp until weaning, the sows' daily feed allowance was increased by 2% to ensure that there was feed in the trough for the main part of the 24 h to encourage pigs to start eating solid feed. Pigs did not receive weaner diet before weaning, but they had free access to the sow diet during the entire lactation period.

Post-weaning period

In each batch, half of the DB and TN litters were randomly allocated to one of the two weaning methods, STAY or MOVE. This resulted in the following total number of pens and pigs per pen in each weaning method × genetic hybrid combination: STAY × DB: four pens (12 ± 2 pigs/pen), MOVE × DB: four pens (25 ± 3 pigs/pen), STAY × TN: four pens (14 ± 2 pigs/pen), and MOVE × TN: four pens (26 ± 2 pigs/pen). Litters assigned to the STAY treatment stayed in the farrowing pens for loose-housed sows after the sow was removed out of sensory range of detection by the pigs. Litters assigned to the MOVE treatment were moved to one of four identical 5.40 × 2.45 m two-climate weaner pens (approximately 0.51 m^2/pig) with approximately 33% slatted, 33% drained, and 33% solid floor in the same weaner unit. The solid floor was roofed by a manually adjustable fiber panel positioned approximately 85 cm above the floor level (Jyden A/S, Denmark) providing two zones in the pen (a cooler slatted and drained floor area and a warmer and darker area under the roof). The water trough was of the same type as used in the farrowing pens. Two weaner feeders (TR4, Rotecna®, Spain) were placed next to each other in the partially slatted floor area providing eight 14 × 18 cm feeding places per weaner pen. On the day of weaning, experimental pigs were (average ± SD) 26 ± 1 days old and weighed 7.4 ± 1.9 kg (DB: 7.0 ± 2.0 kg; TN: 7.8 ± 1.8 kg). To accommodate all-in all-out farrowing for hygiene purposes, weaning took place on 1 day per week, as close as possible to the day when litters were 28 days old.

Pigs were weaned at 14:00 h. Litters in treatment STAY were locked inside the pig creep area while the sow was moved out of the farrowing pen. Lactation diet leftovers were removed from all feed troughs, the water nipple was turned off and a weaner diet was offered. At the time of weaning and every morning post-weaning, all STAY pens were provided approximately 130 g of chopped wheat straw on the solid floor and approximately 400 g of sawdust in the creep area. Litters allocated to treatment MOVE were grouped with another entire litter of the same breed and walked to a conventional weaner pen in another section of the building located approximately 80 m from the farrowing unit. The MOVE pens received double the amount of straw and sawdust (to maintain same provision per animal) placed on the drained floor and on the solid floor, respectively.

The room temperature was increased to 24°C and gradually decreased to 21°C 56 days post-weaning in both treatments. Pigs were *ad libitum* fed a pelleted standardized weaner diet (Prime Midi Piller, DLG, Denmark, metabolizable energy: 14.8 MJ/kg, 19.3% crude protein), with no added zinc oxide, delivered once daily in the morning. No pigs were treated with antibiotics during the experimental period.

Play behavior recording

Play behavior was observed from video recordings on days −1, 1, and 2 relative to the weaning day (day 0). A 2D video camera (model DS-2CD2145FWD-I, Hikvision, China) was placed above each pen with a top-view angle, overlooking the entire pen area, except under the creep area in the farrowing pen and under the cover in the weaner pen, from a height of approximately 2.3 m in the farrowing pen and 2.8 m in the weaner pen. Video data were processed with the software Blue Iris v.5 (Blue Iris Security, USA). Each pig was identified on the video recordings by use of markings on the back made with a livestock marker on days −2, 1, and 2.

Play behavior was observed using behavioral sampling and continuous recording (Bateson and Martin, 2021) of the individual pig from 14:00 h to 22:00 h on each experimental day (−1, 1, and 2) using the Noldus Observer XT 15 (Noldus Information Technology, The Netherlands) software package. Pigs were expected to play with the highest frequency during light hours (07:00 to 22:00 h). However, the hours from 07:00 to 14:00 h included experimental and management procedures disturbing the pig such as general management, marking, and weighing of the pigs, and data sampling. Thus, it was chosen to only observe the pigs during the undisturbed hours from 14:00 h to 22:00 h on each day. The durations of two types of play behavior were recorded: locomotor-rotational play (LOC; Table 1) and social play (SOC; Table 2). When a behavior duration was registered, the observer returned the video to the start time of this behavior and continued the observations from that time point to ensure that no play behavior was missed. If necessary, the identities of the pigs participating in the play were noted to avoid registering the same events twice. The durations of LOC and SOC of the individual pig comprised the sum of durations of all behavioral elements constituting each type of play, respectively, as displayed by the individual pig during the 8-h observation period. Whenever pigs were disturbed (i.e., a human was within the framework of the pen, the pigs were not in the pen, or the pigs were being moved in or out of the pen), this was noted as disturbance, and no behavior registrations were made. The duration of disturbance across pens ranged from 0 to 150 s on day −1, 0 to 47 s on day 1, and 0 to 51 s on day 2.

