Gradual Training of Alpacas to the Confinement of Metabolism Pens Reduces Stress When Normal Excretion Behavior Is Accommodated

Kirrin E. Lund, Shane K. Maloney, John T. B. Milton, and Dominique Blache

All authors are affiliated with the School of Animal Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, Western Australia, in the following positions: Kirrin E. Lund, BSc, is a PhD student; Shane Maloney, BSc, PhD, is a professor; John T. B. Milton, BSc, PhD, is a senior research fellow and director of the Independent Lab Services in Crawley; and Dominique Blache, BSc, MSc, PhD, is an associate professor. Address correspondence and reprint requests to Dr. Dominique Blache, School of Animal Biology (M085), Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, 6009, Western Australia, or email: dominique.blache@uwa.edu.au.

Abstract

Confinement in metabolism pens may provoke a stress response in alpacas that will reduce the welfare of the animal and jeopardize the validity of scientific results obtained in such pens. In this study, we tested a protocol designed to successfully train alpacas to be held in a specially designed metabolism pen so that the animals’ confinement would not jeopardize their welfare. We hypothesized that the alpacas would show fewer behaviors associated with a response to stress as training gradually progressed, and that they would adapt to being in the confinement of the metabolism pen. The training protocol was successful at introducing alpacas to the metabolism pens, and it did reduce the incidence of behavioral responses to stress as the training progressed. The success of the training protocol may be attributed to the progressive nature of the training, the tailoring of the protocol to suit alpacas, and the use of positive reinforcement. This study demonstrated that both animal welfare and the validity of the scientific outcomes could be maximized by the gradual training of experimental animals, thereby minimizing the stress imposed on the animals during experimental procedures.

Key words: animal welfare; behavior; habituation; stress

Introduction

The confinement of an animal is known to alter its normal behaviour, and can be a significant stressor (Bowers et al. 1993; Fraser 2008). The use of metabolism crates in research, where the animal is confined for an extended period of time, is a controversial practice because both the welfare of the animal and the validity of scientific results can be compromised (Fraser 2008). Little has been reported with respect to the reaction to stress of alpacas in metabolism crates. Although some reports on confinement in other species appear in the literature, it is important to bear in mind that large interspecies variability can occur in animals’ capacity to tolerate and adjust to confinement (Bowers et al. 1993; Fraser 2008).

The effect of confinement has been studied in other farm animals such as sheep, pigs, and horses (Bowers et al. 1993; Jaskulke and Manteuffel 2011; Mal et al. 1991). Acclimatization periods and habituation training can assist sheep in adapting to confinement and restraint during handling (Grandin 1989). Sheep that are acclimatized to a procedure or routine generally display reduced physiological and behavioral responses associated with the stress response compared with sheep that experience the same procedure for the first time (Grandin 1997). Alpacas are social animals with a strong group hierarchy (Fowler 1998). Therefore the act of placing them in confinement where they are exposed to a novel environment and have limited social contact is likely to induce changes in behavior and physiology associated with the “stress response” that is seen in other social species such as sheep (Bowers et al. 1993; Done-Currie et al. 1984).

The main impetus that led to our study was the need to conduct nutrition experiments, which require alpacas to spend approximately 7 days in a metabolism pen so that energy and nitrogen balance can be determined. The aim of the study was to design a protocol to train alpacas successfully to remain in a specially designed metabolism pen that would be used for future nutritional studies (Figure
1). The training protocol and metabolism pens were developed to decrease the welfare compromise associated with the transition from a paddock and group situation to the semi-isolation of the metabolism pen (the pen is not total isolation because an alpaca in the pen can see and communicate with other alpacas). For ethical reasons and safety concerns, we did not compare the behavioral stress response of trained and untrained alpacas, but instead compared the same animals when they were naive to following training. It was expected that the alpacas would gradually show fewer stress-associated behaviors as training progressed, and that they would adapt to the confinement of the metabolism pen.

![Figure 1. The metabolism pen designed for alpacas with sufficient floor space and high sides.](image)

Material and Methods

The University of Western Australia Animal Ethics Committee approved all of the methods described in this article (RA/3/100/877). In the study, the alpacas were gradually trained to the confinement of a metabolism pen. During the training, the alpacas' behavioral response to this stress was monitored and used as a measure of the amount of strain the animal was experiencing.

