

BEHAVIORAL INDICATORS OF PIGS' RESILIENCE TO WEANING STRESS: DEFENSE
CASCADE AND OBSERVING HOME PEN BEHAVIORS

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ABSTRACT

Given that pigs in modern intensive farming systems encounter a range of stressors, it is vital to gain insights into the pig's resilience, which is the ability to return to pre-stress status, across various environmental factors. The aim of the thesis was to assess whether behavioral responses differed between pigs designated as stress-resilient (SR) or stress-vulnerable (SV). We examined the relationships between resilience or vulnerability to weaning stress and 1) pigs' behavioral response to an auditory startle test, and 2) pigs' behavior in the home pen, focusing on their behavioral response to and recovery from weaning stress. In the first study, we conducted a startle test on 7-week-old pigs ($n = 52$) to evaluate their behavioral responses. The data revealed no connection between the response to the auditory startle test and resilience or vulnerability to weaning stress. In the second study, we focused on observing behaviors (d1 & d4 post-weaning) of focal pigs ($n = 52$) within their home pens in response to weaning. We found behavioral differences associated with stress resilience. On d1, SV pigs had more non-injurious contact ($P = 0.0198$) but less lying down behavior ($P = 0.018$) than SR pigs. On d4, SV pigs showed longer fighting behavior ($P = 0.025$) 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 ($P = 0.013$) and drinking behaviors ($P < 0.001$) compared to d4. Conversely, on d4, pigs spent more time feeding ($P = 0.004$) and lying down ($P < 0.001$) when compared to d1. In summary, this thesis enhances our comprehension of evaluating pigs' welfare through their affective state, behavioral responses to challenging situations, as well as physiological resilience, providing opportunities to further improve pig welfare in the future by targeting management and breeding strategies based on resilience.

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TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION	1
CHAPTER 2: ASSOCIATIONS BETWEEN RESILIENCE TO WEANING STRESS AND STARTLE RESPONSE IN NURSERY AGE GILTS	10
CHAPTER 3: ASSESSING THE RELATIONSHIP BETWEEN PIGS' STRESS RESILIENCE AND THEIR BEHAVIOR IN RESPONSE TO WEANING	34
CHAPTER 4: SUMMARY AND CONCLUSIONS	55
REFERENCES	59

CHAPTER 1: INTRODUCTION

LITERATURE REVIEW

Challenges and considerations in modern pig farming

Pork is one of the most popular meat products in the world (FAOSTAT 2018), and demand is expected to increase with the growth of the global economy and population in the future (FAO 2017, Ottosen et al., 2020). There are a wide variety of rearing methods used in the production of pigs (Degré et al., 2007, Bonneau et al., 2011), with the dominant form of pig production systems characterized by closed buildings and slatted floors (Delsart et al., 2020). In modern intensive pig farming, compared to pigs in their natural habitat, there is relative restriction of their ability to wander freely during the day and sleep in a spacious area at night (Fraser, 1984). The ability of pigs to express their full range of species-specific behaviors and engage in voluntary social interaction is undermined when they are confined in pens or stalls (Millet et al., 2005). Hence, although food safety and sensory quality have remained significant for pork consumers, the well-being of production pigs has emerged as a significant area of concern (Millet et al., 2005). In various countries, including Brazil (Yunes et al., 2017), the United States (Sato et al., 2017), Canada (Ryan et al., 2015), and Europe (Krystallis et al., 2009), consumers are indicating their desire for animals to be raised in free-range environments, allowing them unrestricted movement. In both the United States and Europe, there is a growing emphasis on incorporating the "natural" aspect into farming practices, which includes providing pigs with outdoor access (Sato et al., 2017). This type of outdoor system, however, presents a greater challenge to managing feeding, watering, temperature, predators, biosecurity, animal health, and food safety.

It is common for commercially raised animals to experience environmental stress due to

their housing conditions, particularly those that are raised in closed systems, such as pigs that are housed indoors (O'Connor et al., 2010). In this housing system, despite being gregarious animals, pigs can experience social stress when mixed with unfamiliar conspecifics, during weaning when piglets and dams are separated, and during regrouping of pregnant sows later in life (Gimsa et al., 2018). Pigs are often unable to express their strongly motivated behaviors important for their health, reproduction, or welfare such as exploration, social interaction, playing, foraging, maternal care, etc. In most cases, this problem is caused by limited housing space, as pigs are densely stocked on commercial farms (Edwards et al., 1988, Turner et al., 2000), and may be exacerbated when limited environmental resources are present that allow expression of behaviors such as foraging or wallowing (Studnitz et al., 2007). In addition, management procedures such as tail docking, ear notching, mixing, or moving animals can be stressful to pigs. The stressors experienced due to housing systems and management could adversely affect pigs, for example, impairing their growth or immune function and subsequently altering how they express essential behaviors (Ludwiczak et al., 2021).

Weaning techniques are frequently employed to enhance sow productivity within modern intensive farming systems (Tang et al., 2022). This approach can lead to an increased annual number of piglets, improved utilization of breeding equipment, and greater economic gains for breeding enterprises (Campbell et al., 2013). However, weaning is a combination of multiple stressful events occurring early in pigs' lives. Piglets are simultaneously removed from the sow (i.e., maternal deprivation), mixed with unfamiliar pigs, moved into a new environment, approached/handled by humans, and switched from milk to solid food diets (Dudink et al., 2006, Sutherland et al., 2014, Kerschaver et al., 2023). Thus, weaning affects the physiology, immune system, and growth performance of pigs (Brown et al., 2006). Additionally, weaning results in a

high incidence of maladaptive behaviors such as belly nosing or biting limbs, ears, tails, and tails of other pen mates (Oostindjer et al., 2011).

Resilience in pig farming

Over the last decade, there has been interest regarding the resilience and adaptability of animals to environmental influences (Klopčič et al. 2009, Hermes and Dominik 2014). We need to understand pigs' capacity to adapt or be resilient to a broad range of environmental challenges to maintain performance in the face of the multiple stressors pigs are exposed to in production systems (Colditz and Hine, 2016). Three terms with slightly different meanings can be used to describe the ability of pigs to adjust to their surroundings while maintaining consistent performance: 1) Resistance, 2) Tolerance, and 3) Resilience. Resistant animals have been described as possessing immunity against a specific pathogen, actively reducing infection pressure to effectively combat infections (Rodríguez Arriola, 2017). On the other hand, tolerant animals may become infected by the disease, but they experience minimal negative effects as a result of the infection (Råberg et al., 2007). Resistance and tolerance are usually mentioned in relation to infectious diseases, whereas resilience goes beyond that. The concept of resilience to parasitic disease in farm animals was initially recognized by Clunies Ross (1932) 90 years ago. However, in recent years, a broader definition of resilience has been developed in animal and human sciences that encompasses both the response of individuals to disease challenges as well as their responses to environmental and social stressors (Russo et al. 2012, Wu et al. 2013, Hermes and Dominik 2014).

The World Organisation for Animal Health (2014) has introduced a definition of animal welfare, which encompasses resilience: "Animal welfare pertains to how well an animal is adapting to the conditions in its living environment." Pigs with a high degree of resilience are

less likely to develop behavioral and health issues after encountering disturbances and can also recover quickly from negative disease experiences, as compared to pigs that are either resistant or tolerant. Therefore, resilient animals should both experience good welfare themselves and benefit the farmer through sustained production, and resilient pigs should be preferred in the pig farming industry. As there is no way to completely eliminate all challenges that livestock are exposed to, the identification of resilient pigs through physiological, biological, and ethological criteria, followed by the selection of these animals, plays a crucial role in enhancing the fitness, performance, and welfare of farm animals (Colditz and Hine, 2016, Luttman et al., 2023).

Understanding animal welfare; concept and measurement

Animal welfare as a scientific term

Animals have various requirements for survival and well-being, which arise from the multiple functional systems that enable life. Animal motivation is affected by the presence of a need, which in turn leads to behavioral and physiological responses that should result in addressing that need. As a result of these coping mechanisms, the animal is able to maintain mental as well as physical homeostasis. Coping encompasses the routine regulation of bodily functions as well as immediate, high-activity responses, including heightened adrenal activity, increased heart rate, or instinctual flight behaviors. The welfare of animals is undeniably influenced by both the inability to cope and the difficulty in coping (Broom, 1991), and the state of welfare for an individual is determined by its ability to adapt to its environment (Broom, 1986a). In the field of animal welfare research, the main challenge has been to find a way to assess the well-being of animals scientifically and objectively (Dawkins 2003).

Expanding the scope of animal welfare: beyond physical health

It is worth noting that, historically, veterinarians and farmers primarily viewed animal

welfare in terms of the animals' physical health and their living conditions, such as shelter and nutrition (Blood and Studdert, 1988, Hewson, 2003). In the past, studies on animal welfare also concentrated on physical aspects, utilizing physiological indicators like endorphin levels, plasma cortisol, and heart rate to assess how well the animal was adapting to its surroundings (Hewson, 2003).

There are, however, limitations to a view of animal welfare based solely on physical condition and physiological indicators. First, it should be noted that some physiological parameters (e.g., heart rate or plasma cortisol) are difficult to interpret since they can increase under both positive and negative circumstances (e.g., the presence of a mate or of a predator). There is also the confound that genetics and environmental factors can produce desirable physical outcomes even when an animal's mental state has been compromised. Finally, when considering only physical health, we may underestimate the complexity of animal welfare by ignoring other aspects important to animals' quality of life. Therefore, a more comprehensive approach to assessing animal welfare encompasses considerations such as mental health, behavioral patterns, and more. As a result, animal welfare should not be limited to the animal's physical condition but also to considering its mental state. In the five domains framework, originally developed by Mellor and Reid in 1994, the mental state component is critical to welfare as it represents a synthesis of the animal's overall experience. This mental state is interpreted as the overall affective outcome derived from both negative and positive experiences, which are influenced by internal conditions or external circumstances in the first four domains (nutrition, environment, health, and behavior) (Mellor et al., 2015). Affective states refer to sensations, sentiments, or emotional conditions like joy, happiness, contentment, sadness, frustration, anger, and fear (Boissy et al., 2007) that possess a "valenced" quality, signifying they

can be either positive/preferred or negative/aversive in nature. The precise evaluation of emotional or affective states serves as the fundamental basis for scientific inquiries into the mental health and welfare of animals (Mendi and Paul, 2020).

A behavioral approach to studying the emotion and mood of pigs

It is now widely accepted that animals have feelings, such as negative and positive affective states. From an evolutionary standpoint, emotions are seen as adaptive mechanisms shaped through recurrent experiences. Emotions are believed to have originated from fundamental abilities that helped animals avoid threats and seek resources (Panksepp, 1982), playing a crucial role in motivating and guiding behavior (Rolls, 2000). Pigs, being social animals often kept in groups, can experience emotional contagion between individuals, which can influence their welfare (Reimert et al., 2013). Hence, it is essential to precisely measure emotional state and emotional contagion in pigs to effectively evaluate their welfare requirements (Murphy et al., 2014). Behavior has been used as a proxy measurement of emotion because the behavioral aspect enables the animal to react to the stimulus that triggers emotions, and thus, assessing this aspect may provide insight into the intensity and valence of the emotion (Murphy et al., 2014).

Assessing defense cascade response as a tool in animal welfare assessment

In recent years, increasing interest in positive emotions within the fields of neuroscience and psychology has spurred the emergence of novel areas of research, encompassing both theoretical and experimental dimensions. Effectively expressing positive emotions typically involves a temporal sequence: it begins with exposure to a rewarding environment, triggering cognitive evaluations within the central nervous system, and resulting in physiological and behavioral responses (Boissy et al., 2007). Nevertheless, despite the endeavors of pioneering

researchers, there has been relatively limited progress in enhancing our comprehension of positive emotions in comparison to their negative counterparts, like fear (Fraser, 1995, Boissy et al., 2007). Certainly, the study of emotions in humans faces a similar tendency. Just like in animal research, the field of human psychology, particularly in the study of well-being, has been predominantly focused on stress studies. This tendency likely stems from the fact that negative experiences tend to manifest with greater intensity than positive emotions, making them more accessible subjects for investigation (Boissy et al., 2007). Positive emotions stimulate social engagement behaviors, while negative emotions, often triggered by threats, provoke defensive reactions (Lang et al., 2013).