Three experimenters (A, B, and C) were assigned to observe play behavior from video recordings, balanced for genetic hybrid and weaning method. Two experimenters (A and B) observed batch 1, with experimenter A also observing batch 2 together with a third experimenter (C) (Table 3). Each experimenter was

TABLE 1 Ethogram of behavioral elements constituting locomotor-rotational play in domesticated pigs (*Sus scrofa*).

Behavior	Description	Modified from
Locomotor-rotational play	A pig performs one of the locomotor play behavior elements: gambolling or scamper; or one of the rotational play behavior elements: flop, head toss, hop/pivot, turn, or shake straw. Behavioral elements are mutually exclusive and recorded with exact start and stop times. If a pig stops playing for a full second, it is noted as a new event.	
Elements of locomotor-rotational play		
Gambolling	Gallop-like energetic running (the two forelimbs move approximately in phase, followed by the two hind limbs) and hopping in forward motions within the pen environment. Often associated with vigorous ear flapping, moving across a large area of the pen, and occasionally bouncing into other pigs. As part of a bout of gambolling, the pig may turn and change direction. The behavior ends when the pigs move at a slower pace or go out of sight.	Brown et al. (2015)
Scamper	Performing at least two hops forward in rapid succession usually accompanied by ear flapping and sometimes accompanied by head tossing. The behavior ends when the pigs move at a slower pace or go out of sight.	Donaldson et al. (2002); Martin et al. (2015); Zupan et al. (2016)
Flop	A rapid drop from an upright position to sternal or lateral recumbency in which the pig appears to fall by itself and not because of contact with another pig.	Donaldson et al. (2002)
Head toss	Exaggerated lateral movement of the head and neck in the horizontal plane, involving at least one full movement to each side.	Donaldson et al. (2002)
Hop/Pivot	Making a short, bouncing leap on the spot during which all feet are simultaneously lifted off the ground, body oriented in the same direction throughout the hop or rotated rapidly in the horizontal plane.	Newberry et al. (1988); Donaldson et al. (2002); Zupan et al. (2016)
Turn	Rapid turning around the body axis on the spot during which at least one foot is touching the ground.	Zupan et al. (2016)
Shake straw	Perform vigorous lateral movements of head and neck while holding straw, which protrudes from mouth.	Newberry et al. (1988)
Throw straw	Holding straw in the mouth and throwing it through the air by a rapid movement of the head and neck.	Zupan et al. (2016)

trained in observing play until a concordance correlation coefficient (CCC) ≥ 0.80 was achieved between experimenters within each batch. The CCC is an integrated measure of correlation as well as accuracy and precision (Lawrence and Lin, 1989) and was herein interpreted according to the criteria proposed by Hinkle et al. (2003) indicating the level of agreement: negligible (0.0 to 0.3), low (0.3 to 0.5), moderate (0.5 to 0.7), high (0.7 to 0.9), or very high (0.9 to 1.00). The CCC calculations accounted for the total duration of play (i.e., LOC and SOC durations combined) of the individual pig. During the training, each experimenter within a given batch was given the same 1-h video to observe for each of the three housing situations: farrowing pen with sow, farrowing pen without sow, and two-climate weaner pen. After observing these 3 h of

TABLE 2 Ethogram of behaviors constituting social play in domesticated pigs (*Sus scrofa*).

