Effect of transportation during periods of high ambient temperature on physiologic and behavioral indices of beef heifers

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Objective—To determine the effect of transportation during periods of high ambient temperature on physiologic and behavioral indices of beef heifers.

Animals—20 heifers (mean body weight, 217.8 kg).

Procedures—Ten heifers were transported 518 km when the maximum ambient temperature was ≥ 32.2°C while the other 10 heifers served as untransported controls. Blood samples were collected from transported heifers at predetermined intervals during the transportation period. For all heifers, body weights, nasal and rectal temperatures, and behavioral indices were measured at predetermined intervals for 3 days after transportation. A week later, the entire process was repeated such that each group was transported twice and served as the control twice.

Results—Transported heifers spent more time near the hay feeder on the day of transportation, had lower nasal and rectal temperatures for 24 hours after transportation, and spent more time lying down for 2 days after transportation, compared with those indices for control heifers. Eight hours after transportation, the weight of transported heifers decreased 6%, whereas that of control heifers increased 0.6%. At 48 hours after initiation of transportation, weight, rectal temperature, and time spent at various pen locations did not differ between transported and control heifers. Cortisol concentrations were higher 4 hours after initiation of transportation, compared with those determined just prior to transportation.

Conclusions and Clinical Relevance—Results indicated transportation during periods of high ambient temperatures caused transient changes in physiologic and behavioral indices of beef heifers. (*Am J Vet Res* 2013;74:481–490)

Throughout the United States, it is common for cow-calf producers to transport cattle substantial distances to feedlot facilities generally located in the central portion of the country.[1,2] In 2010, > 34 million cattle were slaughtered in the United States,[3] and most of those cattle would have been transported at least once prior to slaughter. Handling prior to, during, and after transportation is stressful for cattle,[4–6] and transportation regulations for cattle are being scrutinized.[7]

Abbreviations

BRD	Bovine respiratory disease
TNF	Tumor necrosis factor

Bovine respiratory disease is one of the most economically important diseases affecting beef feedlot cattle. For immature beef cattle, the incidence of BRD commonly increases during the stress of weaning and transportation to a feedlot.[6,8] Bovine respiratory disease affects 14.4% of all cattle entering beef feedlots,[9] and the immune responses of recently transported cattle are often suppressed[10] because of increased cortisol concentrations.[11]

Factors associated with stress to cattle during transportation include management changes, novelty, social regrouping, ambient temperature, humidity, and

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transit time.[5] Knowles[12] recommends not transporting cattle when the ambient temperature is > 30°C. Because of the constant flow of cattle into feedlots, restriction of the transportation of cattle to when temperatures are < 30°C may cause logistic complications for the beef industry during summer months and some periods of the spring and autumn when high ambient temperatures persist for several consecutive days.[13] These complications may compound the stress endured by feedlot cattle, especially when movement to the feedlot is delayed; cattle exposed to severe or sustained stress may have increased susceptibility to disease.[14] Research to examine the physiologic responses of cattle to transportation during periods of high ambient temperatures is warranted to determine whether restrictions on cattle transportation are necessary.

The objectives of the study reported here were to determine the effects of transportation during periods of high ambient temperature (≥ 32.2°C) on physiologic and behavioral indices of beef heifers. Our hypotheses were that transported heifers would have increased body temperatures, increased concentrations of stress biomarkers in blood, and decreased activity after transportation. Evaluation of these variables may help identify important risk factors for morbidity after transportation of cattle.

Materials and Methods

Animals—Twenty black crossbred beef heifers with a mean ± SD body weight of 217.8 ± 12.1 kg were selected for the study. All heifers were owned by Kansas State University, and all study procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Throughout the study, the heifers were housed as a group in a single pen (12.2 × 24.4 m) and fed a ration calculated to provide each heifer 2.3 kg of ground corn/d with trace mineral and 0.9 kg of alfalfa/d in addition to ad libitum access to brome hay, a supplemental salt block, and water. The heifers were humanely handled during each portion of the study. The health status of the heifers was monitored by the same individual who recorded each heifer's heart rate and respiration rate and assigned a clinical health score to each heifer twice daily throughout the study.

Study design—The study had a double-crossover design. At study initiation, each heifer was matched to another heifer on the basis of weight to form a block of 2 heifers. Then, each heifer within a block was randomly allocated to 1 of 2 groups such that each group contained 10 heifers. On each of 4 days, one group of heifers was transported 518 km in a livestock trailer (2.1 × 6.1 m; stocking density, 170 kg/m^2) while the other group served as untransported controls. The study protocol was repeated such that each group was transported twice and served as controls twice. The days during which cattle were transported were selected by a predetermined criterion that the maximum ambient temperature was forecasted to be ≥ 32.2°C.

A wireless remote weather station was installed at the research facility to allow continuous monitoring of the environmental conditions where the heifers were housed. On the morning of each day selected for transportation, none of the heifers were fed grain until after the transportation process was initiated, and the heifers being transported did not have access to feed or water during the 8-hour period of transit and processing. The heifers being transported were loaded into the livestock trailer at 8:00 AM (hour 0). Those heifers were then transported for 4 hours (noon; hour 4) and approximately 259 km to a remote livestock-working facility where they were unloaded and worked through a chute system so that body weights and nasal and rectal temperatures could be measured and venous blood samples could be obtained. Then, the heifers were reloaded into the livestock trailer and transported 4 hours back to the research facility, arriving at approximately 4:00 PM (hour 8). Three days after the first group of heifers was transported, the study groups were crossed over, and the 10 heifers that served as controls during the first transportation day were transported. The entire process was repeated once such that each group of heifers was transported twice and served as controls twice.

Measurement of rectal, nasal, and surface temperatures—For each heifer, rectal temperature was measured with a rapid equilibration thermal probe.[a] Radiofrequency thermal sensors[b] were implanted at a depth of approximately 2 mm in the submucosa of the nasal mucosae on the dorsal and medial aspects of the left and right nares approximately 100 mm caudal to the alar cartilage. Each sensor contained a radiofrequency transponder that, when initiated by an electronic signal from a reading device,[c] sent the temperature (± 0.1°C) back to the reading device, where it was recorded into an electronic database. A high-definition thermal sensor camera[d] was used to record surface temperatures of the right and left nares, nasal planum, and cornea at hours 0 and 8. The mean temperature for the left and right nares was calculated, and this value was used for subsequent statistical analyses. Rectal and nasal temperatures were recorded from all heifers immediately prior to (hour 0) and at 4 (transit midpoint), 8 (transit end), 10, 12, 14, 16, 18, 20, 22, 24, 36, 48, and 56 hours after initiation of each transportation period.

