Effect of maternal exercise on calf dry matter intake, weight gain, behavior, and cortisol concentrations at disbudding and weaning

R. A. Black,* B. K. Whitlock,† and P. D. Krawczel*1
*Department of Animal Science, The University of Tennessee, Knoxville 37996
†Department of Large Animal Clinical Sciences, The University of Tennessee, Knoxville 37901

ABSTRACT

The objective of this study was to determine the effect of maternal physical activity during late gestation on calf dry matter intake, weight gain, behavior, and cortisol concentration during disbudding and weaning. Fifty-five Holstein and 5 Jersey × Holstein crossbred calves were enrolled into the study during gestation. Calves were born from pregnant, nonlactating Holstein (n = 58) and Jersey × Holstein crossbred (n = 2) dairy cows. Cows were assigned to either confinement (n = 20 cows; 13 female calves, 7 male calves), exercise (n = 20 cows; 8 female calves, 12 male calves), or pasture (n = 20 cows; 11 female calves, 9 male calves) treatments at dry-off from January to November 2015. Enrollment in treatment was balanced by parity (1.8 ± 0.9), projected mature-equivalent fat-corrected milk (13,831 ± 2,028 kg/lactation), dam breed, and projected calving date. Cows assigned to confinement remained in the pen throughout the dry period. Cows assigned to exercise were walked 5 times/wk at a targeted 1.5 h at 3.25 km/h. Cows assigned to pasture were turned out 5 times/wk for a targeted 1.5 h/d. Treatments were terminated on the expected due date or at signs of calving. Calves were removed from cows immediately once observed by farm staff and subsequently weighed and moved into a straw deep-bedded hutch. Data loggers were attached to the rear fetlock of each calf −3 d to +6 d relative to disbudding and weaning to monitor changes in lying behavior. Calves were weighed on d −7, −5, −3, −1, 0, 1, 3, 5, and 7, and grain was weighed the 7 d preceding and following disbudding and weaning. Blood was collected 24 h before and 0, 1, and 4 h after disbudding and d −1, 0, 1, and 2 relative to weaning to determine cortisol concentrations. Data were analyzed using mixed linear model in SAS (SAS Institute Inc., Cary, NC). Calf weight gain decreased the day after disbudding and calves tended to have elevated cortisol concentrations 1 h after disbudding, regardless of maternal treatment. Calf weight gain decreased the day of and after weaning; calves had elevated cortisol concentrations the day after weaning, regardless of treatment. Behavior did not differ by treatment at disbudding, but calves from pasture cows lay down for less time compared with confinement and exercise maternal treatments and less frequently than exercise maternal treatments at weaning. More research investigating the significance of lying time and restlessness around stressful events is needed to further understand the implications of such behavioral responses.

Key words: maternal exercise, calf performance, cortisol, behavior

INTRODUCTION

The maternal environment plays a tremendous role in fetal growth and development during gestation, and manipulations during this time can either improve or impair calf performance. Prenatal heat stress can alter endocrine dynamics, reduce immune function, reduce calf birth and weaning weight, and potentially reduce the milk yield potential of calves (Collier et al., 1982; Tao et al., 2012; Strong et al., 2015). Prenatal stress during cow transport can reduce cortisol clearance during stressful events, altering the physiological response to stress (Lay et al., 1997). Further, undernutrition of cows during the first trimester resulted in calves with potentially suboptimal fertility, enlarged aortic trunk size, and increased blood pressure (Mossa et al., 2013). Therefore, stress during pregnancy can likely cause impaired performance of calves early in life and potentially into their productive lives.

Exercise, or planned, structured, and repetitive physical activity (Caspersen et al., 1985), may also play a role in prenatal stress. Although studies have not investigated this concept in cattle, prepartum exercise and its effect on offspring have been studied in humans, albeit minimally. In a Healthy Start study examining physical activity during gestation, a negative linear trend between fat mass and energy expenditure...
was noted, indicating reduced neonatal adiposity with increased physical activity without decreasing neonatal fat-free mass (Harrod et al., 2014). Similarly, women participating in heavy exercise up to or beyond 28 wk of gestation delivered smaller infants (Magann et al., 2002). However, in neither study did researchers follow infant growth or observe infant responses to stressful events, such as weaning.

In dairy cattle, exercise during late gestation previously resulted in improved physical fitness and performance. Exercising heifers 4 to 8 wk prepartum for a distance of 1.6 km at 5.5 km/h improved ease of parturition, placental release, and feed efficiency (Barker et al., 1975). Exercising nonlactating dairy cows during the final 70 d of parturition every other day using a mechanical walker led to reduced heart rate during exercise, faster return to resting heart rate after exercise, and lower L-lactate concentrations (Davidson and Beede, 2009). However, exercise increased cortisol during and post exercise in calves and pregnant heifers (Kuhlmann et al., 1985; Arave et al., 1987; Piguet et al., 1994). If the increase in cortisol concentration denotes that exercise causes distress, it may impose similar negative effects to calves in utero, as seen with prenatal heat stress, transportation stress, and malnutrition. Further, the application of exercise via mechanical walker in a production system is not practical on commercial dairy farms because of required labor. Alternatives that encourage physical activity, such as pasture access, offer a more realistic management practice for dairy producers.