Various instinctual behaviors, such as fear, anger, and disgust, are associated with negative emotions (McDougall et al., 1908). To evaluate an animal's negative emotional state, a defense cascade response has been employed as a potential indicator. From an evolutionary perspective, the components of the defense cascade represent primitive emotional states—coordinated patterns of motor, autonomic, and sensory responses—that can be automatically activated in the face of danger (Kozłowska et al., 2015). The defense cascade process follows a relatively universal sequence observed in various species, including pigs: commencing with arousal, marked by an immediate response to stimuli, followed by fight or flight, moments of freezing (sometimes involving vigilant monitoring of the source of danger), and ultimately returning to either prior behavior or adopting a defensive posture that can lead to a state of collapsed or quiescent immobility (Statham et al., 2020). Variations in defense cascade responses are evident, and these variations may serve as novel indicators of emotional valence and, thus, well-being. It is likely that this variability is a result of a combination of psychological factors, including temperament, environmental conditions, and genetic predispositions, that have an

impact on an animal's present emotional state. For instance, increased defense cascade (startle) responses have been observed in humans (Vrana, 1994; Bradley et al., 2001), rodents (Koch, 1999), and monkeys (Winslow et al., 2002) when they experience negative emotional states. Consequently, the defense cascade response offers a comprehensive assessment of how specific individuals, with their unique experiences and genetic backgrounds, are impacted by their current circumstances (Statham et al., 2020).

Pigs exhibit distinctive defense cascade behaviors when they are unexpectedly startled. This typically consists of a sudden, full-body startle reaction with a shift to a tense standing posture, occasionally accompanied by a barking vocalization (Blackshaw et al., 1998). Subsequently, pigs enter a phase of immobility or freezing, during which they seem to be attentively monitoring or trying to identify the origin of the disturbance. These reactions conclude either when the pig flees to escape from the situation or returns to its previous activities. Thus, the defense cascade could be a potential indicator of pigs' affective states, and variation in their response could provide information about affective valence and hence welfare (Statham et al., 2020).

Aim and outline of this thesis

In this thesis, we investigated the connection of an animal's stress resilience to its behavioral responses. While the defense cascade response and normal behavior of animals have been widely used as indicators of welfare, research considering resilience as an important component in modern farming systems is still lacking. In Chapter 1, we explored the connection between pigs' startle response (defense cascade) and their resilience to weaning. The hypothesis was that pigs would respond differently to an auditory stimulus during a startle test depending on whether they were classified as stress-resilient or stress-vulnerable at the time of weaning. In

Chapter 2, we investigated how pigs' behavior during the weaning process related to their resilience during this transitional phase. We hypothesized that pigs would have different behavioral response to and recovery from weaning and this would be related to their physiological stress resilience at weaning. Developing behavioral indicators to identify resilient pigs could prove beneficial in selecting robust animals for future breeding and production, ultimately improving their overall welfare.

CHAPTER 2: ASSOCIATIONS BETWEEN RESILIENCE TO WEANING STRESS AND STARTLE RESPONSE IN NURSERY AGE GILTS

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ABSTRACT

Resilience is the capacity of animals to return quickly to their pre-stress status following a disturbance, including social, physical, and/or disease challenges. Understanding the impact of individual resilience on behavior is key to improving the performance and welfare of farm animals. The goal of the study was to assess whether behavioral response to an auditory stimulus during a startle test (acute stress) differed between pigs designated at weaning (27 +/- 2d of age) as stress-resilient (SR) or stress-vulnerable (SV). Blood samples were collected from female piglets (n = 170) from 26 litters surrounding weaning at multiple time points. Using serum cortisol levels from these samples, two female pigs from each litter (n = 52) were classified as either SR (n = 26) or SV (n = 26) and used for the startle test. The startle test was conducted when pigs were 7wk-of-age while they were housed in the nursery room. We assessed startle magnitude score (SM) and time to resume home pen behavior as indicators of the pigs' behavioral responses. In addition to stress resilience designation, pigs' relaxed-tense score (RT), orientation to stimulus (OS), and pen position were also observed which may affect startle response. Our data suggest no relationship between behavioral response to an auditory startle test and resilience or vulnerability to weaning stress. The difference in the type of stressors needs to be considered as an acute, simple auditory stimulus was used for the startle test and a longer-lasting, multi-modal weaning stress was used for initial resilience designation. However, the startle test could be a relatively easy way to assess the fearfulness of pigs on farms, as it requires no training of pigs and can be conducted in the home pen, but further methodological

improvement is required.

INTRODUCTION

Stress can be defined as any aversive stimulus, while fear can be defined as a motivation that impels fleeing, escaping, or showing defensive behavior in a dangerous or threatening situation (Barrows, 2011). Fear is an emotional state that triggers a stress response, allowing animals to avoid potentially hazardous situations and activities (Casey, 2022). According to Seabrook (1990), this undesirable emotional state may also have adverse effects on farm animal welfare, growth, and reproduction, thus affecting the well-being of individuals (Boissy and Bouissou, 1995). Although stress and fear are not identical, they involve similar mechanisms within the central nervous system (Levine, 2008), indicating a correlation between them. Commercially farmed animals, such as pigs, are typically housed indoors, subjecting them to a variety of challenges throughout their lives due to the presence of numerous concurrent environmental stressors (O'Connor et al., 2010). Especially, weaning is an acute social stress occurring early in pigs' lives. Piglets experience aversive conditions during the weaning process, which involves encounters with various stressors in both their physical and social surroundings. Some notable instances include being introduced to unfamiliar conspecifics (Jensen and Yngvesson, 1998), undergoing handling procedures that trigger fear towards humans (Hemsworth et al., 1987; Hemsworth, 2003), and being exposed to novel environments (Erhard and Mendl, 1999).

Considering the multiple stressors pigs are exposed to in production systems, we need to understand pigs' capacity to adapt or be resilient to a broad range of environmental challenges to maintain performance. Resilience encompasses not only the response of the individual to disease or parasitic challenges but also the individual's response to environmental and social stressors

including those that evoke fear (Wu et al., 2013; Hermes et al., 2015). Resilient pigs demonstrate minimal susceptibility to disturbances or quickly restore their physiological, behavioral, cognitive, health, affective, and production state to their original (pre-challenge) condition (Colditz and Hine, 2016). The capacity to be resilient is likely to be key to improving the performance and welfare of farm animals.

Various behavioral assessments to assess fear have been developed for use under farm conditions, including novel object tests, novel arena tests, handling tests, startle tests, and emergence tests (Forkman et al., 2007; Statham et al., 2020). In the current study, we conducted a startle test to measure pigs' innate behavioral fear response, termed the defense cascade (DC) response, which encompasses strong instinctive reactions following unexpected stimuli. Defense cascade responses include primitive affective states that are elicited automatically in the context of danger (Kozłowska et al., 2015). It proceeds in a relatively common pathway across species including pigs: arousal (immediate response (startle) to the stimuli), fight or flight, freezing (including monitoring the source of danger in some cases), and finally resumption of either previous behavior or defensive motion resulting in collapsed/quiescent immobility (Statham et al., 2020; Kozłowska et al., 2015; Levine, 2008). There is a variation in defense cascade response (e.g., potentiated or attenuated startle responses) depending on the individual's experience, temperament, current social and physical environment, and also current affective state. For example, potentiated (i.e., increased) defense cascade (startle) responses have been observed in humans (Vrana, 1994; Bradley et al., 2001), rodents (Koch, 1999), and monkeys (Winslow et al., 2002) when they are in negative affective states. Thus, the defense cascade could be a potential indicator of animals' affective states, and variation in the response could provide information about affective valence and hence welfare (Statham et al., 2020). However,

no research has yet been conducted on how an animal's stress resilience affects its defense cascade response, including in pigs.

Young animals, including pigs, often exhibit an instinctive fear response when exposed to abrupt auditory stimuli or disruptions within their surroundings (Steimer, 2022). Both fear (Statham et al., 2020) and stress responses (Muráni et al., 2010) show interindividual variation due to temperamental traits, affective states, experience, and genetic factors. Therefore, we hypothesized that pigs would have different behavioral responses to an auditory stimulus during a startle test (acute stress) depending on whether they were designated as stress-resilient or stress-vulnerable at weaning. Therefore, the objective of our study was to describe the associations between physiological resilience to weaning stress and behavioral defense cascade responses in pigs following the startle test.

MATERIALS AND METHODS

Animals and Housing

All experimental animals in this study were housed at the Michigan State University Swine Teaching and Research Center in East Lansing, MI, USA. Housing procedures and selection of animals used for this study were described previously (Luttman et al., 2023). Female pigs (n= 52) from 26 litters across three farrowing groups were used (purebred parity 2 or 3 Yorkshire dams bred to PIC359 sire line (PIC, Hendersonville, TN, USA). Selected litters had at least 5 gilts and consisted of an average of 7 gilts (range 5 – 12 gilts per litter), (Rep 1 = 4 litters, Rep 2 = 13 litters, Rep 3 = 9 litters). Pigs were housed with their littermates and dams in farrowing crates prior to weaning. At weaning (27 +/- 2d of age) animals were moved to identical nursery rooms where they were placed in 1.6m x 1.4m pens (n=8 pigs/pen) with slatted-metal flooring (Fig. 1). Each new social group in the nursery pen consisted of groups of 2-3 littermates chosen based on

comparable weights (mean = 8.38 kg; min = 4.95 kg, max = 11.91 kg). Non-study females of appropriate weight were used as necessary to maintain equivalent stocking density across all pens.

Pigs were provided *ad libitum* access to feed that met or exceeded the nutritional requirement for pigs of this age and weight. Water was also provided *ad libitum*, with one nipple drinker in each pen. The pigs received full LED light for 8 hours per day, and half-light from auxiliary LED light for the remaining 16 hours per day. The handling of the pigs prior to the experiment consisted of teeth clipping, ear notching, tail docking, and injection (iron) on day 2 after birth.

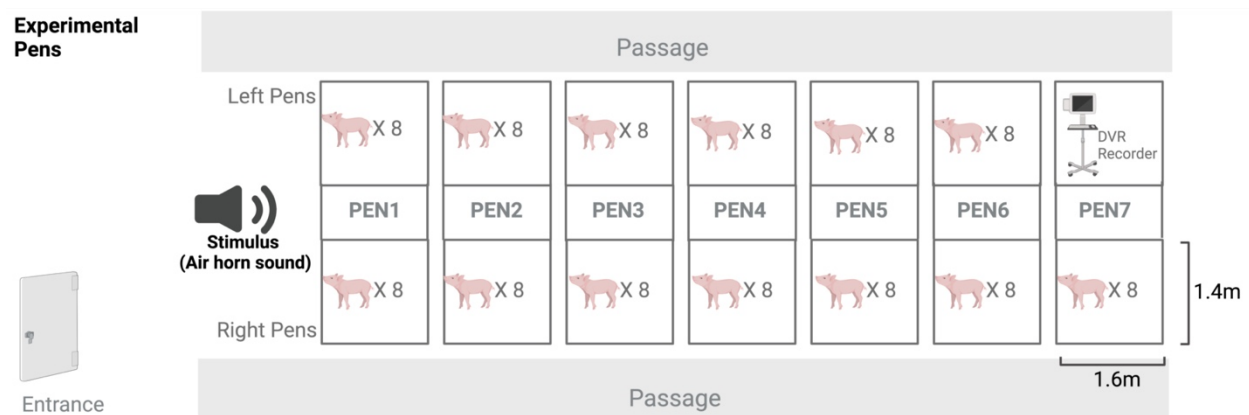


Figure 2.1. Layout of the nursery rooms used for housing the pigs and conducting the test. The center two rows (bold outline) were used to house experimental animals. All pens were the same size. Pens were numbered, with higher numbered pens being further from the location where the air horn was sounded

Video recording

Cameras (4K Motorized Varifocal HD IP Bullet Security Camera, Lorex, Linthicum, MD) were fixed in place on the ceilings above the pens at least one day prior to any video recording. Camera lenses were cleaned, and connections were checked one day prior to video recording. Recordings were made to a DVR (4K Ultra HD NVR, Lorex, Linthicum, MD).

