

Evaluation of stress and acclimation to new environments, procedures or equipment in Göttingen Minipigs using behavioral and physiological stress indicators

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Introduction

Swine, including domestic farm breeds and miniature breeds, are increasingly utilized in biomedical research because of similar anatomical and physiological traits to humans. Despite the benefits of swine models, specific accommodations have been made to work with laboratory swine due to their particular social, behavioral and husbandry needs. It is well established that swine are highly social and intelligent animals that require substrate for successful behavioral management because of their hard-wired rooting behavior (Ellegaard et al. 2010; Grandin 2014; Panepinto 2014). Therefore, if the swine model is being used as a dermatologic or surgical model, the research/veterinary group must make thoughtful considerations to prevent irritation, bleeding or infection of the wound site. Single-housing on slatted hard flooring without substrate may have been the standard alternative to maintain the integrity of a wound care dressing and bandage. However, barren enclosures, single housing and hard surfaces without substrate do not provide cushion, thermoregulation, rooting or social opportunities (Grandin 1986, 2014, 2015, 2016; Panepinto 1986, 2001, 2009, 2014, 2015, 2016; Smith and Swindle 2006). To that end, it is important that animal welfare and behavioral studies are conducted to assess an animal's wellbeing within the specifications set forth by a research environment.

This paper describes a collaborative effort of animal vendors, equipment manufacturers, researchers, veterinarians and swine behaviorists in developing a protective jacket for swine which will endure manipulation, be easy to apply and remove by handlers, minimize stress, and provide comfort. We believe that this team approach of consulting experts in the field ensures the highest standard of care for our research animals.

New environments, procedures or equipment can be major stressors for an animal (Ellegaard et al. 2010; Grandin 1986, 1997). Both short term and long-term stress factors have been shown to interfere with an animal's normal physiological parameters resulting in compromised immune responses and reproduction cycles (Grandin 1986; Swindle et al. 1994). Chronic stressors include long term single housing and housing swine in barren environments with no substrate or manipulanda limiting opportunities for animals to express species appropriate behaviors. Acute stressors include short term restraint for blood draw without prior conditioning or transport (Grandin 1997). Both types of stress factors have the potential to reduce the

wellbeing of the animal and therefore should be adequately identified and addressed.

To assess the psychological wellbeing of swine, a simple behavioral scoring system is used (Table 1) for gauging the animal's emotional state upon arrival to a facility and subsequently, throughout study procedures. To evaluate the animal's physiological state, salivary cortisol samples can be collected using SalivaBio's Children's Swab method.

Table 1:

Swine Behavioral Scoring System	
Description: Upon entering the enclosure and shutting the door, the observer sits within 2 meters from the entrance and starts the timer. Scores are defined by the time taken for each animal to approach the observer.	
Score	Definition
1	swine approaches the observer after 10 minutes
2	swine approaches the observer between 6 and 10 minutes
3	swine approaches the observer within 5 minutes

The goal of this project is to evaluate stress in swine during conditioning, placement, and wearing of a specifically designed protective jacket that is durable, thermoregulating, and comfortable for use in medical and research procedures. Through communications with Lovelace Biomedical veterinarians and principle investigators, Marshall BioResources (animal vendor), Lomir BioMedical (equipment manufacturer) and swine behaviorists (Temple Grandin and Linda Panepinto) refinement efforts were two-fold. First, to evaluate two types of Lomir custom designed protective jackets for swine during dermatology or surgical care and secondly, to assess the welfare of the animal during the process of conditioning and wearing the jacket using behavioral and physiological stress indicators.

Lovelace Biomedical collaborated with Lomir BioMedical to design a swine protective jacket to assess ease of use, comfort, and durability. Neoprene fabric was used for the jackets' material for flexibility and comfort (Mino et al. 2013). Using Lomir's swine measurement form located on their website (<https://www.lomir.com>), each pig was measured for establishing a well-fitted protective jacket. Two jackets were developed using the same neoprene material but with different closure types to assess ease of use for the handler when applying or



Image 1: Lomir custom swine protective jacket #1 with zipper and Velcro closure



Image 2: Lomir custom swine protective jacket #2 with Velcro closure only

removing from the pig as well as durability and long-term wear (Images 1 and 2).