Behavioral data acquisition—Prior to initiation of the study, a remote location–monitoring tag[e] was applied to the left ear of each study heifer to record heifer behavior and activity as described.[15] Briefly, the tag transmitted information about the heifer's location (ie, X [length] and Y [width] coordinates) within the pen to fixed wireless sensors at the periphery of the pen, which relayed the information to a computer database where it was stored for analysis.[f] The heifer's coordinates were recorded at 1-second intervals throughout the study, and each set of coordinates was identified with a time stamp. A data mining software program[g] was used to compare each set of coordinates for a heifer with the known X and Y coordinates of specific locations (grain feeder, hay feeder, waterer, and shelter) within the pen, and each set of heifer coordinates were dichotomously (yes or no) classified as being within a 0.3-m radius of each location. The time stamps on the coordinates were evaluated to determine the amount of time a heifer spent at a particular location within the pen during a given period. Data obtained from heifers during transportation were not included in the statistical analyses.

Each heifer also had a commercial accelerometer[h] and pedometer[i] applied to the lateral aspect of the right hind limb just proximal to the metatarsophalangeal (fetlock) joint. The accelerometer and pedometer were placed within a neoprene sleeve and affixed to the limb with a strap. The accelerometer recorded triaxial (X, Y, and Z axes) directional forces with an axis range of ± 10 g and recorded 100 measurements/s.[16] The accelerometers were programmed with validated settings[17] such that X, Y, and Z acceleration and mean and maximum vector magnitude were recorded at 5-second intervals. Values for the mean force of gravity and vector magnitude were calculated by summing the values for force of gravity and acceleration (values for X, Y, and Z axes combined), respectively, and dividing each by the number of measurements (ie, 5-second intervals) recorded during a specified time period. The maximum vector magnitude was the highest combined value for acceleration during the 5-second interval. Every 7 days throughout the study, the accelerometers were briefly removed from the heifers so that data could be downloaded into a computer database and then were reapplied. Data obtained via the accelerometers were analyzed with a data-mining software program[g] to determine the amount of time each heifer spent standing, lying down, or walking during each 5-second interval, which was then aggregated by day. Data obtained from accelerometers during transportation were analyzed separately from data obtained from acclerometers during the remainder of the observation period.

For each heifer, the number of steps/d was determined via the pedometer, which contained a 2-D accelerometer that monitored the up and down movement of the limb. Along with the accelerometers, the pedometers were briefly removed from the heifers every 7 days so that data could be downloaded into a computer database and then were reapplied. Although behavioral data were obtained throughout the study, behavioral activity was analyzed for a period of only 3 days after initiation of each transportation period.

Body weight—For each transportation period, all heifers were individually weighed immediately prior to (hour 0) and at 4, 8, and 48 hours after initiation of transportation. For each heifer at 4, 8, and 48 hours, respectively, the percentage change in body weight was calculated as follows: ([body weight at hour 0 – body weight at the time of interest]/body weight at hour 0) × 100%.

Blood sample collection and analyses—During each transportation period, blood samples (12 mL) were collected via jugular venipuncture from the heifers being transported immediately prior to (hour 0) and at 4, 8, 24, 36, 48, and 56 hours after initiation of transportation. The blood samples were immediately transferred to a 6-mL serum separator tube and 6-mL tube continuing potassium EDTA. All blood samples were centrifuged at 1,500 × g for 10 minutes. A 2-mL aliquot of serum or plasma was separated from each sample, placed in a cryovial, and frozen at –80°C until analyzed for serum cortisol and TNF-α concentrations or plasma substance P concentration.

Serum cortisol concentration was determined via a solid-phase competitive chemiluminescent enzyme immunoassay[j] as described.[18] The immunoassay's lower limit of detection was 5.5 nmol/L; therefore, the results for serum samples with cortisol concentrations < 5.5 nmol/L were recorded as 5.5 nmol/L.

Serum concentration of TNF-α was determined via a commercial ELISA[k] modified for use with bovine serum. The primary and secondary antibodies used for the assay were goat anti-bovine TNF-α and biotinylated goat anti-bovine TNF-α, respectively. Horseradish peroxidase–labeled streptavidin[j] and a tetramethylbenzidine–hydrogen peroxide solution[l] were used for antibody detection and color development. The TNF-α concentration was calculated by subtraction of the absorbance value at 540 nm from the absorbance value at 450 nm and comparing that value with the curve for the TNF-α standard that was run on the same plate as the serum sample.

Substance P concentrations in plasma samples obtained at 0, 4, 8, 24, and 48 hours (substance P concentrations were not determined for plasma samples obtained at 36 and 56 hours) were determined via a commercial immunoassay kit[m] that had been validated for use with bovine plasma.[19] Plasma samples were extracted by means of C18 cartidges[n] prior to immunoassay analysis, and the assay used a polyclonal anti–substance P antibody. The concentration of substance P in plasma samples was inversely proportional to the intensity of the color detected at a wavelength of 405 nm. Any plasma sample with a substance P concentration outside of the standard curve was not included in statistical analyses.

Statistical analysis—Data were imported into 1 of 2 commercial statistical software packages[o,p] for analyses. Distributions of each variable were visually evaluated for normality, and when data appeared to be nonnormally distributed, that variable was logarithmically transformed ($\log_{10}$). Mixed regression models were used to evaluate potential relationships between continuous outcome variables (ie, nasal temperature, rectal temperature, surface temperatures, heart rate, and respiratory rate) and independent variables of interest, which included transport status, trial hour, and the interaction between transport status and trial hour. All analyses included random effects for each heifer and transportation day to account for a lack of independence caused by repeated measures. For transported heifers only, the effect of trial hour on cortisol, substance P, and TNF-α concentrations, respectively, was analyzed by the use of a mixed regression model.

Logistic regression models were used to determine the probability of proximity to a specific location (grain feeder, hay feeder, waterer, or shelter) within the pen or engagement in a specific activity (standing, lying down, or walking). Independent variables included transport status, trial day, and the interaction between transport status and trial day. A first-order autoregressive correlation structure was defined to account for the repeated measures on heifers over time in all analyses.[20] Multivariable regression models were constructed by the use of backward selection in a stepwise procedure, and the final multivariable model for each outcome included only variables with a type 3 likelihood ratio test $P < 0.05$. Within each trial day, differences between transported and control heifers were evaluated via *t* tests, and the level of significance was set at $P < 0.01$ a priori to account for multiple comparisons.

Results

All heifers remained healthy throughout the study. The maximum ambient temperature exceeded 32.2°C on each transportation day (**Table 1**). Mean respiratory

Table 1—Mean, maximum, and minimum temperature and humidity and maximum heat index for each of 4 days during which 10 beef heifers were transported 518 km while another 10 heifers served as untransported controls.