Selection of SR and SV pigs

In a previous study (Luttman et al., 2023), our group selected focal pigs as stress-resilient (SR) and stress-vulnerable (SV). Briefly, blood from all gilt pigs from 26 litters ($n = 170$) was sampled three times surrounding weaning: 1 day before weaning (baseline), on the day of weaning (acute), and 4 days after weaning (recovery). Cortisol values from acute and recovery stages of each gilt were converted to values representing the percent change from baseline at each stage (Luttman et al., 2023). Next, total recovery value was calculated as the difference between the relative acute and relative recovery values to summarize the gilts' recovery over time. Within each litter, gilts were then ranked by total recovery value. The gilt with the highest total recovery value in the litter was selected as the SR focal pig and the gilt with the lowest total recovery value was selected as the SV focal pig (i.e., 2 focal pigs from each litter). We used these focal pigs for our current study.

Startle test procedure

The startle test was conducted when pigs were 7wk of age while they were housed in the nursery. Pigs were marked on their backs in the morning at least 2h before the startle test. Videos were recorded for 1.5h prior to the test, during the test, and for 1.5h after the test concluded. The startle test occurred between 1110-1130 in each repetition. The experimenter entered the test room and sat quietly by the door for 10 minutes before delivering the startling stimulus to allow the pigs to return to normal behavior. The experimenter then sounded an air horn (SeaSense, Urbandale, IA) for 5 seconds. The observer remained still and stationary for 10 minutes or until the pigs were observed behaving normally before exiting the room. Following the test, data was collected using the recorded video.

Data recorded during startle test

Relaxed-tense scores (RT)

Relaxed-tense score was a rating scale used to demonstrate the pigs' degree of underlying behavioral relaxation prior to the test (Statham et al., 2020). To standardize across repetitions, the pigs were scored right after the observer entered the room, which was 10 minutes prior to delivering the auditory stimulus. The act of entering the room itself created various stimuli that pigs were already accustomed to as part of routine farm management but ensured pigs were awake and responding to a standardized set of environmental stimuli when given an RT score. This rating scale ranged 1-3 with a larger number indicating a more tense state. (See Figure 2.A, modified from Statham et al., 2020).

Startle response behaviors

To assess the behavioral startle response of SR and SV pigs, pigs' behaviors were observed immediately after the stimulus. The ethogram of response behavior is presented in Table 1.

Table 2.1. Ethograms of pigs' startle response and home pen behaviors

Ethogram of response behavior		
Behavior	Behavior type	Description
Run away	Event	Running quickly to the other/opposite side of the pen in response to the stimulus
Walk away/sidestep	Event	(Walk away) Walking slowly/normally to the other/opposite side of the pen in response to the stimulus (Sidestep) Sideways movement of a few steps in response to the stimulus

Table 2.1 (cont'd)

Stand up	Event	Going from laying/sitting to standing up, all four legs are in contact with the floor in same location
Sit up	Event	Going from laying to sitting up, straighten front legs to lift head/upper body, hindquarters remain on the floor in same location
Freeze	Status	Becoming temporarily immobile in response to the stimulus, the whole-body stationary with ear prick Standing motionless, but the pig's head and nose move slightly (up and down, sideways) to detect the source of danger
Monitor the environment	Status	Head up rather than head down, sniffing the air May be softer ear tension than in the freeze position Usually occurs after the freezing, could be accompanied by intermittent steps
Return to home-pen behavior	Outcome	Returning to any normal home-pen behaviors while exhibiting a relaxed posture
Ethogram of home pen behavior		
Behavior	Description	
Sleeping	Lying with eyes closed, not performing any other behaviors	
Eliminating	Defecating or urinating	
Feeding-drinking	Pig's mouth and head are in the feeder or on the drinker.	
Non-injurious Contact	(Allo-grooming) touching other pig's head, ears, tail, legs, or rump with a nose disk, possibly gentle manipulation with snout (nibbling) and mouth but not biting injuriously	
	(Nosing body) touching or nudging the body of a pen mate with a snout except for head, ears, and anogenital region with a snout. 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 a snout	
	(Nose-to-nose contact) touching another pen mate's snout with a snout	
	(Nosing anogenital) touching, rubbing, or licking the anogenital region of a pen mate with a snout	
Injurious Contact	(Belly nosing) Nosing, nudging another pig's belly with repetitive up and down snout movements	

Table 2.1 (cont'd)

	(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
Competition	Head knocking, biting, pushing, mounting, pawing or other aggressive interaction to remove penmates from front of the feeder/drinker so pig delivering actions gets access to feed/water
Mounting	Pig standing on hind legs with both front legs on another pig's body.
Playing	(Social play) Scampering, pivoting, running, head tossing, flopping or hopping together with at least one other pig (Solitary play) Same as above but on own
Locomotion	(Run) Running--moving quickly--around pen but not showing other indicators of play (e.g., head toss, pivot or scamper) (Jump) Vertical, rather than horizontal, movement above ground. May go from laying to standing as in part of the startle response. May be against side of pen pushing up from hind legs. (Walk) Slow movement in a forward direction, not repetitive pacing
Standing	Standing with four legs on the floor, not performing any other behaviors
Sitting	Straighten front legs to lift head/upper body, hindquarters remain on the floor, not performing any other behaviors
Lying down	Lying on the floor with eyes open, possibly also interacting with pen mates, floor, walls or other pen elements
Exploring	Investigating surrounding environment by nudging, rooting, sniffing, scratching, or chewing with at least one pen mate or alone

Home pen behaviors

Behaviors of all pens of pigs were observed using video recorded 1 hour before the observer entered the room to develop an ethogram covering the range of behaviors pigs could engage in as they returned to normal behavior following the startle test. Possible outcome behaviors seen in the home pen included sleeping, eliminating, feeding-drinking, non-injurious contact, injurious contact, competition, mounting, playing, locomotion, standing, sitting, lying

down, and exploring (ethogram provided in Table 1). Each pig's behavior following its recovery from the startle test was recorded to evaluate if SR and SV pigs returned to different types of normal behaviors following the test.

Startle magnitude score (SM)

A startle magnitude scale was created based on the response behaviors. To determine the SM score, two factors were used 1) immediate response behavior (i.e., degree of pigs' response) and 2) whether they returned to normal behavior within 1 minute (i.e., speed of pigs' recovery). The scale was rated 0-4 from least to most intense. Pigs with a score of 0 expressed no reaction in response to the stimulus. Scores of 1-3 were assigned when pigs returned to home-pen behaviors within 1 minute, with severity depending on which specific response behaviors they exhibited. A score of 4 was assigned if a pig did not resume normal home-pen behaviors within 1 minute following the stimulus regardless of what their immediate response behavior was following the air horn. A detailed description of the startle magnitude scores is provided in Table 2 and Figure 2.B.

Table 2.2. Startle Magnitude Score (SM)

Score	Strongest reaction seen	Behavior description
4	Stay frozen/monitoring	Did not resume any normal home-pen behaviors within 1 min. (Freeze) Becoming temporarily immobile in response to the stimulus, the entire body stationary with ear prick. (Monitoring) Standing motionless, but the pig's head and nose may move slightly (up and down, sideways) to detect the source of danger, head up rather than head down, sniffing the air, may be softer ear tension than in the freeze position, usually occurs after the freezing, could be accompanied by intermittent steps

Table 2.2 (cont'd)

3	Run away	Running quickly to the other/opposite side of the pen in response to the stimulus
2	Walk away/ side step	(Walk away) Walking normally (slowly) to the other/opposite side of the pen in response to the stimulus (Sidestep) Sideways movement with a few steps in response to the stimulus
1	Posture change in same location	(Sit up) Going from laying to sitting up, straightening front legs to lift head/upper body, hindquarters remain on the floor in same location (Stand up) Going from laying/sitting to standing up, all four legs straighten to lift body from floor, all feet are in contact with the floor, pig remains in same location
0	No startle reaction	Behavior did not change in response to the stimulus

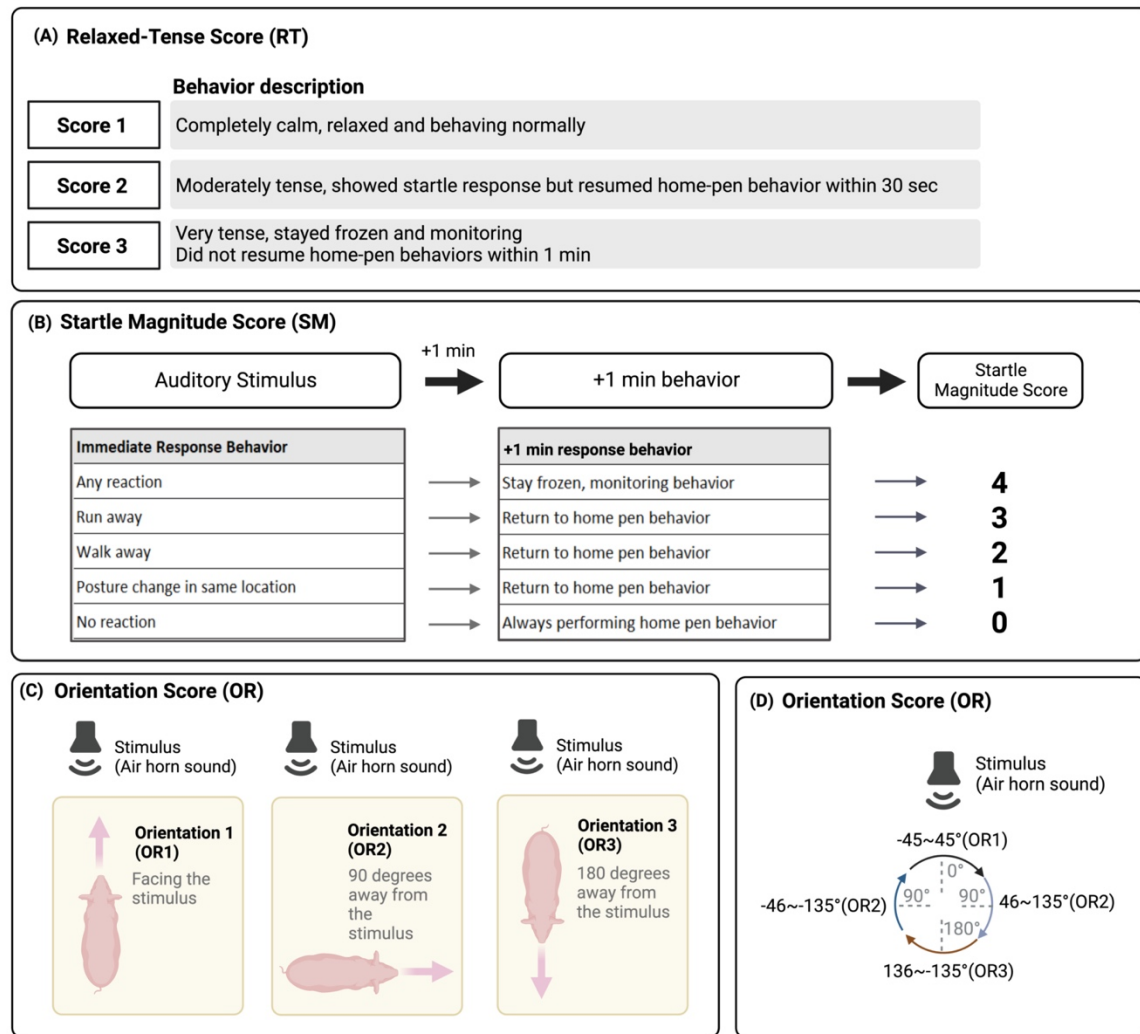


Figure 2.2. (A) The RT score was used to assess the degree of underlying behavioral relaxation of the pigs prior to the test. A more tense state was indicated by a larger number. (B) In order to determine a pig's SM, two factors were used: 1) examine the pig's immediate response to the startle stimulus; and 2) observe whether the pig returned to home pen behavior within 1 min or not. When a pig expressed no reaction immediately after the stimulus, it was assigned an SM score of 0. Pigs assigned SM scores from 1-3 showed responses to the stimulus as described above and, specifically, returned to home pen behavior within 1 min. Regardless of any initial response, a pig was allocated a score of 4 if it did not resume home pen behavior by 1 minute. (C) A schematic diagram of how observers assigned orientation scores focusing on the animal's head to identify whether the pig's senses (eyes, ears) were oriented toward or away from the location of the startling stimulus. (D) From -45° to $+45^{\circ}$ was considered OR1, $\pm 46^{\circ}$ to $\pm 135^{\circ}$ was considered OR2, and $+136^{\circ}$ to -135° was considered OR3

Orientation Score (OR)

The direction the pigs were facing (i.e., their orientation to the stimulus) was decoded from video immediately before the air-horn sounded. Pigs were allocated into one of three categories based on their orientation (Figure 2. C, D).

