Choice of attractive conditions by beef cattle in a Y-maze just after release from restraint

T. Ishiwata,* R. J. Kilgour,† K. Uetake,*1 Y. Eguchi,* and T. Tanaka*

*School of Veterinary Medicine, Azabu University, Sagamihara 229-8501, Japan; and
†Agricultural Research Centre, New South Wales Department of Primary Industries,
Trangie, New South Wales 2823, Australia

ABSTRACT: To provide useful information on how to moderate posthandling stress, the attractiveness of different conditions to beef cattle just after release from restraint was determined. Angus heifers were individually allowed to enter a choice area after 2 min of restraint in a squeeze chute and to choose between 2 pens. After the heifer had chosen a pen, it could freely access both test pens and the choice area for a further 5 min. In Exp. 1, each heifer was given 1 of the following choices: a pen with 3 familiar heifers (PEERS) vs. a pen with a pile of hay on a metal rack (FOOD; n = 34); PEERS vs. the bare pen (BARE; n = 34); and FOOD vs. BARE (n = 35). When the choice combination was PEERS vs. BARE, more heifers chose PEERS (P < 0.05). When the choice combination was PEERS vs. FOOD, more heifers than expected tended to choose PEERS (P < 0.10), whereas FOOD and BARE did not differ. The latency to choose either pen was shorter if PEERS was 1 of the 2 choices (P < 0.01). After choosing, more heifers entered the PEERS pen than the FOOD (P < 0.05) or BARE (P < 0.01) pens. In Exp. 2, another 86 heifers were given 1 of the following choices: a pen with a familiar handler standing inside (STI) vs. a pen with a novel object (NO; n = 29); a pen with the handler standing outside the pen (STO) vs. NO (n = 29); a pen in which the handler was sitting inside (SI) vs. NO (n = 28). Fewer heifers chose the pen with the human (STI, STO, and SI; all P < 0.01). Except for the choice of STO vs. NO, the number of heifers that had voluntarily chosen either pen was larger than expected (STI and SI; both P < 0.01). The number of times in which the NO pen was entered was greater than the STI and STO (P < 0.01), although the number of times in which the SI and NO pens were entered was not different. More heifers avoided the human, particularly a standing human. In conclusion, just after handling with restraint, returning cattle to the group of peers and not approaching the cattle needlessly should moderate their stress.

Key words: beef cattle, behavior, human-animal relationship, preference test, restraint, social motivation

INTRODUCTION

In normal beef cattle management, isolation from peers and restraint in a squeeze chute are handling conditions often used for such operations as weighing or administration of medication. However, these operations may cause stress to the animals, and this stress may make them agitated and difficult to handle. In the past, many tests have been performed to assess individual differences in behavioral reactions to stress (Grandin, 1993; Boissy and Bouissou, 1995; Le Neindre et al., 1995) and to determine the effects of handling on the human-cattle relationship (de Passillé et al., 1996; Boivin et al., 1998; Munksgaard et al., 2001). These studies produced useful information on the reaction of animals to human handling.

Another aspect of cattle handling worthy of study is that of behavioral responses once the animals are released from the squeeze chute, in particular the attractiveness or aversiveness of the conditions immediately after release. Such a study would provide useful information on how to overcome the aversive effects of handling. Although the Y-maze has indicated that calves need social contacts in their home pen (Raussi et al., 2003), care of animals after unavoidable management procedures has not previously been studied.

The objective of the current study was to determine the attractiveness of different conditions to cattle to moderate posthandling stress. In the first experiment, we investigated the relative attractiveness of hypothesized positive conditions such as peers, food, and a bare
Figure 1. Floor plan of the Y-maze. The test field and the choice area were surrounded by fences 1.6 m high. A circle (●) indicates the position of the choice stimulus when there were 3 peers or a human standing outside the choice pen; a triangle (▲) indicates the position of the novel object suspended on the fence, the food, the seated human, or the human standing inside the pen. The bold full line shows the location of the hemp cover sheets.

pen. In the second experiment, we investigated the relative attractiveness of hypothesized negative conditions such as a human in different postures and positions and a novel object.

