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# Assessing the relationship between pigs' stress resilience and their behavior in response to weaning

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**Introduction:** As pigs are exposed to multiple stressors in production systems, we must understand their ability to be resilient to a range of environmental challenges to maintain production and welfare. Stress-resilience (SR) is the capacity to cope with and recover from stressors while maintaining healthy emotional functioning. In contrast, stress-vulnerability (SV) contributes to and predicts the onset and persistence of mood disorders and pathological processes following exposure to stress.

**Methods:** 52 focal gilts were identified through a physiological marker (cortisol) in a previous study to compare resilience to weaning stress and behavioral responses at weaning. Within pigs' home pen, we observed agonistic behavior, non-agonistic social behavior, and daily maintenance behaviors. Behavior was observed over two 4-hour periods (6 AM to 10 AM): one (D1) and four days postweaning (D4).

**Results:** On D1, SV pigs displayed a higher average frequency of non-injurious contact behavior (P = 0.0198) compared to SR pigs, while SR pigs exhibited a significantly longer average duration of lying behavior (P = 0.018) compared to SV. On D4, SV pigs exhibited a significantly longer duration of fighting behavior (P = 0.025) on average compared to SR pigs. Additionally, a significant effect of time on behavioral adaptation patterns was observed. On D1 post-weaning, pigs spent more time fighting (P < 0.001) and exploring (P < 0.001) and showed more frequent non-injurious contact (P = 0.029) and drinking behaviors (P < 0.001) compared to D4. Conversely, on D4, pigs spent more time feeding (P = 0.005) and lying (P < 0.001) compared to D1.

**Discussion:** Our findings imply that non-injurious contact and lying behaviors immediately after weaning and fighting behavior several days later may be promising indicators of pigs' ability to be resilient to the stress associated with weaning. However, to better understand how pigs change their behavior in response to the stress of weaning, we need standard approaches for measuring

their behavior and evaluating the degree of change. Understanding behavioral variation between SR and SV pigs can facilitate the development of resilience indexes that could be helpful in breeding programs, facilitating the selection of resilient pigs that overcome challenges associated with weaning.

#### KEYWORDS

temporal variation, aggressive behavior, social contact, exploration, maintenance behavior, weaning stress, resiliency

## **1** Introduction

Modern animal welfare definitions emphasize the fulfillment of the Five Freedoms, which include freedom from hunger, discomfort, pain, and distress as well as freedom to express normal behavior (Farm Animal Welfare Council (FAWC), 1993). A more recent perspective underscores the significance of the Five Domains as an evolution of the Five Freedoms (Webster, 2016). This model introduces a structured methodology for detecting compromises in four physical and functional domains - nutrition, environment, health, and behavior - in addition to a single mental domain that encompasses an animal's comprehensive welfare state, particularly in terms of its emotional well-being (Mellor and Beausoleil, 2015). However, animal welfare also goes beyond that. Animals should have the ability to adapt to changes (i.e., resilience) (Colditz and Hine, 2016) and have positive experiences (e.g., social interaction such as playing, allo-grooming, and non-injurious contact; ability to perform strongly-motivated natural behaviors such as exploring, rooting, nesting, foraging, nesting, etc) (Mellor, 2012).

Resilience refers to an animal's capacity to cope with and recover rapidly from disturbances or challenges, ensuring minimal negative effects, and is regarded as a crucial aspect of animal welfare (Colditz and Hine, 2016). Since 1960, commercial pig production has undergone significant changes, leading to many welfare challenges (Siegford, 2024). These changes have shifted housing systems from extensive, outdoor systems and high labor input (family-runs farm) to economically efficient units characterized by minimal labor input, restricted space allowance, indoor confinement, high stocking density, and slatted floor systems with little to no environmental enrichment (Pedersen, 2018; Siegford, 2024). As current husbandry systems often expose pigs to various stressors different from those they evolved to cope with, it is essential to enhance their resilience to prevent cumulative stress and associated health and behavioral issues. Optimizing resilience is significant for the overall welfare and performance of farm animals (Colditz and Hine, 2016). Providing producers, who are responsible for managing overall herd health and overseeing breeding and reproduction, with information on resilience allows them to detect instances of compromised resilience and identify the specific animals involved (Van der Zande et al., 2021). Producers

could also use a better understanding of resilience to manage animals in ways that promotes development of resilience, such as through social learning or allowing them to strengthen bonds with other pigs, including their dams (Buckner et al., 2003; Rutter, 2006). Furthermore, there is evidence of genetic control and heritable variation related to activity of the hypothalamic-pituitaryadrenocortical axis in pigs (Mormede and Terenina, 2012; Larzul et al., 2015). Thus, the capacity to discriminate between pigs exhibiting stress resilience or stress vulnerability could serve as a valuable approach for selecting more resilient pigs for future breeding (Bacou et al., 2017; Luttman et al., 2023).