From consultations with well-known animal behaviorists on acclimation techniques to new equipment and procedures, such as jacket placement, several factors were identified:

1. Start with the best

- Swine should be purchased from an animal supplier with a reputable animal welfare program and dynamic behavioral management program that includes
 - frequent handling of weanlings
 - social housing
 - training animals
 - acclimation of animals to novel experiences on their own terms
- Communicate with animal supplier for selecting previously socially housed swine for ease of transition to a new facility
- Continue social housing compatible swine in pairs or groups

2. Gain the animal's trust

- Upon arrival to the new facility, immediately begin conditioning swine to humans to gain their trust, using positive reinforcement
 - use gentle tone when interacting with naive swine
 - enter pen slowly
 - sit on pen floor
 - allow swine to inspect care staff
- Conditioning swine to novel equipment on their own terms such as, allowing each pig with pen mate to inspect and explore a new area or novel piece of equipment (e.g. sling or protective jacket)
- Clicker or target training swine for
 - increasing their confidence
 - reducing their startle response
 - providing mental stimulation
 - ease of handling

3. Structural Enrichment

- House swine with substrate rather than smooth flooring for
 - traction, as unsecure footing causes stress
 - providing comfort to joints
 - assisting in thermoregulation
 - encouraging hard-wired rooting behavior
 - bedding depth of at least 0.1016 m (about 4 to 5 inches)

4. Comfortable Fitting Equipment

- A swine projective jacket should be designed with the following
 - soft, comfortable quick drying material
 - smooth closures, as rough edges can irritate
 - form fitting

Methods and Materials

For this evaluation, four male Göttingen Minipigs approximately 5 months old with weights ranging from 9.3 kg to 14.4kg were pair housed with original pen mate from Marshall BioResources upon arrival after a veterinarian health assessment. The minipigs were housed in accordance with the Guide (NRC, 2010).

Swine pairs were housed in a 3.048m X 6.096m enclosure (10' X 20') with aspen wood shavings approximately 0.1016m (4 to 5 inches) deep as depicted in Image 3.

All animals had access to water ad libitum, fed twice a day and pens were cleaned daily with new substrate added as needed. Both enclosures included a variety of sensory enrichment, with a minimum of 4 hanging and 4 loose manipulanda per pen, rotated weekly for novelty.

Behavioral Scores were recorded upon entering the enclosure prior to beginning any interaction or acclimation sessions as described in Table 1.

Acclimation Period

- To humans**

From day 2 post arrival, human acclimation sessions began following the recording of individual behavioral scores. No sessions were conducted on weekends. After the swine approached and moved forward to appear purposeful in making contact with the observer (usually with their snout), the observer could attempt to touch the swine under the jaw or on the sides of their body

- To environment**

Within 5 days after arrival, swine were given “walk-a-bout” sessions with pen mate in the center isle of the animal housing building to explore and increase competency of environment which has been shown to reduce fearful behavior

Training Period

- Clicker training began once each animal was acclimated to the handler
- Each pig was taught to touch a target (a plastic spoon or a PVC pole approximately 0.25m to 0.75m long), follow the target to a floor scale, hold snout to target for up to 10 seconds and return to home pen on cue (Image 4)

Lomir Protective Swine Jacket Acclimation

- After swine were compliant with training >80% of the time when given the cue by the handler, both swine jackets were introduced for acclimation. Jackets were placed on the pen floor during AM feeding for swine to inspect on their own terms and removed by the end of the day

Saliva Cortisol Collection

- Cortisol samples were collected from each animal before feeding using Salimetrics collection swabs in the AM and PM over the course of this study and 3 days after jacket evalua-