Statistical analysis

To investigate time to reach the end of the defense cascade (i.e., returning to home pen behavior), a linear mixed effect model was fit with the stress status (SR and SV), orientation, pen position, and relaxed-tense score as fixed effects and pen composition, pen position, and replicate were included as random effects. Pairwise least square mean comparisons (LSD) were used to compare levels within significant factors in the main effects model (Orientation). A mixed-effects ordinal logistic regression model was fit to the startle magnitude score with stress status (SR and SV), orientation, pen position, and relaxed-tense score as fixed effects and pen environment as a random effect. To test whether there was any correlation between outcome behaviors and stress levels, we performed the additional Pearson's chi-squared test. To test the relationship between stress resilience designation and RT score, a Fisher's exact test was used. We set the null hypothesis to expect no difference in RT score levels in each stress level.

RESULTS

Relationship between stress resilience and RT score

Stress resilience was not statistically related to RT scores ($P = 0.2736$, Figure 3).

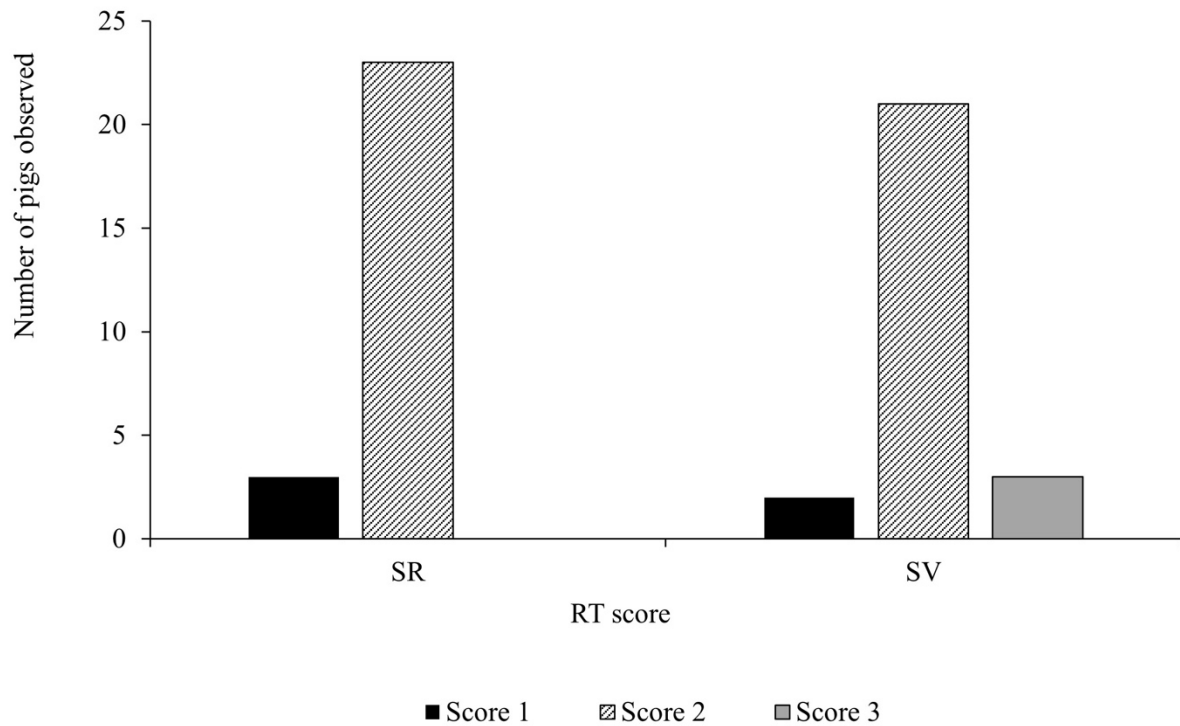


Figure 2.3. The frequency of RT scores (1-3) in stress-resilient and stress-vulnerable pigs

Startle magnitude score

The pigs' stress resilience designation (SR and SV) did not significantly relate to the SM score and OR score also had no influence on SM (Table 3). Pigs with RT score 3 were significantly more likely to have lower SM scores than pigs with RT scores of 1 ($p = 0.0185$). The SM scores for pigs with RT score 2 compared to RT score 1 were not significantly different ($p = 0.2546$). The pigs housed in pens further from the stimulus showed less startle response than pigs housed near the air-horn sound ($p = 0.007$; Table 3).

Table 2.3. The relationship between pigs' startle magnitude score and stress status, orientation, relaxed-tense score, and pen-position factors

Factor¹	Estimate	Standard error	z-value	P-value
Stress (SV)	-0.10	0.65	-0.15	0.8773
Orientation 2 vs 1	0.74	1.00	0.74	0.4592
Orientation 3 vs 1	-0.40	0.75	-0.54	0.5889
RT score 2 vs score 1	-1.33	1.17	-1.14	0.2546
RT score 3 vs score 1	-4.56	1.94	-2.35	0.0185*
Pen-position	-0.82	0.30	-2.70	0.007*

¹Stress: SR and SV designation. Orientation: a direction of animals toward the sound stimulus. A larger number indicates more distance from the stressor. Relaxed-tense (RT) score: ‘relaxed-tense’ state of pigs 10 minutes prior to the auditory stimuli, a larger number indicates a more tense state. Pen-position: location of pens in the nursery room. A larger number indicates more distance from the stimulus. * $P < 0.05$

Time to resume home pen behavior

Stress resilience designation (SR and SV) did not significantly relate to pigs’ time to resume home pen behavior (Table 4). The pigs’ orientation prior to the startling sound were significantly associated ($P = 0.039$) with time to resume home pen behavior. Pigs in OR2 took a longer time (71.98 sec, SEM=12.15) to return to normal behavior compared to OR1 (44.87 sec, SEM = 8.76) and OR3 (46.00 sec, SEM=6.64) ($P < 0.05$, LSD), while no difference was detected between OR1 and OR3 ($P > 0.05$, LSD). Pigs’ time to return to normal behavior was not significantly affected by their relaxed-tense score or pen position (Table 4).

Table 2.4. The relationship between time to return to home pen behavior after the startle test and stress status, orientation, relaxed-tense score, and pen-position factors

Factor¹	Sum of square	Mean of square	NumDF	DenDF	F-value	P-value
Stress	346.4	346.39	1	43.75	0.51	0.479
Orientation	4733.1	2366.56	2	43.14	3.48	0.039*

Table 2.4 (cont'd)

Relaxed-tense score	2373.5	1186.75	2	43.17	1.74	0.186
Pen-position	1370.8	1370.78	1	24.03	2.02	0.168

¹Stress: SR and SV designation. Orientation: direction of animals toward the sound stimulus. A larger number indicates more distance from the stressor. Relaxed-tense score: 'relaxed-tense' state of pigs 10 minutes prior to the auditory stimuli, a larger number indicates a more tense state. Pen-position: location of pens in the nursery room. A larger number indicates more distance from the stimulus. * $P < 0.05$

Outcome behaviors after the startle response ended.

Outcome behaviors consisted of normal home-pen behaviors that pigs resumed following the auditory startle and included competition, eliminating, exploring, feeding, and drinking, locomotion, lying down, and non-injurious contact. Competition, eliminating, and locomotion were only observed once each and always in SR pigs. However, lying down was observed at similar rates between SR (4 times) and SV pigs (5 times). Rates of exploring, feeding, and drinking, and non-injurious contact behaviors did not differ significantly between SR and SV pigs ($P = 0.245$), perhaps due to a relatively small sample size (Figure 4).

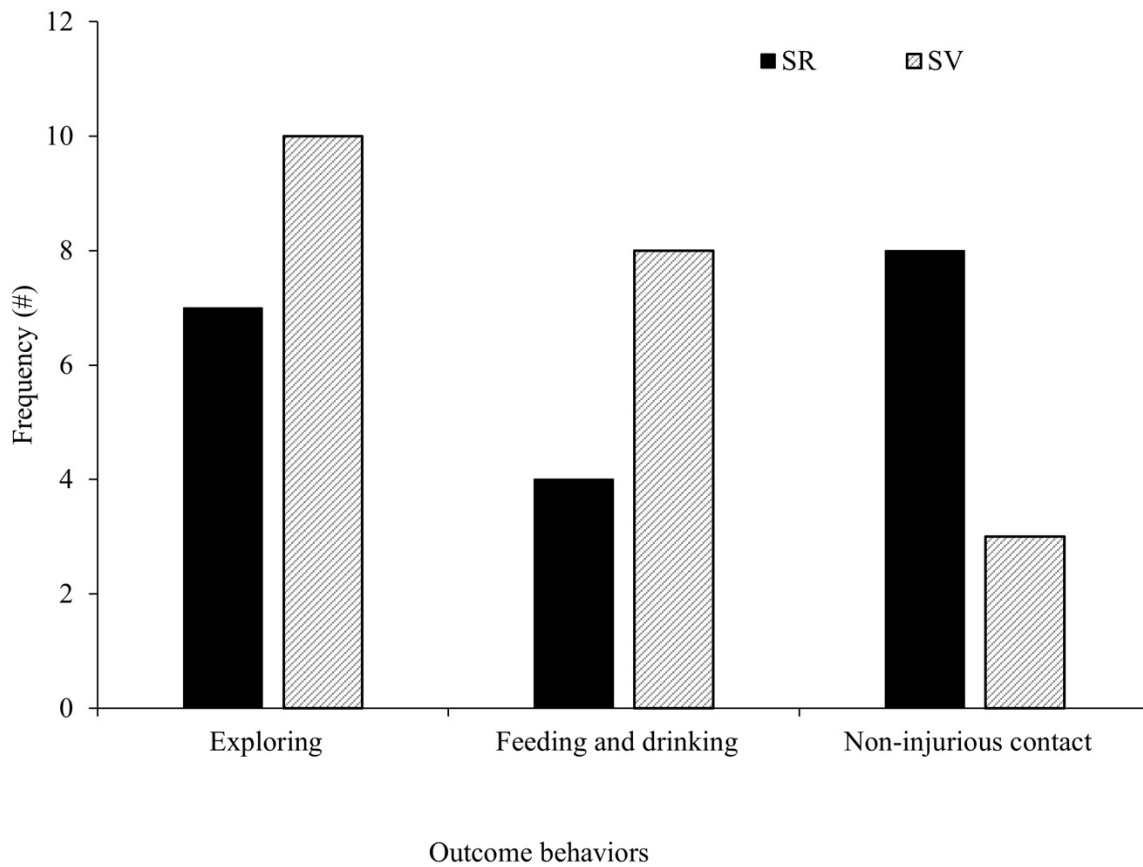


Figure 2.4. Outcome behaviors with biological implication observed after the startle response ended

DISCUSSION

Influence of RT score on SM

A relaxed-tense score (RT) was assigned to pigs 10 minutes before the startle test to evaluate whether the degree of behavioral relaxation before the test affected their SM score. Statham et al., (2020) previously demonstrated such a relationship between a pig's defense cascade response and their relaxed-tense score. In their study, the RT score they created was defined as a subjective rating of how 'relaxed or tense' pigs were immediately before the startle test and was assigned shortly after each individual pig was transferred to the test room. They

found that the animals with a higher RT score displayed less reaction to the startle test. Statham et al., (2020) concluded that the least calm animals paid less attention to their environment, making them less responsive to the test stimulus. Accordingly, they emphasized that pigs should be settled and calm at the point of startle testing (Statham et al., 2020). Therefore, in our current study, we tried to keep the pigs calm before the startle test by entering quietly and waiting 10 minutes before testing. In agreement with the previous study, our findings showed the lowest startle magnitude score was detected in pigs with an RT score of 3, the score representing the highest level of underlying tension. Thus, underlying behavioral tension of the animal should be considered in similar future research since it influenced pigs' behavioral fear responses (i.e., defense cascade responses) (Statham et al., 2020 and in present study).