Allowing pasture access to tie-stall housed cows daily reduced disease treatments by veterinarians, culling, and occurrences of subclinical mastitis during the first 2 wk postpartum, and it improved welfare, as shown by fewer hock lesions, fewer lame cows, and lower mastitis prevalence (Gustafson, 1993; Popescu et al., 2013). While 34.5% of dairy operations in the United States provide access to pasture or an open lot to lactating cows, 60.8% of operations provide it to dry cows (USDA, 2016), making this group of animals more easily targeted for additional physical activity, based on current management practices. The opportunity to move outdoors offers potential health benefits for cows, and determining the effects of physical activity may improve recommendations of physical activity levels during gestation.

Before recommending increased physical activity for late-gestation cows, an understanding of the consequences or benefits to calves should be understood. Although studies have investigated the effects of different stressors during gestation on calves, none have determined how physical activity may influence calves. The objective of this study was to determine the effect of maternal total confinement, pasture access, or forced exercise during late gestation on calf body weight and intake, behavior, and cortisol concentrations during disbudding and weaning. We hypothesized that both exercise and pasture turnout during gestation would reduce calf body weight and intake, alter behavior, and increase cortisol concentrations during disbudding and weaning compared with total confinement.

**MATERIALS AND METHODS**

*Maternal Housing, Treatments, Management, and Activity*

**Animals.** Calves were born from 29 primiparous and 31 multiparous, Holstein (n = 58) and Jersey × Holstein crossbred (n = 2) dairy cows. Crossbred cows and calves were used during the study because of cow availability and farm management practices (i.e., Jersey semen for an easier calving in a small cow). Additionally, breed effects were not expected for the measured outcomes because cows were assigned to either confinement (n = 19 Holstein cows, 1 Jersey × Holstein crossbred cow; 11 female Holstein calves, 2 female Jersey × Holstein crossbred calves, 6 male Holstein calves, 1 male Jersey × Holstein crossbred calf), exercise (n = 19 Holstein cows, 1 Jersey × Holstein crossbred cow; 8 female Holstein calves, 10 male Holstein calves, 2 male Jersey × Holstein crossbred calf), or pasture (n = 20 Holstein cows; 11 female Holstein calves, 9 male Holstein calves) treatments at dry-off from January to November 2015. Enrollment in treatment was balanced by parity (confined Holstein, 1.8 ± 1.0; confined Jersey × Holstein crossbred, 2 ± 0; exercise Holstein, 1.7 ± 0.9; exercise Jersey × Holstein crossbred, 2 ± 0; pasture Holstein, 1.8 ± 0.9), projected mature-equivalent fat-corrected milk (confined Holstein, 13,163 ± 2,048 kg/lactation; confined Jersey × Holstein crossbred, 14,754 ± 0 kg/lactation; exercise Holstein, 14,076 ± 1,881 kg/lactation; exercise Jersey × Holstein crossbred, 15,179 ± 0 kg/lactation; pasture Holstein, 14,105 ± 2,061 kg/lactation), dam breed, and projected calving date (breeding date plus 280 d).

**Housing.** Cows were housed in a naturally ventilated, 4-row head-to-head freestall barn with drive-through feed bunk and deep-bedded sand freestalls at the University of Tennessee’s Little River Animal and Environmental Unit (Walland, TN). Deep-bedded sand freestalls were 2.4 m long and 1.2 m wide with a 1.2-m-high neck rail positioned 1.7 m from the curb and a 0.6-m-high PVC tube brisket board placed 1.7 m from the curb. Fresh sand was added once per week with
manure removed from stalls twice daily before milking the lactating herd (0730 and 1730 h). Fans turned on automatically when temperatures rose above 23°C. Pens measured 12.1 m wide and 19.4 m long, enclosing 24 freestalls and 26 0.6-m-wide headlocks and containing 2 waterers, 1 on each end. Study cows were conjoined unless the pen was split into far-off (dry-off to 2 wk before parturition) and close-up (2 wk before parturition) groups, leaving 12 freestalls and 13 headlocks for each group. Cows were maintained below 80% stocking density, based on headlock and freestall availability.

**Treatments.** Before enrollment, all cows had been housed in the same freestall barn with no previous experience with exercise, aside from pasture access during the dry period before the previous calving. Cows were enrolled into treatments weekly on the day of dry-off. Cows assigned to confinement remained in the pen at all times, except for general management reasons (i.e., cleaning, rebedding stalls) when cows were moved to an adjacent lane for a maximum of 30 min daily. Confined cows were permitted to eat, drink, and move around the pen during exercise times. Cows assigned to exercise were removed from the pen 5 times/wk, Monday through Friday, and walked for 1.5 h at a targeted pace of 3.25 km/h (Davidson and Beede, 2009) beginning at 1200 h on a grooved concrete alleyway within the freestall barn. Cows were walked in a group using the cows’ flight zones and implements (i.e., rattle paddle) to encourage walking. Exercise pace was calculated by the distance walked divided by the total exercise time. During periods of high heat load, determined subjectively through cow heat stress behavior (i.e., increased respiration rate, panting), cows were offered water at a single point along the exercise path from a 19-L bucket. Cows did not have access to feed during the exercise period.

