Virtual fences are not more stressful than conventional electric fences in rotationally stocked beef cattle

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On the Ground

- Virtual fence (VF) technology is rapidly developing and being adopted, but many ranchers and consumers have questions about its effect on the welfare of range beef cattle.
- We conducted two studies using either VF collars or conventional electric fencing to rotationally graze beef cattle. We measured several common physiological and behavioral indicators of stress and correlated these indicators with the number of electric stimuli received from the VF collars.
- Physiological and behavioral indicators of stress were not different between cattle rotated within the two types of fencing. No correlations were evident between the number of electrical stimuli received and stress indicators. From the perspective of cattle welfare, we concluded that continued development and use of VF is warranted.

Keywords: electric, fence, stress, welfare.

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Introduction

New technologies, such as virtual fencing, continually arise and enable innovative ways to manage livestock. Virtual fence (VF) could improve utilization of seasonal forage growth or the reestablishment of pasture biodiversity using exclusion areas,[1] while also allowing for automated herding or mustering of animals.[2] VF has been reported to effectively contain cattle without substantially affecting animal behavior and welfare.[3]

These VF systems incorporate electric stimulus to implement animal control similar to conventional electric fence (EF) that has been adopted by grazing managers worldwide for decades.[4] Electric shock, either in the form of electrified wire fencing or VF collars, can cause some degree of animal discomfort,[5,6] which is necessary to achieve the objective of constraining animal movement. Such stressors in cattle can cause an increase in stress hormones (i.e., cortisol) and blood metabolites such as plasma lactate and nonesterified fatty acid (NEFA) concentrations.[7] These metabolites may result in decreased animal performance because they elicit a change in animal metabolism, especially when due to prolonged exposure to stressors.[7,8] Stress caused by electrical shock has been reported to adversely affect animal behaviors by interrupting normal grazing activities and increasing agitation following electrical shock.[3,4] Changes in animal physiology and behavior should be a repeatable and objective measure of the stress cattle may experience in these systems. We want to be confident that VF systems do not unnecessarily increase stress in cattle. Therefore, our study objective was to evaluate the effects of VF on stress and behavioral responses in beef cattle when compared to EF in a rotational stocking system.

Methods

Pastures

Our research was conducted at the Bluestem Research Range at Oklahoma State University, 14.5 km (9 miles) SW of Stillwater, Oklahoma, USA. The pastures consisted of warm-season perennial grasses, primarily Bermuda grass (*Cynodon dactylon*) and yellow bluestem (*Bothriochloa ischaemum*), and exterior fencing of each paddock was traditional, five-strand barbed-wire fencing.

Study 1—Our pilot study was conducted over 4 weeks, beginning in August 2020 using two, 24-ha (59-acre) pastures. One pasture was assigned to VF and one to EF. In EF, the pasture was divided approximately in half with a double-stranded, electrified, high-tensile wire fence. The fence controller (Gallagher B600 Solar; Gallagher, Riverside, MO) maintained a voltage of 7 kV throughout the study in the EF pasture. The VF pasture was divided approximately in half, using only the VF system (see VF system section below). The VF pasture did not contain any physical interior fencing.

Study 2—Our second study was conducted over 8 weeks, beginning in October 2020 using four pastures ranging in size from 9.4 to 16.5 ha (23 to 41 acres). Each pasture was randomly assigned either VF ($n = 2$) or EF ($n = 2$). In EF pastures, each pasture was divided approximately into four paddocks with a double-stranded, electrified high-tensile wire fence. As in Study 1, the fence controller maintained a voltage of 7 kV throughout the study in the EF pastures. In VF pastures, each pasture was divided into four paddocks (similar to EF) but using only the VF system (see VF system section below). The VF pastures did not contain any physical interior fence.

Animals in both studies were rotated among paddocks at approximately 8:00 a.m. on Mondays. EF animals were manually rotated by a herdsman in a utility vehicle (UTV; Gator XUV835M, John Deere, Moline, IL). VF animals were rotated solely with the VF system. A one-way gate action was used on the day of rotation, where the VF which prevented animals from entering the next rotation paddock was disabled. Once animals crossed into the next assigned paddock, VF was enabled again. If animals attempted to return to the previous rotation, sound and shock patterns were initiated by the VF collar (see VF system section below). Each pasture contained one water tank to provide ad libitum water in either the southeast, southwest, or northeast corner of each pasture, sourced from the rural water district. Ponds were present in three of the four pastures; however, animals were excluded from accessing these ponds by either a single electrified, high-tensile wire or by the VF system. The interior fences in EF pastures were present and functioning from the start of each experiment.

Animals

Study 1—Fifty-five Angus heifers (body weight [BW] = 315 ± 30 kg [694 ± 66 pounds]) were used for our first study. These beef cattle were randomly allocated into an EF ($n = 24$) or VF ($n = 31$) pasture. These cattle had no prior exposure to the VF system and were not accustomed to the pastures; however, the cattle did have prior experience with EF, the herdsman, and UTV. Only the VF cattle wore VF collars.

Study 2—Fifty-nine Angus, Beefmaster, and Angus-Hereford cross mature cows and heifers (BW = 484 ± 84 kg [1067 ± 185 pounds]) were used for our second study. These cows previously had been managed in the pastures used in this experiment or similar pastures and were familiar with the fencing, water tanks, herdsman, and UTV. All cattle underwent at least 2 weeks of training with the VF collars and system, which consisted of VF fences implemented in the same place with the perimeter barbed-wire fencing. Calves were weaned from the cows 4 days prior to the start of the study. Cows were stratified by previous calf status (calf weaned vs. no calf) and breed type (Angus/Angus-Hereford cross, $n = 38$ or Beefmaster, $n = 21$). Within these stratifications, cows were randomly assigned to one of two EF ($n = 15$ and 15) or 1 of 2 VF ($n = 14$ and 15) pastures. All cattle wore a VF collar; however, the VF function on collars in EF pastures was disabled but still recorded Global Positioning System (GPS) location at 5-minute intervals. Because all cattle were collared, the results from this study are relevant to the electric stimulus delivered by the collar and not the effect of wearing a collar.

