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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

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ABSTRACT

Virtual fencing (**VF**) represents a way to simplify traditional pasture management with its high labour and cost requirements for fencing and to make better use of the 'beneficial' agronomic and ecological effects of livestock grazing. In this study, the VF technology (® Nofence, AS, Batnfjordsøra Norway) was used with Fleckvieh heifers to investigate possible welfare impacts on the animals compared to conventionally fenced animals when they were trained to respond correctly to the system. The Nofence® collars (attached to the neck of the heifers) send acoustic signals as a warning when the animals approach the VF line, which was set up by GPS coordinates within the Nofence®-App, followed by an electric pulse when they do not stop or return. The heifers had no experience with VF prior to the study. Two treatments (VF versus physical fencing (**PF**)) were applied to six groups of four heifers each (three groups per treatment) over three 12-day time replicates. One VF line separated the pasture of the VF group into an accessible or non-accessible area. The control group had a PF line. Both groups were equipped with Nofence® collars (deactivated for the PF group). The trial took place on two adjacent paddocks of 1 000 m^2 each following a 12-day schedule which was divided into three sections: visual support of the VF line by a physical barrier (first 2 days), only virtual border without visual support, moving the VF line (on day 8). Each time replicate followed the next successively on different paddocks with two new groups of heifers, which were grazed 5 h daily. During the whole experiment, the behaviour of each of the four animals per group was continuously observed; 2 h a.m., 2 h p.m. Exclusion by the VF line was effective in our trial. None of the heifers crossed the virtual boundary, i.e. the time spent in exclusion zone was zero. The heifers received 2.70 ± 2.63 acoustic signals and 0.30 ± 0.36 electric pulses (mean ± SD) per heifer and hour during all time replicates. Main cattle behaviour on pasture was not affected by the fencing system. Live weight gain, herbage consumption and faecal cortisol metabolites also revealed no significant differences. The duration until the heifers restarted grazing after an electric pulse from the Nofence® collar was significantly shorter than after an electric pulse from the physical fence. We can summarise that in our study, cattle well-being on pasture was not negatively affected by VF compared to PF.

Implications

Virtual fencing offers promising future perspectives for more grazing as it simplifies the effort for fencing. The intention of the current study was to investigate the potential impact of virtual fencing technology (® Nofence, Norway) on a range of cattle physiological and behavioural responses as an indicator of the impact on animal welfare. In a replicated experiment over time, virtual and physical fencing was compared using small groups of heifers. There was no evidence for an increased stress level or any negative impact on animal welfare for the virtual fenced heifers compared to the physically fenced heifers.

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Introduction

Grazing animals are essential for maintaining open pastures and achieving nature conservation objectives in grasslands (Tallowin et al., 2005), which has been increasingly recognised, especially in the context of climate change and conservation of biodiversity (Isselstein, 2018).

Precision livestock farming, especially the evolving technique of virtual fencing, opens up new opportunities to facilitate the use of the available pasture land (Stevens et al., 2021) as it proposes to make fencing less laborious (Lomax et al., 2019), more flexible to spatio-temporal dynamics in pasture conditions (Campbell et al., 2018; 2020), more precise and efficient, and gives even more flexibility in grazing management (Langworthy et al., 2021; Verdon et al., 2021). The technology uses GPS signalling to set virtual boundaries and to emit acoustic warnings when animals move towards the virtual barrier that is set via a mobile user interface (e.g. Campbell et al., 2020). If the animal continues moving forward and the barrier is in risk to be crossed, a short-duration electric pulse is emitted, following the acoustic signal which is always played before electric pulses, via a collar which carries the GPS device. Most published virtual fencing trials used the eShepherd® technology (Gallagher, Melbourne, Vic., Australia) and were conducted in Australia (e.g. Campbell et al., 2017; 2019; Keshavarzi et al., 2020; Verdon et al., 2021). These studies tested the applicability of virtual fencing and provided strong evidence that cattle are able to learn the system without negatively affecting animal behaviour and welfare. Brunberg et al. (2017) investigated the ability of ewes with lambs to learn a prototype virtual fencing technology manufactured in Europe by 'Nofence' (® Nofence, AS, Batnfjordsøra Norway), which works similarly to the eShepherd® system. In that latter study, wider application on sheep was not recommended because a high number of electric pulses implied that animal welfare might have been at risk. However, the study used first-generation collars with technical issues, which caused failure of acoustic signalling before emitting electric pulses. After further development, the Nofence virtual fencing technology for goats, cattle and sheep is now commercially available in Norway and UK (Lucia Ribagorda Garcia, personal communication ® Nofence (09/09/2021)). The national animal welfare acts of most EU member states restrict the use of virtual fencing so far.