The main research approaches to measuring welfare in swine have been through assessing productivity, physical health, and physiological indicators such as plasma cortisol, heart rate, and endorphin levels (Hewson, 2003). An alternative metric for the evaluation of swine welfare involves quantifying the frequency and duration of positive behavioral states, such as play (Horback, 2023), as well as negative behavioral states, such as aggressive activity. Due to their omnivorous diet, complex social systems, and utilization of multi-modal communication, pigs require multifaceted sensory stimulation to maintain positive welfare (Horback, 2023). Extensive research has been conducted on various aspects of normal behavior and activity in pigs, including social, agonistic behavior, contact, and daily behavior (Murphy et al., 2014). Furthermore, numerous studies have investigated the behavioral stress response of pigs in relation to challenging situations such as weaning or mixing (Weary et al., 2008). However, no investigation has been conducted into how pigs' stress resilience influences their behavior under stressful conditions.

Physiological changes, such as changes in blood pressure or heart rate, resulting from the activation of the hypothalamicpituitary-adrenal axis (HPA) including the subsequent release of cortisol, are frequently used as indicators of animal welfare (Candiani et al., 2008). These physiological parameters have also been used previously to measure resilience in pigs (Hermesch and Luxford, 2018). In our present investigation, we utilized the approach established by Luttman et al. (2023), which developed a methodology for identifying and characterizing pigs resilient to social stress using cortisol at several time points, to identify SR pigs, which rapidly reverted to their pre-stress status within a few days of weaning, and SV pigs, which failed to exhibit a similar recovery.

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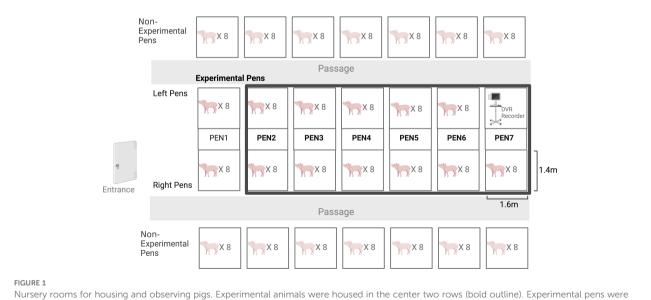
The well-being of animals can be influenced by conditions, and animal welfare is particularly compromised when animals are subjected to stressful circumstances (Dwyer and Bornett, 2004). This study exposed the pigs to an intense stressor, the weaning process. Pigs are typically weaned between 3 and 5 weeks of age (e.g., US: ~3-5 weeks of age; EU: ~4-5 weeks of age; Canada: ~3-6 weeks of age), and are sent to market at around 22-26 weeks of age (or about 6 months). Therefore, market pigs are sent to slaughter approximately five months after weaning. At weaning, piglets simultaneously transition from dependence on maternal milk and living with their litter to new food and social groups. In natural settings, this process occurs gradually, with decreased milk intake and increased solid food consumption and the sow introducing her litter to others in the herd (Weary et al., 2008; Stolba and Wood-Gush, 1989). However, in commercial farming, both of these aspects of weaning typically happens abruptly through separation from the mother, which can lead to high levels of distress behaviors (Weary et al., 2008). Weaning involves social stress (e.g., maternal deprivation, social hierarchy stress), environmental stress (new home pen environment), and abrupt dietary change (from mainly liquid milk to solid food) in addition to physical (human handling and approach) and physiological stress (changes in cortisol levels caused by various stressors). Characterizing behavioral variations between stress-resilient and stress-vulnerable pigs induced by weaning stress has the potential to contribute to future breeding programs by facilitating the selection of robust pigs (Luttman et al., 2023). Further, this knowledge can be used to develop better weaning management practices for swine producers, thereby enhancing overall productivity. Our goal in the present study was to investigate pigs' home pen behavior in the nursery, with a specific focus on examining their behavioral response to and recovery from weaning stress in relation to physiological resilience. We hypothesized that pigs exhibiting stress resilience would display distinct behavioral patterns compared to those showing stress vulnerability, with differences in play behaviors and types of physical contact influenced by time since weaning.

