Evaluation of blood markers of stress in beef cows during exposure to virtual fence stimuli

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On the Ground

- Containing cattle with a virtual fence (VF) has gained considerable attention. VF technology uses auditory and electric stimuli to contain or exclude cattle from predetermined areas, which has raised concerns over cattle welfare.
- We evaluated blood markers associated with stress and inflammatory response when naive cattle were fitted with VF collars.
- We detected no major changes in blood markers. Cattle were able to quickly identify and adapt to VF boundaries and over time reduce the number of stimuli.
- Our results indicate VF technology can contain cattle within a pre-established boundary and does not negatively impact cattle welfare.

Keywords: behavior, cattle, cortisol, ceruloplasmin, haptoglobin, shock.

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Introduction

In the western United States, cattle often graze for long periods of the year on large parcels of public and private rangelands. In these locations, subdividing large pastures into smaller ones is a desired practice, when practical, to intensify grazing management and achieve more uniform animal distribution,[1] therefore assisting in fine fuels management,[2] controlling invasive plant species population,[3] and helping to maintain the structure of wildlife habitat.[4] More intensive livestock systems, such as rotational stocking, rely on fences for pasture and paddock subdivision, aiming to achieve uniform forage utilization and consistent animal performance and nutrient distribution.[5] The containment of cattle in these grazing systems commonly uses traditional fencing (e.g., barbed wire or electric fences), which is costly and labor-intensive to build and maintain.[1,6]

The use of virtual fence (VF), an emerging technology within the growing area of precision livestock management, may offer a less expensive and logistically challenging alternative to traditional fencing. VF can provide flexibility to land managers to allocate desirable areas for grazing or exclusion from livestock, leading to improved natural resources use and livestock management. VF can be defined as a structure serving as an enclosure, or boundary, without a physical barrier, which relies on animal behavioral modification resulting from the animal receiving sensory cues triggered when the animal enters the boundary/containment zone.[7] Typically, an animal in a VF system is fitted with a global positioning system (GPS) device that identifies the VF boundaries and triggers an audible stimulus (AS) followed by an electric stimulus (ES) when the animal crosses pre-established boundaries. The rationale for these sensory cues is based on the premise of conditioning animal behavior using associative learning by combining neutral stimuli (i.e., AS) with aversive stimuli (i.e., ES).[8] Different types of VF have been tested over the years,[8] including systems with collars and an induction cable laid on, or buried in, the ground.[9] Nonautomated collars[10] have cues manually triggered by observers, and automated collars[11-15] have cues automatically triggered by the collars according to proximity sensors or GPS coordinates.

Previous studies demonstrated that VF is highly effective at keeping cattle in designated locations[9,13] or preventing cattle from crossing a boundary,[14-16] following appropriate training to sensory cues. Furthermore, our group has demonstrated the effects on cattle behavior when fitted with a VF collar for the first time are minimal and transient. Animals engage in nondesirable behaviors for a short period of time, but with no readily apparent long-lasting effects or negative associations with the technology.[15] Although the effects on animal behavior seem to be minimal and temporary, the use of sensory cues, especially electric stimuli, could negatively affect cattle physiology, which could impact subsequent cattle performance.[17,18] Stressors elicit coordinated physiologic responses within the body in an attempt to reestablish homeostasis, an energy-costly process for the animal.[19] From a welfare perspective, the effects of this technology should be evaluated through metabolic markers[20] to examine its' effects on animal physiology and particularly on mechanisms related to stress, as such responses can divert energy from other important bodily functions. Collectively, the evaluation of cattle behavior and physiological markers when using VF will provide livestock managers with data needed to make informed decisions related to adopting and using VF technology, ensuring enhanced productivity and sustainability, as well as the welfare and safety of cattle and handlers.

Therefore, our objective was to evaluate the effects on blood markers associated with stress and inflammatory responses using naive cattle fitted with automated VF collars where sensory cues were automatically triggered by cattle location based on GPS coordinates. We hypothesized that the use of VF would not negatively impact these markers, as it was expected cattle would rely primarily on the neutral stimuli (i.e., AS) provided by the VF. To the best of our knowledge, this is the first study to evaluate such parameters.

Materials and methods

Our study was conducted at the Northern Great Basin Experimental Range (NGBER, Riley, OR; 43.4°N, 119.7°W) at an altitude of 1288 m (4,226 feet) during the summer of 2022. All animal handling and care were approved by the Institutional Animal Care and Use Committee of Oregon State University (#2022-0272).

Animal selection and VF collar fitting

Forty mature Angus × Hereford cows (body weight: 595 ± 10.3 kg [1311 lbs ± 22.7]) with or without their calves (n = 21 and 19, respectively for lactating and dry cows) were randomly selected from the Eastern Oregon Agricultural Research Center (EOARC, Burns, OR) herd to be enrolled in a larger study evaluating the use of VF as a land management tool. Our study focused on the training phase (5 days) of the larger study, evaluating the effects of VF on blood markers associated with stress and inflammatory response. The training phase is required for the habituation of cattle to the technology and designated VF boundaries.