	Transportation day			
Variable	**1**	**2**	**3**	**4**
Mean temperature (°C)	33	30	35	36
Maximum temperature (°C)	40	38	42	43
Minimum temperature (°C)	24	24	25	29
Mean humidity (%)	54	67	47	39
Maximum humidity (%)	83	83	69	78
Minimum humidity (%)	22	27	23	27
Maximum heat index (°C)	40	41	41	42

Prior to study initiation, each of 10 heifers was matched to another heifer on the basis of weight to form a block of 2 heifers; then each heifer within a block was randomly allocated to 1 of 2 groups such that each group contained 10 heifers. The study had a double-crossover design such that each group of heifers was transported twice and served as controls twice. There were 3 days between transportation days 1 and 2 and transportation days 3 and 4 and 1 week between transportation days 1 and 3.

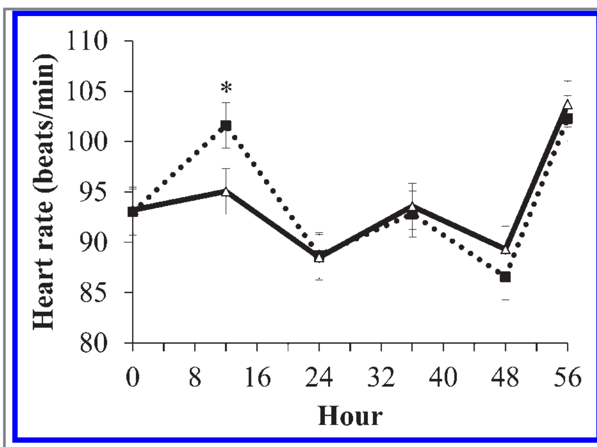

Figure 1—Mean ± SEM heart rate immediately prior to (hour 0) and at 12, 24, 36, 48, and 56 hours after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperatures (≥ 32.2°C). Prior to study initiation, each of 10 heifers was matched to another heifer on the basis of weight to form a block of 2 heifers. Then each heifer within a block was randomly allocated to 1 of 2 groups; therefore, each group contained 10 heifers. The study had a double-crossover design such that each group of heifers was transported twice and served as controls twice; thus, each data point represents the mean of 40 observations. There were 3 days between transportation days 1 and 2 and transportation days 3 and 4 and 1 week between transportation days 1 and 3. Interaction between trial hour and transport status was significant ($P < 0.01$). *Within an hour, values for heifers during transportation and control periods differ significantly ($P < 0.01$).

rate did not differ significantly between transported and control heifers and did not vary significantly during the observation period. Transported heifers had a significantly higher mean heart rate, compared with the mean heart rate for control heifers at 8 hours after initiation of transportation (**Figure 1**). Evaluation of rectal ($P < 0.01$) and nasal ($P < 0.01$) temperature data revealed a significant association between trial hour and transport status (**Figure 2**). Transported heifers had lower mean rectal ($P < 0.01$) temperatures at 12, 14, 20, and 24 hours after initiation of transportation, compared with those of the control heifers. Similarly, transported heifers had lower mean nasal ($P < 0.01$) temperatures at 4, 8, 20, and 24 hours after initiation of transportation, compared with those of the control heifers. The

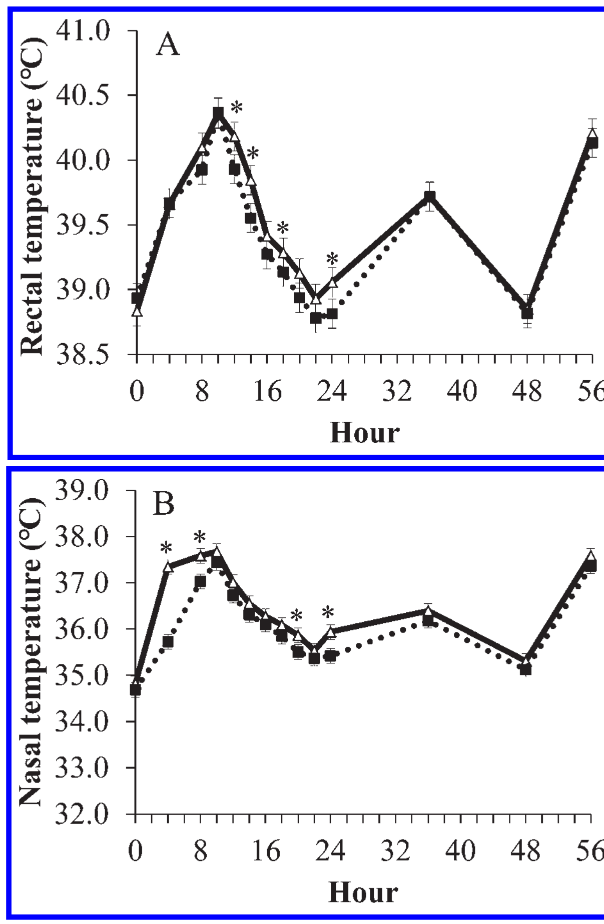

Figure 2—Mean ± SEM rectal (A) and nasal (B) temperatures immediately prior to (hour 0) and at 4 (transit midpoint), 8 (transit end), 10, 12, 14, 16, 18, 20, 22, 24, 36, 48, and 56 hours after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C). Rectal temperatures were measured via a rapid equilibration thermal probe. Nasal temperatures were determined by means of radiofrequency thermal sensors that were implanted at a depth of approximately 2 mm in the submucosa of the nasal mucosae on the dorsal and medial aspects of the left and right nares approximately 100 mm caudal to the alar cartilage. For each heifer, the nasal temperature recorded at each observation represented the mean of the temperature readings from the sensors in both the left and right nares. Notice that the scale of the y-axis differs between panels. Interaction between trial hour and transit status was significant ($P < 0.01$). *See* Figure 1 for remainder of key.