Cows on pasture were moved into a 2.11-ha pasture (pasture 1) from January to April 2015 and a 0.42-ha pasture (pasture 2) from April to December 2015 5 times/wk, Monday to Friday. A different pasture was used based on management factors and proximity to the freestall barn. Pasture 1 was 330 m from the barn to the pasture gate, and pasture 2 was 15 m from the barn to the pasture gate. Cow numbers ranged from 3 to 7 on pasture 1 and from 1 to 6 on pasture 2. Pasture 1 had rolling hills and a 0.75-ha wooded area, and pasture 2 had a shade structure and trees along one side of the fence line. Both pastures were seeded with orchardgrass and KY-31 fescue and managed by the farm manager for a height of 0.3 to 0.5 m. Cows were put on pasture before and returned to the barn after exercising cows from the exercise treatment group. Cows were put on pasture for a target of 1.5 h, excluding travel time to and from the paddock, beginning at 1200 h. Both pastures had access to water and grass.

**Feeding Management.** Cows were fed twice daily at 0730 and 1530 h. Far-off cows were fed a TMR from dry-off to 2 wk before projected parturition consisting of 4.5 kg of ryegrass hay, 3.4 kg of orchardgrass hay, 2.3 kg of corn silage, and 2.7 kg of dry cow grain per cow per day. Close-up cows were fed a TMR up to parturition consisting of 3.6 kg of orchardgrass hay, 1.8 kg of clover, 11.3 kg of corn silage, and 3.0 kg of dry cow grain per cow per day. All cows had ad libitum access to water, except exercise treatment cows during exercise.

**Calving Management.** The dry period lasted 58.0 ± 0 d for the confined Jersey × Holstein crossbred cow (n = 1), 57.3 ± 4.8 d for confined Holstein cows (n = 19), 57.0 ± 0 d for the exercise Jersey × Holstein crossbred cow (n = 1), 57.5 ± 5.7 d for exercise Holstein cows (n = 19), and 60.7 ± 5.6 d for pasture Holstein cows (n = 20). Cows were monitored for signs of calving by farm staff regularly between 0730 and 2100 h and moved into maternity pens by farm staff on their due date or when cows displayed signs that calving was imminent (i.e., restlessness, holding of tail, water breaking, swollen vulva), and treatments were discontinued. Maternity pens were 4.2 × 4.1 m containing a rubber-filled mattress covering the entire pen floor (ProMat Inc., Woodstock, ON, Canada) with no bedding. Each pen had access to water, and cows were fed using a rubber tub twice daily.

**Activity.** Cows were fitted with accelerometers (IceTag, IceRobotics, Edinburgh, UK) 3 d before dry-off that were removed 14 d after calving. Activity was summarized by day from dry-off to the day before calving into daily steps (n/d).

**Calves, Housing, and Management**

Fifty-five Holstein and 5 Jersey × Holstein crossbred calves were enrolled into the study during gestation. Calves were removed from cows immediately once they were observed by farm staff (exact time between calving and removal not recorded). They were then weighed (W-W Paul Scales, Duncan, OK), moved into a straw deep-bedded hutch (RSI Calf Systems, Riverside Plastics Inc., Flemingsburg, KY), and fed 3.8 L of colostrum during 1 or 2 feedings. Calves weighed 39.2 ± 5.4, 42.1 ± 6.8, and 44.3 ± 6.3 kg from confined, exercise, and pasture cows, respectively, at birth. Calves were fed 1.9 L of milk replacer (Calf 26–20 Bov SC ClariFly Medicated, Tennessee Farmer’s Cooperative, La Vergne, TN; Table 1) via bottle to 28 d of age and 2.8 L to 60 d of age twice daily at 0500 and 1500 h. Water and grain
starter (Grain Mix, Tennessee Farmer’s Cooperative; Table 1) were available ad libitum inside the hutch with additional water available outside the hutch.

Calf health was observed thrice daily at feeding and during the daily pen cleaning, with the staff observing for intake (refusal to drink or finish milk), fecal consistency (loose stool), alertness, and eye or nose discharge. If a health concern was noted, the calf’s temperature was taken, it was fed electrolytes (Re-sorb, Zoetis, Parsippany, New Jersey), and it continued to be monitored. If the concern persisted, veterinary consultation was sought.

Passive transfer was routinely evaluated by farm staff using serum total protein; however, because this evaluation was not a part of the current study, data were not available for all study calves. Blood was collected at 3.4 ± 1.8 d of age (n = 37). Five milliliters of blood was collected in serum blood tubes (BD Vacutainer, BD, Franklin Lakes, NJ) and placed on ice. Samples were immediately centrifuged for serum separation, and a sample of serum then placed on a Brix refractometer (Master-Sur/Na Refractometer, Atago U.S.A. Inc., Bellevue, WA) to determine serum total protein (Deelen et al., 2014). Serum total protein levels were 6.5 ± 0.6 g/dL for confined Holstein calves (n = 12), 7.0 ± 0.3 g/dL for confined Jersey × Holstein crossbred calves (n = 2), 7.0 ± 0.4 g/dL for exercise Holstein calves (n = 11), 7.0 ± 0.4 g/dL for exercise Jersey × Holstein crossbred calves (n = 2), and 6.1 ± 0.7 g/dL for pasture Holstein calves (n = 10). Only 1 calf had a value below 5 g/dL (4.7 g/dL; female pasture Holstein), and she later died from illness.