## 2 Methods

### 2.1 Animals and housing

We employed the identical animal settings as those utilized in Luttman et al. (2023). Briefly, pigs were housed at the Michigan State University Swine Teaching and Research Center located in East Lansing, MI, USA. These animals consisted of female pigs from 26 litters obtained by crossing parity 2 or 3 purebred Yorkshire dams with the PIC359 sire line (PIC, Hendersonville, TN, USA). The selected litters contained at least 5 gilts with an average of 7 gilts per litter (range: 5-12 gilts). We used three groups of sows (replicates) that farrowed at different times (Rep 1 = farrowed Sep 12-14, 2021; Rep 2 = farrowed Nov 28 - Dec 3, 2021; Rep 3 = farrowed March 13-18, 2022). Replicate 1 consisted of 4 litters, replicate 2 had 13 litters, and replicate 3 included 9 litters. Only one sex (gilt) was selected to minimize variation since sexually dimorphic stress responses are common in pigs (Fardisi et al., 2023; Pluske et al., 2019). At 4 weeks of age, the pigs of each replicate were weaned by relocating them to identical nursery rooms and placing them in 1.6m x 1.4m pens (n = 8 pigs/pen) with metal slatted flooring (Figure 1). For each new social group created in a nursery pen, groups of 2-3 littermates were included. To maintain an equal stocking density of 8 pigs/pen across all pens, non-study females of comparable weights were added to pens as needed (mean = 8.38 kg; min = 4.95 kg, max = 11.91 kg). Prior to weaning, piglets were marked on their backs using a non-toxic black marker for later identification in video analysis.

The pigs were exposed to full LED lighting for 8 hours each day, followed by 16 hours of reduced lighting provided by auxiliary LED



identical in size. Each pen was assigned a number, with higher numbered pens positioned further from the door to the room. Across all replications, focal pigs were not placed in pen 1.

sources. Pigs were given ad libitum access to a diet that met or exceeded the nutritional requirements for their age and weight. Water was also available ad libitum, supplied through one nipple drinker per pen. Prior to the experiment, the pigs underwent standard handling procedures, which included teeth clipping, ear notching, tail docking, and iron injection on the second day post-birth.

## 2.2 Focal pigs

Fifty-two focal gilts from the litters described above were used. These animals were selected during a previous study in which our group selected one stress-resilient (SR) and stress-vulnerable (SV) pig from each of 26 litters (Luttman et al., 2023). In short, blood was collected from each gilt from 26 litters (n = 170) on three occasions surrounding weaning: 1 day before weaning (baseline), on the day of weaning (acute), and four days after weaning (recovery). For blood sampling, piglets were restrained on a blood collection table by two researchers, with a blood collection time median of 59 seconds and an average of 75 seconds. The cortisol levels measured during the acute and recovery stages for each gilt were converted to percent changes from baseline values at each stage (Luttman et al., 2023). For analysis of the gilts' recovery over time, the difference between the relative acute and relative recovery values was calculated as the total recovery value. Gilts were then ranked based on their total recovery value within each litter. Luttman et al. (2023) visually evaluated the cortisol response pattern of each

| TABLE 1 | Ethograms | of | pigs' | home | pen | behaviors. |
|---------|-----------|----|-------|------|-----|------------|
|---------|-----------|----|-------|------|-----|------------|

gilt within every litter to confirm that the selected focal gilts accurately represented the intended pattern. The gilt displaying the highest total recovery value within a litter was designated as the SR focal pig, while the gilt with the lowest total recovery value was identified as the SV focal pig (resulting in two focal pigs selected from each litter). The cortisol levels in all gilts considered for selection varied from 38 to 210 ng/mL, with an average of 110 +/- 34 ng/mL (Luttman et al., 2023). It is crucial to emphasize that focal pigs were chosen per litter, given the absence of a specified 'normal' or reference range for cortisol levels in pigs, and considering that both the assay method and the pig breed may influence cortisol levels. Also of importance is that SR and SV pigs were of similar weights both during pre- and post-weaning phases (Luttman et al., 2023).