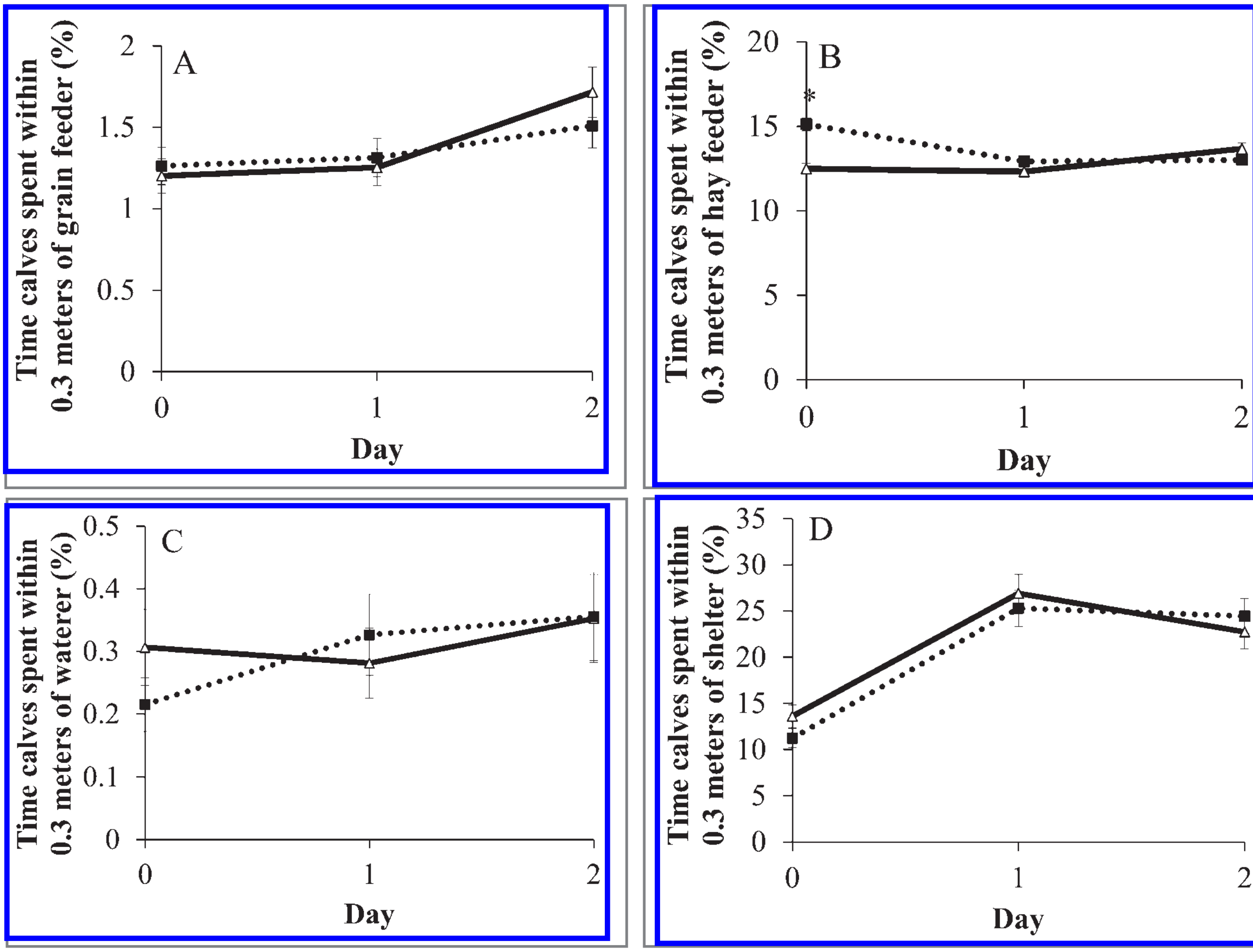

Figure 3—Mean ± SEM percentage of time spent within 0.3 m of the grain feeder (A), hay feeder (B), waterer (C), and shelter (D) the day of (day 0) and for the 2 days after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C). The time spent at specific locations within the pen was determined by measurements obtained from a remote location–monitoring tag that was applied to the left ear of each heifer prior to study initiation. The tag continuously transmitted coordinate data to wireless sensors located at the pen's periphery, which then transmitted the coordinates to a computer database for analysis. Notice that the scale of the y-axis varies among the panels. Interaction between trial day and transit status was significant ($P < 0.01$). *Within an observation day, values for heifers during transportation and control periods differ significantly ($P < 0.01$). *See* Figure 1 for remainder of key.

mean surface temperatures of the nares, nasal planum, and cornea did not vary significantly between transported and control heifers at any time measured. For both transported and control heifers, the mean surface temperatures of the nares ($P < 0.01$), nasal planum ($P < 0.01$), and cornea ($P < 0.01$) obtained at 8 hours after initiation of transportation were significantly higher, compared with those obtained immediately before transportation (hour 0).

A significant interaction was identified between transport status and trial day for the percentage of time heifers spent within 0.3 m of the grain feeder, hay feeder, waterer, and shelter (**Figure 3**). Transported heifers spent significantly ($P < 0.01$) more time near the hay feeder on the day of transportation (day 0) than did the control heifers. Otherwise, the percentage of time spent at a particular pen location did not differ significantly between transported and control heifers during the 3-day observation period after initiation of transportation.

During the 8-hour transportation period, the transported heifers spent a significantly ($P < 0.01$) greater percentage of time walking (mean ± SE, 2.7% ± 0.3%), compared with the percentage of time spent walking (2.0% ± 0.2%) by the control heifers, whereas the control heifers spent a significantly ($P < 0.01$) greater percentage of time lying down (22.0% ± 2.0%), compared with the percentage of time spent lying down (4.0% ± 0.4%) by the transported heifers. A significant interaction was identified between transport status and trial day for the percentage of time heifers spent lying down ($P < 0.01$) and standing ($P < 0.01$). Transported heifers spent a significantly greater percentage of time walking ($P < 0.01$) on the day of transportation (day, 0) and days 1 and 2 after transportation and lying down on days 1 and 2 after transportation, compared with the percentage of time spent walking and lying down by the control heifers during the same period (**Figure 4**). A significant interaction was also identified between transport status and trial day for the number of steps traveled by heifers. The mean number of steps traveled by the transported heifers was significantly greater, compared with the mean number of steps traveled by the control heifers on the day of transportation (**Figure 5**).

Compared with the mean body weight immediately prior to transportation (hour 0), the mean body weight of the transported heifers was decreased at 4 and 8 hours after initiation of transportation, whereas the mean body weight of the control heifers was increased at 4 and 8 hours after initiation of transportation (**Figure 6**). Also, the percentage change in body weight from hour 0 differed significantly between transported and control heifers at 4 and 8 hours after initiation of transportation. However, at 48 hours after initiation of transportation, the mean body weight did not differ between transported and control heifers.

Data for cortisol, substance P, and TNF-α concentrations were not normally distributed; therefore, logarithmic transformations ($\log_{10}$) were applied to the data so that regression analyses could be performed. Heifers had an increased mean serum cortisol concentration at 4 and 8 hours after initiation of transportation, compared with that immediately prior to transportation (**Figure 7**). Conversely, heifers had a decreased mean serum substance P concentration at 24 and 48 hours after initiation of transportation, compared with that immediately prior to transportation. The mean plasma TNF-α concentration did not vary significantly during the observation period.