Calves were disbudded at 25.6 ± 10.0 d of age. Three milliliters of 2% (20 mg/mL) lidocaine was administered at each cornual nerve 5 to 10 min before disbudding. Calves were disbudded using an electrically heated disbudding (Dehorner X50-A, Rhinehart Development Corp., Spencerville, IN) with the iron applied to the horn bud from 10 to 20 s depending on calf age and horn bud size (Graf and Senn, 1999). Calves were abruptly weaned at 02:1 ± 2.4 d of age. Calves were fed a morning bottle at 0500 h and did not receive a bottle at 1500 h. Calves remained in the same hutch from birth until 7 d after weaning and were then commingled into a 0.56-ha paddock with other weaned calves, with access to water, free-choice grain, and an aluminum shelter open on all 4 sides.

**Calf Behavior**

Data loggers (HOBO Pendant G Data Logger, Onset Computer Co., Bourne, MA) were attached to the rear fetlock of calves in a horizontal orientation using bandaging tape (Co-Flex, Andover Healthcare Inc., Salisbury, MA) 3 d before and removed 6 d after disbudding and weaning to monitor changes in lying behavior (Bonk et al., 2013). Data were summarized by day to determine daily lying time using the time of disbudding or the date of weaning at 1500 h as the starting time (0000 h) of the day. Lying time and bout frequency data were also summarized into 3-h periods during the 24 h preceding and following disbudding and weaning (−24 to −22, −21 to −19, −18 to −16, −15 to −13, −12 to −10, −9 to −7, −6 to −4, −3 to −1, 0 to 2, 3 to 5, 6 to 8, 9 to 11, 12 to 14, 15 to 17, 18 to 20, and 21 to 23 h). Periods of 3 h were used to reduce the level of variation that occurred on an hourly basis. The preceding 24 h were also divided into 3-h periods and averaged to create a baseline lying behavior before disbudding and weaning.

**DMI and BW**

Calves were weighed on d −3, −1, 0, 1, 3, 5, and 7 relative to disbudding and weaning. Calves were weighed at the same time daily to reduce variation in weight from feeding and management factors. Starter grain was weighed daily from 3 d before to 7 d after disbudding and weaning to determine daily intake.

**Cortisol Measurement**

Six milliliters of blood was collected via jugular venipuncture in sodium heparin tubes (BD Vacutainer) at −24, 0, 1, and 4 h relative to disbudding (Morisse et al., 1995) and −24, 0, 24, and 48 h relative to 1500 h on the day of weaning (modified from Hickey et al., 2003). After collection, sodium heparin tubes were centrifuged, plasma was separated into microcentrifuge tubes, and tubes were frozen at −80°C until further analysis to

---

**Table 1. Nutrient analyses of calf milk replacer and grain starter fed to calves throughout study period**

<table>
<thead>
<tr>
<th>Feed</th>
<th>DM  %</th>
<th>Protein, % of DM</th>
<th>Fat, % of DM</th>
<th>Fiber, % of DM</th>
<th>Ca, % of DM</th>
<th>P, % of DM</th>
<th>Lasalocid, mg/kg of DM</th>
<th>Diflubenzuron, mg/kg of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk replacer</td>
<td>100</td>
<td>26.0</td>
<td>20.0</td>
<td>0.15</td>
<td>0.75–1.25</td>
<td>0.70</td>
<td>66.1</td>
<td>10.0</td>
</tr>
<tr>
<td>Grain starter</td>
<td>89.7</td>
<td>16.8</td>
<td>3.5</td>
<td>15.2</td>
<td>1.00</td>
<td>0.58</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Journal of Dairy Science Vol. 100 No. 9, 2017
determine cortisol concentration. Plasma total cortisol concentration was determined by a radioimmunoassay procedure (Doherty et al., 2007) using a commercially available kit (ImmunChem Cortisol 125 | RIA Kit, BP Biomedical, LLC, Orangeburg, NY). Inter- and intra-assay coefficient of variation for the low control (7 ng/mL) was 42.9% and 27.6%, respectively, and 27.5% and 9.7%, respectively, for the high control (25 ng/mL).

Statistics

Six calves were not enrolled after birth, including 1 calf that was born dead (pasture: Holstein female), 1 calf that was born alive but then sat on by the dam (pasture: Holstein female), 1 calf that died while being pulled because the calf was very large (63.5 kg; pasture: Holstein male), 2 that were euthanized because of illness (pasture: 1 Holstein female; confinement: 1 Holstein female), and 1 calf that was euthanized at 7 d old because of a broken leg from being pulled during calving (exercise: 1 Holstein female). One calf died from illness from a separate LPS challenge study (pasture: 1 Holstein male) between disbudding and weaning, treatment × period were used as explanatory variables. Values presented are least squares means (LSM) ± standard error of means (SEM) unless otherwise stated. The Kenward-Roger approximation for distribution of degrees of freedom between fixed effects was used and treatment effects were declared significant at \( P \leq 0.05 \), while a trend was assumed for probabilities \( P < 0.1 \) and \( P > 0.05 \). Post hoc means separation for significant main effects was done using Tukey-Kramer’s adjustment of probability values. Data normality was evaluated using the UNIVARIATE procedure of SAS.