Behavior	Description	Modified from
Social play	Energetic mutual interaction between two or more upright pigs initiated by play fighting and may include the following elements: climb, follow/chase, lever, nudge, and push (see below). Pigs display neither biting nor avoidance. The social play ends when the pigs are no longer in contact, or when one pig no longer shows interest, or when they stop follow/chase, or if at least one of the pigs moves out of sight.	
Elements of social play		
Climb	Placing both front hoofs on the back of another pig.	Brown et al. (2015)
Follow/chase	Walking/running (often with physical contact) in the same direction as another pig.	Zupan et al. (2016)
Lever	With the snout lifting another pig so that at least two feet are lifted off the floor.	Donaldson et al. (2002)
Nudge	Use of snout to gently touch another pig's body, not including naso–naso contact.	Brown et al. (2018)
Push	Using head, neck, or shoulders with minimal or moderate force to drive into another pig's body.	Brown et al. (2018)
Play fight	Two pigs mutually push and head-knock each other. A general mild intensity of the performed fighting behaviors and a lack of biting or avoidance distinguish play fight from aggressive fighting.	Brown et al. (2015)

TABLE 3 Breakdown of the number of pens and pigs per pen following a 2 × 2 factorial arrangement of the factors weaning method (litters mixed in a conventional weaning pen; MOVE vs. litter stayed in the farrowing pen after removing the sow; STAY) and genetic hybrid (DanBred; DB vs. Topigs Norsvin TN70; TN) observed by each experimenter on each day (−1, 1, and 2) relative to weaning.

Day	Treatment combination	Experimenter		
		A	B	C
-1	MOVE × DB	4 pens, 11 ± 2	2 pens, 13 ± 1	2 pens, 15 ± 1
	MOVE × TN	4 pens, 13 ± 2	2 pens, 13 ± 1	2 pens, 13 ± 0
	STAY × DB	2 pens, 13 ± 1	1 pen, 14	1 pen, 10
	STAY × TN	2 pens, 12 ± 3	1 pen, 15	1 pen, 15
1	MOVE × DB	1 pen, 28	2 pens, 24 ± 3	1 pen, 24
	MOVE × TN	3 pens, 27 ± 1	—	1 pen, 23
	STAY × DB	2 pens, 13 ± 1	1 pen, 14	1 pen, 10
	STAY × TN	2 pens, 12 ± 3	1 pen, 15	1 pen, 15
2	MOVE × DB	1 pen, 28	2 pens, 24 ± 3	1 pen, 24
	MOVE × TN	3 pens, 27 ± 1	—	1 pen, 23
	STAY × DB	2 pens, 13 ± 1	1 pen, 14	1 pen, 10
	STAY × TN	2 pens, 12 ± 3	1 pen, 15	1 pen, 15

Number of pigs per pen is presented as average ± standard deviation, when more than one pen was observed.

video, CCC was calculated by the Noldus Observer XT software and any disagreement between experimenters was discussed. This process was repeated on 1-h videos until the acceptable CCC was achieved (batch 1: 0.86, batch 2: 0.87, all combinations of experimenters across batches: 0.82). Intra-experimenter agreement was also tested for the experimenter observing both batches, achieving a CCC of 0.84.

Statistical analyses

Statistical analyses were performed in R v.4.1.1 (R Core Team, 2021). p-values < 0.05 were considered significant, and p-values of $0.1 > p \geq 0.05$ were considered tendencies. The durations (s/pig/8-h) of LOC and SOC were analyzed over experimental days at the individual pig level, accounting for the nested structure in data with pigs nested within pens. Because of the percentage of zero observations (day −1: 11%; day 1: 49%; day 2: 26%), LOC was square-root-transformed to fulfill the assumptions of normality and homoscedasticity and analyzed with a linear mixed-effects model with a Gaussian distribution (library glmmTMB v.1.1.2; Brooks et al., 2017). Due to the high percentage of zero observations (day −1: 67%; day 1: 95%; day 2: 92%), SOC was converted into a binary variable (played; did not play) and analyzed with a mixed-effects logistic regression model (library glmmTMB v.1.1.2). Both LOC and SOC models initially included sex (castrated male; female), weaning method (MOVE; STAY), genetic hybrid (DB; TN), day (−1; 1; 2), all possible two-way interactions between weaning method, genetic hybrid, and day, and the three-way interaction among weaning method, genetic hybrid, and day. Batch, pig, pen before weaning, and pen after weaning were included as random effects. Additionally, a continuous-time autoregressive covariance structure was included to account for repeated measures of each pig over days. Model assumptions of normality and homoscedasticity were confirmed through graphical inspection of the residuals. To achieve a satisfactory fit of the model, the initial SOC model was reduced by removing the three-way interaction. Tukey-adjusted *post-hoc* analyses were performed for each model (library emmeans v.1.6.3; Lenth, 2021).