A fundamental requirement for new technologies in the animal sector is that they at least maintain or lead to an improvement in animal welfare. To satisfy this standard, the design and implementation of new technologies need to be adapted to and complement the learning abilities of the animal (Lee et al., 2018). Conditional learning should ensure that the cattle become trained over time to avoid the electrical pulse by reacting to the acoustic signal and, therefore, make the fence system predictable (Butler et al., 2006; Lee et al., 2009; Lee et al., 2018). The basic learning behaviour of a conventional electric fence system and a virtual fence system is the same, the visual/acoustic stimulus is reliably followed by the electrical pulse. Results on potential impact on cattle welfare, so far, indicate no concerning behavioural impacts when virtual fencing groups were compared to control groups confined in paddocks surrounded by standard electric wire fence (Campbell et al., 2017; 2019). Reaching the limits of learning ability and behavioural adaption to environmental constraints can induce chronic stress (Lee et al., 2018). Therefore, extensive determination of changes in normal behavioural time budgets induced by virtual fencing is required to assess possible welfare impacts as one of the so called five freedoms characterising animal welfare, is the freedom to express normal behaviour by ensuring conditions which avoid mental suffering (UK Farm Animal Welfare Council, 1993). Continuous discomfort leads to helplessness and may result in chronic stress, and therefore, it is important to provide welfare assurance by detecting the possible impacts of virtual fencing on livestock (Lee and Campbell, 2021). Evaluation of behavioural time budgets as indicators of animal well-being was done by using sensors e.g. (Campbell et al., 2019) or scan sampling for certain research questions e.g. (Keshavarzi et al., 2020) so far. Continuous animal monitoring of behavioural indicators by observation during the whole time on pasture while using virtual fencing technology is missing so far. Assessments of behavioural indicators can be strengthened by physiological responses in animals as validated indices of negative animal welfare (Mellor, 2016). Therefore, in the current study, the metabolites of stress hormones (glucocorticoids) in faecal samples were measured. This procedure is a powerful tool that provides information on the endocrine status of animals in a non-invasive way (Palme, 2005).

European Studies, concerning small-scaled pastures, documenting the applicability of (Nofence®) virtual fencing in cattle by using continuous animal monitoring are, to our knowledge, missing so far. We set out the current study with the objective to test the feasibility of virtual fencing (hereafter called as '**VF**') systems (Nofence®) to exclude grazing cattle from a virtually set exclusion zone. Furthermore, we approach the knowledge gap regarding consequences in behavioural and physiological responses of grazing heifers with the Nofence® VF system. Our trial with a 12-d schedule could also be seen as a training protocol for future studies. The importance of an appropriate training protocol is highlighted by a study of Verdon et al. (2021) who recommended training cattle to VF for intensive grazing in dedicated paddocks and also by McSweeney et al. (2020). In our study, a shift of the virtual boundary on day 8 ensured a holistic learning of the VF system independent from visual cues. The continuously observed behavioural time budgets of the VF group were compared with those of a group of heifers having a conventional physically fenced (hereafter called as '**PF**') exclusion zone (which was also shifted on day 8). Continuous animal observation, VF collar information, faecal samples, pre- and postgrazing herbage mass, walked steps and individual live weight gain per time replicate were measured to test the following hypothesis in a holistic sense:

(i) VF has a negative effect on grazing heifers compared to PF, which can be measured by a range of behavioural characteristics and physiological responses.

Material and methods

The present study was conducted from August to September 2020 at the experimental farm of the University of Goettingen in Relliehausen, Solling Uplands, Lower Saxony, Germany (51°46′55.9″N, 9°42′11.9″E), 250 m above sea-level and was split into three subsequent time replicates of 12 days (17–28 August, 31 August–11 September, 14–25 September). We examined the ability of Fleckvieh heifers to learn the VF system with Nofence collars (® Nofence, AS, Batnfjordsøra Norway). Average daily temperature and precipitation sums per time replicate, recorded by the German Weather Service 'Deutscher Wetterdienst' at a distance of approximately 21 km from the farm, were 16 °C and 19.8 mm; 18 °C and 19.2 mm and 19 °C and 4.1 mm, for time replicate one, two and three, respectively.