RESULTS

Maternal Treatments and Activity

Exercise cows walked for a mean (±SD) of 1.4 ± 0.1 h at 1.88 ± 0.58 km/h. Pasture cows spent a mean (±SD) of 2.0 ± 0.3 h on pasture and 1.7 ± 0.3 h on pasture 2. Holstein exercise cows took 2,923.5 ± 1,084.5 steps (mean ± SD) daily, and the Jersey × Holstein crossbred exercise cow took 2,459.6 ± 1,006.8 steps daily. Holstein pasture cows took 2,117.7 ± 775.6 steps daily. Holstein confined cows took 1,822.5 ± 803.4 steps daily, and the Jersey × Holstein confined cow took 1,362.6 ± 474.0 steps daily.

Calf DMI and Weight Gain

Actual calf weights were collected on d −2.9, −1.0, 0.1, 1.0, 3.0, 5.0, and 7.0 ± 0.1 relative to disbudding and d −3.1, −1.1, −0.1, 0.9, 2.8, 4.9, and 6.8 ± 0.1 relative to 1500 h on the day of weaning. At disbudding, weight gain was not affected by treatment × day (\( P = 0.90 \)) or treatment (\( P = 0.63 \); Table 2), but was affected by day (\( P < 0.0001 \); Table 3). Dry matter intake at disbudding increased by day (\( P < 0.0001 \); Table 3). Calves from pasture cows had a tendency to consume more feed on d 4 compared with calves from confined cows (0.72 ± 0.10 vs. 0.47 ± 0.10 kg, respectively; \( P = 0.08 \)) but did not differ from calves from exercise cows (0.59 kg; \( P = 0.37 \)). At weaning, weight gain was not affected by treatment × day (\( P = 0.25 \)) or treatment (\( P = 0.83 \); Table 2) but increased by day (\( P < 0.0001 \); Table 3). Treatment × day (\( P = 0.88 \)) and treatment (\( P = 0.14 \); Table 2) did not affect DMI at weaning, but DMI increased by day (\( P < 0.0001 \); Table 3).

Daily Lying Behavior

During the 3 d before and 6 d following disbudding, treatment, day, or treatment × day had no effect on daily lying time (\( P \geq 0.78 \)) or daily lying bout fre-
quency ($P ≥ 0.33$; Tables 2 and 4). No treatment ($P = 0.44$; Table 2) or treatment × day ($P = 0.37$) effect was observed on daily lying bout duration at disbudding; however, day did affect daily lying bout duration ($P = 0.03$; Table 4), and calves spent more time resting the day before disbudding.

During the 3 d before and 6 d following weaning, treatment, day, or treatment × day had no effect on daily lying bout duration ($P ≥ 0.18$; Tables 2 and 4), and treatment × day had no effect on daily lying time ($P = 0.40$) or daily lying bout frequency ($P = 0.44$). Calves from pasture cows spent less time lying compared with calves from exercise and confined cows and had fewer lying bouts compared with calves from exercise cows ($P < 0.01$; Table 2) over the 3 d before and 6 d following weaning. Lying bout frequency did not differ between calves from confined cows (41.4 ± 2.4 bouts/d) and those from pasture (35.6 ± 2.4 bouts/d) and exercise cows (46.7 ± 2.7 bouts/d; $P > 0.10$). Further, both daily lying time and daily lying bout frequency were affected by day ($P < 0.01$; Table 4); calves had fewer and shorter lying bouts following weaning.

### Hourly Lying Behavior

During the 24 h following disbudding, lying time and lying bout frequency over a 3-h period were not affected by treatment ($P ≥ 0.11$) or treatment × period ($P = 0.24$), but were affected by period ($P ≤ 0.01$; Table 5); calves spent less time lying 9 to 11 h following disbudding and had the fewest lying bouts 21 to 23 h following disbudding.

At weaning, treatment did not affect lying time ($P = 0.49$) or lying bout frequency ($P = 0.98$) over a 3-h period and treatment × period did not affect lying bout frequency ($P = 0.29$); however, both behaviors were affected by period ($P < 0.01$; Table 5), and calves spent less time lying and had fewer bouts 15 to 17 h following weaning. Further, lying time over a 3-h period was affected by treatment × period ($P = 0.02$; Table 6); pasture calves spent less time lying than exercise calves 9 to 11 h following weaning and had the fewest lying bouts 21 to 23 h following weaning.