MATERIALS AND METHODS

Animals and Test Procedure

Research protocols were approved by the Animal Experiment and Care Committee of Azabu University, Sagamihara-shi, Japan. Azabu University has the Animal Welfare Assurance number (A5393-01) of the Office of Laboratory Animal Welfare (OLAW), National Institutes of Health, Bethesda, MD.

A total of 189 Angus heifers (12 mo of age, 391.5 ± 33.0 kg) were used in Exp. 1 (n = 103) and 2 (n = 86). Heifers were born and reared on pasture at the Agricultural Research Centre, Trangie, New South Wales, Australia. Heifers were continually kept on pasture. Because of drought conditions prevailing at the time of study, heifers were managed as a single group and fed a drought ration every 2 d. This means that heifers had some contact with humans several times weekly. The diet consisted of 50% alfalfa hay and 50% grain (either oats or barley) and was fed at a rate of 9 kg-heifer⁻¹·d⁻¹. To ensure that all heifers had access to the drought ration, they were kept on pasture at a relatively high stocking rate of approximately 1 heifer/1.5 ha. Each morning of the experiment, a group of approximately 30 heifers was removed from the main group and moved to the yard complex where the Y-maze testing was carried out.

The Y-maze was constructed of metal fence panels 1.6 m in height and covered with sheets made from hemp to minimize the effects of outside distraction. During testing, heifers did not have visual or physical contact with cattle other than the heifers used as the choice stimulus. The Y-maze consisted of a forcing pen, single-file alley (0.9 × 11.8 m), a squeeze chute (0.9 × 2.75 m), a choice area, and 2 choice pens divided by the fence panel covered with hemp sheets (6.5 × 8.5 m each).

The floor of the choice area and the choice pens was bare earth (Figure 1).

Heifers were used once during the testing procedure. Immediately before testing, heifers were placed in holding pens adjacent to the forcing pen. Each heifer was then moved down the alley that led from the forcing pen and moved into the squeeze chute individually by 1 familiar handler. The handler came close to the heifer from the outside of the alley and tapped the rump of the heifer to encourage it to enter the squeeze chute. The test heifer was confined in the squeeze chute for 2 min without restraint in the head gate, which was simply used as the front gate of the chute to contain the heifer. For the first approximately 30 s of the 2 min, the handler stood in front of the heifer.

After the 2 min of confinement, the front gate was opened and the heifer was released. All heifers were allowed to voluntarily leave the chute, and they were never touched by the handler. After the heifer left the chute, it could enter the choice area, from where it could choose 1 of the 2 pens. If the heifer entered neither of the 2 pens within 5 min, the handler entered the choice area near the beginning of the chute and walked slowly behind the heifer until it entered one of the choice pens. This handler walked along the center line of the choice area to avoid influencing the choice made by the test heifer. Choice was defined as all feet of the heifer entering one of the choice pens, the choice area, or the chute. Consequently, 1 test period was for up to 12 min; 2 min for restraint, up to 5 min to choose, and 5 min for free movement.

From the time of release from the chute until completion of the test, movement of the heifer was recorded by a video camera that was attached to the point at which both the test area and the choice area could be recorded. From the video tape, the choice pattern (voluntarily or forced), the pen first chosen, the latency to choose, the numbers of times that heifers entered each
pen, and the time spent in each pen and choice area were recorded by the same person.

**Experiment 1.** To investigate the relative attractiveness of hypothesized positive conditions, each heifer was given 1 of the following choice combinations: 1) a pen containing 3 familiar heifers (PEERS) vs. a pen with a pile of hay on a metal rack measuring 1.2 × 0.65 × 0.65 m (FOOD; n = 34); 2) PEERS vs. a bare pen (BARE; n = 34); or 3) FOOD vs. BARE (n = 35). Familiar heifers were selected from the heifers already tested. During testing, familiar heifers that were a choice stimulus remained in a pen measuring 6.5 × 4.3 m adjacent to the test pen and separated by a barrier through which they could have visual and physical contact with the test heifer (Figure 1). Heifers used as the choice stimulus were changed every 3 or 4 tests. The metal rack containing hay was installed in the test pen at the midpoint of the side barrier opposite the entry gate (Figure 1).