VF system

The VF system (Vence, vence.io; Merck & Co., Inc., Rahway, NJ) consisted of collars using GPS technology, a base station, a cloud server, and an end-user software interface. VF boundaries were defined through the Vence software, and the outlined parameters and instructions were communicated wirelessly to each collar through the base station. The VF system used audio and electrical cues administered as cattle attempted to penetrate a VF boundary. The width of the VF boundary for which sensory cues were administered was determined in the Vence computer software as a stimulus zone (50 m [164 feet]) from the VF fence line and an additional auditory zone (5 m [16 feet]) for a total of 55 m (180 feet) of VF boundary. An auditory tone (4 kHz; experienced by the animal at 75 dB for 0.5 seconds) was emitted by the VF collar as an animal approached the VF boundary (the auditory zone). If the animal responded to the auditory cue and turned away from the VF boundary, no other stimulus was applied. However, if an animal continued toward the VF boundary after receiving the auditory cue, a short electric pulse was applied to the animal's neck through the VF collar (at 800 V for 0.5 seconds). If the animal remained in the VF boundary after receiving both an auditory and electric stimulus, a pattern of 0.5 seconds of sound, 1.5 seconds of no stimulus, 0.5 seconds of electric stimulus, and then 2.5 seconds of no stimulus would continue for 100 seconds. After 100 seconds, there was a 180-second period of no stimulus. This pattern would continue for approximately 20 minutes; at which time built-in fail-safes would disable any further stimuli until the collar was manually reset by the manager. Each of the 0.5-second stimuli were recorded in the database.

VF implementation

On day 0 of each study, cattle entered the assigned pastures. Cattle in EF pastures were immediately stocked into one of the rotation paddocks. VF cattle were immediately placed into a 48-hour training period in which no interior VF lines were activated. During the first 24 hours, cattle were subjected to a 10-m (32 feet) stimulus zone in the same position as the perimeter fence line. Cattle were also excluded from any ponds using the VF system. At 24–48 hours the previous exclusions were applied, with the addition of a 5-m (16 feet) auditory zone added to the interior boundary of the stimulus zone. This caused cattle to first pass through an auditory zone and then stimulus zone prior to reaching the physical, barbed-wire fence.

After the 48-hour training period, VF along the perimeter of the VF pastures were deactivated and the VF defining the interior paddocks were activated. Due to this acclimation period, during the first 48 hours, VF cattle had access to a much larger pasture (24 or 58 ha [59 or 143 acres]) compared to cattle in the EF pastures. After the first 48 hours, cattle in both treatments had access to pastures of approximately the same size.

Sample collection

Study 1—A subset of cattle was randomly selected ($n = 18$; $n = 9$ per treatment) to wear an additional, custom-built GPS collar (Knight collars[9,10]) and a pedometer[11] (IceQube, IceRobotics Ltd.; Edinburgh, Scotland). The pedometers were placed on the rear right leg of selected cattle on day 0. Data from the Knight collars are not presented here, but we mention the presence of the collars because wearing the collar may be a source of stress.

Feces were collected from all cattle via rectal palpation on day 0 and day 27 to measure corticosterone concentration.[12,13] Fecal samples were stored in airtight sample bags at −20°C (−4°F) until corticosterone analysis was completed. Additionally, once per week, fecal samples from cattle in the subset group were collected from the pasture while the herd was grazing. Fecal samples were obtained by observing cattle then collecting a sample from a fresh feces pile produced by each animal. Also at this time, a weekly fecal composite was created for each of the two treatments by combining samples of 20 fresh feces from each pasture.

To measure cortisol levels, hair was shaved from the tip of the tail switch with clippers equipped with a surgical blade; hair was shaved as close to the skin as possible.[12,14] Hair was shaved on day 0 to remove existing hair and was not collected; on day 28, hair grown over the study period was shaved and collected for analysis. Hair was stored in airtight sample bags at −20°C (−4°F) until cortisol analysis was completed.

Study 2—A subset of the 59 cattle ($n = 16$; $n = 4$ per pasture) were randomly selected and fitted with a pedometer as described previously. Feces were collected from all 59 cattle via rectal palpation on day 0 and day 55 to measure corticosterone levels. Fecal samples were stored as described previously. Additionally, once per week, fecal samples from the subset of cattle wearing pedometers were collected from the pasture while the herd was naturally grazing. This fecal collection was conducted in the same fashion as described previously. Also at this time, a weekly fecal composite from each of the four pastures was taken from samples of 20 fresh feces. Hair was shaved from each animal while in the chute on day 0, and day 55 to determine cortisol concentration. Hair was shaved and stored as described previously.

Blood was collected from each of the 59 cattle via coccygeal venipuncture on day 0 and day 55 (Red top BD Vacutainer; Franklin Lakes, NJ). Samples were placed on ice after collection and transported to the laboratory in Stillwater, Oklahoma. A wooden stir stick was used to release the blood clot before centrifuging samples. Blood tubes were centrifuged at 3,000 × g for 25 minutes at 4°C (39°F; Sorvall RC6; Thermo Scientific, Waltham, MA). All day-0 samples required recentrifuging at 5,000 × g. Serum was collected and stored at −20°C (−4°F) until lactate and NEFA analysis.

Laboratory analysis

Serum samples were thawed at room temperature immediately before lactate and NEFA analysis. Serum L-lactate was analyzed using an immobilized enzyme system (YSI Model 2950 D; YSI Inc., Yellow Springs, OH). NEFA concentrations were quantified using a commercial kit (HR Series NEFA HR2; Wako Pure Chemical Industries, Osaka, Japan) following manufacturer instructions. Hair cortisol analysis was performed using methods described by Koren et al.[15] modified by Moya et al.[12]; during which, samples were saturated with methanol, incubated, and the supernatant was evaporated to dryness. Cortisol was then isolated with the use of a commercial RIA kit (MP Biomedicals; Irvine, CA). Fecal corticosterone analysis was performed using the method described by Foote.[13] Fecal glucocorticoid metabolites were extracted and analyzed for corticosterone concentrations in duplicate using a commercial RIA kit. Intra- and interassay coefficients of variability (CV) were 3.16% and 5.13%, respectively.