Cows had no previous experience with VF and were familiar with grazing in sagebrush (*Artemisia tridentata*) rangelands pastures with permanent fences during spring, summer, and early fall, with winter-feeding of hay meadow. Cows were cohorts within the same herd and were familiar with each other and the working and holding facilities where the study was conducted. Our study design aimed to evaluate the initial physiological response of cattle when first fitted with VF collars. For the duration of the study, each cow was fitted with a unique VF collar (Vence; Merck & Co., Inc., Rahway, NJ). The VF collar is comprised of three main components: 1) a hardware box containing all GPS, ES, and AS components hanging from the neck of the animal; 2) two metal chains used to hold the hardware box, which can be adjusted at the top of a cow's neck, thereby respecting the individual neck diameter with the chain on the right side of the hardware box delivering the ES; and 3) a double plastic chain link used to join the two sides of the chains using a plastic zip tie. The link rests on top of the cow's neck.

VF boundary and management zones

Cows were fitted with VF collars on day 0 and moved to a paddock with an established VF boundary. The VF boundary was created using Herd Manager (Vence; Merck & Co., Inc., Rahway, NJ), a cloud mapping software that allows the user to design the boundaries of the VF and its corresponding management zones using GPS coordinates. The system communication was accomplished via a solar or AC-powered base station, GPS, cellular connection, and the Herd Manager software. The base station used a radio signal to communicate user-designed boundaries to the VF collar worn by the cattle. The VF collars were powered by a lithium battery and capable of monitoring animal locations at user-defined intervals.[14]

The paddock where the VF boundary was established was square-shaped (approximately 130 m x 100 m [426 × 328 feet]) and had a traditional 5-strand barbed-wire fence perimeter. The VF boundary was created inside of this paddock and contained two management zones, which followed the length of the paddock (approximately 130 m [426 feet]). These zones were created from the traditional fence inward to the paddock: 1) the AS management zone, where AS was automatically triggered when the animal crossed the boundary; and 2) the ES management zone, where ES was automatically triggered. In each ES-triggered event, the cow received an electrical stimulus of 0.33 joules, which is less than a standard electric fence (0.5-1.0 joules) discharge. The management zones changed daily to achieve a final boundary of approximately 30 m (98 feet), where 5 m (16 feet) was for the AS management zone and 25 m (82 feet) was for the ES management zone. Specifically, on day 0, no management zones were activated (local acclimation), and cows could walk freely in the paddock without receiving any stimuli (Fig. 1A). On day 1, a 20-m ES management zone was activated (Fig. 1B), which increased to 30-m on day 2 (Fig. 1C). On day 3, a 5-m AS management zone was activated and included in the VF boundary, resulting in a 25-m ES management zone and a 5-m AS management zone from day 3 to 5 (Fig. 1D). The VF boundary established for the training phase had the objective of confining and maintaining cows within the designated area (i.e., inclusion VF), and the order of stimuli activation was proposed by the manufacturer.