Results

A significant interaction between weaning method and day on LOC was detected (Table 4; Figure 1). On each day, no statistical difference in time spent in LOC between MOVE and STAY was observed. However, performance of LOC in both weaning method groups decreased from days −1 to 1 and STAY pigs increased time spent in LOC from days 1 and 2. A significant interaction between genetic hybrid and day on LOC was also detected (Table 4; Figure 2). On day −1, TN pigs performed LOC longer than DB pigs. From days −1 to 1, performance of LOC decreased in both genetic hybrid groups. Conversely, TN pigs performed more LOC on day 2 than on day 1. A tendency for interaction among weaning method, genetic hybrid, and day on LOC was detected (Table 4; Figure 3).

Irrespective of weaning method or genetic hybrid, the odds of displaying SOC were higher on day −1 than days 1 and 2 [day −1/day 1: (odds ratio ± standard error) 9.4 ± 3.05; day −1/day 2: 6.6 ± 1.98], while there was no statistical difference between days 1 and 2 (0.7 ± 0.27) (Table 4). A significant interaction between weaning method and day on SOC was detected (Table 4; Figure 4). On day 2,

TABLE 4 Test statistics (χ^2 test) and p-values for the duration (s/pig/8-h) of locomotor-rotational play (LOC) and social play (SOC) in weaner pigs used in a 2 × 2 factorial arrangement of treatment factors: weaning method (litters mixed in a conventional weaning pen; MOVE vs. litter stayed in the farrowing pen after removing the sow; STAY) and genetic hybrid (DanBred; DB vs. Topigs Norsvin TN70; TN), on days –1, 1, and 2 relative to the weaning day.

Variable	Statistics	Sex[1]	Weaning method[1]	Genetic hybrid[1]	Day[2]	Weaning method × Genetic hybrid[1]	Weaning method × Day[2]	Genetic hybrid × Day[2]	Weaning method × Genetic hybrid × Day[2]
LOC	χ^2 test	0.04	0.18	0.06	403.67	3.15	6.80	6.62	5.17
	p-value	0.828	0.666	0.804	< 0.001	0.075	0.033	0.036	0.075
SOC	χ^2 test	3.77	0.24	0.21	72.75	0.69	20.56	5.76	—
	p-value	0.051	0.623	0.651	< 0.001	0.404	< 0.001	0.056	—

[1]Degrees of freedom = 1; [2]Degress of freedom = 2.

STAY pigs had a higher probability of displaying SOC than MOVE pigs. A tendency for interaction between genetic hybrid and day on SOC was detected (Table 4; Figure 5). Castrated male pigs tended to show higher odds of displaying SOC than female pigs (1.5 ± 0.33; Table 4).

Discussion

Pre-weaning period

Our finding that TN pigs engaged more in LOC compared with DB pigs before weaning may be linked to the respective breeding goals, and consequent pig characteristics at birth, of each genetic hybrid. Litters originating from DB sows feature a high number of live-born pigs with low birth weight and low rectal temperature within 24 h after farrowing. Conversely, TN sows typically farrow slightly smaller litters composed of heavier live-born pigs with higher rectal temperature 24 h post-farrowing than the DB genetic hybrid (Schild et al., 2020). Reduced birth weight and reduced rectal temperature are accompanied by lower body energy reserves and higher sensitivity to cold (Pedersen et al., 2011), jeopardizing the ability of pigs to reach the teats and delaying the first suckling event (Le Dividich and Sève, 2000; Quiniou et al., 2002). The consequence of this cascade is increased risk of mortality and reduced weight on the expected day of weaning (Pedersen et al., 2015), which may decrease pigs' resilience to abrupt, earlier than natural, weaning (Wolter and Ellis, 2001; Collins et al., 2017). In the current study, TN pigs were heavier than the DB pigs on the day of weaning. Additionally, Winters et al. (submitted), accounting for a larger population of pigs (including the current study's population), reported higher average daily weight gain during the week prior