Statistical analysis

All data were analyzed in R.[16] Descriptive statistics were summarized for Study 1. Study 2 was analyzed as a completely randomized design using analysis of variance (ANOVA) with pasture ($n = 4$) as the experimental unit. Dependent variables were physiological variables and behavior measures provided from pedometers (mean step count, standing time, lying bouts, and motion index). Weekly cortisol data and daily behavior measures were analyzed with a repeated-measures ANOVA model with independent variables of fence type, day, and the interaction. Electric stimulus (0.5-second stimulus) count from VF cattle in both studies were summarized and counts from cattle with complete data from pedometers were correlated (Pearson method) with behavioral and physiological data. Effect size was calculated as Cohen's d using a pooled standard deviation.

Results and Discussion

Electric stimuli

In general, cattle received fewer electric stimuli over time (Fig. 1). In all days, <100% of cattle received an electric stimulus (Fig. 2). Data on the frequency of auditory stimuli received by the cattle were not available. A weakness of our study is that we do not have data on the interactions of cows in EF pastures with the physical electrical fencing, but we assume from experience and previous research[17] that these interactions would be less frequent than stimuli delivered by VF collars. We can-

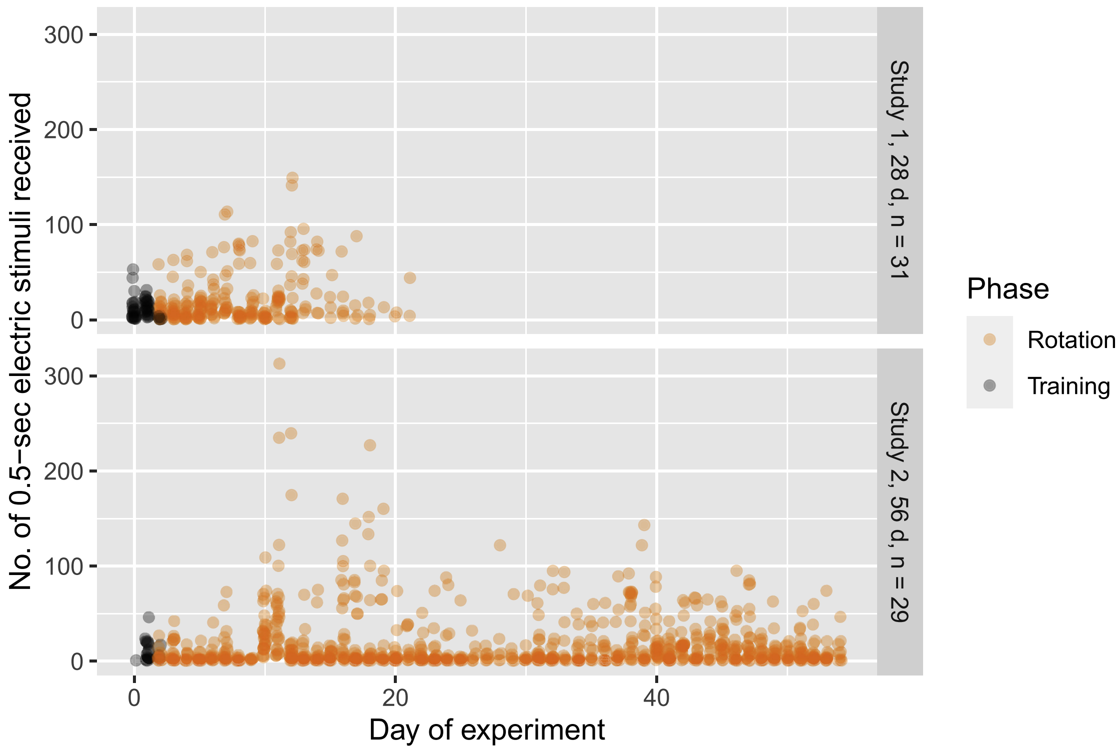

Figure 1. Distribution of 0.5-sec electric stimuli received over time by virtually fenced beef cattle at Bluestem Research Range at Oklahoma State University, 14.5 km (9 miles) SW of Stillwater, Oklahoma, USA, during August 2020 and October 2020. A 48-hour training phase was part of each study prior to the cattle being rotated weekly by the virtual fence. Dots are partially transparent to illustrate the overlap of datapoints.

