J. Dairy Sci. 96:1–11
http://dx.doi.org/10.3168/jds.2013-6944

Repeated mixing and isolation: Measuring chronic, intermittent stress in Holstein calves[1]

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ABSTRACT

Objectives of this study were to determine the physiological effects of psychological stress applied to dairy calves and to test if molasses consumption could be used to validate that a stressed condition was achieved. Twenty male calves (3 wk old) received jugular catheters and were randomly assigned to control (CTR; n = 4 pens of 1 calf per pen) or social stress treatments (STR; n = 4 pens of 4 calves per pen). The STR treatment included 5 cycles of 24-h isolation followed by regrouping with unfamiliar animals for 48 h (over 15 d). An ACTH challenge (0.1 IU/kg of body weight) was used to determine adrenal fatigue. Peak and total cortisol concentrations were greater for STR calves until the ACTH challenge. After the ACTH challenge, CTR calf cortisol increased and STR calf cortisol continued to decrease, suggesting adrenal fatigue. The number of calves that became positive for fecal shedding of *Salmonella* after the acute stress of being moved and the number of calves that were positive after the move decreased with each move. Fifty-six percent of STR calves changed from negative to positive for shedding after the first move compared with 18.75% of STR calves remaining negative after the third move. Difference in fecal shedding of *Enterobacteriaceae* from samples taken before and after moving calves on d 6 was less than that on d 2, 3, and 5. Leukocyte counts were not different, but trends for day effects were detected for neutrophil and monocyte percentages. Molasses consumption was greater for STR calves on d 2 and 11, as was total consumption. Latency to lie after eating also increased as the study progressed; STR calves required more time to lie after eating on d 12 than on d 3, and latency to lie was greater for STR than CTR on d 4, 8, 12, and 14. The STR calves also stood more than the CTR calves in the 4-h afternoon period on d 4, 5, 7, and 14. However, during the 4-h morning observations on d 14 (ACTH challenge), CTR calves stood more than STR calves. This model induced chronic stress, as characterized by adrenal fatigue, which was confirmed by molasses consumption and behavior changes. Therefore, molasses consumption could be used to confirm social stress in experimental models.
Key words: bacteria, cortisol, dairy calf, stress

INTRODUCTION

Dairy calf management, as recommended by the Babcock Institute of the University of Wisconsin (Wattiaux, 2000), includes a period of isolation followed by regrouping. To reduce pathogen load originating from the adult animals or from cross-contamination among calves (McGuirk, 2008), dairy farmers frequently remove neonates from their dams between 1 and 12 h postpartum and rear them in individual hutches or pens (USDA-APHIS, 2008). Following this period of isolation, heifers are typically weaned at 6 to 8 wk of age and placed into groups. Male calves used for dairy beef or veal are now grouped earlier (4 to 6 wk), with emphasis on social needs of calves, and are periodically regrouped according to size.

After weaning, calf management then follows a routine of repeated regrouping to maximize group homogeneity (Garnsworthy, 2005). Each of these activities—weaning (Hickey et al., 2003), transportation (Fazio and Ferlazzo, 2003), isolation (Moberg, 2000), mixing, and change of housing (Veissier et al., 2001)—is known to elevate circulating cortisol concentrations, an indicator of acute stress.

Acute stress is the physiological response to physical or psychological threats to an animal's homeostasis. As the animal perceives a threat, corticotropin-releasing factor is produced in the hypothalamus, initiating a cascade of events along the hypothalamic-pituitary-adrenal (**HPA**) axis. Stimulation of corticotropin-releasing factor receptors located in the anterior pituitary gland produces a release of ACTH and glucocorticoids such as cortisol from the adrenal gland. As part of the

Received April 19, 2013.
Accepted July 20, 2013.
[1]Mention of trade names or commercial products in this paper is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.
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allostatic control of stress, cortisol regulates the release of glucose from the liver and inhibits the production of ACTH in the pituitary, ultimately decreasing the production of cortisol (Douglas, 2005). Mild acute stimulation of the HPA axis causes a transient spike in metabolism and the temporary suspension of digestion, growth, and repair (Sapolsky, 2004). If an animal has sufficient reserves, it can recover from these physiological changes.