Preliminary Halter and Lead Training

Ten alpacas underwent halter training and were used in this study. They were sourced from local breeding facilities where they had been housed in a paddock environment and were rarely yarded. The alpacas had received little to no training before being used in the experiment. Upon arrival at Shenton Park Research Station at the University of Western Australia, we allowed the alpacas 1 week to become accustomed to their new environment. We kept them in a paddock with an open-sided shelter. This paddock was the location for the training periods, and when they were not in the metabolism pens, we permitted the alpacas to roam freely in the paddock. Before beginning the metabolism pen training protocols, the alpacas were familiarized with the handlers and underwent halter and lead training. The same method of halter training was used for all of the alpacas (Table 1). Each training session lasted approximately 15 to 20 minutes although the time taken for each step of the protocol varied according to the progress made by each individual alpaca. Generally, we introduced a new step every 2 days, and we modified some sessions according to the progress made by the individual alpaca. Within 10 days, the majority of the alpacas were accustomed to the halter and would walk on the lead without pulling. We offered food rewards when a training step was successfully completed, and we used this method of positive reinforcement throughout the entire training process.

<table>
<thead>
<tr>
<th>Training step</th>
<th>Description of method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alpaca caught and held by handler. Gently rubbed over neck and shoulders.</td>
</tr>
<tr>
<td>2</td>
<td>Alpaca introduced to the halter; allowed to sniff, halter gently placed in front of</td>
</tr>
</tbody>
</table>
3 Once nosepiece was in place, strap behind ears was fastened. Alpaca taught to stand quietly and allow halter to be removed.

4 Alpaca was led in straight line, encouraged not to pull. Introduced to “walk on” and “stand” commands.

5 Alpaca was led in a more complicated trajectory (e.g., weaving, over obstacles).

Metabolism Pen Training

Monitoring During Training

The level of stress that an animal perceives can be assessed using either physiologic or behavioral indicators, or using both (Baldock and Sibly 1990; Grandin 1997). For the present study, we chose behavioral indicators to assess the response to stress because they are noninvasive and/or impose no additional stress on the animal. They also provide an immediate measure of the response to stress and therefore constitute a more useful and practical assessment during this experiment. Alpacas display a number of behavioral reactions to stressful situations such as holding their tail above their back, spitting, rolling their head, making loud screaming vocalizations, and flaring their nostrils (McGee-Bennett 2001). During the introduction of an alpaca to a metabolism pen, we defined a stressed alpaca by the following possible behavioral indicators of stress: repetitively lying down and getting up over short time intervals, attempts to escape, kicking toward the pen, restlessness or pacing around the pen, loud vocalizations, excessive alertness defined by the ears pricked forward and flared nostrils, or pushing against the sides of the pen. We quantified the incidence of these behavioral responses during the training of the alpacas and used the data as a guide to the degree of success and subsequent progression through the training. During constant monitoring, we recorded the alpaca’s behavior every 5 minutes or when the alpaca showed signs of distress. If an alpaca showed three or more of the stress signals over two time monitoring periods (every 5 minutes to every 2 hours depending on the training step), we removed the animal from the metabolism pen and retrained from the previous training step. Throughout the study, whenever one alpaca was in the metabolism pen, we kept its companions in a small yard that was constructed around the pen, or in other metabolism pens placed close by, so that all of the animals remained in contact with each other.

Training Protocols

The study consisted of two parts. First, we conducted a preliminary study to assess a training protocol designed from knowledge of the social behavior of alpacas. We used four alpaca wethers (2-2.5 years old) who were successfully accustomed to the metabolism pens using the training protocol (see Protocol 1 below) within 11 days. During step 6, we arranged for the construction of the metabolism pen under the open-sided shelter so that the alpacas had sufficient cover while in the pen. We left in the pen the alpaca’s water bucket and daily food ration of milled barley straw, along with a roughage-based pellet (Macco 101 pellet, Macco Feeds Australia, Williams, Western Australia) and dry, granulated sugar cane molasses (Palabind, Probio tec). We used dry molasses as a food reward during the other steps of the protocol.

Protocol 1

1. We marked out an area of ground with the same dimensions as the metabolism pen (1.6 × 1.6 meters) using metal pickets ~ 1.5 meters high and rope. The alpacas were individually confined in this makeshift pen for 30 minutes while a handler standing several meters away constantly monitored the animals. We repeated this process two or three times until the animals settled and showed no stress-associated behaviors.
2. We left the alpacas in the makeshift pen for 2 hours under constant supervision.
3. We placed the flooring of the metabolism pen with the alpacas in a small yard where they spent half a day exploring...
and become familiar with it.

4. We restricted the alpacas to the flooring for 2 hours with constant monitoring. For practical reasons, we modified this step during the training so that the handler led the alpacas over the flooring and left them standing on the floor for progressively longer periods of time (10, 30, and 60 seconds).