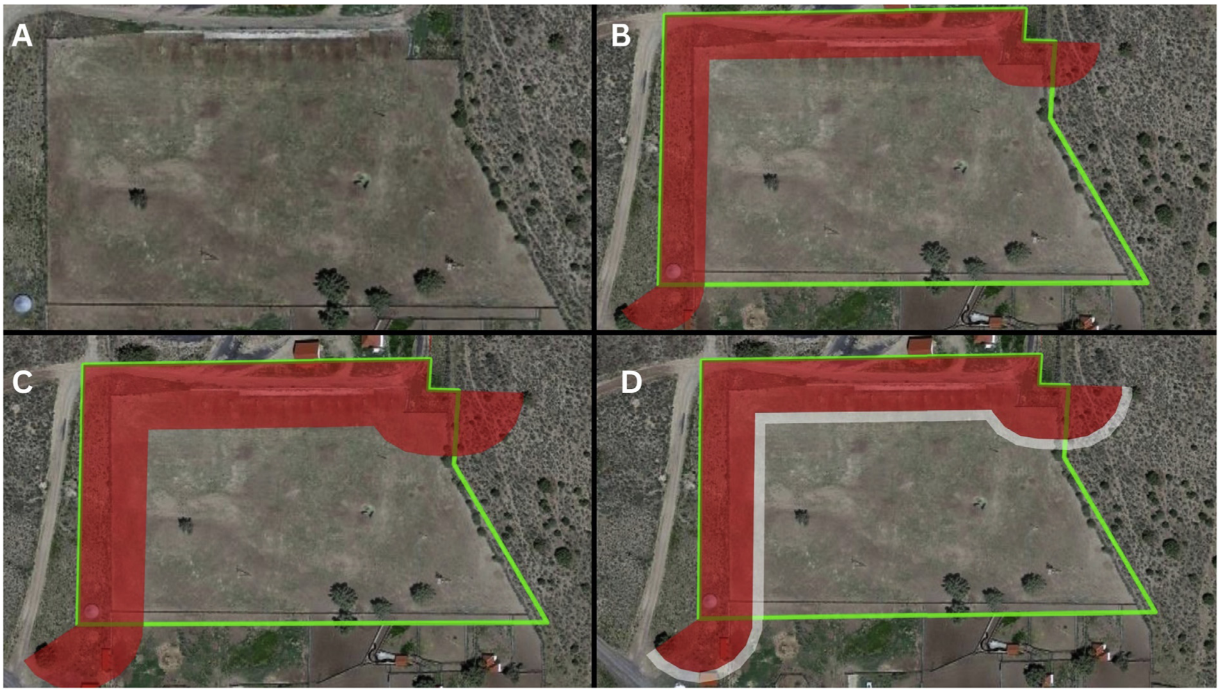

Figure 1. Schematic representation of virtual fence management zones over time at the Northern Great Basin Experimental Range, Riley, Oregon during the summer of 2022. Cows were managed in this area from day 0 to 5 of the study. A, The training area with no management zones activated. Cows could walk freely in the paddock without receiving any stimuli. B, A-20 m electric stimulus (ES) management zone activated. C, A 30-m ES activated management zone. D, A 25-m ES management zone and 5-m audible stimulus (AS) management zone.

AS and ES were automatically triggered when a cow moved into the respective management zones based on GPS coordinates. When both management zones were present, the sensory stimulus began with sound only, a 0.5-second tone followed by 1.5-second pause. This pattern repeated for 60 seconds, followed by a cool-down period (no stimuli) of 180 seconds. If a cow remained within the management zones, a combination of AS and ES were applied based on location or due to time spent into the management zones. The ES differed depending on the trigger for the stimulus (timing or location) and was 0.5 seconds in duration followed by either a 1.5 second or 2.5 second pause.

Each VF collar logged the time stamp, GPS coordinates, and AS and ES triggered by the collar. At the end of our study, the data were accessed from Vence and processed using a Python pipeline created for this type of data as previously used by our research group.[14-16]

Data collection

On day 0, cows were brought to the working facility, and a blood sample was collected before equipping the cows with VF collars. A second blood sample was collected on day 5. Blood samples were collected via jugular vein puncture using commercial heparinized vacuum tubes (BD Vacutainer, 10 mL; Becton, Dickinson and Company, Franklin Lakes, NJ). Blood samples were placed on ice immediately following collection, transported to the lab, and centrifuged at 2,500 × g for 30 minutes at 4°C (39°F) for plasma harvest. Plasma samples were frozen at −20° C (−4° F) on the same day of collection and stored at –80°C (–112°F).

On days 0 and 5, body weight (BW) was collected, and body condition score (BCS) was assessed. Additionally, chute score, chute exit velocity, and collar fit score (only day 0) were recorded for each cow. Body condition score was collected by 3 trained technicians using a 1 to 9 scale, where cows scored 1 were considered emaciated, while cows scored 9 were deemed overconditioned. Chute score was collected for each cow by 3 trained technicians. The score was assessed as cows entered the chute and after the cow's head had been caught. The chute score was given according to Arthington et al.[21] using a 1 to 5 scale, where cows were classified as: 1 = calm, no movement; 2 = restless shifting; 3 = constant shifting with occasional shaking of the chute; 4 = continuous movement and shaking of the chute; and 5 = violent and continuous struggling. Chute exit velocity was collected for each cow and was calculated by determining the speed of the cow exiting the squeeze chute by measuring the rate of travel over a 1.6 m (5.2 feet) distance with an infrared sensor (FarmTek Inc., North Wylie, TX). Collar fit score was noted for each cow by 3 trained technicians immediately after the cow left the chute following 30 seconds of observation. Collar fit score was previously used by our group to quantify the cows' immediate reaction to collaring.[15] Collar fit score was developed on a scale 1 to 5, based on cattle locomotion and head and neck positioning. Cows were classified as: 1 = unalarmed and unexcited, walking slowly; 2 = slightly alarmed and excited, moving moderately quick; 3 = moderately alarmed and excited, moving quickly; 4 = very alarmed and excited, moving quickly and shaking head; and 5 = extremely alarmed and excited, moving quickly, shaking the head, and jumping. It is important to note that collar fit score was given immediately after cattle exited the chute, and therefore no stimuli were applied at the time of scoring.

Laboratory analysis

Plasma samples were analyzed for cortisol, ceruloplasmin, and haptoglobin, due to their crucial role in stress and inflammatory response. These parameters are widely used as a means to evaluate animal health and welfare.[19,22,23] Plasma cortisol concentrations were measured in a single run using chemiluminescent enzyme immuno-assays (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). The intra-assay coefficient of variability was 2.75%. Plasma ceruloplasmin oxidase activity was measured in duplicate samples using colorimetric procedures described by Demetriou et al.[24] Ceruloplasmin concentrations are expressed as mg/dL as described by King.[25] The intra- and inter-assay coefficients of variability were 2.70% and 10.4%.

Plasma haptoglobin concentrations were determined in duplicate samples by a biochemical assay measuring haptoglobin-hemoglobin complex by the estimation of differences in peroxidase activity.[26] Results were obtained as arbitrary units resulting from the absorption reading at 450 nm (0.00002 inches; VersaMax Tunable EXT). The same quality control standards used in the biochemical assay were analyzed by quantitative determination of bovine haptoglobin in plasma (bovine haptoglobin ELISA test kit; Life Diagnostics, Inc., West Chester, PA). The concentrations of haptoglobin, based on the ELISA assay, ranged from 0.03 (0.004 oz/gal; low control) to 0.95 mg/ml (0.13 oz/gal; high control) with an intra-assay CV of 1.26%. The ELISA standard curve was used to convert the arbitrary units obtained from the biochemical procedures into mg/mL with the least detectable value of 0.03 mg/mL.[27] The intra- and inter-assay coefficients of variability were 2.15% and 8.10%, respectively.