### Table 2. Calf weight gain, DMI, and daily lying behaviors at disbudding and weaning for confined, exercise, and pasture calves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Confined</th>
<th>Exercise</th>
<th>Pasture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disbudding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight gain, kg/d</td>
<td>0.95 ± 0.14</td>
<td>1.11 ± 0.13</td>
<td>0.96 ± 0.14</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>0.44 ± 0.09</td>
<td>0.49 ± 0.01</td>
<td>0.50 ± 0.10</td>
</tr>
<tr>
<td>Lying time, h/d</td>
<td>17.4 ± 0.3</td>
<td>17.5 ± 0.4</td>
<td>17.2 ± 0.4</td>
</tr>
<tr>
<td>Lying bouts, no./d</td>
<td>24.8 ± 1.9</td>
<td>27.9 ± 2.5</td>
<td>28.2 ± 2.2</td>
</tr>
<tr>
<td>Lying bout duration, min/bout</td>
<td>44.6 ± 2.7</td>
<td>43.2 ± 3.5</td>
<td>39.5 ± 3.1</td>
</tr>
<tr>
<td>Cortisol concentration, ng/mL</td>
<td>3.72 ± 0.47</td>
<td>3.81 ± 0.47</td>
<td>4.40 ± 0.47</td>
</tr>
<tr>
<td><strong>Weaning</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight gain, kg/d</td>
<td>1.74 ± 0.14</td>
<td>1.84 ± 0.15</td>
<td>1.86 ± 0.16</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>2.10 ± 0.13</td>
<td>2.44 ± 0.14</td>
<td>2.41 ± 0.14</td>
</tr>
<tr>
<td>Lying time, h/d</td>
<td>16.5 ± 0.3abc</td>
<td>16.7 ± 0.3a</td>
<td>15.7 ± 0.3b</td>
</tr>
<tr>
<td>Lying bouts, no./d</td>
<td>25.4 ± 1.7</td>
<td>23.5 ± 1.8</td>
<td>28.2 ± 1.7</td>
</tr>
<tr>
<td>Lying bout duration, min/bout</td>
<td>41.4 ± 2.4abc</td>
<td>46.7 ± 2.7a</td>
<td>35.6 ± 2.4b</td>
</tr>
<tr>
<td>Cortisol concentration, ng/mL</td>
<td>4.29 ± 0.51</td>
<td>5.31 ± 0.51</td>
<td>4.37 ± 0.51</td>
</tr>
</tbody>
</table>

* Different superscripts within a row indicate significant differences between treatments ($P < 0.01$).

### Table 3. Calf weight gain and DMI at disbudding and weaning

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−3</td>
</tr>
<tr>
<td>Disbudding</td>
<td></td>
</tr>
<tr>
<td>Weight gain, kg/d</td>
<td>0.89abc</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>0.32ab</td>
</tr>
<tr>
<td>Weaning</td>
<td></td>
</tr>
<tr>
<td>Weight gain, kg/d</td>
<td>2.10abc</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>1.29a</td>
</tr>
</tbody>
</table>

* Different superscripts within a row indicate significant differences between days ($P < 0.05$).

1Day, relative to disbudding or to weaning.
Cortisol Measurement

Actual blood collection occurred at −24.0 ± 1.8, 0 ± 0, 1.1 ± 0.1, and 3.8 ± 0.3 h relative to disbudding and −26.9 ± 2.3, −2.8 ± 2.3, 21.2 ± 2.2, and 45.5 ± 2.3 h relative to 1500 h on the day of weaning. Treatment and treatment × time did not affect cortisol concentration at disbudding or weaning (P ≥ 0.40; Table 2). Cortisol concentration was lowest 4 h after disbudding (1.93 ± 0.49 ng/mL) compared with h −24, 0, and 1 (6.00 ± 0.48, 5.23 ± 0.49, and 6.82 ± 0.49 ng/mL; P < 0.0001) and tended to be higher 1 h after disbudding compared with immediately after (P = 0.09). Calves also had higher cortisol concentrations the day after weaning (10.25 ± 0.46 ng/mL) compared with the day of weaning and 2 d after (2.59 ± 0.51 and 1.92 ± 0.47 ng/mL; P < 0.0001).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hour period,1 h</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disbudding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lying time, h/d</td>
<td></td>
<td>17.3</td>
<td>17.5</td>
<td>17.0</td>
<td>17.3</td>
<td>17.4</td>
<td>17.5</td>
<td>17.4</td>
<td>17.2</td>
<td>17.4</td>
<td>17.4</td>
</tr>
<tr>
<td>Lying bouts, no./d</td>
<td></td>
<td>42.1</td>
<td>41.5</td>
<td>38.9</td>
<td>43.6</td>
<td>42.0</td>
<td>42.8</td>
<td>44.1</td>
<td>42.3</td>
<td>44.2</td>
<td>42.7</td>
</tr>
<tr>
<td>Lying bout duration, min/bout</td>
<td></td>
<td>27.0b</td>
<td>28.5ab</td>
<td>30.9a</td>
<td>26.4b</td>
<td>27.7b</td>
<td>26.9b</td>
<td>25.4b</td>
<td>26.3b</td>
<td>25.4b</td>
<td>25.9b</td>
</tr>
<tr>
<td>Weaning</td>
<td></td>
<td>16.6ab</td>
<td>16.6ab</td>
<td>16.5ab</td>
<td>16.1bc</td>
<td>16.8a</td>
<td>16.3ab</td>
<td>15.9a</td>
<td>16.3ab</td>
<td>15.9a</td>
<td>15.8a</td>
</tr>
<tr>
<td>Lying bouts, no./d</td>
<td></td>
<td>42.5ab</td>
<td>44.9a</td>
<td>44.8a</td>
<td>44.8a</td>
<td>44.0a</td>
<td>38.3a</td>
<td>39.2bc</td>
<td>38.6bc</td>
<td>36.7</td>
<td>38.5bc</td>
</tr>
<tr>
<td>Lying bout duration, min/bout</td>
<td></td>
<td>26.1</td>
<td>26.0</td>
<td>24.3</td>
<td>23.6</td>
<td>24.6</td>
<td>27.1</td>
<td>25.8</td>
<td>26.7</td>
<td>27.1</td>
<td>25.8</td>
</tr>
</tbody>
</table>
| a–cDifferent superscripts within a row indicate significant differences between day periods (P < 0.05).  
1Day, relative to disbudding or to weaning.