**Experiment 2.** To investigate the relative attractiveness of hypothesized negative conditions, another 86 heifers were each given 1 of the following choice combinations: 1) a pen with a familiar handler standing inside (STI) vs. the pen with a novel object (NO; n = 29); 2) a pen with the same human standing outside the pen (STO) over the fence vs. NO (n = 29); or 3) a pen with the same human sitting inside the pen (SI) vs. NO (n = 28). During testing, the familiar handler as choice stimulus was standing in the test pen or the adjacent pen or he was sitting on a stool in the test pen (Figure 1). The person used in the test had some contact with the test heifers for 1 wk before this study. During this prior contact, heifers were moved down the alley and into the squeeze chute individually. The handler was also the same person encouraging the test heifer into the chute before the test and standing in front of the test heifers during restraint. The novel object was an orange-painted tire, 40.6 cm in diam., suspended by a rope 50 cm above the ground at the midpoint of the side barrier opposite the entry gate (Figure 1).

**Allocation of the Choice Stimuli.** In any 1 choice pairing, the choice stimuli were equally allocated to the right or left pens to be balanced for side of the maze. The choice combination was changed every 3 or 4 heifers so that it was not on the same side. It took 3 d to complete each experiment. The testing lasted from 0800 to 1200 h and 1300 to 1800 h each day. The time of each combination was allocated randomly during the morning tests and during the afternoon tests. During tests involving food, heifers to be tested were kept in the pasture adjacent to the test facility to prevent their motivation for hay from changing as a result of time off feed.

**Statistical Analysis**

In both experiments, the numbers of heifers choosing each pen and the number of heifers choosing voluntarily or after force were analyzed with the χ² test considering no choice (50% of heifers tested) as an expected number of heifers. The latency to first choice was analyzed using the Mann-Whitney U test, because the data were not normally distributed. The latency to choose a particular choice combination was analyzed using the Kruskal-Wallis test for nonnormally distributed data or a 1-way factorial ANOVA for normally distributed data. If the effects were significant, posthoc testing was performed with Tukey’s studentized range test. Comparisons among the numbers of times that heifers entered each pen were made using the Wilcoxon matched-pairs test. The time spent in each pen and choice area was analyzed using 1-way ANOVA or the Kruskal-Wallis test. If the effects were significant, posthoc testing was performed with Tukey’s studentized range test.

**RESULTS**

**Experiment 1**

The number of heifers choosing the PEERS pen was greater (P < 0.05) than expected when the choice combination was PEERS (70.6%) vs. BARE (29.4%). The number of heifers choosing the PEERS pen tended to be greater (P < 0.10) than expected when the choice combination was PEERS (64.7%) vs. FOOD (35.3%). There was no difference between the number of heifers choosing the FOOD pen and the expected value when the choice was FOOD (54.3%) vs. BARE (45.7%).

The latency to choose a particular choice condition (PEERS, FOOD, or BARE) was not affected by the choice condition on the other side of the Y-maze. However, latency (±SD) to choose was longer (P < 0.01) when the choice combination was FOOD vs. BARE (153.8 ± 128.2 s) than when it was PEERS vs. FOOD (57.8 ± 72.7 s) or PEERS vs. BARE (71.1 ± 88.5 s). Furthermore, fewer (P < 0.01) heifers than expected had to be forced to choose when the choice combination was PEERS vs. FOOD (forced choice: 1 heifer or 2.9% and voluntary choice: 33 heifers or 97.1%) vs. PEERS vs. BARE (forced choice: 4 heifers or 11.8% and voluntary choice: 30 heifers or 88.2%). No difference was observed between the number of heifers that had to be forced and the expected value when the combination was FOOD vs. BARE (forced choice: 14 heifers or 40.0% and voluntary choice: 21 heifers or 60.0%).