Statistical analysis

All data collected by technicians (BCS, chute score, and collar fit score) were averaged among technicians for each cow. The number of AS and ES triggered was summarized for each cow and day.

Cow was considered the experimental unit. Cow performance and blood marker data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC; version 9.4). The model statement included the effects of day and the respective variable. Day was included in the repeated statement with cow as subject. Cow parity (lactating vs. dry cows) was included in the model and removed as no significance was detected ($P \geq 0.11$).

AS and ES were characterized as zero-inflated count data; thus, the UNIVARIATE procedure was used to evaluate data normality, which resulted in a non-normal distribution with the Shapiro-Wilk test (0.66, 0.25, AS and ES, respectively; $P < 0.01$). Therefore, AS and ES data were analyzed using the analysis of variance model using a negative binomial distribution (data mean and variance differed) in PROC GLIMMIX. Data are presented in the original scale for easier interpretation.

Data were separated using PDIFF when a significant F-test was detected. Results are reported as least squares means, except for AS and ES (raw average count data). Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $P \leq 0.10$.

Table 1
Cow performance and temperament variables evaluated at virtual fence collar fitting (day 0) and upon the end of the virtual fence training phase (day 5).

Item	Day 0*	Day 5*	SEM	*P* value
Body weight† (kg)	595	594	10.30	0.89
Body condition score‡	5.70	5.70	0.07	1.00
Chute score§	1.25	1.30	0.05	0.11
Exit velocity‖ (m/s)	1.20	0.94	0.05	0.001
Fit score¶	4.39	.	1.28	.

Note: These data were collected at the Northern Great Basin Experimental Range, Riley, Oregon during the summer of 2022. SEM indicates standard error of the mean. Significance was set at $P \leq 0.05$.

* Forty mature Angus × Hereford cows were collared with VF collars on day 0 and exposed to evolving VF boundaries until day 5.

† Cows were weighed using a commercial scale on both days.

‡ Body condition score was evaluated on both days by 3 trained technicians using a 1 to 9 scale, where cows scored as 1 were considered emaciated, and cows scored as 9 were considered overconditioned.

§ Chute score was collected for each cow by 3 trained technicians. The score was given as cows entered the chute and after the cow's head had been caught. Chute score was given according to Arthington et al.[21] on a scale from 1 to 5, where cows classified as 1 were calm, no movement, and cows classified as 5 were violent and continuously struggling in the chute.

‖ Chute exit velocity[17] was collected for each cow in each run and was calculated by determining the speed of the cow exiting the squeeze chute by measuring the rate of travel over a 1.6-m distance with an infrared sensor (FarmTek Inc., North Wylie, TX).

¶ Collar fit score was collected for each cow by 3 trained technicians immediately after the cow left the chute, upon 30 seconds of observation, according to Ranches et al.[15] Collar fit score was developed on a scale from 1 to 5 where cows classified as 1 were unalarmed and unexcited, walking slowly, and cows classified as 5 were extremely alarmed and excited, moving quickly, shaking the head, and jumping.

Results

No effects over time ($P \geq 0.89$) were observed for BW or BCS of cows collared with VF (Table 1). Similarly, no effects were observed for the chute score ($P = 0.11$); however, chute exit velocity changed between days 0 and 5 ($P = 0.001$). Cows exited the chute more slowly on day 5 when compared with the day cows were collared with VF (day 0; Table 1). The collar fit score was not statistically evaluated over time, as cows were collared only once; however, the collar fit score on day 0 was, on average 4.39, indicating cows were slightly uncomfortable (4 = very alarmed and excited, moving quickly and shaking

Table 2
Cow blood markers associated with stress and inflammatory response were analyzed at virtual fence collar fitting (day 0) and upon the end of the virtual fence training phase (day 5).

Item	Day 0*	Day 5*	SEM	*P* value
Cortisol† (μg/dL)	2.70	2.50	0.13	0.12
Ceruloplasmin‡ (mg/mL)	28.30	29.30	0.87	0.11
Haptoglobin§ (mg/mL)	0.40	0.45	0.01	<0.0001

Note: These data were collected at the Northern Great Basin Experimental Range, Riley, Oregon, US during the summer of 2022. Significance was set at $P \leq 0.05$.
SEM indicates standard error of the mean.
* Forty mature Angus × Hereford cows were collared with VF collars on day 0 and exposed to evolving VF boundaries until day 5. Blood samples were collected from each cow from the jugular vein on both days. Blood samples were placed on ice immediately after collection and centrifuged at 2,500 × g for 30 minutes at 4°C for plasma harvest. Plasma samples were frozen at −20° C in the same day of collection, and stored at −80°C.
† Plasma cortisol concentrations were measured using chemiluminescent enzyme immuno-assays (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA).
‡ Plasma ceruloplasmin oxidase activity was measured in duplicate samples using colorimetric procedures described by Demetriou et al.[24] Ceruloplasmin concentrations are expressed as mg/dL as described by King.[25]
§ Plasma haptoglobin concentrations were determined in duplicate samples by a biochemical assay measuring haptoglobin-hemoglobin complexing by the estimation of differences in peroxidase activity.[26]