Animals

This experiment utilised 24 heifers (Fleckvieh), aged 14–16 months with an initial live weight of 320–451 kg. None of the animals were experienced with the VF technology prior to the study. These heifers were born at the experimental farm and therefore habituated to the environment. They were divided into six groups of four heifers each evenly distributed according to their age, live weight and a modified weighing (temperament) score adapted from Geburt et al. (2015). These groups were randomly assigned to the experimental treatments and time replicates, i.e. either VF or PF and a time replicate. The respective average live weights (kg) ± SD in advance of the trial were as follows: time replicate one VF: 415.5 ± 39.24, PF: 409.3 ± 36.55; time replicate two VF: 421.8 ± 30.03, PF: 398.3 ± 48.09; time replicate three: VF: 413.5 ± 39.69, PF: 418.3 ± 31.98. Each heifer was marked individually with animal spray colour (Raidex®, Dettingen/Erms, Germany) on the back. As the heifers had no access to pasture before the start of the study, a habituation period of at least 14 days was given on a pasture surrounded by a common PF-system (posts with electric fence tape) adjacent to the trial area. Three days before each 12-day time replicate started, the fencing system treatment groups (VF and PF) were separated and then equipped with Nofence collars (® Nofence AS, Batnfjordsøra Norway) and IceTag accelerometers (Ice-robotics Ltd, Edinburgh, Scotland) (Fig. 1). Between equipping and the start of a time replicate, the groups were separately housed at *ad libitum* access to water and hay. After each experimental time replicates, all sensors were removed and the heifers returned to a neighbouring pasture after final weighing.

Virtual fencing system

The Nofence technology is based on a battery/solar-powered collar (weight: 749 g) and an application (**App**) for diverse clients (PC, Smartphone, Tablet). The collar has an integrated Global Positioning System along with sound generators and electric pulse generators, which are connected to a neck chain via two electrodes. Virtual boundaries can be set using the Nofence App. If an animal approaches the virtual boundary, an acoustic signal is emitted as a tone rising in pitch. If the animal does not react to the acoustic signal and continues moving towards the virtual boundary, the acoustic signal (82 dB at 1 m) is followed by a short-duration electric pulse (0.2 J at 3 kV Duration = 1.0 s) (source: https://www.nofence.no/en/product/cattle 25/01/2022). If the animal shows the desired response and turns away from the virtual boundary, no further stimuli (acoustic signals or electric pulses) are emitted. The system is based on associative learning/operant conditioning and should, therefore, be controllable and predictable for the animal. The electric pulse will only be emitted if all tones of the warning signal (increasing in pitch, duration 5–20 s, depending on whether the animal continues to ignore the warning or responses as desired) are played by the collar. The kind of desired response depends on which collar mode is activated. In teach mode, the animal only has to turn its head to stop the acoustic signal. The movement is registered by the accelerometer in the collar and allows an immediate response to the animals' action to ensure successful learning of the VF system. Collar transition to operating mode takes place when the animal has responded correctly to 20 consecutive acoustic signals, without receiving an electric pulse. After switching to the operating mode, the animal has to walk at least 2 m away from the virtual boundary into the virtual pasture to stop the acoustic signal. In both modes, if the animal ignores the acoustic signal and continues moving towards the virtual boundary, it will receive a maximum of three electric pulses if it does not react to the acoustic signal before each electric pulse. After that, the collar sends the notification "the animal has escaped" to the owner and continues to monitor the animal's location, but the animal will not receive any further acoustic signals or electric pulses. If this animal crosses the virtual boundary to re-enter the virtual pasture, the collar will return to normal functionality without manual interference. (All technical information is taken from the Nofence manual (2020) and personal communication (Natascha Grinnell, Nofence 19 April 2022)). The collars were attached to the neck of all heifers in the trial. Collars were deactivated for the PF groups and activated during the daily grazing time (5 h) for the VF groups. The GPS sensors of activated collars recorded positions in 15-minute intervals if the animal was at least 30 metres away from the virtual boundary. When the heifer approached the boundary, the frequency of recorded GPS positions automatically increased up to four positions per second at a distance <3.5 m to the boundary.