**DISCUSSION**

The objective of this study was to determine the effect of maternal physical activity on calf BW gain, DMI, behavior, and cortisol concentrations during stressful life events. Calves gained less weight the day of weaning and the day after and had elevated cortisol concentrations the day after weaning, regardless of treatment. However, calves from pasture cows lay down for less time compared with confined and exercise maternal treatments and less frequently than exercise maternal treatments.

**Disbudding**

Maternal treatment did not alter the response of calves to disbudding and all calves had similar weight gain, DMI, and behavioral and physiological responses. Small sample size may have influenced a lack of differences. Although previous studies have reported differences in behavior and physiology from disbudding (Boandl et al., 1989; Graf and Senn, 1999), behavior from weaning (De Paula Vieira et al., 2010), and physiology from prenatal stress (Lay et al., 1997; Mossa et al., 2013), with fewer than 20 animals per treatment in the current study, exercise may not have caused as much stress or been as distressful to cows for a similar change in physiological and behavioral measures to result.

**Table 5.** Hourly lying behaviors of calves at disbudding and weaning

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0-2</th>
<th>3-5</th>
<th>6-8</th>
<th>9-11</th>
<th>12-14</th>
<th>15-17</th>
<th>18-20</th>
<th>21-23</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disbudding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lying time, min/3 h</td>
<td>147.3a</td>
<td>131.8bc</td>
<td>139.5bc</td>
<td>125.5c</td>
<td>139.1ab</td>
<td>135.0bc</td>
<td>126.7bc</td>
<td>147.6a</td>
<td>5.2</td>
</tr>
<tr>
<td>Lying bouts, no./3 h</td>
<td>2.30ab</td>
<td>2.48a</td>
<td>1.72bc</td>
<td>1.96abc</td>
<td>1.51c</td>
<td>1.65c</td>
<td>2.07bc</td>
<td>1.44c</td>
<td>0.25</td>
</tr>
<tr>
<td>Weaning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lying time, min/3 h</td>
<td>130.0a</td>
<td>123.3b</td>
<td>122.6b</td>
<td>122.0a</td>
<td>126.9b</td>
<td>148.1a</td>
<td>128.5b</td>
<td>131.6bc</td>
<td>5.2</td>
</tr>
<tr>
<td>Lying bouts, no./3 h</td>
<td>2.59a</td>
<td>2.09bc</td>
<td>1.61bc</td>
<td>1.78bc</td>
<td>2.11ab</td>
<td>1.46a</td>
<td>2.08bc</td>
<td>2.64a</td>
<td>0.22</td>
</tr>
</tbody>
</table>

\*Different superscripts within a row indicate significant differences between hour periods (P < 0.05).
1Hour, relative to disbudding or to 1500 h weaning.
mination, rubbing, and head jerks 4 h after disbudding (Morisse et al., 1995; Graf and Senn, 1999; Grøndahl-Nielsen et al., 1999). Calves increased lying bout duration and tended to reduce lying bout frequency the day before disbudding. However, this behavior was potentially a result of the initial response of calves to blood collection because their first exposure was 24 h before disbudding. Handling may impose stress on calves (Boandl et al., 1989; Wohlt et al., 1994); however, researchers attempted to use low-stress handling during blood collection (no chute, blood collection within pen while standing with only 1 or 2 people) to minimize a confounding cortisol response.

Further, although behavior differed, total daily lying time remained the same, indicating calves compensated for the changes in behavior. Morisse et al. (1995) demonstrated similar lying times in calves during the 24-h period before and following disbudding. This finding indicates that, although disbudding is stressful, calves display the majority of behavioral modification during the 2- to 4-h period after disbudding (Morisse et al., 1995; Petrie et al., 1996; Graf and Senn, 1999) and later compensate for important behaviors, such as lying, which is important in young, growing animals that need greater amounts of sleep (Rechtschaffen, 1998; Siegel, 2005; Hänninen, 2007). This possibility can be further evidenced through an increase in lying bout frequency during the 3 to 5 h following disbudding compared with 6 to 8 h without a difference in lying time between those 2 periods. However, calves spent the most time lying immediately after disbudding and 21 to 23 h following. The period following disbudding was accompanied with a high number of lying bouts, indicating calves were likely expressing distress behavior (Morisse et al., 1995). However, during the 21 to 23 h following, lying bout frequency became less, indicating calves likely returned to normal behavior.

An increase in lying bouts following disbudding was likely indicative of a stress response as cortisol concentration tended to increase from 0 to 1 h after disbudding. These results are similar to those previously reported (Laden et al., 1985; Morisse et al., 1995; Petrie et al., 1996) indicating a cortisol spike 30 min to 1 h after disbudding with concentrations returning to basal 4 to 24 h later (Morisse et al., 1995). In the current study, cortisol concentrations 4 h after disbudding fell below those 24 h prior. While this decline may be a depletion of glucocorticosteroids from storage, otherwise termed as “shock” (Selye, 1955; Friend, 1991), it is more likely an adaptation of the calf to handling. The alteration of behavior due to the initial blood collection indicates that calves were likely stressed, and the final sample at 4 h may be a more accurate representation of basal cortisol concentrations.