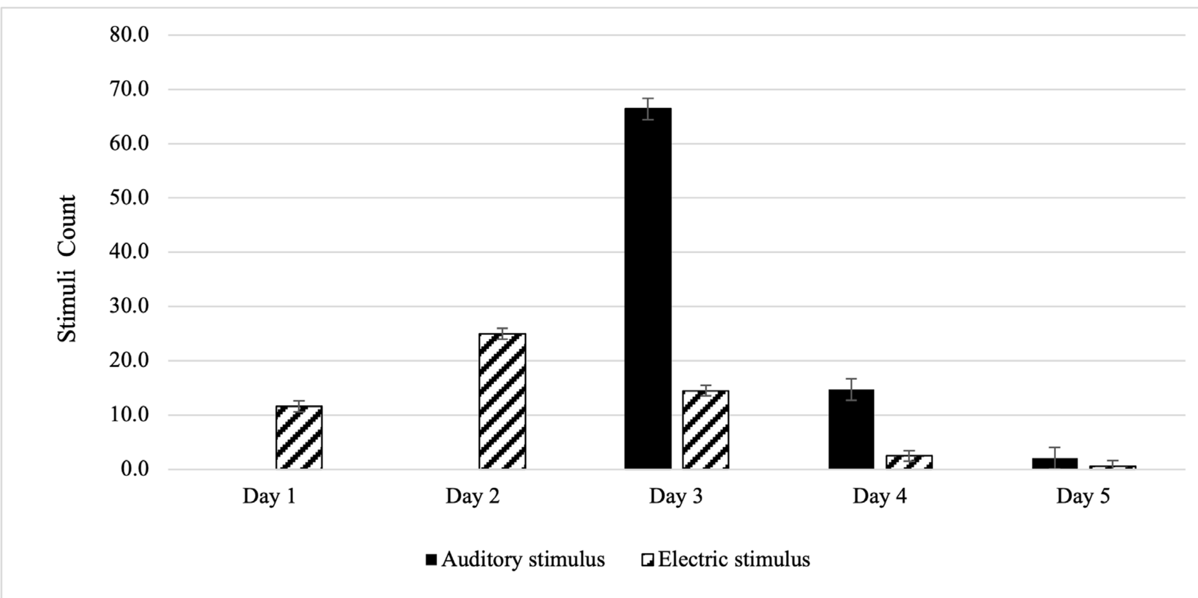

Figure 2. Count of audible stimulus (AS) and electric stimulus (ES) received by cows during the training phase (day 0-5) in the virtual fence (VF) boundaries at the Northern Great Basin Experimental Range, Riley, Oregon during the summer of 2022. ES in the VF was activated on day 1, and AS was activated on day 3. Count of stimuli over time is presented as the average stimuli per cow per day.

head; Table 1), which is similar to what has been previously reported by our group.[15]

No changes over time ($P \geq 0.11$) were observed for plasma concentration of cortisol or ceruloplasmin for VF collared cows. In contrast, plasma haptoglobin concentration of VF collared cows changed over time and was greater ($P < 0.0001$) on day 5 compared with day 0 (Table 2).

Each VF collar logged the time stamp, GPS coordinates, and the number of AS and ES stimuli to each cow. The count of stimuli over time was summarized and presented as the average daily stimuli per cow (Fig. 2). AS were activated from day 3 to 5, and ES were activated from day 1 to 5. Both AS and ES counts changed over time ($P < 0.0001$). Auditory stimuli were greatest on day 3 (the first day of AS) and decreased over time, reaching approximately two stimuli per cow per day on day 5 (2.03 count). The ES followed a similar pattern where the greatest ES were observed on days 2 and 3, decreasing over time and reaching less than one ES per cow per day on day 5 (0.56 count). The count of ES on day 1 was slightly lower ($P = 0.006$) than on day 2 and 3 (11.5, 24.9, and 14.4 ES count for days 1, 2, and 3, respectively).

Cow location during the training phase was determined using VF collar GPS coordinates. Overall, cow confinement within the VF boundaries was 93%, suggesting cows spent most of their time within the VF boundaries during the training phase. By days 4 and 5, 96% and 97% of cows' locations were reported within the VF boundaries, in agreement with the decreased number of AS and ES over time.

Discussion

Changes in BW or BCS were unlikely to be observed due to the duration of our study and were likely to happen only if the collaring process was extremely stressful. Similarly, considering the category of cattle used in our study, mature beef cows, no changes were expected for the temperament parameters evaluated (unless the collaring process was stressful), as these cows were familiar with the working facilities and accustomed to being managed and handled multiple times. The reduction in chute exit velocity observed on day 5 is more likely a result of the good handling practices adopted by our group and the familiarity of cattle with the working conditions[18,28] rather than an effect of being collared with the VF collar.