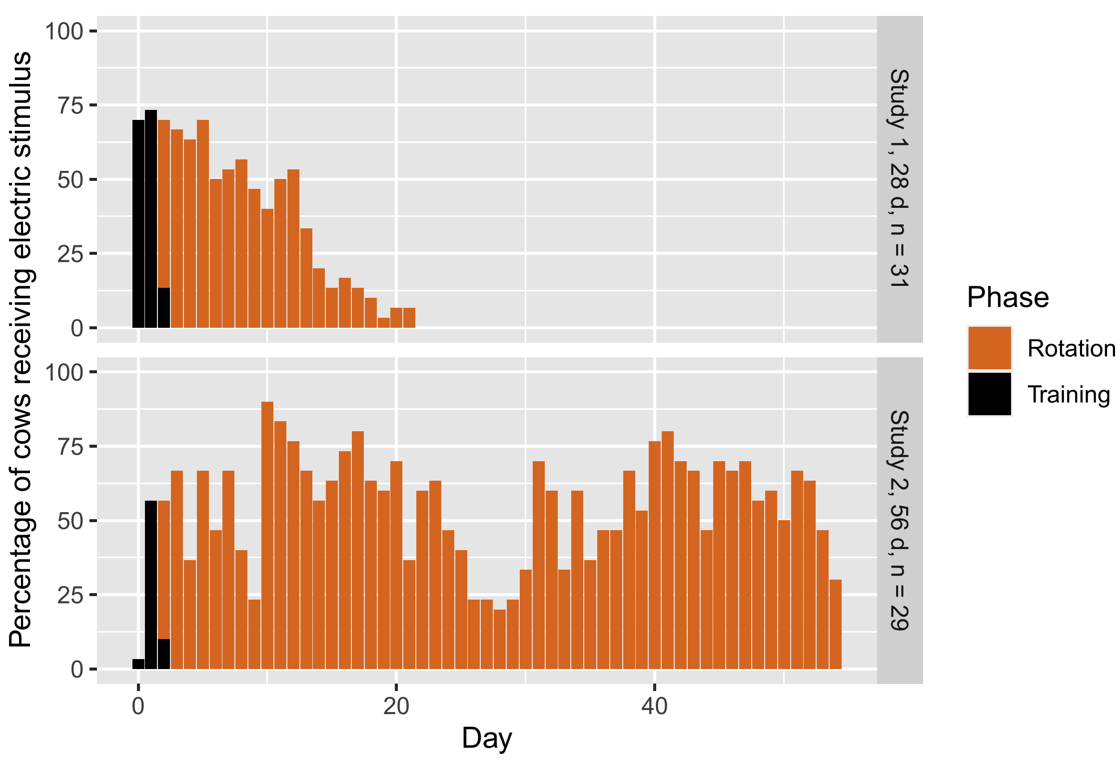

Figure 2. Percentage of beef cattle receiving at least one electric stimuli per day by a virtual fence (VF) collar at Bluestem Research Range at Oklahoma State University, 14.5 km (9 miles) SW of Stillwater, Oklahoma, USA, during August 2020 and October 2020. A 48-hour training phase was part of each study prior to the cattle being rotated weekly by the VF. Cattle in Study 1 ($n = 31$) had no prior experience with VF, whereas cattle in Study 2 ($n = 29$) had a 2-week exposure to VF prior to the study.

Table 1
Summary of 0.5-second electric stimuli received by beef cattle, per individual, by virtually fenced (VF) cattle at Bluestem Research Range at Oklahoma State University, 14.5 km (9 miles) SW of Stillwater, Oklahoma, USA, during August (Study 1) and October (Study 2) 2020.

	Study 1: 28 days	Study 2: 56 days
Max.	506	2306
Min.	0	0
Mean	174	506
SD	155	571

Max, maximum; Min. minimum; SD, standard deviation.
Cattle in Study 1 ($n = 31$) had no prior experience with virtual fence, whereas cattle in Study 2 ($n = 29$) had a 2-week exposure to virtual fence prior to the study.

not determine if stress responses are due to frequency of interactions with either VF or EF. We can only address the type of fence used.

Behavior variables

Study 1 was a pilot study, and only descriptive statistics were reported (Tables 1 and 2). Values in each treatment were comparable to published values (see Cortisol and Corticosterone and Serum metabolites sections below). In Study 2, daily step count was affected by an interaction between fence type and the quadratic effect of day ($P < 0.001$; Fig. 3). Step count generally decreased in the VF pastures over the study period. Standing time in Study 2 was not different between treatments ($P = 0.59$; Fig. 3). However, standing time for both treatments decreased over the study period ($P = 0.005$). Interestingly, the spike in standing time on day 11 was due to an ice storm on October 26, 2020[18] because cattle were unable to graze the standing forage and were fed hay. This observation gave us confidence that the pedometer data was measuring behavior changes in cattle due to their environment, and any effect of VF on standing time was much less than the effect of this storm. Lying bouts did not differ among treatments ($P = 0.68$; Fig. 3). Like step count, motion index was affected by an interaction between fence type and the quadratic effect of day ($P = 0.002$; Fig 3). Motion index is defined as a measure of an animal's activity relative to acceleration and energy expenditure.[19] Motion index is affected by the duration of activity and the extent of leg movement, making the motion index indicative of overall activity.[20] Our results indicate VF cattle exhibited more motion early in the trial, even though standing and lying times did not differ due to fence type.

The behavioral activity of cattle is often indicative of animal comfort and well-being.[21] Grazing cattle spend most of their time either resting, ruminating, or grazing (normally 90.95% of daily activity).[22] Behavioral activities of the cattle monitored in Studies 1 and 2 were not indicative of cattle stressed or experiencing discomfort based on previous research.[22–24] Large increases or decreases in animal motion are direct stress responses. Grandin[23] reported cattle are more active when excited or stressed, and others have reported cattle placed in stressful situations spend less time ruminating and lying and more time standing.[24,25] Cattle in Studies 1 and 2 did not exhibit large deviations from normal activities of grazing cattle because no large increases in standing time, step count, and motion index were observed (Fig. 3). Cattle in our studies also exhibited no decrease in number of lying bouts. Our results indicate VF was not more stressful than EF.

Cortisol and corticosterone

Hair cortisol concentrations at the end of Study 1 were 0.40 ± 0.32 pg/mg for EF and 0.37 ± 0.15 pg/mg for VF (Table 2). Hair cortisol concentrations in Study 2 did not differ by fence type on day 0 ($P = 0.16$, $d = 0.6$) nor day 56 ($P = 0.34$; $d = -0.3$; Table 3). Hair cortisol concentrations decreased over the study period (i.e., 8 weeks), but no differences were found in the magnitude of change from day 0 and day 56 due to fence type ($P = 0.14$, d = 0.6). Effect size was moderate in these cases, but in some cases, VF was greater than EF, and in other cases the opposite was true (i.e., negative *d*). The numerical decrease in cortisol concentrations over the study period could be attributed to residual cortisol concentrations reported in the day 0 samples, as previous management unrelated to our study may have resulted in cortisol deposition in the day 0 samples.