5. We gradually added the sides of the metabolism pen, and we left the alpacas in the constructed pen for 30 minutes.

6. We gradually increased the time the alpacas spent in the metabolism pen from 2 to 4, 8, and 24 hours.

The behavior of the four alpacas indicated that the gradual construction of the metabolism pen sufficiently desensitized the alpacas to confinement. This desensitization of the animals enabled them to become familiar with the pen and to remain in the pen with apparent lack of concern for long periods of time (Table 2).

Table 2 Assessment of training success achieved with each step of Protocol 1*

<table>
<thead>
<tr>
<th>Training step</th>
<th>Degree of success and observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>All of the alpacas stayed in pen. Animals had to remain haltered because the rope fence did not always prevent them from leaving the defined area. No signs of any perceived stress; escape initiated out of boredom rather than fear.</td>
</tr>
<tr>
<td>2</td>
<td>Alpacas remained haltered. No signs of any perceived stress.</td>
</tr>
<tr>
<td>3</td>
<td>Repeated over 3 days. All alpacas observed exploring the flooring; were content to lie down near it and eat food from it.</td>
</tr>
<tr>
<td>4</td>
<td>All alpacas were comfortable with being led on and off the flooring. No signs of any perceived stress.</td>
</tr>
<tr>
<td>5</td>
<td>Two opposing sides added; all alpacas led over with no signs of perceived stress. Third side added; alpacas accepted gradual confinement with no signs of perceived stress. Fourth side added; some signs of perceived stress were evident. One alpaca circled the pen, looking for a way out, and attempted to escape several times by pushing and banging on the sides of the pen. This animal was removed from the metabolism pen and calmed down before starting again from the beginning of the training step. Second attempt was more successful; alpaca discovered his food and ate, which resulted in less circling and no attempts at escape.</td>
</tr>
<tr>
<td>6</td>
<td>All alpacas appeared comfortable. As time increased, the alpacas were observed to refrain from urinating and defecating until they were released. When required to stay in pen for ≥ 8 hours, the alpacas urinated and defecated. Some animals appeared to experience some discomfort, evident by small humming noises</td>
</tr>
</tbody>
</table>
and sniffing at the flooring. When the animals did defecate, their feces were solid and packed together unlike the animals’ normally excreted individual pellets. Consequently, the feces did not fall through the flooring of the metabolism pen to be trampled and spread over the floor, making collection for nutritional studies difficult and inaccurate.

*See text for a description of Protocol 1.

After the preliminary study, we developed a more flexible, modified protocol (see Protocol 2 below) in which we altered some of the training steps originally used to suit the progress of an individual alpaca and the degree of success achieved with each step. We then conducted a second study using six different alpaca wethers of similar age to test Protocol 2. The alpacas completed Protocol 2 within 6 to 7 days.

**Protocol 2**

1. We placed the flooring of the metabolism pen in a small yard where the alpacas spent half a day exploring and becoming familiar with it.
2. We led the alpacas across the flooring until we observed no behavioral indications of stress.
3. Gradually, we added the sides of the metabolism pen, and we led the alpacas through the constructed pen.
4. The alpacas spent a full day in the metabolism pen, in sight of peers, under constant supervision.

**Design of the Metabolism Pens**

The metabolism pens that we used in this study were specially designed for alpacas but are suitable for other production animals such as sheep and goats. The welfare of each animal in the pen was a high priority, thus the metabolism pen had a larger floor space \((1.6 \times 1.6 \text{ meters})\) than most conventional metabolism crates. Each pen accommodated one alpaca lying down with its neck stretched out in front, as is their habit (Figure 2). The flooring was situated as low as possible to the ground so that the animal was required to take only one small step up to enter, but the flooring was high enough to accommodate urine and feces collection apparatus underneath. The flooring was made of nonslip plastic material that is commonly used in piggeries (Stepper flooring, MIK International, Germany). The sides of the crate were made high \((1.2 \text{ meters})\) but still allowed the animal to see its peers.

![Figure 2](image)

**Figure 2.** The metabolism pen has sufficient floor space to accommodate an alpaca lying down with its neck stretched out in front.

**Results**

Five of the six alpaca wethers were successfully trained to the metabolism pen using Protocol 2. One alpaca was excluded from the training phase because he showed no improvement during basic handling procedures such as being haltered and walked on the
lead. We decided that any attempt to put him near the metabolism pen would impose a considerable stress and be dangerous to the animal and to the handler.