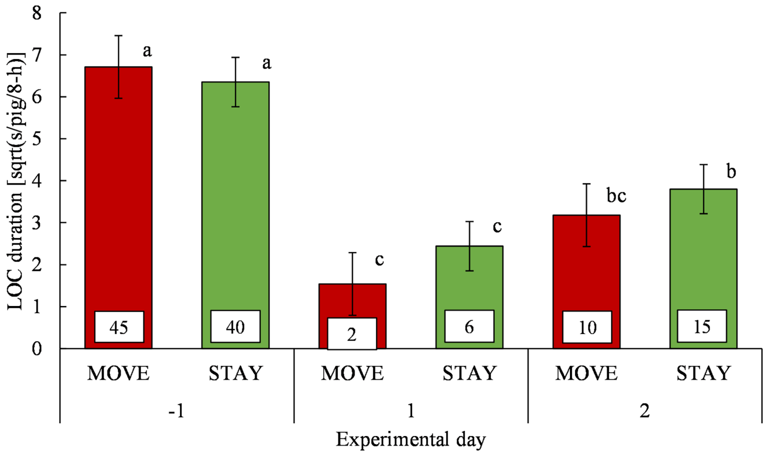

FIGURE 1
Time spent performing locomotor-rotational play (LOC), in s/pig/8-h, in pigs moved to a weaner pen (MOVE; red bars) or kept in the farrowing pen (STAY; green bars) after weaning, between 14:00 h and 22:00 h on days –1, 1, and 2 relative to weaning. The bars represent least squares means and the error bars indicate standard error. Estimates and standard error bars were square root transformed prior to analysis, and the back-transformed least squares means are shown within a box on the top of each bar. Different superscript letters indicate statistical difference at $p < 0.05$ both between and within days.

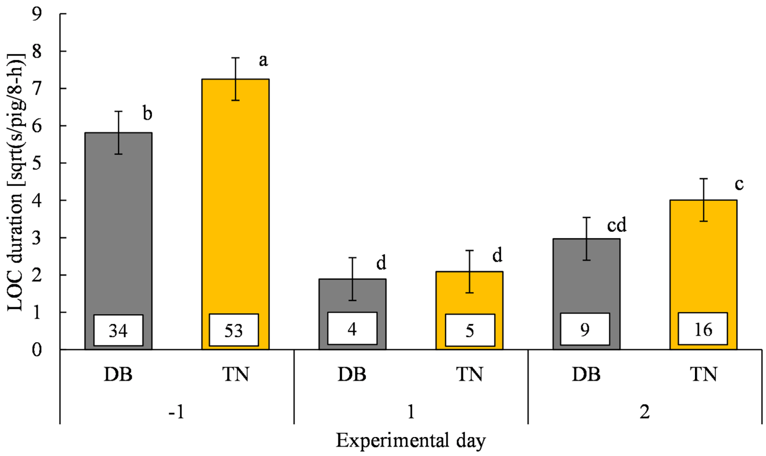

FIGURE 2
Time spent performing locomotor-rotational play (LOC), in s/pig/8-h, in DanBred pigs (DB; gray bars) or Topigs Norsvin TN70 pigs (TN; yellow bars) between 14:00 h and 22:00 h on days –1, 1, and 2 relative to weaning. The bars represent least squares means and the error bars indicate standard error. Estimates and standard error bars were square root transformed prior to analysis, and the back-transformed least squares means are shown within a box on the top of each bar. Different superscript letters indicate statistical difference at $p < 0.05$ both between and within days.

to the weaning day and higher weight at weaning in TN pigs than in DB pigs. As play behavior has been demonstrated to be sensitive to negative physical and physiological conditions (Siviy and Panksepp, 1985; Newberry et al., 1988), our findings support our hypothesis that TN pigs would be more playful than DB pigs because of their higher weights prior to weaning. Had the litter size in DB and TN groups not been equalized to the number of functional teats, the weight gain of the DB pigs would likely have been less uniform, and lower, due to increased within-litter competition for teats. Consequently, the difference in time spent playing between the two genetic hybrids could have been greater if litter size had not been harmonized to a similar number of suckling piglets in the two genetic hybrids. Commercial farms using DB sows increasingly allow a higher number of piglets suckle the sow than the number of her functional teats to manage the large litter size, a strategy that results in increased within-litter competition and reduced weight gain of suckling piglets (Kobek-Kjeldager et al., 2020a; Kobek-Kjeldager et al., 2020b).