Hair cortisol concentrations from both Studies 1 and 2 were within reported reference ranges, (0.76 to 28.95 pg/mg in multiparous cows).[26] Other researchers have reported hair cortisol concentration ranges as low as 0.30 to 5.31 pg/mg in Angus cross bulls.[12] The wide range in concentrations may be due to breed, sex, physiological state of study cattle, and lab-to-lab analysis variation.[12,27,28] Hair cortisol concentrations reported for Studies 1 and 2 are indicative of unstressed cattle (Tables 2 and 3).

Fecal corticosterone concentrations reported for Study 1 were 140 ± 79.6 ng/g for VF and 128 ± 56.7 ng/g for EF at final collection (day 28). Overall, corticosterone concentrations increased on day 28 when compared to day 0 (Table 2). These results were evaluated with greater robustness in Study 2. Fecal corticosterone concentrations reported in Study 2 did not differ by fence type on day 0 ($P = 0.46$, $d = -0.2$) nor day 56 ($P = 0.51$ $d = -0.2$; Table 3). Again, fecal corticosterone concentrations increased over the study period, though the difference between concentrations from day 0 and day 56 were not significant ($P = 0.66$; $d = 0.1$ Table 3). No difference by fence type was observed for weekly fecal corticosterone concentrations ($P = 0.79$; Fig. 4) nor for weekly corticosterone composite concentrations ($P = 0.16$).

Fecal corticosterone concentrations from Studies 1 and 2 were within concentration ranges reported for cattle.[29] Fecal corticosterone concentrations in Study 1, with the exception of day 28, were similar to basal fecal cortisol metabolite concentrations (13.5 to 97.7 ng/g) in previous research where lactating cows were subjected to transportation, loading and unloading, or no handling.[29] Concentrations from day 28 were greater than basal cortisol metabolite levels[29] but were on the lower end of the range reported for peak

Table 2

Summary of behavior and physiology observations of beef cattle in Study 1 at Bluestem Research Range at Oklahoma State University, 14.5 km (9 miles) SW of Stillwater, Oklahoma, USA, during August 2020.*

	Virtually fenced						Electrically fenced						Cohen's d‡
	n†	Mean	SD†	CV†	Min.	Max.	n*	Mean	SD	CV	Min.	Max.	
Steps per day	7	4,656	1,345	0.29	1,911	11,522	7	4,440	985	0.22	2,085	8,334	0.2
Lying bouts per day	7	13.5	3.75	0.27	6.0	31	7	11.3	2.77	0.25	5.0	20	0.7
Standing time, min. per day	7	740	83.5	0.11	533	1,084	7	744	79.4	0.11	516	1,063	–0.1
Motion index§	7	19,848	6,129	0.31	7,613	52,722	7	18,494	4,867	0.26	7,287	41,155	0.3
Hair cortisol, pg/mg													
d 28	13	0.37	0.15	0.39	0.13	0.59	9	0.40	0.32	0.80	0.01	0.86	–0.1
Fecal corticosterone, ng/g													
day 0	11	77.9	46.8	0.60	11.5	158	8	73.8	34.1	0.46	14.4	113	–0.1
day 7	11	96.5	65.6	0.67	22.8	177	8	67.4	37.8	0.56	31.1	131	0.6
day 14	11	59.5	33.4	0.56	23.9	96.9	8	39.6	32	0.81	13.3	92.1	0.6
day 21	11	86.0	33.5	0.39	46.4	133	8	75.2	28.6	0.38	28.3	99.5	0.4
day 28	11	140.0	79.6	0.57	50.5	296	8	128	56.7	0.44	21.9	180	0.2

CV, coefficient of variation; Max., maximum; Min., minimum; SD, standard deviation.

*Cattle were rotated in either virtually or electrically fenced pastures weekly for 4 weeks.

†Number of cattle for which data was available.

‡Effect size calculated using pooled SD.

§Motion index per day; activity relative to acceleration and energy expenditure reported by the pedometer algorithm (IceQube, IceRobotics Ltd.; Edinburgh, Scotland).