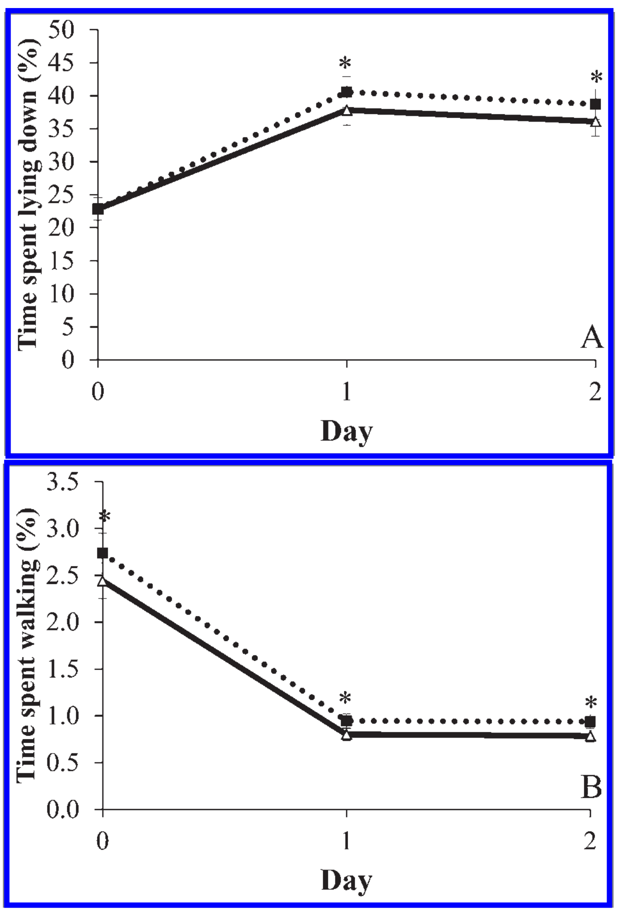

Figure 4—Mean ± SEM percentage of time spent lying down (A) and walking (B) the day of (day 0) and for the 2 days after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C). Data were obtained via accelerometers that were applied to all heifers on the lateral aspect of the right hind limb just proximal to the metatarsophalangeal (fetlock) joint. The accelerometers were programmed such that acceleration along X, Y, and Z axes and mean and maximum vector magnitude were recorded at 5-second intervals, and the data obtained were aggregated by day. Notice that the scale of the y-axis differs between panels. Interaction between trial day and transit status was significant ($P < 0.01$). *Within an observation day, values for heifers during transportation and control periods differ significantly ($P < 0.01$). *See* Figure 1 for remainder of key.

Discussion

Results of the present study indicated that beef heifers transported during periods of high ambient temperatures (≥ 32.2°C) had a transient decrease in rectal and nasal temperatures and body weight. Transported

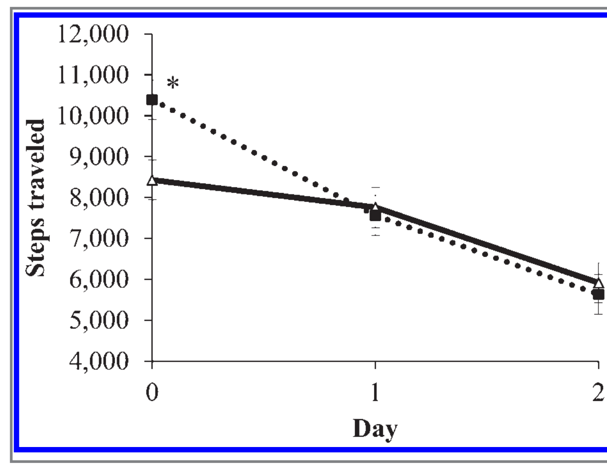

Figure 5—Mean ± SEM number of steps traveled the day of (day 0) and for the 2 days after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C). Data were obtained via pedometers that were applied to all heifers on the lateral aspect of the right hind limb just proximal to the metatarsophalangeal (fetlock) joint. Interaction between trial day and transit status was significant ($P < 0.01$). *Within an observation day, values for heifers during transportation and control periods differ significantly ($P < 0.01$). *See* Figure 1 for remainder of key.

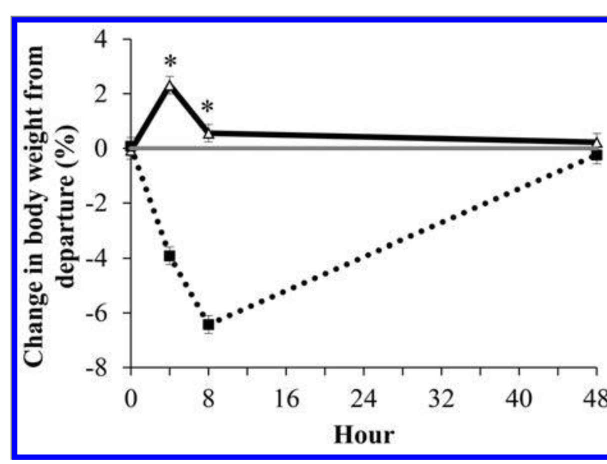

Figure 6—Mean ± SEM percentage change in body weight immediately prior to (hour 0) and at 4 (transit midpoint), 8 (transit end), and 48 hours after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C). *Within an hour, values for heifers during transportation and control periods differ significantly ($P < 0.01$). *See* Figure 1 for remainder of key.

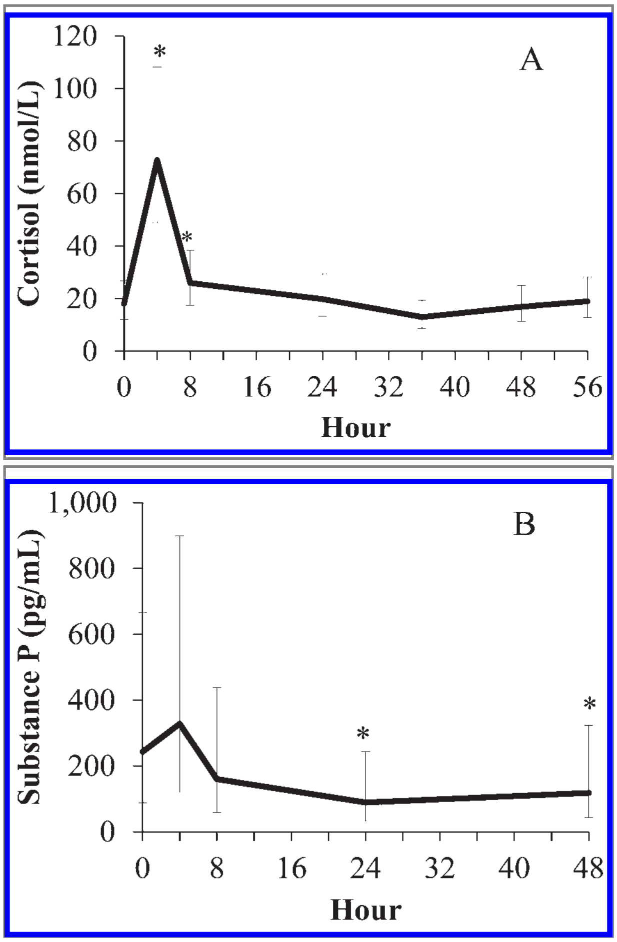

Figure 7—Mean and 95% confidence intervals for serum cortisol (A) and plasma substance P (B) concentrations immediately prior to (hour 0) and at 4 (transit midpoint), 8 (transit end), 24, 36, 48, and 56 hours after initiation of transportation for 20 beef heifers that were transported 518 km during periods of high ambient temperature (≥ 32.2°C). Plasma substance P concentration was not determined at 36 and 56 hours after initiation of transportation. Serum cortisol concentrations were determined via a solid-phase competitive chemiluminescent enzyme immunoassay and plasma substance P concentrations were determined via a commercial immunoassay. *Value differs significantly ($P < 0.01$) from that at hour 0. *See* Figure 1 for remainder of key.