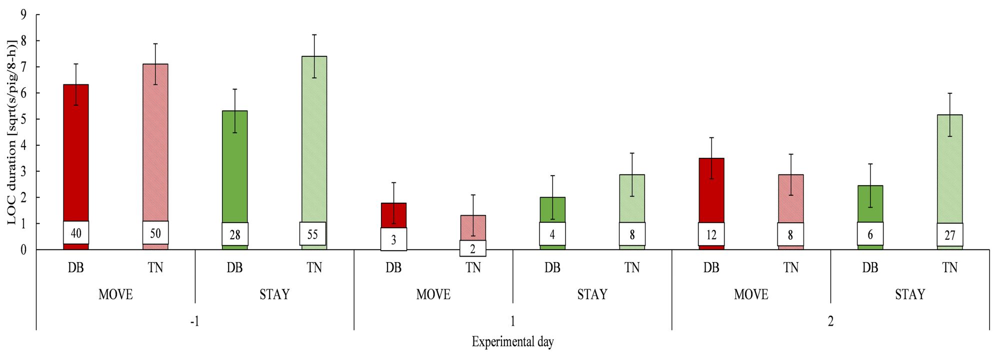

FIGURE 3
Time spent performing locomotor-rotational play (LOC), in s/pig/8-h, in pigs moved to a weaner pen (MOVE; red bars) or kept in the farrowing pen (STAY; green bars) and DanBred pigs (DB; solid bars) or Topigs Norsvin TN70 pigs (TN; striped bars) between 14:00 h and 22:00 h on days –1, 1, and 2 relative to weaning. The bars represent least squares means and the error bars indicate standard error. Estimates and standard error bars were square root transformed prior to analysis, and the back-transformed least squares means are shown within a box on the top of each bar.

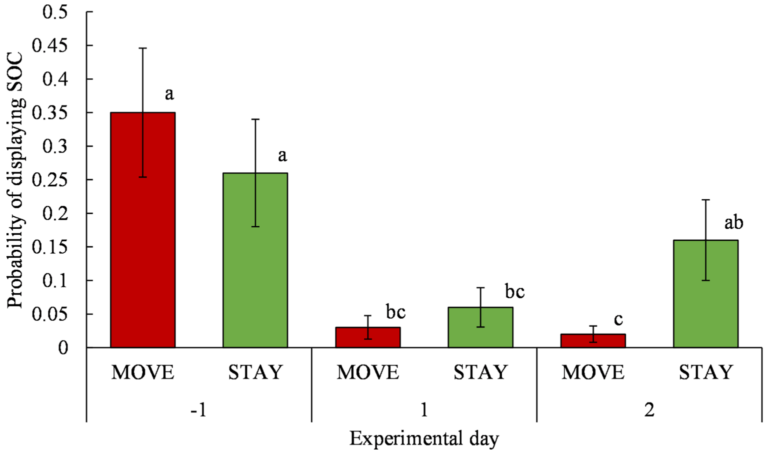

FIGURE 4
Probabilities of displaying social play (SOC) in pigs moved to a weaner pen (MOVE; red bars) or kept in the farrowing pen (STAY; green bars) after weaning, between 14:00 h and 22:00 h on days −1, 1, and 2 relative to weaning. The bars represent probability, and the error bars indicate standard error. Estimates and standard error bars were back-transformed from the model using the inverse link function. Different superscript letters indicate statistical difference at $p < 0.05$ both between and within days.

Post-weaning period

Performance of play behavior was lower on the first days after weaning compared to the day before weaning, irrespective of genetic hybrid or weaning method. This pattern in performance of play is in accordance with previous studies on play behavior in pigs around weaning (e.g., Donaldson et al., 2002; Hötzel et al., 2010; Colson et al., 2012). It has been suggested that pigs of the age used in this study are still nutritionally dependent of the sow's milk, and an abrupt separation from the sow and change to solid feed, likely jeopardizes their welfare, as reported in other animal species (e.g., dairy calves, Krachun et al., 2010; deer fawns, Muller-Schwarze et al., 1982). Our findings corroborate the understanding that early weaning challenges the welfare of pigs, who are too young and immature to cope easily with simultaneous stressors associated with this management practice involving separation from the sow, abrupt transition from milk to solid feed, and social mixing in a novel environment.