## 2.3 Video and data collection

Cameras (4K Motorized Varifocal HD IP Bullet Security Camera, Lorex, Linthicum, MD) were mounted on the ceilings above the pens, at least 24 hours before recording. Prior to each recording, the camera lenses were thoroughly cleaned, and the connections were carefully inspected to ensure optimal performance. Recordings were captured and stored using an NVR system (4K Ultra HD NVR, Lorex, Linthicum, MD). For the present study, behavioral observations were performed during two 4-h periods with respect to the weaning day (D0): one day after weaning (D1) and four days post-weaning (D4). On D1 and D4,

| Behavior type          | Behavior              | Description  |  |
|------------------------|-----------------------|--|--|
| Agonistic behavior     |                       |  |  |
| State                  | Fighting              | Any activity indicative of agonistic behavior or social conflict. Includes mutual aggressive interaction between two or more piglets that may result in injuries on the body of one or both piglets. Agonistic behaviors include: mutual pushing (parallel or perpendicular), biting, chasing, mounting, head-to-head knocks, head-to-body knocks, ramming or pushing of the opponent with the head, or lifting others by pushing the snout. |  |
| Event                  |                       | Contact by one pig results in a negative reaction from the recipient pig, indicating this was a painful or unpleasant contact behavior.  |  |
|                        | Injurious Contact     | (Injurious biting) Chewing or biting the ear, tail, vulva, or body part of another pig in a way that causes a pain withdrawal response or visible skin damage.   |  |
|                        |                       | (Belly nosing) Nosing, nudging another pig's belly with repetitive up and down snout movements   |  |
| Non-agonistic behavior |                       |  |  |
| Event                  |                       | The recipient should not react negatively to the touch, indicating this was a non-injurious contact behavior. Touching behavior includes allo-grooming, nosing body, nosing head, nose-to-nose contact, nosing anogenital and etc.   |  |
|                        | Non-injurious contact | (Allo-grooming) touching other pig's head, ears, tail, legs, or rump with nose disk, possibly including gentle manipulation with snout (nibbling) and mouth but not biting injuriously.  |  |
|                        |                       | (Nosing body) touching or nudging the body of a pen mate with snout, not including contact<br>with head, ears, and anogenital region. If repetitive nosing of belly occurs, score as Belly Nosing.   |  |
|                        |                       | (Nosing head) touching or nudging the head and/or ears of a pen mate with snout.   |  |

(Continued)

#### TABLE 1 Continued

| Behavior type          | Behavior   | Description  |  |  |
|------------------------|------------|--|--|--|
| Non-agonistic behavior |            |  |  |  |
|                        |            | (Nose-to-nose contact) touching another pen mate's snout with own snout.   |  |  |
|                        |            | (Nosing anogenital) touching, rubbing, or licking the anogenital region of a pen mate with snout.  |  |  |
| Daily behavior         |            |  |  |  |
| State                  | Feeding    | Pig's mouth and head are in the feeder suggesting ingestion of feed is occurring   |  |  |
| State                  | Lying down | Lying on the floor in any posture (sternal or lateral recumbency), may be sleeping (lying with eyes closed), lying inactive or simultaneously engaged in other behaviors such as interacting with pen mates, floor, walls or other pen elements while lying. |  |  |
| State                  | Exploring  | Investigating surrounding environment by nudging, rooting, sniffing, scratching, or chewing alone or with one or more pen mates  |  |  |
| Event                  | Drinking   | Pig's mouth is seen touching the drinker or head is positioned in such a way that indicates drinking is occurring  |  |  |
| State                  | Playing    | (Social play) Scampering, pivoting, running, head tossing, flopping or hopping together with at least one other pig  |  |  |
|                        |            | (Solitary play) Same actions as above but done on own  |  |  |

videos were watched for 4 hours from 6 AM to 10 AM, and data were recorded using a continuous sampling method. The analysis focused on observing behaviors within pigs' home pen, specifically agonistic behavior, non-agonistic social behavior, and daily behavior. The ethogram of target behaviors is presented in Table 1.