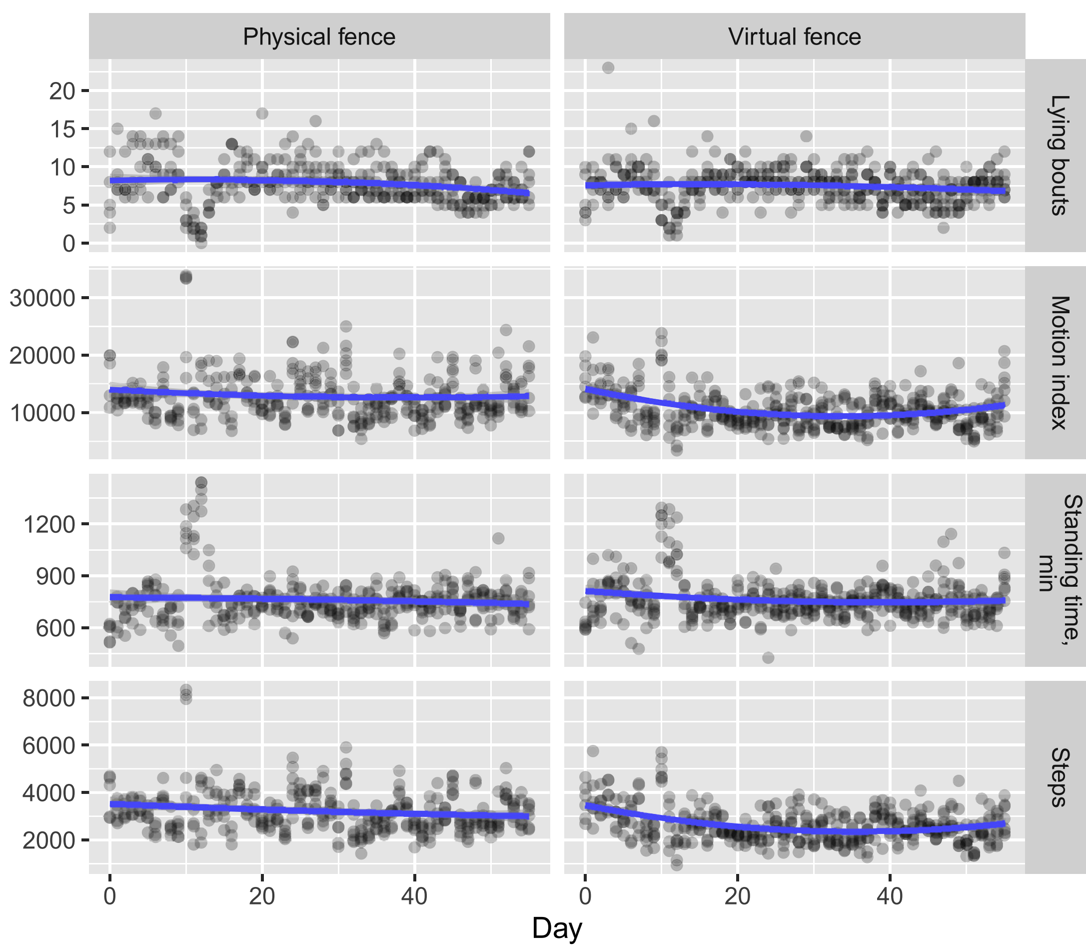

Figure 3. Effects of virtual fencing (VF) on daily behavior of beef cattle in Study 2, with a LOESS regression model (blue line). Treatments were 2 VF pastures or 2 physical electrically fenced (EF) pastures ($n = 8$ cattle per treatment). The fence type by quadratic effect of day interaction was significant for steps and motion index ($P < 0.002$), indicating cattle that were VF were more active at the initiation of the study. Study 2 took place at Bluestem Research Range at Oklahoma State University, 14.5 km (9 miles) SW of Stillwater, Oklahoma, USA, during October 2020. Motion index is activity relative to acceleration and energy expenditure reported by the pedometer algorithm (IceQube, IceRobotics Ltd.; Edinburgh, Scotland).

Table 3
Effects of virtual fencing on cortisol metabolite concentrations of beef cattle in Study 2 at Bluestem Research Range at Oklahoma State University, 14.5 km (9 miles) SW of Stillwater, Oklahoma, USA, during October 2020.

	Virtually fenced	Electrically fenced	SE	*P* value	Cohen's *d**
n, pastures (cattle)	2 (29)	2 (30)	—	—	—
Hair cortisol, pg/mg					
day 0	0.52	0.29	0.072	0.16	0.6
day 56	0.09	0.17	0.046	0.34	–0.3
delta, day 56–day 0	0.43	0.13	0.09	0.14	0.6
Fecal corticosterone, ng/g					
day 0	168	224	43.7	0.46	–0.2
day 56	223	265	37.5	0.51	–0.2
delta, day 56–day 0	–56	–4	74.0	0.66	0.1
Lactate, mg/dL					
day 0	36.1	37.6	4.9	0.85	–0.1
day 56	42.3	40.9	7.8	0.91	0.1
delta, day 56–day 0	–6.2	–4.2	7.6	0.86	0.1
NEFA, mEq/L					
day 0	452	346	16.8	0.04	1.2
day 56	367	428	84.2	0.65	–0.1
delta, day 56–day 0	85	–72	91.7	0.35	0.3

NEFA, nonesterified fatty acid concentration; SE, standard error.
*Effect size calculated using calculated SD as SE * $n^{0.5}$

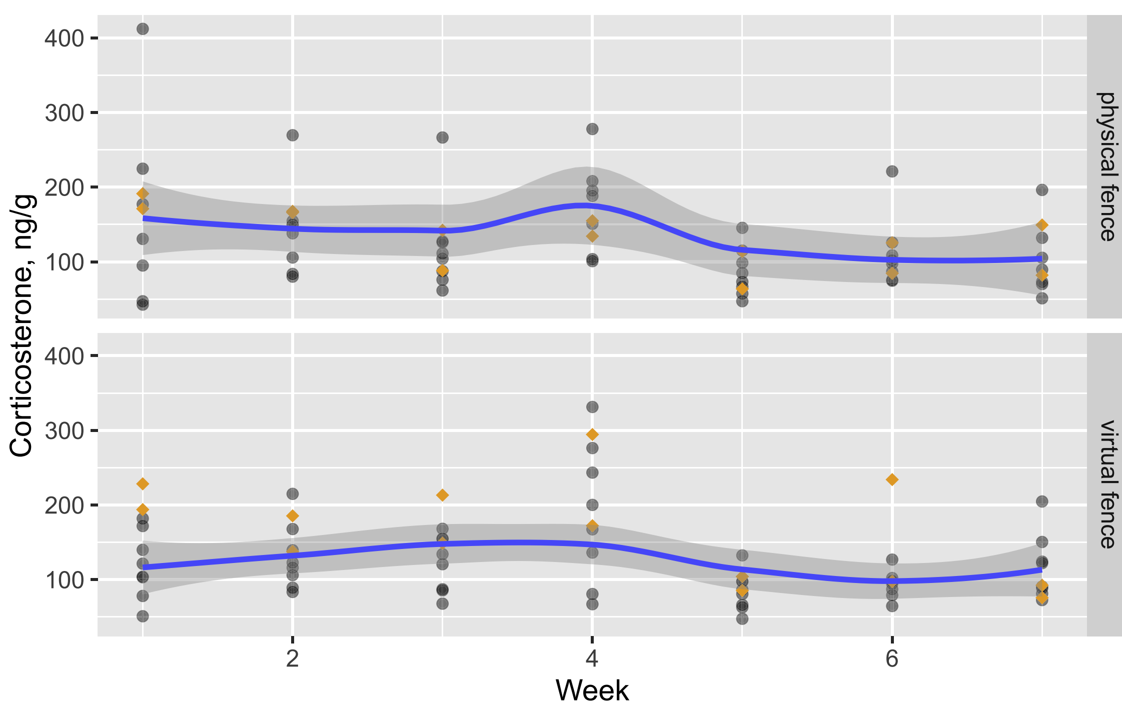

Figure 4. Effects of virtual fencing (VF) compared to physical electric fencing (EF) on weekly fecal corticosterone concentrations of individual beef cattle (grey points, 8 cattle in each fence type) in Study 2, with a LOESS regression model (blue line) with standard error bands (grey shading). Fence type was not significant $P = 0.79$. Orange diamonds are composite samples of 20 fresh fecal pats in each pasture; fence type effect $P = 0.16$. Study 2 took place at Bluestem Research Range at Oklahoma State University, 14.5 km (9 miles) SW of Stillwater, Oklahoma, USA, during October 2020.