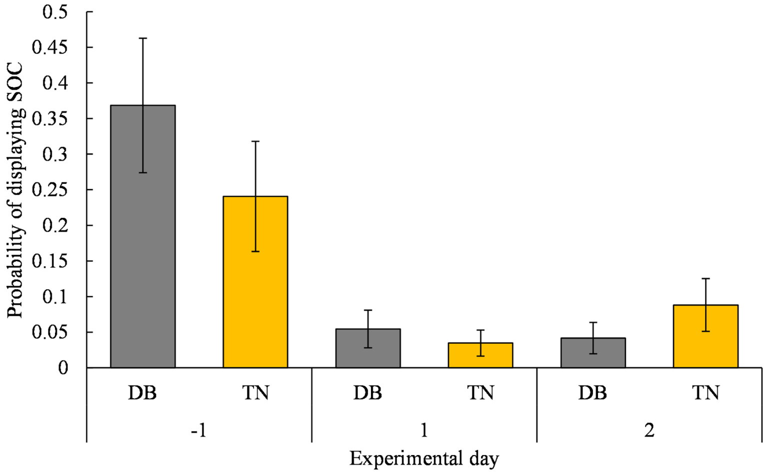

FIGURE 5
Probabilities of displaying social play (SOC) in DanBred pigs (DB; gray bars) or Topigs Norsvin TN70 pigs (TN; yellow bars) between 14:00 h and 22:00 h on days −1, 1, and 2 relative to weaning. The bars represent probability, and the error bars indicate standard error. Estimates and standard error bars were back-transformed from the model using the inverse link function.

Our results did not support the hypothesis that TN pigs would be more playful than DB pigs after weaning. The TN pigs showed a steeper decrease in time spent performing LOC from days −1 to 1 than the DB pigs, suggesting that the TN group was more affected by the disappearance of the sow and abrupt dietary shift. Conversely, from days 1 to 2, the TN pigs showed a steeper increase in duration of LOC compared to the DB pigs, suggesting a faster recovery from this management practice. Additionally, we found that the STAY pigs also displayed a steeper increase in time spent in LOC from days 1 to 2 and a higher engagement in SOC on day 2 than the MOVE pigs. These findings suggest that weaning pigs in the farrowing pen for loose-housed sows and, hence, avoiding social mixing in a new environment can reduce weaning stress. Social mixing requires establishment of new dominance relationships, which consequently involves aggression commonly leading to body injuries (Meese and Ewbank, 1973), and physiological and psychological stress (Campbell et al., 2013; Peden et al., 2018). Previous studies found increased levels of salivary cortisol, indicating stress, in pigs moved to a new environment or socially mixed, or both, after weaning (e.g., Merlot et al., 2004; Colson et al., 2012). Accordingly, the activation of stress pathways may result in intestinal and immunological dysfunction compromising feed intake, growth, and resistance against pathogens (Le Dividich and Sève, 2000; Pié et al., 2004; Moeser et al., 2012). Altogether, these potential negative consequences related to social mixing in a novel environment as part of conventional weaning could have constituted a welfare threat to the pigs (Held and Špinka, 2011) and have reduced their motivation to engage in play. Meanwhile, weaning pigs in the farrowing pen and, hence, avoiding placement in an unfamiliar environment and social disruption by mixing litters may have limited the negative effects of this management procedure. Other stressors were still present, such as maternal separation and abrupt dietary shift (e.g., Hillmann et al., 2003; Martin et al., 2015), likely explaining the reduction in time spent playing on day 1 in pigs subjected to this treatment.

In this study, the level of play recorded was low compared to previous studies examining play in (weaner) pigs (e.g., Hötzel et al., 2010; Camerlink et al., 2021). Methodological differences may provide a partial explanation. For instance, our SOC definition required two or more pigs to mutually engage in social play, while the above-mentioned studies did not require the social play to be mutual. Furthermore, there can be circadian differences in play behavior (Ocepek et al., 2020) that may explain differences in results between studies. Thus, for a more accurate interpretation and comparison of results within and between studies, it may be advisable to express time spent playing relative to the time active during the observation periods in future studies. Furthermore, due to low occurrence, we were unable to analyze the SOC durations and had to analyze the data as a binary response per 8-h period. Using one-zero sampling with short intervals over more time periods per day could have been more efficient for capturing treatment differences in play behavior.

Our results show that LOC durations rose from days 1 to 2, especially in STAY pigs and TN pigs. If pigs had been observed for a longer period following weaning, we may have seen a level of play similar to, or higher than, the pre-weaning level, and the effects of weaning method, genetic hybrid, or both might have been stronger. For instance, Camerlink et al. (2021) reported a lower level of social play in weaner pigs during the first 48 h and a subsequent increase from day 3 relative to weaning. Moreover, Donaldson et al. (2002) and Brown et al. (2018) observed higher levels of locomotor play in pigs at days 3 and 5 post-weaning compared with the pre-weaning period. Together with these studies, our results suggest that the adverse effects of weaning on pigs are relatively short-lived given the tendency to increase time spent performing locomotor play. However, Donaldson et al. (2002) and Brown et al. (2018) could not tease apart post-weaning effects from increased pen space availability after weaning, or from a possible age effect, given that locomotor play has been observed to peak at approximately 4 to 5 weeks of age (Newberry et al., 1988). Hence, it would also be complex to disentangle the effects of the genetic hybrid and weaning method from these other aspects (i.e., space and age).