The collar fit score created by our group is a scoring system used to evaluate the comfort level of cattle when fitted with VF collars for the first time. In our study design, the collar fit score was evaluated only once, therefore, evaluating changes in score over time was not possible. The values we observed are similar to previously published literature by our group. Cows seemed to be alarmed and excited as an effect of the wearable device. However, this behavior was transient, as reported by Ranches et al.[15] and does not seem to impact or negatively affect cattle behavior over time.

Blood markers evaluated were cortisol, ceruloplasmin, and haptoglobin, which were specifically chosen, as these blood parameters are often associated with stress and the initiation of inflammatory responses in animals.[19,23,28] Cortisol is known as the "stress hormone" and cortisol plasma concentrations can quickly increase (i.e., within minutes[29,30]) upon interactions with physical, psychological, or chemical stressors.[19,28] Ceruloplasmin and haptoglobin are positive acute phase proteins associated with the inflammatory response, and similar to cortisol, plasma ceruloplasmin and haptoglobin concentrations increase after animal exposure to an insult. The increase in plasma concentration of both acute-phase proteins is slower when compared with cortisol, and the peak in plasma ceruloplasmin and haptoglobin concentrations is often observed between 24 to 48 hours after the initial insult.[19,31]

In our study, plasma cortisol and ceruloplasmin concentrations remained similar over time, suggesting the use of VF by cows may not influence those markers. However, plasma haptoglobin concentration increased from day 0 to 5 and could indicate a mild inflammatory response. Nonetheless, considering the ES as the major stressor encountered by cows in our study and the time of blood collections adopted by our group, it is likely that the observed increase in plasma haptoglobin concentration is related to the nature of this protein rather than a major and complex inflammatory response caused by the ES triggered by the VF collars. This is because plasma haptoglobin concentration can increase up to three-fold and remain elevated for up to 2 weeks.[32] In fact, the handling practices during the collaring process and the wearing of the collars could have influenced our outcome, more than the actual ES. Similar to our findings, Hamidi et al.[33] evaluated cortisol concentrations in feces of heifers fitted or not with VF collars and found no differences in fecal cortisol concentrations between the two groups. Additionally, Campbell et al.[13] compared fecal cortisol concentrations of steers fitted with VF and steers contained by electric tape fence and found no differences in the fecal cortisol concentration between the two groups. Also, comparing electric tape fence and VF, Verdon et al.[34] evaluated the milk cortisol concentration of lactating dairy cows and found no difference in milk cortisol concentration due to fence type. Confessore et al.[35] evaluated hair cortisol concentration of grazing beef cows contained in different VF configurations, where the initial hair collection was conducted with deactivated VF and the final hair collection was conducted when the VF was activated. These authors reported no changes in hair cortisol concentration over time. Finally, Jeffus[36] evaluated cortisol concentration in the hair and feces of cattle in rotational grazing with VF or physical fence, and found no cortisol concentrations in hair or feces nor changes in behavior. Although these studies differ in procedures to evaluate cortisol concentration and type of cattle, collectively, these studies, combined with our work, suggest the use of VF technology does not negatively affect cattle physiology, specifically cortisol, and consequently does not appear to negatively impact cattle welfare. To the best of our knowledge, we are the first group to evaluate plasma concentration of acute-phase proteins of cattle using a VF system; therefore, we are limiting the discussion of these variables, as more research is warranted.

AS and ES were quantified in our study as the count of stimuli per cow per day. The count of stimuli triggered by the VF observed herein agrees with previous observations by our group following the same pattern, where the number of stimuli triggered decreased over time. During the 5 days of acclimation and exposure to the VF boundaries, the ES reduction was approximately 97% from the peak stimuli to the last day in the training area, while the AS decreased 98% in the same period. Although the percentage of stimuli reduction is numerically greater for AS, the actual count of stimuli triggered by the VF collars on the last day of the study was greater for the AS (2.03 vs. 0.56, respectively), suggesting that once trained, cattle were relying primarily on the AS when in a VF setting.[11,13,37] The number of stimuli triggered by the VF collars have been previously reported by others; however, direct comparisons of these variables are challenging, as VF collar configurations differ, likely resulting in a different number of stimuli over time. Cattle breed, category, temperament, and personality might also play a role in the number of stimuli triggered over time. Some cattle may receive more stimuli than others as a function of the rate of learning, which is reported to be variable and possibly influenced by previous exposure to stimuli.[8,10,11,37]

Regardless of these differences, a growing body of literature has reported that the number of stimuli triggered over time decreases for ES and AS which fits with our findings. Campbell et al.,[13] working with automated VF collars fitted to beef steers, reported that all steers interacted with the VF boundaries at the beginning of the 4-week study and interactions with VF boundaries decreased over time. Lomax et al.[38]

working with grazing dairy cattle fitted with automated VF collars similarly reported a decrease in stimuli triggered by VF collars over a 6-day grazing trial. Aaser et al.,[39] working with mature beef cows fitted with automated VF collars in a 139 day grazing trial, also reported a reduction in number of stimuli over time. However, in Aaser et al.[39] VF boundaries were modified, resulting in some fluctuation in the number of stimuli triggered, indicating cattle were capable of identifying and responding to new VF boundaries.

In summary, because of the ability of cattle to quickly learn to identify VF boundaries and respond positively to VF stimuli, as noted by the decrease in stimuli triggered by VF collars over time, no major negative effects were observed in the physiological blood markers evaluated in our study or in BW or BCS.