concentrations, 75.2 to 797.3 ng/g. Corticosterone concentrations from Study 2 were above the basal (11.8 to 154.2 ng/g) or below the peak (258.1 to 677.1 ng/g) fecal cortisol metabolite concentrations,[29] where cattle were challenged with adrenocorticotropic hormone. Although day-56 corticosterone concentrations were increased compared to day 0 in Study 2 (Table 3), the effect of fence type was not statistically significant ($P \geq 0.16$, $d < 0.2$) in day 0, day 56, weekly samples, or weekly composites, which suggests VF was not more stressful than EF.

Serum metabolites

Serum lactate concentrations in Study 2 did not differ by fence type on day 0 ($P = 0.85$ $d < -0.1$) nor day 56 ($P = 0.91$ $d < 0.1$; Table 3). Lactate concentrations in beef cattle have been reported to range from 9 to 115 mg/dL (0.01 to 0.14 ounces/gallon).[30,31] Mitchell et al.[32] reported elevated serum lactate concentrations in cattle post-transportation of 42 mg/dL (0.05 ounces/gallon), which are similar to day 56 concentrations from Study 2. Although day-56 lactate concentrations were elevated from normal compared to previous research,[29] the change in concentration between day 0 and day 56 was not statistically significant by fence type ($P = 0.86$, $d < 0.1$).

Lactate is produced in the muscle through anaerobic glycolysis as a result of the conversion of pyruvate to lactic acid via lactate dehydrogenase.[31] Increases in lactate concentrations have been reported in cattle undergoing a stress event[33,34] and are strongly related to the intensity of a stressor.[35] Therefore, lactate data reflect cattle in Study 2 were undergoing some stress response; however, lactate concentrations were only slightly elevated in comparison to previous research,[34] and fence type did not affect the magnitude of change.

NEFA concentrations were different by fence types on day 0 ($P = 0.04$, $d = 1.2$) but not on day 56 ($P = 0.65$; $d = -0.1$; Table 3). Although elevated NEFA on day 0 could logically be caused by the weaning event shortly before day 0,[36] cattle were randomly assigned to treatment on day 0, within physiological stage; therefore, we cannot explain why there was a difference in NEFA concentrations between treatments. We suspect this is a spurious difference. Fence type did not affect the change in NEFA concentrations between day 0 and day 56 ($P = 0.35$, $d < 0.1$).

NEFA are released from adipose tissue in response to the presence of corticosteroids and catecholamines. Increased NEFA concentrations have been reported to induce inflammatory responses in cattle.[37] NEFA concentrations in Study 2 were slightly increased compared to the published range of normal NEFA concentrations, which was reported to be no greater than 200 mEq/L.[38] However, they did not approach the 700 mEq/L level associated with increased disease susceptibility.[39] The increase in NEFA concentrations reported in our study may not be a stress response but a physiological state of the cattle used in Study 2. It is presumed that as cattle progress through gestation, adipose tissues mobilize, and thereby increase serum NEFA concentrations.[40] Importantly for the objective of our study, fence type did not affect the change in NEFA concentration over the course of our study ($P = 0.35$).

Correlations

Previous research indicates movement of cattle is associated with a stress response.[24] Cattle deemed to be excited or stressed have a higher basal concentration of catecholamines and cortisol.[41] Cattle deemed “flighty” or “excitable” are more active, and cattle expressing increased cortisol concentration spend less time ruminating and more time standing.[23,24,42] To our knowledge, there are few direct comparisons of cortisol metabolites and the behavior we documented in our study. However, if the cattle in our study were experiencing chronic stress from VF, we expected to find positive associations between some of the behaviors, cortisol metabolites, and blood metabolites.

Most of the behavior variables measured in our studies were not correlated with the cortisol metabolites in either study (Fig. 5). We did, however, observe in Study 1 that day-0 fecal corticosterone concentration and standing time were correlated ($R = 0.97$), while hair cortisol concentration was correlated with step count ($R = 0.70$) and standing time ($R = 0.99$). However, in Study 2, there were no positive correlations between the behavior variables associated with a stress response (step count and motion index; $P > 0.49$) and the cortisol metabolites measured on day 0 and day 56.

Cattle behavior has also been associated in the literature with the blood metabolites measured in Study 2. Lactate is associated with the stress response in cattle because stressors increase the rate of anaerobic glycolysis resulting in an increase in lactate production in the muscle.[7,31] However, lactate is most often correlated with motion.[34,43,44] In a feedlot setting, both flight speed (the speed at which cattle exit confinement) and animal movement around a test arena were correlated with cortisol, NEFA, and lactate concentrations.[34]

We expected lactate concentrations and standing time would be negatively correlated. However, in Study 2, the coefficient was nonsignificant ($R = 0.41$, $P = 0.24$). Additionally, no other behavior variables measured in Study 2 produced the expected positive correlation with the physiological variable measured. Like standing time, lying bouts tended towards being correlated to day 56 NEFA concentrations ($R = 0.54$, $P = 0.10$), which could be related to increased standing time caused by the ice storm that occurred during Study 2. However, NEFA concentrations were not elevated during this time. Cattle may have relied on circulating NEFA for energy[45] when the cattle were experiencing cold stress.

Moya et al.[12] reported correlations between hair cortisol and glucocorticoid concentrations, but these correlations were neither strong nor consistent in our studies. Similarly, Tallo-Parra et al.[14] reported a significant correlation between fecal cortisol metabolites and white hair collected from dairy cattle but no correlation with black hair collected from the same cattle. We think additional validation research needs to be done before these variables can be relied on to understand stress in cattle.