Influence of pen position on SM

The pigs located further from the stimulus showed less startle response than pigs housed near the air-horn sound. Closer proximity to a fear-inducing stimulus has been related to an increased possibility of startle reaction due to the nearness of potential danger and therefore harm (Lang et al., 2000). The expected effect of proximity was obvious in our study, as pigs adjacent to the auditory stimulus who were exposed to a greater magnitude of sound, showed an increased startle response. To improve on farm home pen startle tests in future, the startling stimulus should be administered uniformly to all pigs in order to control the impact of proximity.

Influence of orientation on time to return to normal behavior

We gave pigs an orientation score based on the direction of their face because the ears of pigs have a forward orientation and are not as mobile as the ears of prey animals such as sheep, cattle, or horses. The pigs' orientations prior to the startling sound were significantly associated with the time it took them to resume home pen behavior. In our current study, when pigs were

oriented at an angle of $\pm 46^\circ$ to $\pm 135^\circ$ (OR2, side on), they took longer to return to normal behavior compared to when they faced directly away from (OR3) or toward (OR1) the stimulus.

It could be assumed that pigs facing away from the stressor (OR3) would be more likely to respond most strongly as they must both orient to and process the stressors or potential danger before finding escape routes or expressing another defensive response. In fact, Statham et al. (2020) previously found that when pigs faced away from a startling stimulus, they responded more strongly than those facing the stimulus. In addition, they found stimuli with a pronounced visual component (e.g., rapid movement) induced a stronger startle response compared with stimuli that were mainly auditory (e.g., loud sound). Multimodal stimuli, which animals perceive through more sensory organs, would thus stimulate more brain areas to evoke a stronger response (King and Calvert et al., 2001). As our study solely used a purely auditory stimulus (after allowing for pigs to acclimate to the sight of the tester standing in the room), this may explain why our OR results did not align with theirs. Pigs facing sideways when presented with an auditory stimulus may have heard the sound loudly but could not locate the stimulus as readily as those facing forward.

Influence of stress resilience designation

The primary objective of this study was to investigate whether behavioral responses to a startling auditory stimulus differed between SR and SV pigs. However, we were unable to show a clear relationship between pigs' startle response including their RT score, time to resume home pen behavior, SM score, and outcome behavior, and their physiological classification as SR or SV. As described previously, focal pigs from each litter were classified as stress resilient (SR) and stress vulnerable (SV) using serum cortisol levels evaluated surrounding weaning (Luttman et al., 2023). As described above, multi-modal, complex, and longer-lasting stressors

simultaneously existed surrounding weaning. However, a simple and brief acute auditory stimulus was used for the startle test. Therefore, we need to acknowledge the difference between these two types of stressors. Additionally, there was an approximately 3-week gap between the initial classification of piglets according to their stress resilience at weaning and the startle test. Young pigs develop and grow rapidly in three weeks, and they also have more experiences and learn from these. Early life experiences can create considerable changes in an animal's stress response that persist into adulthood (Barker, 1996; Phillips, 2002; Heim and Nemeroff, 2001). Even brief periods of stress during the early days of life in rodents can have significant impacts on later behavioral regulation including conditioned fear responses (Walker et al., 2017). Luttman et al. (2023) also advise caution when using cortisol to characterize an animal's stress response. Cortisol's effects on metabolism vary with different stressors (Martínez-Miro' et al., 2016) and can be influenced by circadian rhythm and sampling methods. However, cortisol remains a suitable biomarker for studying HPA axis responses to weaning or social stress (Guevara et al., 2022). Therefore, taking these factors into account, physiological and behavioral responses to different stressors do not always perfectly align due to individual variations ranging from genetics to personality to experience.

Outcome behavior after freeze

In our study, we observed the outcome behaviors that pigs showed when their defense cascade response ended. Outcome behaviors are the normal home pen behaviors that occurred after freezing. Our focal pigs showed competition, eliminating, exploring, feeding and drinking, locomotion, lying down, and non-injurious contact behaviors as their defense cascade responses ended. The differences in outcome behaviors were not statistically significant, perhaps due to the relatively small sample size, but they are biologically interesting. Non-injurious contact was

defined in the ethogram as touching another pig's part of the body gently without biting injuriously. This behavior includes allogrooming, nose-to-nose contact, nose other's head, body, or anogenital regions, which are considered part of social behavior (Camerlink et al., 2012). Positive social contact, for example, touching or allogrooming, is known to stimulate the reward system of the brain and prompt the release of the neuropeptide oxytocin (Pellis and Pellis, 2010, Rault, 2012). Oxytocin is a neuropeptide that is essential in regulating social cognition and behavior (Kumsta and Heinrichs, 2013). In human and rodent models, oxytocin decreases stress levels (Uvnäs-Moberg, 1998) and it is also involved in the adaptation to changing environments and in the establishment of social relationships. This may indicate that oxytocin plays a critical role in expressing resilience and has persistent effects on developing it (Feldman, 2020, Takayanagi and Onaka, 2022). However, as far as we are aware, no study has been conducted previously in the swine model; therefore, further study is required to determine the relationship between oxytocin and resilience in pigs.

Startle test as a tool

Various behavioral assessments have been developed to experimentally examine fear and the stress associated with fear, including novel object tests, novel arena tests, emergence tests, handling, and human approach tests (Forkman et al., 2007; O'Malley et al., 2018; Luttman et al., 2023). In the present study, we used the startle test with an unexpected, loud noise as a stimulus to measure pigs' instinctive behavioral response (DC response) to an auditory stimulus. The DC response has been proposed as a method for assessing the affective state, which is one of the most important determinants of animal welfare (Carreras et al., 2017; Statham et al., 2020). Components of the DC response are regulated by the individual's affective state (Statham et al., 2020). It is common for humans and rodents in negative affective states to exhibit potentiated

increased startle magnitudes and freeze durations. For example, rats selected for an anxiety and depression-like phenotype show a higher level of freezing in aversive conditioning tests (Widman et al., 2019). This variation is likely to represent a combination of influences on the animal's present affective state, encompassing temperamental and environmental factors. Consequently, the DC response can offer a concise overview of how specific individuals, with specific experience and genetic predispositions, are being impacted by their current circumstances (Statham et al., 2020). In other words, evaluating variation of DC response through a startle test provides potential new indicators of affective valence and hence welfare. Therefore, the strong instincts of pigs enable us to assess their behavioral fear response as an outward expression of their internal state.

In addition, because there is no requirement for special equipment or need to train pigs or handlers to perform it, a startle test could be implemented quickly and easily under a variety of conditions. Therefore, it could be relatively easy to perform under farm conditions in the pigs' home pen though subsequent decoding of video could be time consuming (Wurtz et al., 2019). However, the DC response represents a motor-autonomic sensory reaction that follows a standardized pattern which includes arousal, freezing, flight or fight, tonic immobility, collapsed immobility, and quiescent immobility. Therefore, in future it should be possible to develop a method of live observation that can be applied in real time on farm, thus avoiding the time-intensive process of later decoding video.

Within the scope of this study, our stressor was limited to an acute auditory stimulus. However, to further enhance the methodology, multimodal stimuli should be incorporated into the startle test that encompass both visual and auditory components to engage more of the sensory cortex. This approach would be expected to elicit a more robust startle response and

contribute to the overall effectiveness of the study.

CONCLUSION

In this study, we assessed the relationship between behavioral response to an auditory startle test and resilience or vulnerability to weaning stress. The SM score and time to resume home pen behavior were measured as indicators of the pigs' behavioral responses.

We predicted that SR pigs, those with more capacity to be minimally affected by and recover quickly from challenges, would show lower SM scores and resume normal behavior quicker than SV pigs. However, findings from our study did not support these hypotheses. Stress designation at weaning had no relationship to SM score or time to return to home pen behavior following presentation of an acute stressor in a startle tests. Overall, physiological and behavioral responses do not always perfectly align due to variations among individuals ranging from genetics to personality (e.g., temperament) to experiences (e.g., social context, management and/or husbandry), which could impact the physiological and behavioral responses of individuals differently. Another potential reason could be differences in the nature of the weaning and startle test stressors. The complexity of understanding pigs' responses indicates a need to carefully consider the limitations of using simplistic parameters to assess animal welfare.

However, the startle test could be a practical way to assess the fearfulness of pigs on farms, as it requires no training of pigs and can be conducted in the home pen. Fear is a primitive emotion necessary for survival. Responses to the startle (defense cascade) are important as an evolutionarily-relevant proxy measurement for affective valence and hence welfare. Therefore, we can use pigs' strong instinctive reactions to assess their behavioral fear response in relation to new factors such as SR and SV through the startle test. In conclusion, the startle test could be a meaningful method, but further methodological improvement is required to enable instantaneous

data collection and use multimodal stimuli to induce a stronger startle response.

CHAPTER 3: ASSESSING THE RELATIONSHIP BETWEEN PIGS' STRESS RESILIENCE AND THEIR BEHAVIOR IN RESPONSE TO WEANING

ABSTRACT

Considering the multiple stressors pigs are exposed to in production systems, it is essential that we understand a pig's ability to adapt or be resilient to a broad range of environmental challenges in order to maintain performance, production, and welfare. In the current study, we utilized 52 focal gilts identified through a physiological marker (cortisol) in a previous study to compare resilience to weaning stress and behavioral responses at weaning. Within the pigs' home pen, we observed agonistic behavior, non-agonistic social behavior, and daily maintenance behaviors. We conducted behavioral observations over two 4-hour periods (from 6 AM to 10 AM): one day after weaning (d1) and four days post-weaning (d4). We found behavioral differences associated with stress resilience. On d1, stress-vulnerable (SV) pigs displayed a higher average frequency of non-injurious contact behavior ($P = 0.0198$) compared to SR, while stress-resilient (SR) pigs exhibited a significantly longer average duration of lying down behavior compared to SV ($P = 0.01796$). On d4, SV pigs exhibited a significantly longer duration of fighting behavior on average when compared to SR pigs ($P = 0.0246$). 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 ($P = 0.013$) and drinking behaviors ($P < 0.001$) compared to d4. Conversely, on d4, pigs spent more time feeding ($P = 0.004$) and lying down ($P < 0.001$) when compared to d1. Our findings imply that non-injurious contact, lying down behaviors observed immediately after weaning, and fighting behavior several days later may serve as 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 to develop 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 robustness indexes that could be helpful in breeding programs, facilitating the selection of resilient pigs that overcome challenges associated with weaning.