The weaning method of keeping pigs in the farrowing pen and, hence, keeping litters intact across weaning may support occurrence of play and prevent or, at least, limit aggressive behavior (Puppe et al., 1997). However, social mixing is unavoidable during the production cycle and later rather than early social mixing can increase the duration of fights and severity of injuries during the establishment of a new hierarchy (e.g., Pitts et al., 2000; D'Eath, 2005). D'Eath (2005) stated that between 5 and 12 days of age is the most suitable time to mix pigs as they are minimally prone to fight or bully other pigs. Accordingly, Weary et al. (2008) recommend social mixing of pigs at this age due to the lower susceptibility to aggression and fear compared to later life stages (Hillmann et al., 2003). As reviewed by Weary et al. (2008), increasing the weaning age, making the solid diet more attractive, or reducing pigs' access to the sow for some hours daily prior to sow removal, so that pigs may achieve partial nutritional independence before separation from the sow, are additional strategies for reducing weaning stress. We encourage future studies using play behavior as an indicator of how well pigs recover from weaning depending on weaning practices.

Conclusions

Our study contributes to the understanding of effects of weaning on pigs and suggests welfare-oriented improvements. The use of a genetic hybrid with characteristics related to increased viability resulted in an increased performance of LOC before weaning. Weaning pigs in the farrowing pen and, hence, avoiding moving to an unfamiliar environment and social disruption by mixing litters resulted in an increased occurrence of SOC play on day 2 after weaning. Additionally, pigs belonging to the genetic hybrid with higher weaning weight and pigs kept in the farrowing pen displayed a steeper recovery in performance of LOC from days 1 to 2 relative to weaning.

Our experimental pigs reduced their time spent playing when several sources of stress were introduced, such as removal of the sow, sudden dietary change, social mixing, and placement in a novel environment, supporting the idea that play behavior is typically suppressed by welfare threats (Burgdorf and Panksepp, 2007; Held and Špinka, 2011). Earlier studies similarly found a drop in play behavior in adverse situations (e.g., disbudding in dairy calves, Mintline et al., 2013; low milk allowance in dairy calves, Jensen et al., 2015; poor weight gain in weaner pigs, Brown et al., 2015). That play behavior is reduced by such welfare threats means that suppression of play behavior can indicate the absence of positive affective states and thus poor animal welfare. However, it is the association with positive experiences (e.g., that animals seek out opportunities to play and solicit play) that qualifies play behavior as a positive welfare indicator. Keeping animals in environments that allow and stimulate play behavior thus promotes positive welfare because it gives the animals the opportunity for positive experiences. Evidence of play behavior increasing when favorable conditions become even more favorable would support play behavior as a positive welfare indicator (Ahloy-Dallaire et al., 2018). Hence, we encourage future studies to examine the validity of play behavior as a positive welfare indicator by use of physiological (e.g., plasma oxytocin), behavioral (e.g., body postures), and cognitive measures (e.g., cognitive bias tests, motor/sensorial lateralization) (as reviewed by Ahloy-Dallaire et al., 2018 and Kremer et al., 2020). Such an examination would allow for stronger inference regarding affective states and animal welfare.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the animal study because the animal experimental procedures and care of animal under study were carried out in accordance with the Ministry of Food, Agriculture and Fisheries, The Danish Veterinary and Food Administration under act 474 of 15. May 2014 and executive order 2028 of 14. December 2020, and under consideration of the ARRIVE Guidelines (Du Sert et al., 2020).

Author contributions

GF, ML, JW, MJ, and LP: conceptualization, investigation, and methodology. GF and ML: data curation, visualization, and writing. GF: formal analysis and software. ML, MJ, and LP: funding acquisition and resources. ML and LP: project administration. MJ and LP: supervision. GF, ML, JW, MJ, and LP: writing, review, and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

We declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2022.909038/full#supplementary-material

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