Fig. 1. Fleckvieh heifer equipped with Nofence neckcollar, CowManager Earsensor (left ear) and IceTag accelerometers (hind right leg) and marked with a coloured number after preparation for the trial.

IceTag accelerometers

IceTag accelerometers were attached to the hind leg (right side) and recorded continuously walked steps for each heifer. Walked steps were used to detect variation in locomotion caused by the fencing system.

Experimental design and collecting data

Throughout each 12-day time replicate, both groups of heifers were grazed on a pasture separated into paddocks each 1 000 m^2 in size, daily between 10 a.m. and 5 p.m. (for 5 h). On the first and the last day of each time replicate, they were grazed for two hours. The first activation of the VF technology took place on the first day on pasture for each VF group. For each time replicate, new paddocks were used, which were not previously grazed. Water was supplied *ad libitum*. After the daily pasture access, the two groups were housed separately with *ad libitum* access to hay and water in a shelter adjacent to the trial site. Each paddock was fenced with a commonly used electric fence and was divided into an accessible and non-accessible area. The electric fence device was commercially available (® Siepmann, Herdecke, Germany) with a pulse energy of up to 4.1 joules (ex-device) at contact, varying according to electric wire conductivity and distance to the device itself. One VF line, which was set up by GPS coordinates using the Nofence app, was established to separate the paddock of the VF group. The division into accessible and non-accessible pasture areas was implemented by electric fence for the control group. Due to the non-accessible pasture area, the total paddock size was reduced to 866.5 ± 32.7 m^2 (overall average). Each 12-day time replicate had the following schedule (VF): On the first day, visual support of the VF by a complete PF serving as a visible barrier; only fence posts with the fence wire removed on day two; only VF without any visual support (day three to day eight), and increasing the accessible area by moving the VF line on day eight to provide new herbage (Fig. 2). On day eight, the accessible area of the PF group was increased as well for providing new herbage by moving the PF line. Both fence lines were shifted for 3 m.

Cattle behaviour

During the whole experiment, individual behaviour of each of the four heifers was continuously observed for 2 h in the morning and 2 h in the afternoon by one observer per group. Main behavioural classes were recorded using the app 'Observasjonslogger' by Morten Sickel (see Table 1 for the ethogram). Counted steps per heifer were retrieved from the IceTag accelerometers. Individual daily live weight gain was retrieved from weighing before and after each time replicate.

Estimated herbage consumption from pasture

On the very first date before the start of the study, 50 compressed sward height (**CSH**) measurements were taken across the whole experimental area using a rising plate meter (30 cm diameter, 200 g plate weight; (Castle, 1976)) in order to quantify herbage availability for adequate paddock sizes to fulfil dietary requirements of each group. Three manually cut herbage samples of known CSH were taken randomly across the experimental area to determine the average herbage availability using linear regression in an approach similar to (Şahin Demirbağ et al., 2009) with an R^2 of 0.97 between CSH and herbage DM. Herbage DM was determined after drying the samples at 60 °C for 48 h. Herbage availability during the experimental time replicates was measured on training day one (pregrazing), day 8 (mid-grazing), and day 12 (postgrazing) of each time replicate in two to four randomly distributed locations per paddock by manual cutting close to soil surface (1 cm) in a round steel frame with an area of 706 cm^2 per sampling location. Based on the difference between pre- and postgrazing herbage DM availability, the group-wise average herbage consumption over 12 days was determined. The assessments consequently refer to the minimum herbage consumption per 5-hour grazing period rather than actual herbage consumption because regrowth during grazing was not assessed.

Analysis of faecal cortisol metabolites

Faecal samples were collected from the heifers immediately after spontaneous defaecation during the daily observation time on day eight and day 12, i.e. mid- and postgrazing, respectively.

Table 1
Ethogram of objective cattle behaviour on pasture continuously recorded by one observer per group (PF treatment and VF treatment) during daily training time.

Cattle behaviour	Definition
Grazing	The heifer walks slowly (grazing step) or stands while picking up grass with her mouth.
Lying	The flank/belly of the heifer touches the ground.
Standing	The heifer remains at one point. All four legs are fully extended vertically on the floor.
Social behaviour	Any interaction with each other: rank fights, mutual grooming etc.
Comfort behaviour	Any behaviour for body care of the individual heifer: stretching the limbs, scratching the ear with the back feet etc.
Locomotion	Running/walking of the heifer on the pasture with head up without herbage intake.