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 (Brambell Report, 1965, 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 Reid, 1994, 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 (Kendrick, 2007, Yeates and Main, 2008, 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 (Broom, 1986, Colditz and Hine, 2016). As current husbandry systems often expose pigs to various stressors, 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; Guy et al., 2012). Providing producers with information

on resilience allows them to detect instances of compromised resilience and identify the specific animals involved (Van der Zande et al., 2021). Furthermore, the capacity to discriminate between pigs exhibiting stress resilience or stress vulnerability could serve as a valuable approach for selecting resilient pigs for future breeding (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 (Candiani et al., 2008, Horback, 2022), 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, 2022). Extensive research has been conducted on various aspects of normal behavior and activity in pigs, including social, agonistic behavior, contact, and daily behavior (e.g., 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 (e.g., 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 the activation of the hypothalamic-pituitary-adrenal axis (HPA) and the subsequent release of cortisol, are frequently used as indicators of animal welfare (Candiani et al., 2008). Physiological parameters have also been used to measure resilience (Hermesch and Luxford, 2018), and Luttman et al., (2023) developed a methodology for identifying and characterizing pigs resilient to social stress. In our present investigation, we utilized the approach established by Luttman et al. (2023) 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.

The well-being of animals can be influenced by conditions, and animal welfare is particularly compromised when animals are subjected to stressful circumstances (Dwyer & Bornett, 2004). This study exposed the pigs to an intense stressor, the weaning process, which 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, with a specific focus on examining their behavioral response to and recovery from weaning stress in relation to physiological resilience. For this purpose, we explored behavioral differences between focal pigs that exhibited stress resilience and stress vulnerability. We also evaluated the effect of time since weaning on behaviors of interest, including play and types of physical contact.

MATERIALS AND METHODS

Animals and Housing

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). Replicate 1 consisted of 4 litters, replicate 2 had 13 litters, and replicate 3 included 9 litters. Before weaning and mixing, the pigs were housed in a farrowing room with their littermates and dams. Two days after birth, pigs underwent teeth clipping, ear notching, and tail docking, and received an iron injection. 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 included (mean = 8.38 kg; min = 4.95 kg, max = 11.91 kg). Pigs were provided with a diet formulated to meet the needs of nursery-stage pigs and water ad libitum. Lighting conditions consisted of 8 hours of full LED light and 16 hours of half-light from auxiliary LED lights. On the day of weaning, two vaccine injections (Erysipelas and Porcine Circovirus Type 2) were administered. Prior to weaning, piglets underwent the process of ear tagging for identification and were marked on their backs using a non-toxic black marker for later identification in video analysis.

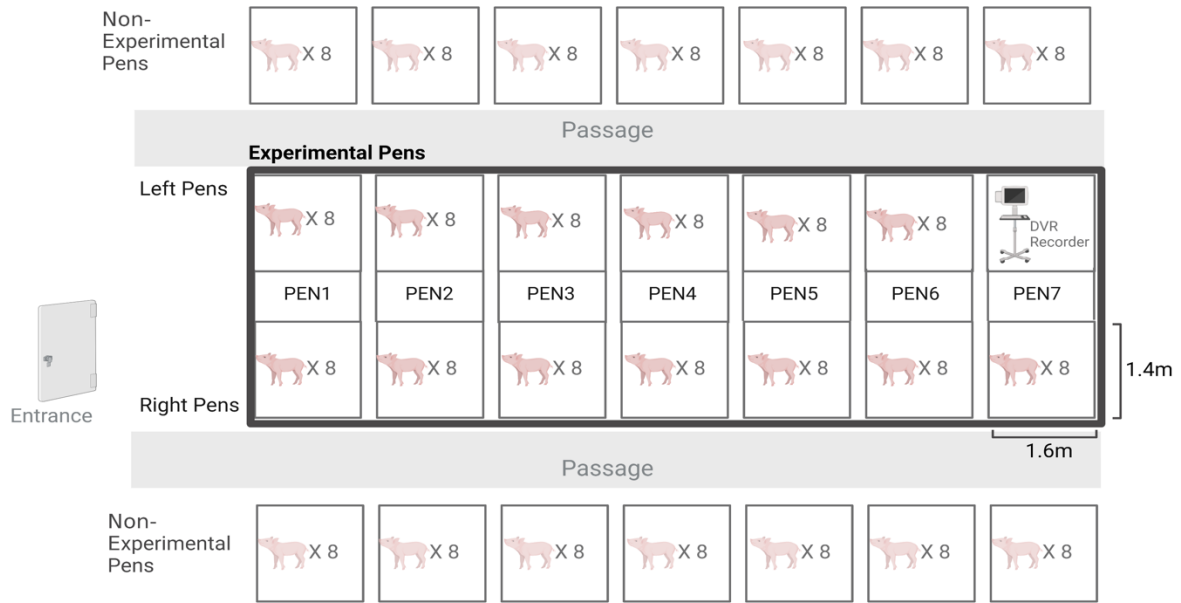


Figure 3.1. Nursery rooms for housing and observing pigs. Experimental animals were housed in the center two rows (bold outline). All pens in the nursery rooms were identical in size. Each pen was assigned a number, with higher numbered pens positioned further from the door to the room

Focal Pigs

Fifty-two focal gilts were used from a previous study in which our group selected stress-resilient (SR) and stress-vulnerable (SV) pigs (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). 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. 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).

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 (from 6 AM to 10 AM) with respect to the weaning date (d0): one day after the weaning (d1) and four days post-weaning (d4). 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.

Table 3.1. Ethograms of pigs' home pen behaviors

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	Injurious Contact	Contact by one pig results in a negative reaction from the recipient pig, indicating this was a painful or unpleasant contact behavior. (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

Table 3.1 (cont'd)**Non-agonistic behavior**

Event	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. The recipient should not react negatively to the touch, indicating this was a non-injurious contact behavior.
		(Nosing body) touching or nudging the body of a pen mate with snout, not including contact with head, ears, and anogenital region. If repetitive nosing of belly occurs, score as Belly Nosing. The recipient should not react negatively, indicating this was a non-injurious contact behavior.
		(Nosing head) touching or nudging the head and/or ears of a pen mate with snout. The recipient should not react negatively, indicating this was a non-injurious contact behavior.
		(Nose-to-nose contact) touching another pen mate's snout with own snout. The recipient should not react negatively, indicating this was a non-injurious contact behavior.
		(Nosing anogenital) touching, rubbing, or licking the anogenital region of a pen mate with snout. The recipient should not react negatively, indicating this was a non-injurious contact behavior.

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

Video analysis

Three decoders collected video 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 half-way 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. These values indicate substantial agreement (0.81 – 0.99) for the duration and near perfect agreement (0.61 – 0.80) for frequency.

Statistical analysis

A one-way ANOVA for Randomized Complete Block Design was fit using R v4.2.1 (R Core Team, 2022; Vienna, Austria) to examine the relationship between behavior and stress resilience designation (SR or SV) at two specific time points (d1 and d4) after weaning. To assess the effect of stress resilience designation (SR or SV) and the passage of time since weaning and the interaction of these factors on behavior, a repeated measures ANOVA model was employed. For each model, the response variable was pigs' home pen behavior (duration or frequency) (as listed in Table 1). A pig's stress resilience designation (SR or SV) was a fixed effect and day of observation was the repeated measure in the model. Random effects included pen position, pen composition, litter, and replicate. Data from observations of solitary and social play behaviors on both d1 and d4 included many zero data points. We conducted a goodness-of-fit test to confirm the suitability of employing a zero-inflated model to these play data rather than a standard linear model, and the result supported the use of zero-inflated data ($P < 0.01$). Therefore, the zero-inflated model was applied with the assumption that the responses follow a generalized gamma distribution, as the variables are continuous.

RESULTS

Relationship between behavioral difference and stress resilience designation at two specific time points

Lying down behavior on d1, non-injurious contact on d1, and fighting behavior on d4

were significantly different between SR and SV pigs (Table 2 and Figure 2). No other significant effects of stress resilience designation were detected for the remaining target behaviors. On d1, statistically significant differences were observed in two behaviors: non-injurious contact ($P = 0.0198$) and lying down ($P = 0.01796$) (Table 2). Specifically, pigs categorized as SV exhibited a greater frequency of non-injurious contact behavior on average compared to SR (Figure 2). Conversely, SR pigs demonstrated a significantly longer duration of lying down behavior on average (Figure 2). On d4, SV pigs showed a significantly longer duration of fighting behavior on average compared to SR pigs (Table 2 and Figure 2, $P = 0.0246$).

Table 3.2. Behavioral Differences between SR and SV Pigs on Day 1 and Day 4 (mean $\pm$ SEM)

^a Behavior denoted either in duration in seconds (s; fighting, feeding, lying down, exploring, solitary play, and social play) or frequency (#; injurious contact, non-injurious contact, and drinking)

^b These behaviors were analyzed using the zero-inflated model. However, due to overconvergence, coefficients could not be estimated based on pigs' stress designation, except for social play on Day 4, which did not differ significantly between SR or SV ($P = 0.237$)

*Value is significantly different from that of SR group pigs ($P < 0.05$)

Day 1	Stress resilience designation	
Behavior ^a	SR	SV
Fighting (s)	283.81 $\pm$ 71.60	333.307 $\pm$ 56.51
Injurious contact (#)	10.35 $\pm$ 1.56	14.115 $\pm$ 2.02
Non-injurious contact (#)	83.04 $\pm$ 7.90	110.346 $\pm$ 9.12*
Feeding (s)	1286.62 $\pm$ 192.42	1190.576 $\pm$ 128.61
Lying down (s)	9251.31 $\pm$ 263.42	8616.69 $\pm$ 235.62*
Exploring (s)	1600.42 $\pm$ 156.14	1898.00 $\pm$ 181.66
Drinking (#)	17.92 $\pm$ 1.06	19.50 $\pm$ 1.34
Solitary Play (s) ^b	0.23 $\pm$ 0.11	4.58 $\pm$ 2.61
Social Play (s) ^b	46.77 $\pm$ 18.02	22.88 $\pm$ 12.62
Day 4	Stress resilience designation	
Behavior ^a	SR	SV
Fighting (s)	21.65 $\pm$ 5.84	37.42 $\pm$ 5.36*
Injurious contact (#)	15.81 $\pm$ 2.51	11.19 $\pm$ 1.25
Non-injurious contact (#)	71.5 $\pm$ 4.79	77.31 $\pm$ 45.35
Feeding (s)	1964.65 $\pm$ 123.07	1769.00 $\pm$ 174.92

Table 3.2 (cont'd)

Lying down (s)	10466.77 ± 333.16	10223.04 ± 272.08
Exploring (s)	1111.54 ± 94.47	1149.31 ± 110.69
Drinking (#)	11.85 ± 1.15	11.92 ± 0.95
Solitary Play (s) ^b	3.34 ± 1.11	0.62 ± 0.30
Social Play (s) ^b	12.38 ± 6.05	4.81 ± 2.39