heifers also spent more time at the hay feeder on the day of transportation, walking during the 3 days after initiation of transportation, and lying down on days 1 and 2 after initiation of transportation, compared with time spent at the hay feeder, walking, and lying down by control heifers that were not transported during the same period. Transported heifers also had increased serum cortisol and substance P concentrations at 4 hours after initiation of transportation, compared with those immediately before transportation. To our knowledge, the present study was the first to monitor both physiologic and behavioral variables in cattle immediately before, during, and after transportation.

The transient decrease in the mean rectal and nasal temperatures of transported heifers for 24 hours after transportation was unexpected and differs from results of other studies.[21,22,q] Given that the environmental conditions were similar for both transported and control heifers, we attributed thermoregulatory differences between the treatment groups to the continuous air flow within the trailer, which allowed the transported heifers to dispel body heat via convection. Infrared thermography is an effective tool for monitoring body temperature in cattle.[23] In the present study, we were unable to detect significant variation in the surface temperatures of the nares, nasal planum, and cornea in the transported heifers, although our ability to detect such variations may have been confounded by the environmental conditions.[r]

Behavioral changes were expected in the heifers during and after transportation. On the day of transportation, all behavior data were affected because of the frequency with which body weight and temperature measurements were obtained. The amount of time that heifers spent standing during transit in the present study was similar to that for heifers of a similar age in another study.[24] Some of the heifers of the present study lay down during transit, which suggested that cattle will lie down while being transported if they have sufficient room to do so. The stocking density of the livestock trailer used in the present study was well below that recommended by the Farm Animal Welfare Council and USDA.[25,26] The fact that the percentage of time that the transported heifers spent walking during the 8-hour transportation period was greater, compared with that of the control heifers, was probably associated with the movement of heifers during loading and unloading of the trailer as well as movement of the heifers within the trailer during transit. The significantly greater number of steps traveled by the transported heifers, compared with the number of steps traveled by the control heifers on day 0, was also likely a reflection of heifer movement during transit. The pedometers used in the present study recorded the number of steps each heifer traveled during a 24-hour period, and unfortunately, we were unable to separate the data obtained during the 8-hour transportation period from the rest of the data obtained on day 0.

Regarding the time heifers spent at specific locations within the pen, the only change in behavior detected after transportation was an increased percentage of time transported heifers spent near the hay feeder on the day of transportation, compared with that for control heifers. We anticipated that the transportation of heifers during periods of high ambient temperature would result in stress and less time spent by the heifers at the feeders in a manner similar to that described in other studies[27,28] involving feedlot heifers, in which morbid heifers spent significantly less time at a grain feeder, compared with healthy control heifers. In the present study, the transported heifers may have spent more time at the hay feeder on the day of transportation in an attempt to recover from the period during which they did not have access to feed. However, because of the limitations of the behavior-monitoring system used, we cannot confirm that the heifers were actually eating during the time they spent at the hay feeder.

Interestingly, transported heifers spent approximately 3% more time lying down and 0.1% more time walking during the 3 days immediately after transportation than did the control heifers. This finding suggested that after transportation, heifers were walking when they were not lying down. The biological importance of this is unknown and warrants further investigation. Regardless, the results of the present study indicated that transportation of healthy feedlot heifers during periods of high ambient temperature did not have a detrimental effect on their behavior during the immediate 3-day period after transit and that the use of accelerometers was a sensitive and effective method for monitoring behavior in cattle.

The amount of weight cattle lose during transportation varies.[29] The distance cattle are transported has been associated with the percentage of weight loss, and the greatest proportion of weight is lost during the first hours of transit.[30–32] In the present study, the percentage of weight lost by heifers during transportation was similar to that lost by feeder steers in another study.[33] The percentage of body weight lost by heifers during transportation in the present study was most likely the result of the withholding of feed and water in addition to excretion of feces and urine and moisture lost via respiration and sweating.

For cattle, it is important to monitor weight loss during transportation because the amount of weight lost is positively associated with the risk of developing BRD.[34] The percentage of weight lost by cattle during transportation has been used as a determinant for the metaphylactic treatment of cattle entering feedlots.[35,36] The distance cattle were transported has also been positively associated with BRD morbidity and negatively associated with average daily gain.[37] In another study,[38] the percentage of weight lost was greater when cattle were transported during periods of high ambient temperature (18° to 34°C), compared with that when cattle were transported during periods of low ambient temperature (–6° to 16°C). Results of yet another study[39] indicate a positive association between the total amount of weight lost during transportation and body weight immediately prior to transport and ambient temperature. In the present study, heifers were transported during periods of high ambient temperature (≥ 32.2°C), and weight loss was transient; by 48 hours after initiation of transportation, the mean body weight for the transported heifers did not differ significantly, compared with that immediately before transportation.

In animals, the physiologic response to fear or stress is the release of cortisol from the adrenal cortex via stimulation of the hypothalamus and pituitary gland.[40] In the present study, we expected serum cortisol concentration to increase in the heifers during transportation because of the novelty of the situation and the increased handling that was required for sample collection. The increase in mean cortisol concentration at the midway (hour 4) point of transit, compared with that immediately prior to transit, was similar to results of other studies.[28,41] However, the decrease in mean cortisol concentration between the midway point and the end (hour 8) of transportation was unexpected and may have been caused by the lack of novelty for the heifers after being reloaded onto the trailer and transported for the second time within a short period of time. Results of research by Grandin[4] indicate that individual animal factors such as previous experience influence the physiologic and behavioral responses to stressful events. The implementation of animal-handling procedures that decrease stress should result in decreased cortisol release and improve animal welfare.[42] Also, the increase in mean serum cortisol concentration in heifers midway through transit may have been associated with the decrease in nasal and rectal temperatures as an effect of weight loss and peripheral vasoconstriction.