Abbreviations: PF = physical fence; VF = virtual fence.

Samples were first cooled after collection and then frozen (−18 °C) within eight hours after sampling. Before analysis, faecal cortisol metabolites (**FCMs**) were extracted from the (defrosted) faeces. A portion of the wet faeces (e.g. 0.5 g) suspended in 5 mL of 80% methanol was shaken and centrifuged and faecal cortisol metabolites were measured in an aliquot of the supernatant via an 11-oxoaetiocholanolone enzyme immunoassay (**EIA**). This EIA measures 11,17-dioxoandrostanes, a group of cortisol metabolites, and has been developed in the laboratory of R. Palme (for details of the assays including cross-reactions, see Palme and Möstl (1997)). Intra- and inter-assay coefficients of variation of a low and high pool sample were below 10 and 15%, respectively. FCM concentrations in cattle faeces reflect the cortisol secretion about 12 h earlier (Palme et al., 1999).

Time elapse between electric pulse and grazing

The time in seconds until a heifer restarted grazing, as it is known as main behaviour for cattle on pasture (Kilgour, 2012), after receiving either an electrical pulse from the VF collar or an electric pulse from the physical fence in both the PF and VF groups was used in order to indicate any immediate direct impacts on behavioural patterns. The severity of the response to the pulse was consequently measured as the duration of interruption of usual behaviour. The data were retrieved from the observational data (electrical pulse from the PF) and the Nofence collar reported data (electrical pulse from the VF collar).

Statistical analysis of results

Statistical analyses were carried out using the software R (R Core Team, 2020). For each target variable, linear mixed effect models were calculated using the package 'nlme' (Pinheiro et al.,

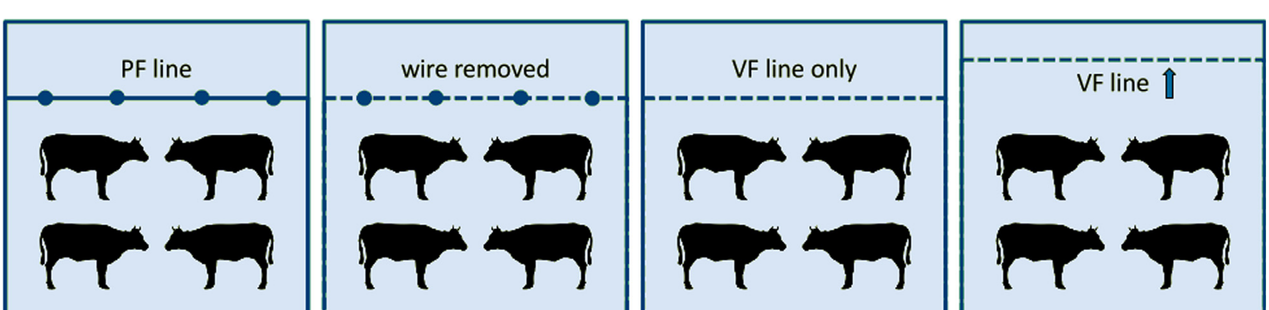

Fig. 2. Sections of the 12-day time replicates with grazing Fleckvieh heifers (virtual fencing (VF) group): day one complete physical fence (PF) + VF line, day two only the fence posts of the PF + VF line, day three to seven only VF line, day eight to twelve, the VF line was shifted for 3 m.

2018). By visual inspection of quantile–quantile plots, the normality of the residuals was checked. Plots of residuals vs fitted values and residuals vs predictor values were used to evaluate the variance homogeneity. For significant influencing factor levels, multiple contrast tests according to Tukey's HSD test with Sidak's method of confidence level adjustment were conducted in the 'emmeans' package (Barton, 2018) following the analyses of variances.

IceTag accelerometers

The measured steps during pasture access time (obtained from IceTag accelerometers) were aggregated to mean steps per hour (and animal) and were evaluated on the fixed effects of fencing system (n = two levels) and day on pasture (n = 12 levels) as well as their interaction. The individual animal nested in the time replicate was used as a random factor (n = 12 replicate animals per fencing treatment in total with four animals per fencing treatment × time replicate).