## 2.4 Video decoding

Three decoders collected data from 52 individual focal pigs using recorded video footage. Prior to starting analysis, all researchers underwent training to minimize observational errors and enhance reliability. The Observer XT (Noldus, Wageningen, The Netherlands) program was utilized for the decoding of behaviors using video recordings. A test of interobserver reliability was performed halfway through the video analysis. The interobserver reliability was calculated using Cohen's Kappa, resulting in average values of 0.94 for duration and 0.78 for frequency, indicating near perfect and substantial agreement, respectively.

## 2.5 Statistical analysis

To assess the effect of stress resilience designation (SR or SV), the passage of time (Day; D1 and D4) since weaning, and the interaction of these factors on behavior, a two-way ANOVA with repeated measures and an incomplete block design was conducted using R v4.2.1 (R Core Team, 2022). Due to the unbalanced design of the data, we used the lmer function to fit the model for continuous data from the lme4 package in R to fit the model with covariates as block effects. A generalized linear mixed model with a negative binomial distribution was used to analyze the count data. We then applied a Type II ANOVA with Wald chi-square tests using the Anova function from the car package. Wald chi-square tests are suitable for evaluating main and interaction effects without requiring balanced data, though they differ from traditional ANOVA F-tests. We employed an incomplete block design since our experimental design was unbalanced (i.e., pens did not include equal numbers of SR and SV as nursery pen compositions were determined before the pigs were classified). In this model, a pigs' stress resilience designation (SR or SV) and time were the fixed effects. The response variable was pigs' home pen behavior (duration or frequency) (as listed in Table 1). Random effects included pen position, pen composition, litter, and replicate. Pen composition refers to the unique social group within each pen, which was different for each repetition while pen position remained constant. Observations of solitary and social play behaviors on both D1 and D4 contained excessive zeros, prompting us to assess whether these data were suitable candidates for a zero-inflated model. We conducted a goodness-of-fit test using the testZeroInflation function from the DHARMa package, which confirmed significant zero-inflation (P < 0.01). As the variables were continuous, we applied a zero-inflated model assuming a generalized gamma distribution. This approach reflected the structure of the data, which we describe as zero-augmented to capture its characteristics accurately. Figures showing mean and standard error of the mean (SEM) were generated in R using grouped summaries derived from the raw data. For each group, the mean was calculated, and SEM was obtained by dividing the standard deviation by the square root of the sample size. This method was consistently applied to both continuous (duration) and discrete (frequency) data.

## **3** Results

Our analysis revealed that fighting behavior was influenced by both factors (stress resilience designation and day) and also

demonstrated that there was a significant interaction effect between the two factors (Table 2). Pigs with a stress-vulnerable designation showed a greater duration of fighting behavior compared to those with stress-resilience designation, with the difference in behavior duration between the two groups being smaller on D4 than on D1 (Figure 2). Additionally, a tendency for an interaction effect was observed between the two factors on injurious contact behavior (P = 0.055; Table 2, Figure 3) with injurious contact behavior increasing over time for SR pigs and decreasing over time for SV pigs. However, injurious behavior was not affected by stress resilience designation alone (Table 2) while a tendency was found for injurious contact to increase from D1 to D4 (P = 0.069, Table 2, Figure 3).

There were several behaviors including fighting behavior significantly influenced by the day such as feeding, lying down, exploring, and drinking behaviors (Table 2). On D1 post-weaning, pigs exhibited a higher frequency/duration of non-injurious contact, exploring, and drinking behaviors compared to D4 (Figure 4).

| TABLE 2  | Behavioral differences by stress resilience designation, day | уð |  |  |  |
|--|--|----|--|--|--|
| stress resilience designation x day interaction. |  |    |  |  |  |