In the present study, we also evaluated plasma substance P concentration as a potential biomarker for transportation stress in cattle. Substance P is a neuropeptide that modulates the dorsal root nociceptive neurons and can be detected in areas involved with pain and stress.[43] Results of another study[19] indicate that substance P concentration is an effective biomarker for pain in calves. Concurrent evaluation of cortisol and substance P concentrations may be beneficial because of the different mechanisms by which each biomarker is released, which may allow for the quantification of the magnitude or severity of stress in cattle. Although the neurophysiologic processing of pain and stress may differ, there is a cross-link between cortisol and substance P concentrations. Cortisol release follows a circadian rhythm with increased secretion occurring with the onset of daylight.[44] Conversely, release of substance P does not follow a circadian rhythm[19]; thus, it may be a more sensitive biomarker for stress when sampling frequency is limited. In the present study, only the short-term effects of transportation on substance P concentration in cattle were evaluated. Also, blood samples were not obtained from control heifers because of logistic limitations; therefore, cortisol and substance P concentrations could not be compared between transported and control heifers. Further research is necessary to elucidate the mechanism of cortisol and substance P release in cattle exposed to pain and stress in various situations.

For the heifers of the present study, the mean serum TNF-α concentration did not vary significantly at any sample collection time, compared with that immediately prior to transportation. In other studies,[45–48] TNF-α concentration either increased or decreased after exposure of animals to heat stress or transportation. The TNF-α concentration results of the present study suggested that there was a lack of a proinflammatory response in heifers that were transported during periods of high ambient temperatures, and this was unexpected.

Extrapolation of the results of the present study to the general feedlot cattle population should be done with caution because the stocking density (170 kg/m^2) of the trailer during transportation was less than half the recommended maximum stocking density (360 kg/m^2) for livestock trailers[25] and did not reflect conditions under which cattle are commonly transported in the United States. Moreover, the location of heifers within a semitruck trailer during transportation has been associated with the subsequent morbidity rate of those heifers.[49]

Results of the present study indicated that beef heifers that were transported during periods of high ambient temperature (≥ 32.2°C) had transient changes in body temperature and weight, serum cortisol and plasma substance P concentrations, and behavior. However, none of the study heifers developed detrimental health effects during the observation period after transportation. To our knowledge, the present study was the first to evaluate nasal mucosal temperature and plasma substance P concentration in beef heifers during and after transportation.

a. Pavia Rectal Temp thermometer, Pavia Sales Group Inc, Plymouth, Minn.
b. Bio-Thermo LifeChip, Destron Fearing, South Saint Paul, Minn.
c. Pocket Reader, Destron Fearing, South Saint Paul, Minn.
d. ThermaCAM S65, FLIR Systems, Wilsonville, Ore.
e. Ubisense Series 7000 compact tag, Ubisense, Denver, Colo.
f. Steggles P, Gschwind S. The Ubisense Space Platform. Advances in pervasive-computing (abstr), in *Proceedings*. 3rd Int Conf Pervasive Comput 2005;191.
g. Insightful Miner, Insightful Corp, Seattle, Wash.
h. GP1 SENSR, Reference LLC, Elkader, Iowa.
i. NL-800, New-Lifestyles Inc, Lees Summit, Mo.
j. Immulite, Siemens Medical Solutions, Los Angeles, Calif.
k. Bovine TNF-α, Pierce, Rockford, Ill.
l. Substrate Solution, R&D Systems Inc, Minneapolis, Minn.
m. SP Correlate-EIA, ELISA kits, Assay Designs Inc, Ann Arbor, Mich.
n. Sep-Pak Vac 3cc C18 SPE, Waters Corp, Milford, Mass.
o. JMP, version 9, SAS Institute Inc, Cary, NC.
p. SAS, version 9.2, SAS Institute Inc, Cary, NC.
q. Stevens DG, Camp TH. Vibration in a livestock vehicle (abstr), in *Proceedings*. Am Soc Agric Biol Eng 1979;10.
r. Gomez A, Vergara C, Cook NB, et al. Is thermography a possible new method to evaluate body temperature in fresh cows? (abstr), in *Proceedings*. Annu Meet Am Assoc Bovine Pract 2011;191.