The stress of disbudding is also illustrated by the reduction in daily weight gain and plateauing of DMI on d 0 and 1. In contrast, Laden et al. (1985) demonstrated that, at 4-wk intervals, disbudded calves grew at similar rates to those that did not experience disbudding, and Grøndahl-Nielsen et al. (1999) observed no differences in feed intake or weight gain during the 7 d before and after disbudding for disbudded and confined calves. Calves from pasture cows did tend to consume more feed 4 d after disbudding. However, calves from pasture cows did not gain more weight and ate similar amounts on the following day indicating that the tendency may be more driven by the changes in performance over day than the maternal treatment. Further, as this occurred 4 d following disbudding and did not continue, the biological relation to disbudding is likely minimal or irrelevant and may instead be related to management.

Weaning

Although maternal treatment did not affect ability of calves to cope with weaning through weight gain, DMI, and physiologic measures, calves born from pasture cows exhibited modified behavior at weaning. The period of weaning typically offers a variety of stressors as calves are exposed to new environments, diets, and social relationships. These stresses cause both physio-

### Table 6. Lying time of calves from confined, exercise, or pasture cows during the 3-h periods following weaning

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hour period, 1 (h)</th>
<th>0-2</th>
<th>3-5</th>
<th>6-8</th>
<th>9-11</th>
<th>12-14</th>
<th>15-17</th>
<th>18-20</th>
<th>21-23</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confined, min/3 h</td>
<td>128.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>128.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>122.0&lt;sup&gt;x&lt;/sup&gt;</td>
<td>121.0&lt;sup&gt;x,x&lt;/sup&gt;</td>
<td>145.8&lt;sup&gt;b,x&lt;/sup&gt;</td>
<td>149.0&lt;sup&gt;y&lt;/sup&gt;</td>
<td>136.9&lt;sup&gt;x&lt;/sup&gt;</td>
<td>119.3&lt;sup&gt;x&lt;/sup&gt;</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>Exercise, min/3 h</td>
<td>127.9</td>
<td>123.0</td>
<td>123.3</td>
<td>143.2&lt;sup&gt;x&lt;/sup&gt;</td>
<td>124.6&lt;sup&gt;y&lt;/sup&gt;</td>
<td>144.3</td>
<td>132.5</td>
<td>129.1&lt;sup&gt;y&lt;/sup&gt;</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>Pasture, min/3 h</td>
<td>134.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>118.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>122.5&lt;sup&gt;x&lt;/sup&gt;</td>
<td>101.7&lt;sup&gt;y&lt;/sup&gt;</td>
<td>110.2&lt;sup&gt;b,y&lt;/sup&gt;</td>
<td>151.0</td>
<td>116.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>146.5&lt;sup&gt;y&lt;/sup&gt;</td>
<td>8.8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a–c</sup>Different superscripts within a row indicate significant difference between periods (P < 0.05).
<sup>x,y</sup>Different superscripts within a column indicate significant difference between treatments (P < 0.05).

1Hour, relative to disbudding or to 1500 h weaning.
Although cortisol did not statistically differ between maternal treatments, calves from exercise cows demonstrated a higher numerical concentration of cortisol 24 h after weaning. Therefore, while calves from both pasture and exercise cows experienced behavioral modifications around weaning, a combined assessment of behavior and cortisol may allow hypothesizing that the higher frequency of lying bouts exhibited by exercise calves was a behavior more indicative of hunger than the shorter lying duration of pasture calves. Calves exposed to heat stress during late gestation experienced reduced immune function (Tao et al., 2012), while those exposed to prenatal stress during transport experienced reduced cortisol clearance and impaired stress adaptation (Lay et al., 1997). Future research may consider the use of pasture access for implementation of physical activity in late-gestation dairy cows to reduce the risk of negative behavioral and physiological adaptation. However, more research investigating the significance of lying time and restlessness around stressful events and with a larger sample size of calves is needed to better understand the implications of such behavioral responses.

CONCLUSIONS

Maternal treatment did not affect the ability of calves to cope with disbudding because calves displayed similar weight gain, DMI, and behavioral and physiological responses. However, calves from pastured cows displayed shorter lying time than calves from confined and exercise cows, potentially highlighting increased stress from weaning. In contradiction, calves from exercise cows exhibited more restlessness compared with calves from pasture cows. Future research may consider the use of pasture access for implementation of physical activity in late-gestation dairy cows to reduce the risk imposed on calves from increased stress, while potentially improving cow health. However, more research investigating the significance of lying time and restlessness around stressful events is needed to better understand the implications of such behavioral responses.

ACKNOWLEDGMENTS

The authors thank Nicole Eberhart, Gregory Sean Stapleton, Heather DeAnna Ingle, Holly Evens, Lindsay Wick, Kala Kyker, and Brittany Nettles (Depart-
ment of Animal Science, University of Tennessee) for assistance during sample collection and laborious cow exercise. We also thank Charlie Young, Mark Lewis, and the University of Tennessee Little River Animal and Environmental Unit staff for support throughout the study. This research was supported by USDA Hatch.

REFERENCES