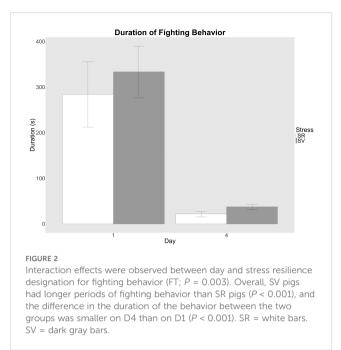
| Behavior                              | Factors             | Chi-square | p-value            |
|---------------------------------------|---------------------|------------|--------------------|
|                                       | Stress <sup>1</sup> | 8.975      | 0.003*             |
| Fighting<br>(Duration)                | Day                 | 15.532     | <0.001*            |
|                                       | Interaction         | 11.881     | <0.001*            |
| Injurious                             | Stress              | 0.114      | 0.736              |
| Contact                               | Day                 | 3.300      | 0.069 <sup>t</sup> |
| (Frequency)                           | Interaction         | 3.685      | 0.055 <sup>t</sup> |
| Non-injurious                         | Stress              | 0.851      | 0.356              |
| Contact                               | Day                 | 4.763      | 0.029*             |
| (Frequency)                           | Interaction         | 1.775      | 0.1828             |
|                                       | Stress              | 0.341      | 0.560              |
| Feeding<br>(Duration)                 | Day                 | 7.708      | 0.005*             |
|                                       | Interaction         | 0.094      | 0.759              |
|                                       | Stress              | 0.346      | 0.556              |
| Lying down<br>(Duration)              | Day                 | 16.202     | <0.001*            |
|                                       | Interaction         | 0.373      | 0.541              |
|                                       | Stress              | 0.195      | 0.659              |
| Exploring<br>(Duration)               | Day                 | 13.549     | <0.001*            |
| · · · · · · · · · · · · · · · · · · · | Interaction         | 0.054      | 0.816              |
|                                       | Stress              | 0.416      | 0.519              |
| Drinking<br>(Frequency)               | Day                 | 77.780     | <0.001*            |
|                                       | Interaction         | 0.572      | 0.449              |

<sup>1</sup> Stress resilience designation (SR and SV) is denoted as 'Stress' in the table.

\*Significant difference due to factors (stress resilience designation and day) for each behavior, with P < 0.05 set as threshold for significance.

<sup>t</sup>Tendency for significant differences, with P > 0.05 and < 0.10.

Model intercept estimates, coefficient, and confidence intervals are presented in Supplementary Table 2.



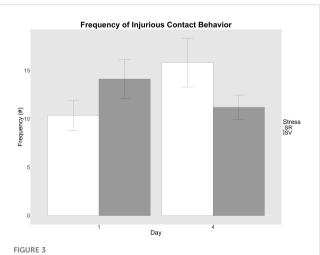
However, pigs on D4 showed longer duration of feeding and lying down behaviors when compared to D4 (Figure 4B).

Due to overconvergence, coefficients could not be estimated for solitary or social play behaviors based on pigs' stress designation, except for social play on Day 4, which did not differ significantly between SR or SV (P = 0.237).

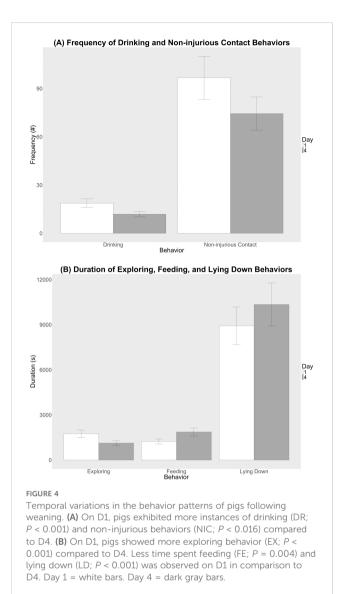
## 4 Discussion

### 4.1 Temporal variations in behavior

The current study revealed significant temporal variations in the behavioral patterns of pigs following weaning, encompassing



A tendency for interaction effects was observed between day and stress resilience designation on frequency of injurious contact behavior (INC; P = 0.055) as SR pigs performed more injurious contact over time while SV pigs performed less injurious contact over time. SR = white bars. SV = dark gray bars.



fighting, non-injurious contact, feeding, lying down, exploring, and drinking behaviors. Behavioral patterns in newly weaned piglets have been extensively investigated, and our findings are in accordance with the outcomes of previous studies.

Under natural conditions, the process of weaning takes place gradually in pigs, and it is completed about 16 weeks after birth (Jensen, 1988). However, under managed conditions, such as in an intensive production environment, weaning occurs abruptly as piglets are moved from the farrowing environment to nurseries, out of contact with their dam. Piglets that undergo abrupt weaning must deal with a negative energy balance due to a combination of low intake of solid feed and high levels of activity. Newly weaned piglets are initially restless in their new environment (Widowski et al., 2008) and exhibit high levels of aggressive and exploratory behaviors, which subsequently decline over time (Besteiro et al., 2018; Bornett et al., 2000). In the current study, we observed more active behaviors on D1, particularly in behaviors such as fighting, non-injurious contact, and exploration compared to D4. Less activity on D4 could be explained in relation to the establishment of a stable social hierarchy occurring within a few days following weaning, which facilitates the stabilization of activity levels after the fourth to fifth day post-weaning, as demonstrated in both our current study and a previous study (Besteiro et al., 2018). This observation could also potentially be attributed to the higher durations of lying down on D4 compared to D1 observed in the present study.