References

1. Feuz DM, Umberger WJ. Beef cow-calf production. *Vet Clin North Am Food Anim Pract* 2003;19:339–363.
2. Mintert J. Beef feedlot industry. *Vet Clin North Am Food Anim Pract* 2003;19:387–395.
3. USDA National Agricultural Statistics Service. *Livestock slaughter 2010 summary*. Washington, DC: USDA, 2011. Available at: usda01.library.cornell.edu/usda/nass/LiveSlauSu//2010s/2011/LiveSlauSu-04–25–2011.pdf. Accessed Apr 12, 2012.
4. Grandin T. Assessment of stress during handling and transport. *J Anim Sci* 1997;75:249–257.
5. Swanson JC, Morrow-Tesch J. Cattle transport: historical, research, and future perspectives. *J Anim Sci* 2001;79(suppl E):E102–E109.
6. Fike K, Spire MF. Transportation of cattle. *Vet Clin North Am Food Anim Pract* 2006;22:305–320.
7. United States Department of Transportation. *Regulatory guidance: applicability of the Federal Motor Carrier Safety Regulations to operators of certain farm vehicles and off-road agricultural equipment*. Washington, DC: Federal Motor Carrier Safety Administration, 2011. Available at: www.fmcsa.dot.gov/rules-regulations/administration/rulemakings/notices/FMCSR-Farm-vehicles-Off-Road-Agricultural-Equipment.aspx. Accessed Apr 12, 2012.
8. Edwards A. Respiratory diseases of feedlot cattle in central USA. *Bovine Pract* 1996;30:5–7.
9. USDA APHIS Veterinary Services. *Part III: health management and biosecurity in US feedlots, 1999*. Fort Collins, Colo: USDA National Animal Health Monitoring System, 2000. Available at: www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot99/Feedlot99_dr_ParPart.pdf. Accessed Apr 12, 2012.
10. Mackenzie AM, Drennan M, Rowan TG, et al. Effect of transportation and weaning on humoral immune responses of calves. *Res Vet Sci* 1997;63:227–230.
11. McEwen BS, Biron CA, Brunson KW, et al. The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. *Brain Res Rev* 1997;23:79–133.
12. Knowles TG. A review of the road transport of cattle. *Vet Rec* 1999;144:197–201.
13. USDA APHIS. *Changes in the US feedlot industry: 1994–1999*. Washington, DC: USDA, 2000;1–42.
14. Moberg GP, Mench JA. *The biology of animal stress: basic principles and implications for animal welfare*. Wallingford, Oxfordshire, England: CAB International, 2000;1–21.
15. White BJ, Anderson DE, Renter DG, et al. Clinical, behavioral, and pulmonary changes in calves following inoculation with *Mycoplasma bovis*. *Am J Vet Res* 2012;73:490–497.
16. *GP1 progammable accelerometer user manual*. Elkader, Iowa: Reference LLC, 2007.
17. Robert B, White BJ, Renter DG, et al. Evaluation of three-dimensional accelerometers to monitor and classify behavior patterns in cattle. *Comput Electron Agric* 2009;67:80–84.
18. Coetzee JF, Gehring R, Bettenhausen AC, et al. Attenuation of acute plasma cortisol response in calves following intravenous sodium salicylate administration prior to castration. *J Vet Pharmacol Ther* 2007;30:305–313.
19. Coetzee JF, Lubbers BV, Toerber SE, et al. Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. *Am J Vet Res* 2008;69:751–762.
20. Agresti A. *An introduction to categorical data analysis*. New York: John Wiley and Sons Inc, 1996;340–341.
21. Grigor PN, Cockram MS, Steele WB, et al. Effects of space allowance during transport and duration of midjourney lairage period on the physiological, behavioural and immunological responses of young calves during and after transport. *Anim Sci* 2001;73:341–360.
22. Tennessen T, Price MA, Berg RT. Comparative responses of bulls and steers to transportation. *Can Vet J* 1984;64:333–338.
23. Stewart M, Webster JR, Schaefer AL, et al. Infrared thermography as a non-invasive tool to study animal welfare. *Anim Welf* 2005;14:319–325.
24. Kent JE. The effect of road transportation on the blood constituents and behaviour of calves. I. Six months old. *Br Vet J* 1983;139:228–235.
25. Farm Animal Welfare Council. *Report on the European Commission proposals on the transport of animals*. London: Department for Environment, Food and Rural Affairs, 1993.
26. USDA Agricultural Marketing Service. *Cattle and swine trucking guide for exporters*. Washington, DC: USDA, 1999. Available at: www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3008268. Accessed Apr 12, 2012.
27. Sowell BF, Bowman JGP, Branine ME, et al. Radio frequency technology to measure feeding behavior and health of feedlot steers. *Appl Anim Behav Sci* 1998;59:277–284.
28. Sowell BF, Branine ME, Bowman JG, et al. Feeding and watering behavior of healthy and morbid steers in a commercial feedlot. *J Anim Sci* 1999;77:1105–1112.
29. Warriss PD. The handling of cattle pre-slaughter and its effects on carcass and meat quality. *Appl Anim Behav Sci* 1990;28:171–186.
30. Coffey KP, Coblentz WK, Humphry JB, et al. Review: basic principles and economics of transportation shrink in beef cattle. *Prof Anim Sci* 2001;17:247–255.
31. Cole NA, Camp TH, Rowe LD Jr, et al. Effect of transport on feeder calves. *Am J Vet Res* 1988;49:178–183.
32. Cernicchiaro N, White BJ, Renter DG, et al. Effects of body weight loss during transit from sale barns to commercial feedlots on health and performance in feeder cattle cohorts arriving to feedlots from 2000 to 2008. *J Anim Sci* 2012;90:1940–1947.
33. Coffey KP. Effects of gathering time on weight and shrink of steers grazing smooth bromegrass pastures. *Prof Anim Sci* 1997;13:170–175.
34. Camp TH, Stevens DG, Stermer RA, et al. Transit factors affecting shrink, shipping fever and subsequent performance of feeder calves. *J Anim Sci* 1981;52:1219–1224.
35. Nickell JS, White BJ. Metaphylactic antimicrobial therapy for bovine respiratory disease in stocker and feedlot cattle. *Vet Clin North Am Food Anim Pract* 2010;26:285–301.
36. Sanderson MW, Dargatz DA, Wagner BA. Risk factors for initial

respiratory disease in United States' feedlots based on producer-collected daily morbidity counts. *Can Vet J* 2008;49:373–378.
37. Cernicchiaro N, White BJ, Renter DG, et al. Associations between the distance traveled from sale barns to commercial feedlots in the United States and overall performance, risk of respiratory disease, and cumulative mortality in feeder cattle during 1997 to 2009. *J Anim Sci* 2012;90:1929–1939.
38. Phillips WA, Juniewicz PE, VonTungeln DL. The effect of fasting, transit plus fasting, and administration of adrenocorticotropic hormone on the source and amount of weight lost by feeder steers of different ages. *J Anim Sci* 1991;69:2342–2348.
39. González LA, Schwartzkopf-Genswein KS, Bryan M, et al. Factors affecting body weight loss during commercial long haul transport of cattle in North America. *J Anim Sci* 2012;90:3630–3639.
40. Molony V, Kent JE. Assessment of acute pain in farm animals using behavioral and physiological measurements. *J Anim Sci* 1997;75:266–272.
41. Crookshank HR, Elissalde MH, White RG, et al. Effect of transportation and handling of calves upon blood serum composition. *J Anim Sci* 1979;48:430–435.
42. Speer NC, Slack G, Troyer E. Economic factors associated with livestock transportation. *J Anim Sci* 2001;79(suppl E):E166–E170.
43. DeVane CL. Substance P: a new era, a new role. *Pharmacotherapy* 2001;21:1061–1069.
44. Thun R, Eggenberger E, Zerobin K, et al. Twenty-four-hour secretory pattern of cortisol in the bull: evidence of episodic secretion and circadian rhythm. *Endocrinology* 1981;109:2208–2212.
45. Suganuma T, Irie K, Fujii E, et al. Effect of heat stress on lipopolysaccharide-induced vascular permeability change in mice. *J Pharmacol Exp Ther* 2002;303:656–663.
46. do Amaral BC, Connor EE, Tao S, et al. Heat stress abatement during the dry period influences prolactin signaling in lymphocytes. *Domest Anim Endocrinol* 2010;38:38–45.
47. Kluger MJ, Rudolph K, Soszynski D, et al. Effect of heat stress on LPS-induced fever and tumor necrosis factor. *Am J Physiol* 1997;273:R858–R863.
48. Arthington JD, Eichert SD, Kunkle WE, et al. Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. *J Anim Sci* 2003;81:1120–1125.
49. White BJ, Blasi D, Vogel LC, et al. Associations of beef calf wellness and body weight gain with internal location in a truck during transportation. *J Anim Sci* 2009;87:4143–4150.

Correction: Evaluation of the efficacy and safety of single administration of 4.7-mg deslorelin acetate implants on egg production and plasma sex hormones in Japanese quail (*Coturnix coturnix japonica*)

In the article "Evaluation of the efficacy and safety of single administration of 4.7-mg deslorelin acetate implants on egg production and plasma sex hormones in Japanese quail (*Coturnix coturnix japonica*)" (*Am J Vet Res* 2013;74:316–323), the results section of the structured abstract should have read as follows:

Results—Egg production was significantly decreased in the treatment group, compared with the control group, from 2 to 12 weeks after implant injection. Egg production ceased in 6 of 10 quail in the treatment group (mean duration of cessation, 70 days). Plasma androstenedione and 17β-estradiol concentrations were significantly lower on day 29 in the treatment group than in the control group. On day 180, 17β-estradiol concentration was lower in control than in treated birds. No clinically relevant lesions were detected in either group at necropsy.