The alpacas showed no behavioral signs of stress during step 1 of the training protocol, when they explored the flooring of the metabolism pen. Initially, most of the animals sniffed at the floor and then ignored its presence. After approximately 1 hour, one alpaca walked over the floor.

During step 2, we observed stress behaviors within the first 10 minutes of the training sessions. Three of five alpacas progressed to the next training step after one 20-minute session (Figure 3). The other two animals that repeated the training step showed five incidents, collectively, of excessive alertness in the first 10 minutes of the session. We also recorded restlessness in the form of pulling on the lead rope on three occasions (Table 3).

Table 3 Collective number of incidents of stress-associated behaviors expressed by all alpacas during each step of Protocol 2.* (Click anywhere on table for larger version.)

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (mins)</th>
<th>Stress Indicators</th>
<th>Total no. of incidents</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Half day</td>
<td>Unsettled</td>
<td>Escape attempt</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Dashes indicate that no behavioural response was observed.

During the first 10 minutes of step 3 of the training protocol, some alpacas demonstrated restlessness and excessive alertness. However, only two training sessions were needed for all of the animals to complete this step (Table 3).

Initially, the alpacas were restless when they were left in a metabolism pen for a day (step 4). One animal initially attempted to escape by pushing on the sides of the pen; however, all animals settled within 1 hour and ate their daily food ration. Throughout the day some animals made soft vocalizations, often in response to another animal. After 9 hours, most of the alpacas appeared to be unsettled and restless, and we observed repeated standing up, lying back down, pacing around their pen, and sniffing at the floor. It was evident that they were uncomfortable. Because alpacas typically defecate and urinate at communal dung heaps, their behavior suggested that they needed to defecate and urinate but were unwilling to do so. One animal attempted to escape by rearing. On the second attempt at escape, that alpaca appeared to urinate accidentally, which apparently prompted him to stand in the body position associated with excretion, after which he did urinate and defecate. After urinating and defecating, the alpaca reverted back to being calm and showed no further overt behaviors associated with perceived stress. The behavior of this animal suggested that the presence of feces in the pen could be used as a stimulus to trigger defecation. The feces from that animal’s pen were transferred into
the other pens in an attempt to trigger a response from the other animals (see Discussion). This method was successful in changing the alpaca’s learned behavior of using communal dung heaps. After all of the animals had urinated and defecated, they showed no further behaviors associated with perceived stress, and they continued to urinate and defecate normally.

Discussion

The protocol developed in this study was successful at introducing alpacas to specially designed metabolism pens, and it led to a progressive decline in the occurrence of behavioral responses to stress that can be experienced by animals subjected to confinement. The alpacas adapted to the confinement of the metabolism pens and showed fewer behaviors associated with a response to stress as the training progressed. The success of the protocol may be attributed to the progressive nature of the training in which each alpaca was presented with one stressor at a time and given time to become comfortable with that stressor before proceeding to the next training step. Compared with a review of behavioral principles of sheep handling in which Hutson (2000) concluded that gradual training and handling can reduce the stress of handling in sheep, it is apparent from our results that his conclusions pertain also to alpacas. Gradual exposure to novelty can allow animals to become accustomed to stimuli that may otherwise prompt stress-associated behaviors (Grandin 1997). In this study, we exercised care to ensure that the alpacas appeared to be comfortable with each training step before we guided them to progress to the next step. For example, we did not introduce them to the flooring of the metabolism pen or lead them over it until they had walked calmly on the lead and had responded to verbal commands from their handler.

We also tailored the training protocol to the alpacas by using information and observations of alpaca behavior. These animals are naturally inquisitive and social, and they should find exploring novel objects a positive experience when kept in a stable group (Fowler 1998; Tennessen 1989). For that reason, we allowed the alpacas to explore the metabolism pen flooring in their own time when it was left in their paddock. By keeping them together as a group and letting them approach the flooring by themselves, it appeared that we effected a reduction in the stress of having a novel object in their yard. Similarly, because of their social nature, we had the metabolism pen designed to allow the alpacas to see each other, even when they were lying down as they do in the field. The freedom to express normal behavior is regarded as one of the “five freedoms” used to assess animal welfare (Farm Animal Welfare Council 1992). We designed the metabolism pen to be more accommodating of the normal behavior of alpacas than conventional metabolism crates, which tend to isolate the animal completely by blocking visual contact with conspecifics and restricting the amount of floor space to such a degree that the animal cannot easily turn around. It appears that in this study, the welfare of the animals was not compromised.