Cattle behaviour

Cattle behaviour from observations was analysed as relative duration of the respective behaviour per observation day (usually-four hours daily). Each target variable was bound on a logit-scale in order to improve the normality of residuals before analysis. Walked steps refer to the absolute counts and were therefore not logit-bound. Each behaviour was assessed using the fixed effects of fencing system and day on pasture as well as their interaction. The individual animal nested in the time replicate was used as a random factor.

Time elapse until grazing

In total, n = 156 electrical pulses from the VF collars and n = 93 electrical pulses from the physical fence were recorded across time replicates and fencing system treatments. These data points were included in one generalised least square model that estimated the effect of pulse type on the time until grazing was continued after having received one of the electrical pulse classes during the observation periods. Data were log-transformed (log naturalis) before analysis in order to improve the normality of residuals.

Estimated herbage consumption from pasture

Estimated herbage consumption was analysed as group-wise average herbage intake (g DM m^{-2}) and was evaluated using the fixed effects of fencing system and day of sample within each time replicate as well as their interaction. The time replicate was used as a random factor.

Individual daily live weight gain (g $head^{-1}$ d^{-1}) of the heifers was evaluated using the fixed effect of fencing system. The individual animal nested in the time replicate was used as a random factor.

Faecal cortisol metabolites

Concentrations (ng/g) of FCMs were evaluated using the fixed effects of fencing system and day of sample (day 8 and day 12) within each time replicate as well as their interaction. The individual animal nested in the time replicate was used as a random factor.

Results

No heifer crossed the VF line during the experiment. This information was measured via automatically stored collar data, as no heifer was classified as 'escaped' during the daily pasture access time. These automatic records were confirmed via continuous animal observation as no heifer entered the defined exclusion zone. The heifers received 2.70 ± 2.63 acoustic signals and 0.30 ± 0.36 electric pulses (mean ± SD) per heifer and hour during all time replicates (see Tables S1 and S2 (Appendix) for further details). This represents a relationship of 9:1 (acoustic signals:electric pulses). In our trial, exclusion was effective with a rate of 100% to exclude heifers from the defined exclusion zones, see Fig. 3 for GPS positions of the VF group.

Cattle behaviour on pasture

Behaviour observed

Differences in observed behaviour between PF and VF animals depended mostly on the day on pasture (for all behaviours observed) in interaction with the fencing system for all variables but grazing (Table 2). The time budgets for grazing were lowest on days one, seven and twelve and highest on day five (Table 3), which is why the day had a significant main effect for grazing time. Only on day ten, differences between the two groups in social behaviour became significant (Table 3). The VF heifers' daily time budgets for social behaviour were larger than for the PF heifers on that day. Comparisons of means of locomotion revealed that the heifers of the PF group were more active in this respect than the heifers of the VF group on days two, three, five, ten and eleven (Table 3). The average difference was 1.14 ± 0.19% (estimated mean ± SE). The lying time on day five and day eleven was greater for the VF group compared to the PF group, while day nine showed the opposite (Table 3). Daily time budgets (estimated mean ± SE) for the two groups were 3.3 ± 0.57% (VF) and 3.1 ± 0.76% (PF). The VF heifers spent more time standing than the PF heifers on day eight (Table 3), while on day eleven, the PF heifers spent more time standing than the VF heifers (Table 3), explaining the significant interaction between fencing system × day on pasture. Only on day ten, the PF heifers showed a significantly larger proportion of comfort behaviour than the VF heifers (Table 3), despite the significant interaction between fencing system × day.

Time elapse from electric pulse until grazing

The latency to graze following an electric pulse was significantly (P = 0.015) influenced by the type of pulse. After having received an electric pulse from the VF collar, the time (estimated means ± SE) until grazing was significantly shorter (22.0 ± 2.6 s) than after an electric pulse from the physical fence (33.6 ± 4.2 s) (Fig. 4).

Walked steps per hour based on IceTag accelerometer data

On average, the heifers of the PF group walked 384 ± 120 steps per hour and the heifers of the VF group 372 ± 129 (arithmetic mean ± SD) steps per hour. Walked steps per hour were significantly affected by the interaction of fencing system × day (Table 2). However, comparisons of means revealed no significant difference between the two treatments. In all time replicates, the heifers of the PF group tended to walk more steps per hour and the walked steps per hour increased by time replicate for both treatments. In time replicate one, the heifers walked 280 ± 62 (PF group) and 267 ± 52.5 (VF group) steps per hour. In time replicate three, the heifers walked most: PF group 478 ± 88.7 steps per hour, VF group 472 ± 108 steps per hour (arithmetic mean ± SD).