Conclusions

Our objective was to evaluate the effects on blood markers associated with stress and inflammatory responses when cattle were fitted with automated VF collars. We hypothesized that the use of VF would not negatively impact these markers, as it was expected that cattle would rely primarily on the neutral AS triggered by the VF collars. Our hypothesis was supported by our findings. No major changes in blood makers were observed over time when cattle were fitted with automated VF collars. Furthermore, the decrease in the number of stimuli received over time indicated cattle quickly identified and avoided the VF boundaries.

Collectively, our findings suggest a high efficacy for the use of VF as a tool to manage cattle without apparent negative effects on cattle welfare as measured by physiological markers. Nonetheless, longer-term studies using VF collars for cattle containment are warranted to further explore the effects of VF on cattle welfare.

Declaration of competing interest

The authors certify that they have no financial interest in the subject matter discussed in the manuscript.

CRediT authorship contribution statement

Juliana Ranches: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Chad Boyd:** Investigation, Methodology, Resources, Writing – review & editing. **Rory C. O'Connor:** Investigation, Methodology, Writing – review & editing. **Matheus Ferreira:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Aline Cristine Rezende dos Santos:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Gracia Maria Puerto Hernandez:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Dustin Johnson:** Investigation, Methodology, Writing – review & editing. **David Bohnert:** Investigation, Methodology, Resources, Writing – review & editing.

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References

1. Knight KB, Toombs TP, Derner JD. Cross-fencing on private US rangelands: financial costs and producer risks. *Rangelands*. 2011; 33(2):41–44. doi:10.2111/1551-501X-33.2.41.
2. Davies KW, Boyd CS, Bates JD, Hulet A. Dormant season grazing may decrease wildfire probability by increasing fuel moisture and reducing fuel amount and continuity. *Int J Wildl Fire*. 2015; 24(6):849–856. doi:10.1071/WF14209.
3. Davies KW, Bates JD, Perryman B, Arispe S. Fall-winter grazing after fire in annual grass-invaded sagebrush steppe reduced annuals and increased a native bunchgrass. *Rangel Ecol Manag*. 2021; 77:1–8. doi:10.1016/j.rama.2021.03.001.
4. Boyd C, Beck J, Tanaka J. Livestock grazing and sage-grouse habitat: impacts and opportunities. *J Rangel Appl*. 2014; 1:58.
5. Sollenberger LE, Newman YC, Macoon B. Pasture design and grazing management. *Forages*. 2020:803–814. doi:10.1002/9781119436669.ch44.
6. Bishop-Hurley GJ, Swain DL, Anderson DM, Sikka P, Crossman C, Corke P. Virtual fencing applications: implementing and testing an automated cattle control system. *Comput Electron Agric*. 2007; 56(1):14–22. doi:10.1016/j.compag.2006.12.003.
7. Anderson DM, Estell RE, Holechek JL, Ivey S, Smith GB. Virtual herding for flexible livestock management – a review. *Rangel J*. 2014; 36(3):205–221. doi:10.1071/RJ13092.
8. Umstatter C. The evolution of virtual fences: a review. *Comput Electron Agric*. 2011; 75(1):10–22. doi:10.1016/j.compag.2010.10.005.
9. Umstatter C, Morgan-Davies J, Waterhouse T. Cattle responses to a type of virtual fence. *Rangel Ecol Manag*. 2015; 68(1):100–107. doi:10.1016/j.rama.2014.12.004.
10. Verdon M, Lee C, Marini D, Rawnsley R. Pre-exposure to an electrical stimulus primes associative pairing of audio and electrical stimuli for dairy heifers in a virtual fencing feed attractant trial. *Animals*. 2020; 10(2):217. doi:10.3390/ani10020217.
11. Campbell DLM, Lea JM, Haynes SJ, Farrer WJ, Leigh-Lancaster CJ, Lee C. Virtual fencing of cattle using an automated collar in a feed attractant trial. *Appl Anim Behav Sci*. 2018; 200(May 2017):71–77. doi:10.1016/j.applanim.2017.12.002.
12. Campbell D, Lea J, Farrer W, Haynes S, Lee C. Tech-savvy beef cattle? How heifers respondto moving virtual fence lines. *Animals*. 2017; 7(12):72. doi:10.3390/ani7090072.
13. Campbell DLM, Lea JM, Keshavarzi H, Lee C. Virtual fenc-