When PEERS was one of the choices, heifers entered the PEERS pen more times than they entered the FOOD pen (P < 0.05) or the BARE pen (P < 0.01) (Figure 2, panel 1). However, when the choice was FOOD vs. BARE, there was no difference in the number of times heifers entered either pen (Figure 2, panel 1). In fact, when the choice was FOOD vs. BARE, heifers spent more (P < 0.01) time in the choice area than in either of the choice pens, and when the choices were PEERS vs. FOOD and PEERS vs. BARE, heifers spent more time in the choice area than in the FOOD pen (P < 0.05) or the BARE pen (P < 0.01; Figure 3, panel 1).
Choice test after restraint in beef cattle

Figure 2. (1) Mean (±SD) number of times that heifers entered each choice pen after choosing in Exp. 1 (panel 1) and mean (±SD) number of times that heifers entered each choice pen after choosing in Exp. 2 (panel 2). Different letters indicate a difference in each choice combination; a,bP < 0.05; A,BP < 0.01; and NS = not significant. PEERS = a pen with 3 familiar heifers; FOOD = pen with a pile of hay on a metal rack; BARE = bare pen; STI = pen with a human standing inside the pen; STO = pen with a human standing outside the pen; SI = pen with a human sitting inside the pen; NO = pen with the novel object.

Experiment 2

The number of heifers choosing the pen containing the human was smaller than expected, regardless of the position or posture of the human (standing inside: 6 heifers or 20.7% vs. novel object: 23 heifers or 79.3%; standing outside: 5 heifers or 17.2% vs. novel object: 24 heifers or 82.8%; sitting inside: 7 heifers or 25.0% vs. novel object: 21 heifers or 75.0%; P < 0.01). No differences in latency to choose were observed among any of the choice combinations. Very few heifers had to be forced to choose when the human was inside the pen, either standing (forced choice: 3 heifers or 10.3% and voluntary choice: 26 heifers or 89.7%; P < 0.01) or seated (forced choice: 5 heifers or 17.9% and voluntary choice: 23 heifers or 82.1%; P < 0.01). However, when the human was standing outside the pen, there was no difference between the number of heifers that had to be forced and the expected value (forced choice: 11 heifers or 37.9% and voluntary choice: 18 heifers or 62.1%).

Figure 3. Mean (±SD) length of time heifers spent in each choice pen or the choice area after choosing in Exp. 1 (panel 1) and mean (±SD) length of time heifers spent in each choice pen or the choice area after choosing in Exp. 2 (panel 2). Different letters indicate a difference in each choice combination; a,bP < 0.05 and A–C P < 0.01. PEERS = a pen with 3 familiar heifers; FOOD = pen with a pile of hay on a metal rack; BARE = bare pen; STI = pen with a human standing inside, STO = pen with a human standing outside the pen; SI = pen with a human sitting inside the pen; NO = pen with the novel object.

In the choice combinations in which the human was standing, heifers entered this pen fewer times than the pen containing the novel object (both P < 0.01), but when the human was seated, there was no difference between the number of times the heifers entered the pen with the human or with the novel object (Figure 2, panel 2). Regardless of the choice combination, the time spent in the choice area was longer than that spent in either choice pen (all P < 0.01; Figure 3, panel 2).

DISCUSSION

In Exp. 1, the preference of heifers for peers was demonstrated by their initial choice, their readiness to choose, and their behavior during the test after they had made their first choice. As regards their first choice, heifers chose peers over the bare pen, with food intermediate between these two. Readiness to choose was shown by their lower latency to choose when peers were one of the choices and very few heifers needing to be forced by the human handler to choose. Once the heifers had made their choice, they entered the pen containing the peers more frequently.

Although our study found that cattle preferred to return to the company of the familiar heifers following
handling, many other studies have shown the influence of the presence of peers while handling procedures are being conducted. Grignard et al. (2000) showed that the visual presence of peers calmed the behavior of calves during handling in a novel environment. Presence of peers has decreased distress behavior of heifers in a novel object test (Boissy and Le Neindre, 1990) and novel environment test (Veissier and Le Neindre, 1992). Furthermore, Boissy and Le Neindre (1997) reported that separation from peers induced behavioral and physiological responses to stress. These studies suggested the preference of peers in forced experimental conditions. Our study indicates the preference of peers as shown voluntarily by the experimental heifers.