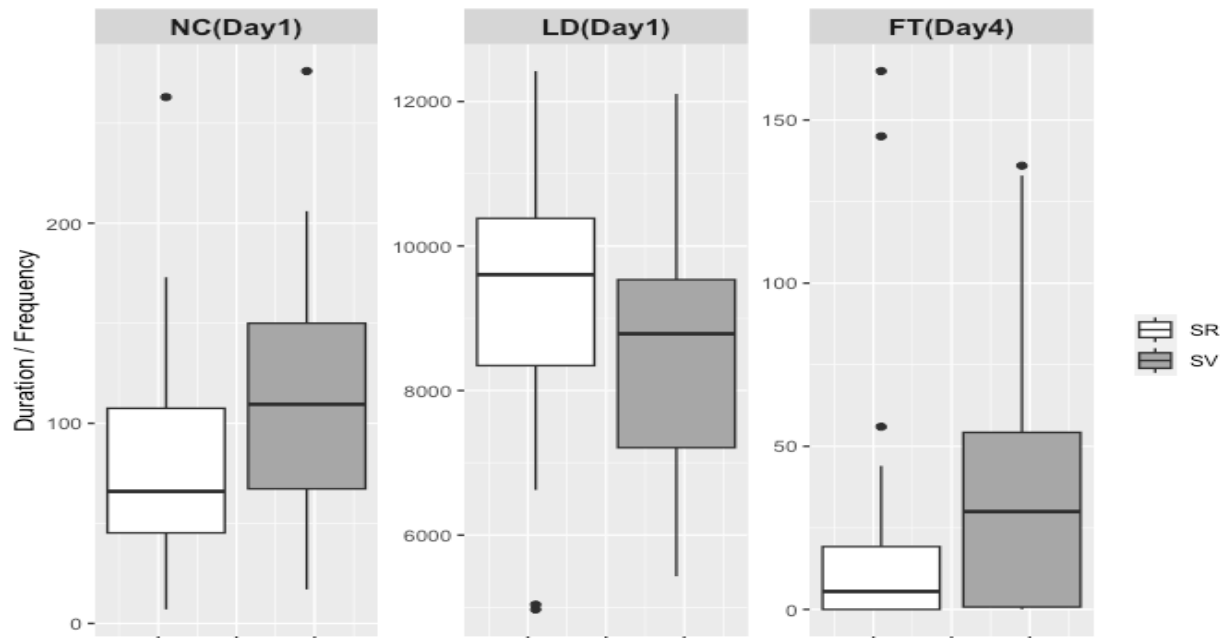


Figure 3.2. Comparison of Behavior Distribution Influenced by Stress Resilience Designation. The box plot displays the distribution of behaviors (duration or frequency) that were significantly impacted by pigs' physiological stress resilience designation. NC: non-injurious contact (frequency) ($P = 0.02$), LD: Lying down (duration) ($P = 0.018$), FT4: fighting (duration) ($P = 0.025$)

Association between behavioral differences and stress resilience designation over time

Our analysis revealed no significant interaction between the factors of stress and time across all observed behaviors (Table 3). Additionally, pigs with a stress-vulnerable designation consistently displayed higher NC behavior levels than those with stress-resilience designation. There were several behaviors influenced by the passage of time since weaning including fighting, non-injurious contact, feeding, lying down, exploring, and drinking behaviors (Table 3). On d1 post-weaning, pigs exhibited a higher frequency/duration of fighting, non-injurious

contact, exploring, and drinking behaviors compared to d4. However, pigs on d4 showed more duration of feeding and lying down behaviors when compared to d1.

Table 3.3. Behavioral Differences by Stress Resilience Designation, Day & Stress Resilience Designation x Day Interaction

Behavior	Factors	p-value
Fighting	Stress ¹	0.323
	Day	<0.001*
	Interaction	0.791
Injurious contact	Stress	0.809
	Day	0.563
	Interaction	0.060
Non-injurious contact	Stress	0.007*
	Day	0.013*
	Interaction	0.219
Feeding	Stress	0.556
	Day	0.004*
	Interaction	0.814
Lying down	Stress	0.097
	Day	<0.001*
	Interaction	0.577
Exploring	Stress	0.376
	Day	<0.001*
	Interaction	0.427
Drinking	Stress	0.629
	Day	<0.001*
	Interaction	0.593

¹ 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

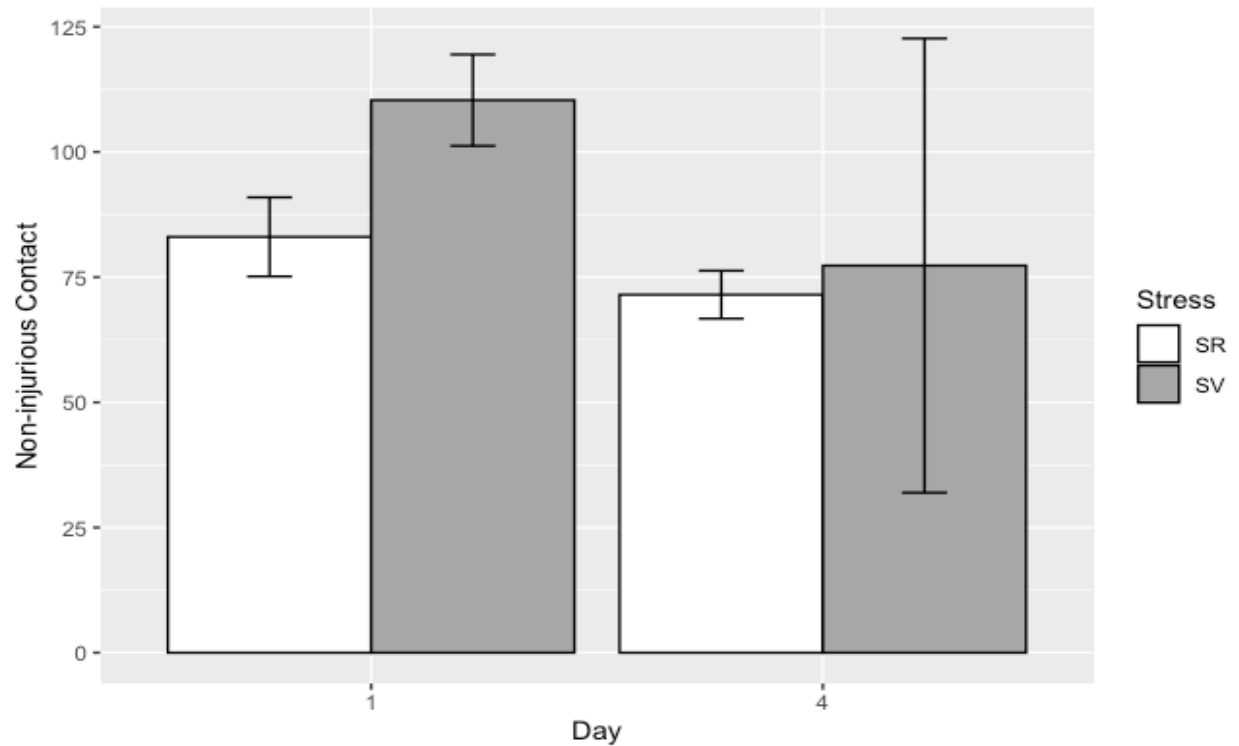


Figure 3.3. Effect of Day and Stress Resilience Designation on Non-injurious Contact (NC) Behavior. There was no interaction between the two factors. On d1, there was a higher occurrence of NC behavior observed in pigs as compared to d4 ($P = 0.007$). SV pigs exhibited a higher frequency of non-injurious contact behavior on both observed dates compared to SR pigs ($P = 0.013$)

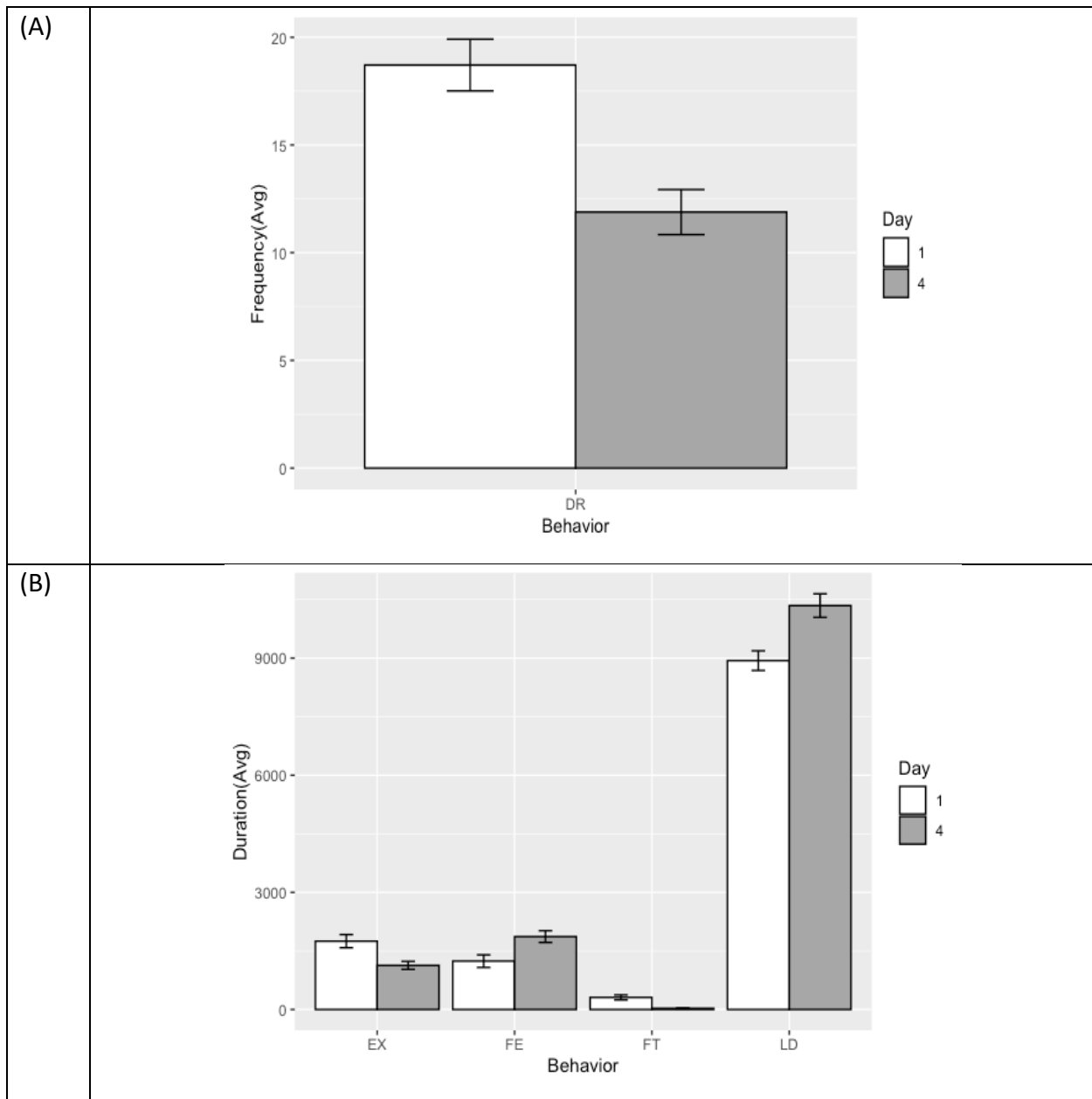


Figure 3.4. Temporal variations in the behavior patterns of pigs following weaning. DR: drinking (frequency), EX: exploring (duration), FE: feeding (duration), FT: fighting (duration), and LD (lying down). (A) On d1, pigs exhibited more drinking behavior ($P < 0.001$) compared to d4. (B) On d1, pigs showed more exploring ($P < 0.001$), fighting ($P < 0.001$), and non-injurious contact ($P = 0.013$) behaviors compared to d4. Less feeding behavior ($P = 0.004$) were observed on d1 in comparison to d4

DISCUSSION

Exploring the relationship between behavioral difference and stress resilience designation at two specific time points following weaning

In this study, we observed variation in a range of behavioral responses to weaning and, as expected, some of these behavioral responses related to the pigs' physiological stress resilience designation. In previous studies of pigs' behaviors, nosing was found to precede or follow most physical interactions among pigs (Camerlink and Turner, 2013). In our study, SV pigs displayed more non-agonistic behaviors on d1 compared to SR pigs. Nosing (which was a key element of our non-injurious contact behavior category) is often considered an affiliative behavior but encompasses several types of non-agonistic behaviors in pigs (Erhard et al., 1997, Goumon et al., 2020). This behavior includes 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 (*Sus scrofa*) (Camerlink and Turner, 2013). However, because 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 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 for and the social functions of the performance of nosing behavior remain unclear (Camerlink and Turner, 2013, Portele et al., 2019, 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. In other cases, nosing behavior may indicate stereotypic or displaced exploratory behavior resulting from limited environmental stimuli (Colson et al., 2006) and has been recognized as a behavioral indicator of stress (Colson et al., 2006). Hence, it could be inferred that pigs with stress vulnerability exhibit a higher frequency of stress-related behavioral responses, which could be considered effective behavioral indicators of stress such as belly nosing, and manipulating other piglets (ears, tails, or other body parts) (Dybckjaer, 1992) as compared to pigs with stress resilience following weaning stress. The frequency of these stress-related behaviors was higher in SV pigs than in SR pigs on d1, suggesting that non-agonistic behavior might be associated with the subsequent occurrence of more fighting behavior observed on d4 in SV pigs.