Although we developed the training protocol for alpacas, it was evident during our study that some behaviors that are characteristic of the species were more difficult to overcome. As mentioned above, alpacas defecate and urinate on communal dung heaps (Fysh 2003). When the alpacas were required to remain in the metabolism pens for extended periods, they initially displayed no signs of an agitated state but later became agitated and restless. Given the subsequent behavior of the alpacas, we conclude that this restlessness was due to their resisting the micturition and defecation reflexes because they did not have access to a communal heap. It was possible, however, to modify this learned behavior and train the alpacas to defecate and urinate within a few hours of being in the pen by transferring fresh feces into the pen from a pile in the paddock or in another metabolism pen. This action appeared to act as a stimulus to release the inhibition of the excretion reflexes. After the initial excretion, the alpacas continued to defecate and urinate in the pens regardless of the amount of time they spent out of the metabolism pens between training sessions. This outcome was important because the objective of keeping animals in a metabolism pen is usually so that feces and/or urine can be collected. It also highlights the importance of the relationship that animals have between olfactory cues and behavior. It is clear from this experiment that rather than having clean and disinfected pens, the animals required the presence of some feces in the metabolism pens for them to perform natural functions. From a regulation and ethical view, keeping animal facilities very clean or sterile can likely affect the behavior of the animal and inadvertently cause stress.

The training protocol may also have been successful because we used positive reinforcement, mainly in the form of food. Sheep can be trained to accept restraint voluntarily with the assistance of grain as a reward (Grandin 1989). Similarly, Hutson (2000) has recommended that the aversive and stressful nature of handling could be reduced by using food rewards. In the present study, the alpacas appeared to remember that when they behaved in a particular way, they would be rewarded. The gradual absence of behavioral responses to stress suggested that the animals had a positive experience.
Although, according to our measures, the training protocol was successful in introducing alpacas to the metabolism pens and reducing the potential stress of being confined within the pen, it must be noted that we used only behavioral indicators to assess the response to stress. Other authors have suggested that it is best to use both behavioral and physiological indicators such as cortisol or heart rate (Arzamendia et al. 2010; Grandin 1997). However, behavioral indicators alone were a practical and immediate measure of the response to stress in this study. Because cortisol concentration was not immediately available, it was not an appropriate stress indicator in this study. Cortisol concentrations may have confirmed the response to stress by the animal, although there is evidence to suggest that cortisol may not always show a response during handling, particularly when the animals are habituated (Andrade et al. 2001; Lay et al. 1992). Likewise, heart rate measurements can be confounded by activity and may not provide an accurate measure of stress during the training. Moreover the training protocol was spread over a few weeks so that it was not practical to equip each alpaca daily with devices to measure heart rate or with indwelling cannulae to take serial blood samples. Both of these procedures can themselves act as stressors.

Changes in behavior are the first symptoms that can indicate the state of an animal’s well-being (Dellmeier 1989). The nature of the training relied heavily on the handler recognizing when an alpaca showed reluctance to the task he was being asked to do and therefore being able to remove the animal from the stressful situation. Studies on the response to confinement in other species, such as horses, have also used behavior as the sole indicator of animal well-being (Mal et al. 1991). Likewise, a correlation between behavioral observations and physiological measures that reflect the physiological response to stress has been identified in cattle (Stockman et al. 2011).

Our study also highlights that although the training protocol worked for most of the alpacas, some animals may not become accustomed to handling or novel experiences regardless of the amount of time the handler spends in taming the animal. During this study, we excluded one alpaca from the training to the metabolism pen because he continued to show signs of stress during basic handling sessions. This animal’s continued adverse reaction may be attributed to its previous life experiences, its genetic makeup, or an interaction between the two. Often an animal’s previous experience can influence its response to stress (Grandin 1997). In addition, genetic factors such as temperament may influence an animal’s resistance to stressors and their ability to be trained (Grandin 1997).

Conclusions

Overall, the training protocol that we developed to train alpacas to the confinement of metabolism pens was successful in reducing the incidence of the animals’ stress-associated behaviors while in the pens. We largely attribute this success to the gradual progression of the training steps and our tailoring of the protocol to suit alpacas by using positive reinforcement. The study demonstrates that when it is necessary to confine large experimental animals, such as during metabolic experimentation, it is clearly advantageous to develop adequate procedures for minimizing the animals’ stress. By implementing such procedures, it is possible to maximize both animal welfare and the validity of scientific outcomes.

Acknowledgments

We thank Dr George Jackson and Jenny Jackson from Banksia Park Alpaca Stud and Peter and Carolyn Richards from Suncloud Alpaca Stud for the loan of the animals. We also thank Raymond Scott and greatly appreciate his assistance in designing and building the metabolism pens.

References