Correlations between hair cortisol and fecal corticosterone concentration in previous research[12,14] do not follow a consistent pattern. Similarly, in Study 1, hair cortisol concentrations were not correlated with fecal corticosterone concentrations. Further, fecal corticosterone concentration measured across weeks for Study 1 did not display a consistent pattern when measured in individuals versus the composite sample. In Study 2, no correlations were observed between day-0 hair cortisol concentrations and any other cortisol metabolite collection dates. However, hair cortisol concentrations on day 56 were correlated with fecal corticosterone concentrations from only week 2 ($R = 0.63$, $P = 0.02$). Like Study 1, fecal corticosterone concentration measured across weeks in Study 2 were inconsistent when comparing individuals to the composite sample.

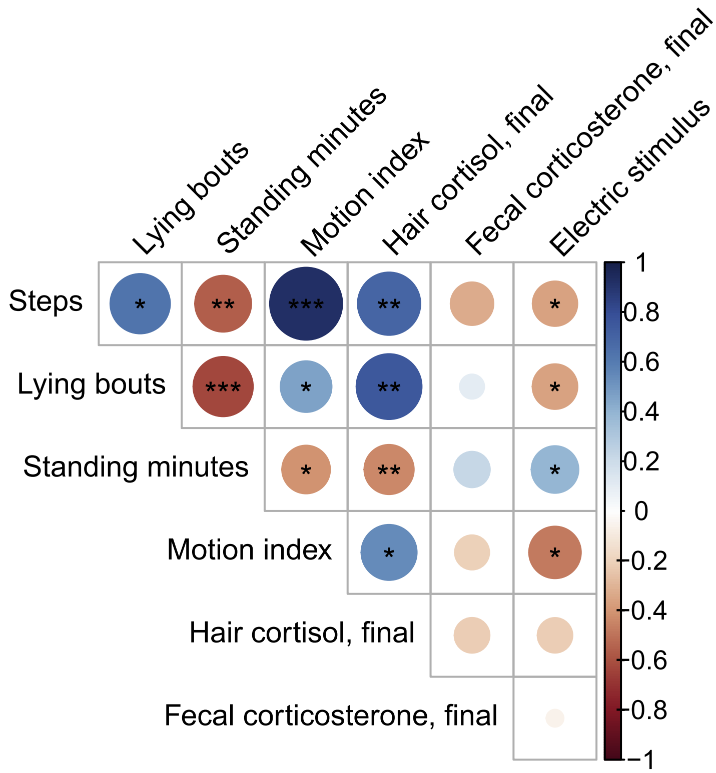

Figure 5. Correlations among beef cattle behavior during either a 28- or 56-day trial (i.e., lying bouts, standing minutes, and motion index), cortisol concentration in hair or feces at the end of the trial, and total electric stimuli received during the trial in 14 virtually fenced (VF) cattle. Size and color of bubble indicates size and sign of the Pearson correlation coefficient. Asterisks indicate significance of correlation coefficients (*** P <0.001 ** P < 0.1 * P < 0.05). Electric stimuli count was not correlated with stress hormones and negatively correlated with the motion index. Motion index; activity relative to acceleration and energy expenditure reported by the pedometer algorithm (IceQube, IceRobotics Ltd.; Edinburgh, Scotland). Research took place at Bluestem Research Range at Oklahoma State University, 14.5 km (9 miles) SW of Stillwater, Oklahoma, USA, during August 2020 and October 2020.

Correlations between blood metabolite and cortisol metabolite concentrations were inconsistent in Study 2. No correlation was found between either measure of hair cortisol or NEFA concentrations. However, Burdick Sanchez[45] showed positive correlations between blood cortisol and NEFA concentrations. Final lactate concentrations were negatively correlated with both measures of fecal corticosterone concentration, similar to Chen et al.[46] in which lactate in rat cells was inhibited by exposure to corticosterone. Also contrary to Gross et al.[47] we found a negative, though nonsignificant, correlation between fecal corticosterone and NEFA concentrations in Study 2.

Cattle experiencing electric stimulation are likely to exhibit more movement and move away from the stimulus. Markus et al.[4] reported cattle subjected to electric stimulation displayed behaviors such as head shaking and a change in movement speed, direction, or body position. Grazing cattle not experiencing a stress response spend most of their time grazing or ruminating, comprising up to 90–95% of daily activity.[22] We expected cattle experiencing an electric stimulus from the VF would display movement and behaviors different than those of cattle not experiencing a stimulus, and their behaviors would be correlated with the number of electric stimuli received.

In Study 1, the total electric stimulus count was positively correlated with standing time ($R = 0.83$, $P < 0.05$), but there was no correlation with any other behavior measured. As expected, stimulus count in Study 1 was also positively correlated with hair cortisol concentration on day 28 ($R = 0.79$, $P < 0.05$). In Study 2, stimulus count was positively correlated with step count and motion index (expected), and with standing time (not expected). Given the results from Study 1, we expected stimulus count would be positively correlated with the cortisol metabolites measured in Study 2. However, only negative correlations were observed between stimulus count and final concentrations (day 56) of hair cortisol, fecal corticosterone, NEFA, and lactate. These negative correlations indicated that the stimulus received by cattle in the VF treatment in Study 2 did not result in a measurable increase in stress.

Applications

Taken together, our results indicate behavior and physiological metabolites were not affected significantly by fence type, and the correlations that would be expected if VF caused stress were either absent or inconsistent. Therefore, using a VF system to contain and rotate cattle was not more stressful to the livestock than EF, which is the industry standard. Because no significant increase in stress was evident, further research, development, and use of VF is warranted.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. The authors except T.P. certify that they have no financial interest in the subject matter discussed in the manuscript. T.P. is an employee of Vence Corp. and was associated with management decisions regarding the topic of this manuscript.

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