In our present study, SV pigs spent more time fighting on d4 in comparison to SR pigs. Numerous studies have investigated agonistic behaviors, including fighting and the establishment of a social hierarchy among unfamiliar piglets from different litters following the mixing process during weaning (Signoret et al., 1975, Clouard et al., 2023). Most agonistic behavior among unfamiliar pigs occurs within the initial 24-hour period, with an order of dominance typically forming within 48 hours in the group (Meese and Ewbank, 1973a, Zayan et al., 1991). In particular, the dominant pig in the group may be obvious in 30 to 60 minutes following mixing (Meese and Ewbank, 1973a, Zayan et al., 1991).

Once the dominance order is established, the highest-ranking pig often takes precedence over others without fighting, particularly in competitive situations such as obtaining food

resources (Signoret et al., 1975). Meese and Ewbank (1972) and Escibano et al. (2015) reported that the top animal rarely changed its position. However, pigs of middle or lower rank may exhibit signs of instability in their dominance hierarchy, and spontaneously change rank. It is possible that SR piglets in the current study developed a stable place in their social hierarchies sooner than SV pigs. This finding also could suggest that SR pigs quickly achieved top-ranking positions. Conversely, SV pigs might be more likely to occupy middle or lower rank positions, as indicated by the longer time taken to stop fighting (i.e., to establish a stable social hierarchy). However, it is important to note that direct observation of dominance order was not performed in the current study. Future research incorporating this data would provide valuable insights into the potential association between stress resilience, the speed of establishing a stable social hierarchy, and rank within that hierarchy.

In the current study, lying down behavior encompassed both actual sleeping and lying awake, and a longer duration of lying behavior was observed in SR pigs on d1. Both lying and sleeping are classified as “comfort behaviors” and therefore have significant characteristics (Blackshaw, 1981) as these behaviors are employed as a means to evaluate pig welfare (Nasirahmadi et al., 2017). Additionally, sleep is crucial for the brain development of young terrestrial mammalian species and plays a vital role in coping with their environment, and therefore well-being. It could be interpreted that SR pigs have more properties that make them more able to cope with difficult situations such as weaning stress in contrast with SV pigs. However, it is crucial to be cautious when interpreting lying down behavior positively, as increased inactivity is a common symptom of stress (Broom, 1996). Right after weaning, there was an increase in lying down behavior, and factors such as fatigue resulting from fighting, seeking maternal presence, or possible reduced food intake in their new environment might have

contributed to this change (Colson et al., 2006). Additionally, information about sleep and lying behavior in pigs is limited and has not been explored recently (Meddis, 1975, Kuipers and Watson, 1979). Consequently, further research is needed, especially in situations involving stressful situations.

Temporal variations in behavior

The current study revealed significant temporal variations in the behavioral patterns of pigs following weaning, encompassing 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 level of activity. Newly weaned piglets are initially restless in their new environment (Widowski et al., 2008) and exhibit high levels aggressive and exploratory behaviors, which subsequently decline over time (Whittemore et al., 1978, Besteiro et al., 2018, Bornett et al., 2000). In the current study, we observed more locomotor activity on d1, particularly 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.

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. According to Brooks et al. (1984), during the initial days post-weaning, piglets exhibited significantly 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. 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). Despite the fact that piglets possess these abilities before weaning, intake of solid food is typically minimal prior to weaning in commercial systems (Widowski et al., 2008). 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 (Yang et al., 1981, 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, 2005).

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 behaviors such as non-injurious contact, lying down

immediately following weaning, and fighting behavior several days later are also promising indicators of pigs' resilience to weaning stress. However, at present, it is difficult to accurately interpret behavioral changes in pigs following weaning without an indication of what constitutes appropriate levels of these behaviors. Essentially, there is a need to determine the ideal frequency, duration, and pattern at which these behaviors should appear in order to indicate good welfare. Developing a method to quantify behavioral parameters related to piglet weaning resilience would be helpful in understanding their behavior patterns and providing ethologically meaningful aspects that inform piglets' behavioral responses and adaptations to post-weaning life. Furthermore, this approach could enable the selection of resilient pigs for future breeding and the assessment of welfare.

CONCLUSION

We conducted this study to investigate pigs' behavior in the home pen, focusing on their behavioral response to and recovery from weaning stress and relating it to their physiological resilience (SR and SV) at weaning. Stress-resilient piglets spent more time lying down immediately following weaning (d1), whereas stress-vulnerable piglets showed more non-agonistic behavior on d1 and fighting behaviors on d4 post-weaning. Behaviors such as fighting, non-injurious contact, feeding, lying down, exploring, and drinking behaviors changed in ways that could indicate that piglets' social hierarchy became more stable over time. These behaviors, as they contributed to the establishment of a stable social hierarchy, could be considered as behaviors associated with adaptation to post-weaning life. However, these results were not influenced by stress resilience designation, except for non-injurious contact behavior. The behavioral changes of pigs as they adapt to and recover from stresses associated with weaning can be difficult to interpret without established behavioral indexes or baselines that allow

interpretation with respect to welfare. By developing a method to evaluate behavioral parameters 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.

CHAPTER 4: SUMMARY AND CONCLUSIONS

The overall goal of this research was to gain further insight into the relationship between pigs' physiological stress resilience and their behavioral responses, with a particular emphasis on assessing whether there were variations in behavioral responses among pigs identified as stress-resilient or stress-vulnerable at weaning.

The aim of the first study was to evaluate the relationship between physiological resilience or vulnerability to weaning stress and pigs' behavioral response to a later auditory startle test. We hypothesized that pigs would have different behavioral responses to an auditory stimulus during a startle test (acute stress) depending on whether they were designated as stress-resilient or stress-vulnerable at weaning. In addition, we predicted that SR pigs, which should be capable of recovering from challenges more quickly, would show lower SM scores and resume normal behavior faster than SV pigs. However, in this study, we found that stress designation at weaning showed no correlation with either the SM score or time to return to home pen behavior following exposure to an acute auditory stressor (blast from an air horn) in the startle test.

Various factors in our study may help explain this disconnect. 1) Individual variation: Animal's responses to stressful situations (included those that induce fear) can be influenced by individual differences, ranging from genetic factors to personality traits (e.g., temperament) to life experiences (e.g., social context, management, and husbandry practices). These individual variations may lead to distinct impacts on both physiological and behavioral responses. 2)

Characteristics of the stressors: another possible explanation could be the difference between the types of stressors associated with weaning and the startle test as a long-lasting, multimodal weaning stress was used for resilience determination and an acute, simple auditory stimulus for the startle test. In order to fully understand pigs' responses, it is necessary to consider carefully

the limitations of using simplistic parameters in order to assess animal welfare. 3) Time difference: there was a 3-week gap between when we initially classified the pigs according to their physiological stress resilience and when we conducted the startle test. Young piglets develop and grow rapidly, gaining more experience and learning during this period. Nevertheless, the startle test could be a practical method for assessing fear in farmed pigs, as it has the advantage of not requiring prior training of pigs and can be conducted in the pigs' familiar home pens (thus requiring no handling). Fear is a fundamental emotion crucial for survival, making the pigs' responses to the startle test a valuable indicator of their emotional state and welfare. Therefore, we can use the pigs' natural and strong reactions in the startle test to evaluate their fear-related behavior, particularly in relation to factors like stress resilience (SR) and stress vulnerability (SV). In conclusion, the startle test shows promise as a meaningful tool for evaluating resilience as well as affective state, but it needs further improvement to enable real-time data collection and the use of various stimuli to elicit stronger startle responses.

The aim of the second study was to investigate pigs' home pen behavior, with a specific focus on examining their behavioral response to and recovery from weaning stress in relation to physiological stress resilience or vulnerability at weaning. Our study found that stress-resilient (SR) piglets spent more time lying down immediately following weaning (d1), whereas stress-vulnerable (SV) piglets showed more non-agonistic behavior on d1 and fighting behaviors on d4 post-weaning. In addition, we observed patterns of changes in certain behaviors over time. Changes in behaviors such as fighting, non-injurious contact, feeding, lying down, exploring, and drinking behaviors could indicate that piglets' social hierarchy became more stable over time. These behaviors, contributing to the development of a stable social hierarchy, may be regarded as indicative of adaptation to post-weaning life. However, at present, it is difficult to

accurately interpret behavioral changes in pigs following weaning without an accurate baseline for what constitutes appropriate levels of these behaviors. Essentially, there is a need to determine the ideal frequency, duration, and pattern of behaviors following weaning that indicate good welfare. By developing a method to quantify behavioral parameters relating to the resilience of piglets to weaning, we would be able to better understand how they behave differently between SR and SV pigs and provide ethologically meaningful aspects that would be useful in helping to assess their adaptation after weaning. Through the development of a method for measuring behavioral parameters associated with piglet weaning resilience, we can gain a deeper understanding of the behavioral distinctions between SR and SV pigs. This approach offers ethologically significant insights that are valuable in evaluating their post-weaning adaptation.

Overall, the findings of this research improved our understanding of assessing pig welfare by examining their affective state, behavioral responses to challenging circumstances, and physiological resilience. Especially, our emphasis was on examining the connection between the stress resilience of pigs and their behavioral responses, with a particular focus on identifying potential variations among stress-resilient and stress-vulnerable pigs. In the first study, we found no correlation between stress designation at weaning and the pigs' stress response in the startle test, highlighting the influence of individual differences, including genetics and life experiences. Moreover, the nature of stressors associated with weaning and the startle test may differ, underscoring the need to consider these differences. Additionally, the initial classification of pigs based on their stress resilience at weaning and the startle test were separated by approximately 3 weeks. While further refinement is necessary for the startle test, it holds potential as a practical tool for assessing fear in farm pigs, providing insights into their emotional state and welfare. The

second study examined how home pen behaviors evolve after weaning, showing links to pigs' physiological resilience. These findings suggested better adaptations to post-weaning life by SR pigs, yet clearer criteria for post-weaning behavior related to good welfare are needed.

Future studies could focus on refining the startle test. To eliminate the need for time-consuming video decoding in the future, it is essential to develop a real-time observational method applicable on farms. By utilizing a standardized sequence of motor-autonomic sensory reactions, including arousal, freezing, flight or fight, tonic immobility, collapsed immobility, and quiescent immobility, there is a possibility of achieving this goal. Additionally, through the utilization of multimodal startling stimuli (e.g., auditory and visual components), we anticipate triggering a more pronounced startle response, thereby enhancing the overall effectiveness of the study. Assessing pigs' behavioral changes following stressful situations such as weaning and developing quantitative parameters to enhance our understanding of piglet weaning resilience are important points of future research. Therefore, it may be necessary to consistently observe these behavioral patterns immediate after the weaning, with a larger sample size, to better understand when they indicate good welfare (adaptation) versus poor welfare (failure to cope). These approaches offer ethologically valuable insights, fostering improvements in post-weaning adaptation. Developing behavioral indicators to identify pigs' resilience ultimately enhances pig welfare by targeting management and breeding strategies based on resilience.

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