Although not shown as clearly as the preference for peers, food appeared to be more attractive for heifers than the bare pen. One possibility is that heifers may not have been hungry enough to be highly attracted to the hay, because we took care to ensure that there was not a great difference in time off feed for heifers tested later in the day compared with heifers tested earlier. The preference for food might be shown when a palatable food like molasses-flavored calf starter was provided (Pajor et al., 2003). It is also possible that feeding using a novel feeder in a novel test pen provoked a reaction to novelty rather than a desire to eat, as suggested by the results of Herskin et al. (2003). The inhibition of feeding in a novel environment has also been used to evaluate the response to novelty by Boissy and Bouissou (1988), Veissier and Le Neindre (1992), and Boissy and Bouissou (1995). Likewise, a sheep in a small group would not leave its group to reach a preferred feeding site located further away (Dumont and Boissy, 2000). In this study, we believe that the time spent in the pen with food got shorter because the heifers had to use the novel feeder in the novel test pen to eat hay.

Experiment 2 showed that presence of the human was initially more aversive than the novel object because heifers were more likely to make the novel object their first choice in preference to the human. Although heifers stayed in the choice area away from the human and the novel object after the first choice, they were as likely to enter the pen with the seated human as they were to enter the pen containing the novel object. When the human was standing, even standing outside the pen, the heifers were not as likely to enter this pen than the pen with the novel object.

In other studies in which responses of cattle to humans have been determined, the human was either sitting stationary on a stool (Hemsworth et al., 1996), standing in the test pen (Veissier and Le Neindre, 1992; de Passillé et al., 1995, 1996), or interacting with the test animal in such situations as the docility test (Le Neindre et al., 1995; Grignard et al., 2000, 2001), restraint test (Boivin et al., 1992a,b, 1994), and the sorting test (Boivin et al., 1992a,b, 1994). In some of these previous studies on cattle, the test animals have been observed to interact with the human. In those in which the human was standing, heifers were observed to have sniffed a human (Veissier and Le Neindre, 1992), and de Passillé et al. (1995, 1996) reported that calves made contact with the human. In the case of a sitting human, Hemsworth et al. (1996) found that most cattle approached within 4 m, although a few cattle approached within 2 m. In our study, no heifers made contact with the human, regardless of the posture and position of the human. It is possible that this lack of difference is related to the location of the heifers, because Morita et al. (2001) and Uetake et al. (2003) showed that animals lose their aversion to humans who work inside their enclosure compared with those who work outside. Heifers in our study were reared on pasture with little close human contact.

The response of the heifers to a human in different postures was dissimilar when measured as the number of times that they entered each pen. The fact that our heifers entered the pen with the human sitting inside as many times as the pen with a novel object indicates that the animals perceived the seated human as less of a threat. Hemsworth et al. (1986) and Miura et al. (1996) reported that pigs are far more likely to approach a squatting or lying human than a standing one. They interpreted this as indicating that relatively large objects are threatening to pigs. Also, Kendrick and Baldwin (1989) showed that a human on all fours is less aversive to sheep than a standing human. Behavioral responses to a human in different postures and positions have never been determined in cattle, although behavioral responses to a stationary or moving human have been determined in situations in which cattle were restrained in a squeeze chute or the corner of a test pen (Grignard et al., 2000, 2001). It is possible that, in our study, the sitting person evoked less of a fear response, resulting in heifers perceiving the sitting human and the novel object as equally and lowly aversive.

In conclusion, after a period of restraint in the squeeze chute accompanied by close human proximity, peers were the most attractive condition for heifers, whereas human presence, particularly a standing human, was the least attractive. Therefore, the stress due to isolation from peers and restraint in the chute may be moderated by reuniting cattle with peers as soon as possible and not approaching cattle needlessly.

**LITERATURE CITED**


Choice test after restraint in beef cattle