During acute stress, elevated circulating cortisol concentrations activate glucocorticoid receptors in the liver that increase the expression of glucose-6-phosphatase, which in turn increases the production and secretion of glucose. Acute stress also increases the secretion of adrenaline, which stimulates the production of insulin. During chronic stress, biological reserves become depleted, leading to adrenal fatigue and hypoglycemia.

Another physiological consequence of prolonged ACTH production and chronic elevation of plasma cortisol concentration is the disruption of immune system functions. Cytoplasmic glucocorticoid receptors are found in almost all mammalian cells. The percentages of neutrophils and eosinophils increase with acute stress. In contrast, chronic elevation of ACTH concentration reduces the production of neutrophils (Bilandzic et al., 2006). Chronic suppression of the immune system shifts the leukocyte differential profile, rendering even the humoral immune system inefficient (Moberg, 2000).

In addition to the benefits of elevating the humoral immune response at the expense of cell-mediated immunity during acute stress (Bilandzić et al., 2006), changes in behavior during acute stress are beneficial for the animal. Increased serum cortisol increases the production and secretion of noradrenaline, which heightens attention. However, repeated stimulation of the hypothalamus and activation of amygdala neuronal cells modifies the morphology of dendrites in these brain regions. This altered morphology changes the sensitivity of neurons and may cause anxiety, increased aggression, and other behavioral changes associated with chronic stress (Cook and Wellman, 2004).

One behavioral change noted in studies of chronic stress in mice and rats is a change in voluntary consumption of sucrose (Pothion et al., 2004). Change in sucrose consumption is typically measured as a percentage of total liquid intake in a 2-bottle preference test (pure water vs. 10% sucrose solution; Pothion et al., 2004). This change, relative to baseline or control animals, is accepted as an indicator of stress and is used in rodents to validate experimental stress protocols (Brenes-Sáenz et al., 2006). It is unknown if dairy calves will modify their consumption of high-sugar feedstuffs as a reaction to stress, as seen in mice and rats. However, a preliminary study conducted in our laboratory demonstrated that upon experimentally inducing greater glucose demand with phlorizin, calves increased their molasses consumption (Wilcox et al., 2008). The observation that endogenous glucose demand is reflected in a change in sucrose consumption behavior suggested that stress may also modify the sucrose consumption behavior of dairy calves.

Changes in behavior, shifts in immune system profile, and HPA axis resistance to ACTH challenge are indicative of chronic stress and are evident in animals that have been exposed to repeated, intermittent, mild stress. For example, weekly regrouping of calves during an 11-wk study resulted in a diminished reaction to novel acute stressors and attenuated cortisol response to ACTH challenges, compared with group-stable controls (Raussi et al., 2004). Social isolation and regrouping are mild psychological stressors that may occur frequently over the life of a calf under current management recommendations. The objectives of this study were to determine physiological effects of psychological stress of isolation and regrouping applied to dairy calves and to test if molasses consumption could be used to validate that a stressed condition was achieved.

MATERIALS AND METHODS

Animals

All animal-related protocols were approved by the Animal Care and Use Committee of Purdue University. Twenty 3-wk-old male calves were fed 2 L of colostrum twice within 24 h of birth. Calves with 24-h plasma protein concentrations of >5.5 mg/dL (measured by refractometer, Brix, Atago, Bellevue, WA) were transported from a commercial herd (Strauss Veal Feeds Inc., North Manchester, IN) for 2 h to the Farm Animal Behavior Laboratory of the Livestock Behavior Research Unit (US Department of Agriculture Agricultural Research Service, West Lafayette, IN). Upon arrival of calves, BW and rectal temperature were recorded. Calves were housed for 3 d in individual pens that converted to group pens by removal of partitions. Calves were then catheterized with jugular venous catheters (Cook Veterinary Products, Bloomington, IN), which were maintained with sterile heparinized saline (4 U of heparin/L of saline; Abbott Laboratories, Abbott Park, IL). After 3 d, calves were assigned randomly to pens of group or individually housed calves. Four pens of 1 control (**CTR**) calf each and 4 pens of 4 stressed (**STR**) calves each were used. Two extra calves were kept in individual pens as replacements to maintain group size if STR calves had to be taken off study. Calves were fed a continuation of the diet and standard operating procedures from the veal farms from which