Image 4: Swine training equipment

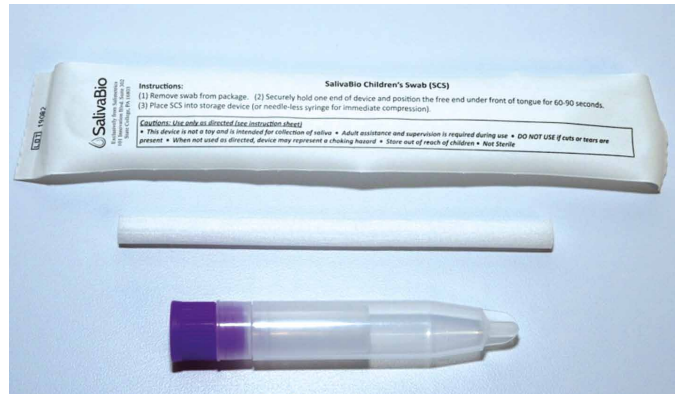


Image 5: Salimetrics saliva collection swab and vial

tion. The manufacturer’s guidelines for collecting saliva was modified from the SalivaBio’s Children’s Swab Method with the following modification for collection with swine (Image 5)

1. The familiar human handler entered the enclosure and sat on the pen floor
2. Swine were randomly selected for individual saliva sample collection
3. The handler held both sides of the sampling swab provided by Salimetrics Saliva Lab to allow the animal an opportunity to mouth the swab voluntarily until saturated with saliva
4. Then the swab was inserted into a pre-labeled vial, sealed, and immediately placed on ice. Within an hour of collection, all samples were stored in a -80C freezer until shipped to Salimetrics for analysis
5. Swine were rewarded with high value food enrichment after collection

Results

- The 4 male Göttingen Minipigs took less than seven days to acclimate to their new environment and human handlers based on their behavioral scores of 3, for three days consecutively
- All swine were clicker trained and compliant to targeting cues >80% of the time within 14 days post arrival
- Swine showed no signs of stress using behavioral scoring for placement and wearing of either type of protective jacket (Images 6 and 7)
- Handlers noted that jacket #1 with a zipper plus Velcro closure remained on swine for the duration of assessment and jacket #2 with Velcro only was removed by the end of the day by swine
- Cortisol analysis for this project is in progress and results are expected to be published later this year

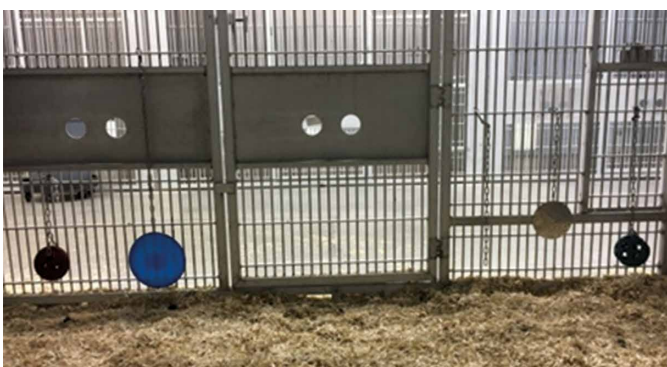


Image 3: Swine enclosure





Image 6:
Göttingen
Minipig in
Lomir Jacket
#1



Image 7:
Göttingen
Minipig in
Lomir Jacket
#2

Conclusion

Communications in the refinement of devices and procedures used in animal medicine and research should include the animal vendor, veterinarians, animal behaviorist and/or manager, principle investigators, equipment designers, and users. Beginning with a reputable animal vendor that is proactive in their commitment for enhancing animal welfare begins the foundation for reducing stress when animals transition to a new environment or novelty. Swine experts recommend gaining the animal's trust prior to beginning new procedures to decrease behavioral signs of excitability and fear. When assessing the welfare of the animal and progress of their acclimation to novelty, it is important to remember, individual behavioral responses may vary between animal subjects. Using a simple scoring system can assist in identifying fearful or timid animals that can benefit from a modification in their conditioning regime as well as evaluate the animal's progress over time. In addition, for collecting cortisol to assess acute stress levels in individual swine, saliva collection is a less invasive technique and shows a stress response within minutes after an event. Swine in well-designed jackets behave normally and with a durable jacket closure system will remain on the animal during social housing.

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