In terms of non-injurious contact behavior, nosing was the main element of our non-injurious contact behavior category. Nosing behavior comes in several forms, including gentle touches (e.g., nose touching any part of another pig as during social grooming) as well as nose-to-nose contact, and it is involved in nearly all social interaction among pigs (Camerlink and Turner, 2013). Nosing is often considered to be an affiliative behavior (Goumon et al., 2020). This behavior is pivotal in strengthening social bonds through communication, acknowledgment, social grooming, and the maintenance of dominance relationships (Spruijt et al., 1992). Hence, it can be postulated that nosing behavior, serving as an indicator of affiliative or social interaction behavior, decreased over time as mutual understanding between individuals increases.

On the other hand, since nosing can take several forms in pigs (Portele et al., 2019), and these are not always differentiated in research studies, it can be difficult to determine absolutely if the type of nosing in each case was likely to be affiliative, neutral, or negative (Candiani et al., 2008). Furthermore, without a clear indication of its role in facilitating positive social interactions or stable social relationships, the underlying motivational reasons and the social function for the performance of nosing behavior remain unclear (Camerlink and Turner, 2013; O'Malley et al., 2022). For these reasons, we examined the recipients' responses (e.g., no reaction, negative or positive reaction) to the giver's behavior in order to distinguish between non-agonistic behavior and agonistic behavior as described in the ethogram. However, definitive categorization was not always possible. Even though we classified the pigs' nose contact behavior as non-agonistic behavior, there is a possibility that it may be a subtle form of agonistic behavior as a previous study by O'Malley et al. (2022) reported a positive correlation between nosing behavior and the duration of both total aggression and initiated aggression. This suggests that nosing behavior could potentially be categorized as a form of agonistic behavior. Due to these reasons, nosing behavior can also be considered as an integral component of fighting behavior, exhibiting similar behavioral patterns as those observed in fighting behavior. In conclusion, we need to be cautious in interpreting nosing behavior, as the valence of this behavior can vary between positive and negative depending on contextual factors.

There were opposite patterns of activity in feeding and drinking in the current study. Piglets displayed less feeding behavior on D1 as compared to D4. Conversely, significantly more drinking behavior was observed on D1 when compared to D4. There is an agreement between these findings and the outcomes of earlier studies. A variety of mechanisms must be in place before weaned piglets can transition from suckling milk to ingesting solid food, including the ability to detect, ingest and masticate food (Widowski et al., 2008). However, despite the fact that piglets possess these abilities before weaning, intake of solid food is typically very minimal prior

to weaning in commercial systems (Widowski et al., 2008). According to Brooks et al. (1984), during the initial days postweaning, piglets exhibited low feed intake, and it takes approximately 2-3 days after weaning for feed intake to increase (Dybkjær et al., 2006), while concurrently, water intake was elevated compared to subsequent days. These observations indicate a limited negative correlation between drinking and feeding behaviors in the early post-weaning period. During the transitional phase from a predominantly liquid milk diet to the assimilation of calories through solid food, piglets may compensate for their lack of solid food consumption by ingesting more water to achieve gastrointestinal fill (Brooks et al., 2001). Additionally, the act of drinking water via a standard nipple drinker might provide some satisfaction to the piglets due to similarities in motor patterns to suckling, which they were familiar with during the nursing period (Torrey et al., 2009).

Our investigation demonstrated that pigs' post-weaning behaviors, such as fighting, non-injurious contact, exploring, lying-down, feeding, and drinking, followed patterns expected in a commercial-style environment.