ing is comparable to electric tape fencing for cattle behavior and welfare. *Front Vet Sci*. 2019; 6(December):1–13. doi:10.3389/fvets.2019.00445.
14. Boyd CS, O'Connor R, Ranches J, et al. Virtual fencing effectively excludes cattle from burned sagebrush steppe. *Rangel Ecol Manag*. 2022; 81:55–62. doi:10.1016/j.rama.2022.01.001.
15. Ranches J, O'Connor R, Johnson D, et al. Effects of virtual fence monitored by global positioning system on beef cattle behavior. *Transl Anim Sci*. 2021; 5(Supplement_S1):S144–S148. doi:10.1093/tas/txab161.
16. Boyd CS, O'Connor RC, Ranches J, et al. Using virtual fencing to create fuel breaks in the sagebrush steppe. *Rangel Ecol Manag*. 2023; 89:87–93. doi:10.1016/j.rama.2022.07.006.
17. Brandão AP, Cooke RF. Effects of temperament on the reproduction of beef cattle. *Animals*. 2021; 11(11):3325. doi:10.3390/ani11113325.
18. Grandin T. Behavioral principles of livestock handling. *Prof Anim Sci*. 1989; 5(2):1–11. doi:10.15232/S1080-7446(15)32304-4.
19. Carroll JA, Forsberg NE. Influence of stress and nutrition on cattle immunity. *Vet Clin Food Anim Pract*. 2007; 23:105–149. doi:10.1016/j.cvfa.2007.01.003.
20. Grandin T. Assessment of stress during handling and transport. *J Anim Sci*. 1997; 75(1):249–257. doi:10.2527/1997.751249x.
21. Arthington JD, Qiu X, Cooke RF, et al. Effects of preshipping management on measures of stress and performance of beef steers during feedlot receiving. *J Anim Sci*. 2008; 86(8):2016–2023. doi:10.2527/jas.2008-0968.
22. Polsky L, von Keyserlingk MAG. Effects of heat stress on dairy cattle welfare. *J Dairy Sci*. 2017; 100(11):8645–8657. doi:10.3168/jds.2017-12651.
23. Ranches J, De Oliveira RA, Vedovatto M, et al. Low moisture, cooked molasses blocks: a limited intake method for supplementing trace minerals to pre-weaned calves. *Anim Feed Sci Technol*. 2021; 273(December 2020). doi:10.1016/j.anifeedsci.2020.114793.
24. Demetriou JA, Drewes PA, Gin JB. Ceruloplasmin. In: Cannon DC, Winkelman JW *Clinical Chemistry*. Harper & Row; 1974:857–864.
25. King J. Ceruloplasmin. In: *Practical Clinical Enzymology*. London: Van Nostrand; 1965:108–110.
26. Makimura S, Suzuki N. Quantitative determination of bovine serum haptoglobin and its elevation in some inflamatory disease. *Japanese J Vet Sci*. 1982; 44(1):15–21.
27. Cooke RF, Arthington JD. Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. *J Anim Physiol Anim Nutr (Berl)*. 2013; 97(3):531–536. doi:10.1111/j.1439-0396.2012.01298.x.
28. Burdick NC, Randel RD, Carroll JA, Welsh TH. Interactions between temperament, stress, and immune function in cattle. *Int J Zool*. 2011; 2011. doi:10.1155/2011/373197.
29. Hernandez CE, Thierfelder T, Svennersten-Sjaunja K, Berg C, Orihuela A, Lidfors L. Time lag between peak concentrations of plasma and salivary cortisol following a stressful procedure in dairy cattle. *Acta Vet Scand*. 2014; 56:61. doi:10.1186/s13028-014-0061-3.
30. Negrão JA, Porcionato MA, de Passillé AM, Rushen J. Cortisol in saliva and plasma of cattle after ACTH administration and milking. *J Dairy Sci*. 2004; 87(6):1713–1718. doi:10.3168/jds.S0022-0302(04)73324-X.
31. Petersen HH, Nielsen JP, Heegaard PMH. Application of acute phase protein measurements in veterinary clinical chemistry. *Vet Res*. 2004; 35(2):163–187. doi:10.1051/vetres:2004002.
32. Gruys E, Toussaint MJM, Niewold TA, Koopmans SJ. Acute phase reaction and acute phase proteins. *J Zhejiang Univ Sci*. 2005; 6 B(11):1045–1056. doi:10.1631/jzus.2005.B1045.
33. Hamidi D, Grinnell NA, Komainda M, et al. Heifers don't care: no evidence of negative impact on animal welfare of growing heifers when using virtual fences compared to physical fences for grazing. *Animal*. 2022; 16(9). doi:10.1016/j.animal.2022.100614.
34. Verdon M, Langworthy A, Rawnsley R. Virtual fencing technology to intensively graze lactating dairy cattle. II: Effects on cow welfare and behavior. *J Dairy Sci*. 2021; 104(6):7084–7094. doi:10.3168/jds.2020-19797.
35. Confessore A, Aquilani C, Nannucci L, et al. Application of virtual fencing for the management of Limousin cows at pasture. *Livest Sci*. 2022; 263(February). doi:10.1016/j.livsci.2022.105037.
36. Jeffus JL. *Evaluation of Animals Sensors and Technology in Grazing Environments*; 2022. https://hdl.handle.net/11244/337104.
37. Lee C, Henshall JM, Wark TJ, et al. Associative learning by cattle to enable effective and ethical virtual fences. *Appl Anim Behav Sci*. 2009; 119:15–22. doi:10.1016/j.applanim.2009.03.010.
38. Lomax S, Colusso P, Clark CEF. Does Virtual fencing work for grazing dairy cattle? *Animals*. 2019; 9(7):429. doi:10.3390/ani9070429.
39. Aaser MF, Staahltoft SK, Korsgaard AH, et al. Is virtual fencing an effective way of enclosing cattle? Personality, herd behaviour and welfare. *Animals*. 2022; 12(7):1–21. doi:10.3390/ani12070842.

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