## 4.2 Exploring the relationship between behavioral differences and stress resilience

In our study, we found that fighting behavior was related to the pigs' physiological stress resilience designation. SV pigs spent more time in fighting behaviors compared to SR pigs. These findings may suggest that SV pigs exhibit stress responsiveness more adversely compared to SR pigs. In commercial pig production settings, a significant number of pigs experience regrouping with unfamiliar conspecifics, typically accompanied by relocation to alternative pens. This mixing of unfamiliar pigs typically leads to intense fighting to establish a new dominance order (Peden et al., 2018), which is known to be highly stressful for pigs (Camp Montoro et al., 2021). SV pigs continued to spend more time fighting compared to SR pigs on D4, which is longer than most pigs take to establish a hierarchy (Yuan et al., 2004), indicative of an ongoing heightened stress response. Further research is needed to determine whether heightened behavioral and physiological (Luttman et al., 2023) responses in these pigs occur in parallel or whether one precedes the other. For instance, the increased duration of fighting among SV gilts that persists over time may contribute to a psychological state of social defeat, consequently affecting their physiological response and leading to their classification as SV pigs. In a previous study, Luttman et al. (2023) observed no significant difference in the body weight of SR and SV pigs during either pre-weaning or postweaning growth phases (Luttman et al., 2023). When these pigs were mixed into new pens, body weight was deliberately considered to create pens with pigs of similar weight to minimize impacts of weight on subsequent behavior, such as on aggression as the pigs established a new social hierarchy. This allowed us to focus more specifically on impacts of SR and SV or SV status in the pen and to minimize the confound of differences in weight contributing to differences in competitive ability.

# 4.3 Understanding individual variability and establishing behavioral benchmarks

Biological parameters (e.g., growth curves, diarrhea scores, and hematological measurements) have been validated in previous research assessing weaning resilience (Revilla et al., 2019). Our results suggest that fighting behavior in the days after weaning is also a promising behavioral indicator of pigs' resilience to weaning stress. However, individual animals act differently for various reasons, including their unique temperaments, behavioral strategies, communication styles, formative experiences, and the unpredictable nature of their environments (von Borell and Raoult, 2024). Understanding these differences is important for studying animal behavior. Based on previous studies, the most practical approach to evaluating variability involves establishing a benchmark for comparison with behavior (Smith, 1960). At present, precise interpretation of post-weaning behavioral changes in pigs presents challenges due to the absence of defined benchmarks distinguishing healthy coping responses from unhealthy manifestations of these behaviors. Thus, there is a necessity to establish adaptive, healthy behavioral ranges for comparative analysis and establishing a connection to degrees and types of stress experienced by pigs and their overall resilience. Furthermore, this approach could enable the selection of resilient pigs for future breeding and more accurate assessment of welfare.

## 5 Conclusion

We conducted this study primarily to investigate pigs' behavior in the home pen, focusing on their behavioral response to and recovery from weaning stress over time and relating it to their physiological resilience (SR and SV). Behaviors such as fighting, non-injurious contact, lying down, exploring, feeding, and drinking changed in ways that could indicate that the social hierarchy of piglets became more stable over time and indicate adaptation to post-weaning life. However, observed behaviors were not associated with stress resilience designation, except for fighting behavior.

These findings indicate that fighting behavior in the days after weaning is a promising behavioral indicator of resilience to weaning stress. However, the behavioral changes of pigs as they adapt to and recover from stresses associated with weaning can be difficult to interpret due to the variability in their behaviors. Therefore, the comprehension of this variability holds significance in the examination of animal behavioral patterns. To evaluate variability, it is essential to formulate a benchmark for behavioral comparison and define a targeted behavioral range for distinguishing between healthy adaptive responses and unhealthy expressions. It may also be worthwhile to establish a correlation between behavioral benchmarks and stress levels in pigs. By developing a method to evaluate behavioral patterns relating to the resilience of piglets to weaning, we would be able to better understand how these differ between SR and SV pigs and provide ethologically meaningful aspects that would be useful in helping to assess their adaptation after weaning.

## 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**

The animal study was approved by Michigan State University Institutional Animal Care and Use Committee (PROTO202100135). The study was conducted in accordance with the local legislation and institutional requirements.

# Author contributions

BL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. AL: Conceptualization, Funding acquisition, Methodology, Project administration, Writing – review & editing. CE: Project administration, Writing – review & editing, Conceptualization, Funding acquisition. NR: Project administration, Writing – review & editing, Resources. SO: Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. JS: Conceptualization, Data curation, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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# **Conflict of interest**

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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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.2024. 1461526/full#supplementary-material

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