Live weight gain

There was no significant effect of the fencing system on the live weight gain of the heifers (Table 4). Daily live weight gain for the VF and the PF groups were 1.4 ± 1.1 and 1.5 ± 1.3 kg d^{-1}, respectively (arithmetic mean ± SD).

Estimated herbage consumption from grassland

The fencing system had no significant effect on the herbage availability, which was affected only by the day of sample

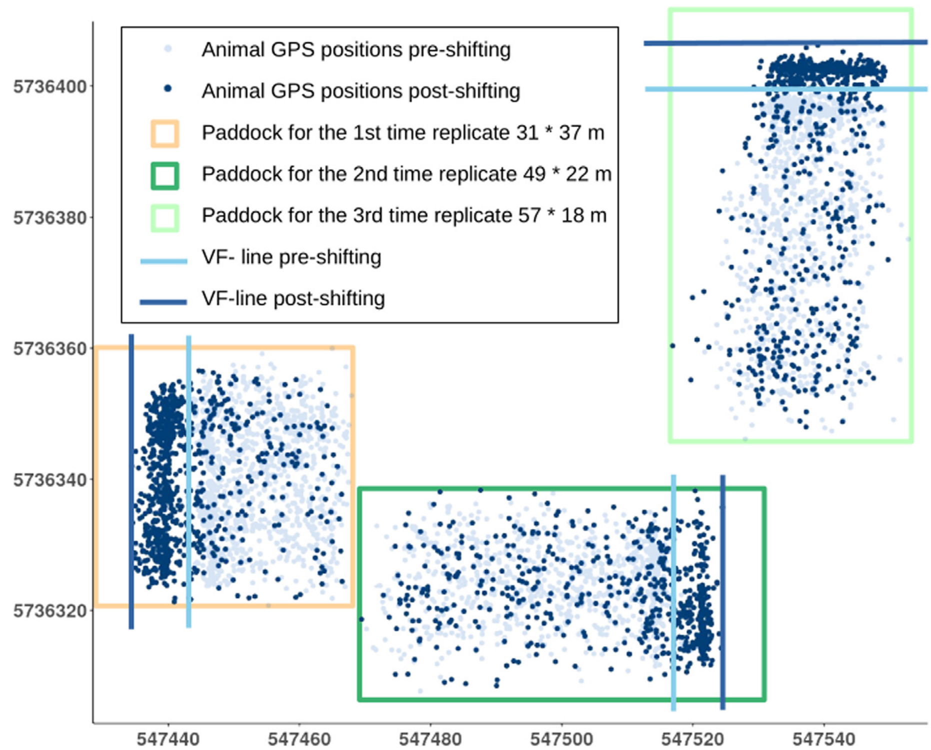

Fig. 3. GPS locations of the virtual fencing (VF) groups of Fleckvieh heifers near the VF line were recorded at four signals per second. Positions apart from the VF line were reported in 15-minute intervals in order to save battery. Shown are the days preshifting the VF line and postshifting the VF line (enlargement of the inclusion zone). GPS positions on the daily used drifts outside of the paddocks have been removed.

Table 2
Output of linear mixed effect models for the analysed parameters of interest to evaluate cattle behaviour and productivity in the virtual compared to the physical (control) fencing system during 12 days of observation over three time replicates (n = 36 days). Shown are *F*-values, degrees of freedom and *P*-values.

Target variable	Fixed and interaction effects	numDF	denDF	*F*-value	*P*-value
Grazing	Fencing system	1	24	0.002	0.97
	Day on pasture	11	238	3.12	0.0006***
	Fencing system × Day on pasture	11	238	1.67	0.08
Social behaviour	Fencing system	1	24	0.73	0.40
	Day on pasture	11	238	1.84	0.049*
	Fencing system × Day on pasture	11	238	2.67	0.003**
Locomotion	Fencing system	1	24	3.11	0.09
	Day on pasture	11	238	2.19	0.016*
	Fencing system × Day on pasture	11	238	3.26	0.0004***
Lying	Fencing system	1	24	0.67	0.42
	Day on pasture	11	109	4.16	<0.0001***
	Fencing system × Day on pasture	11	109	3.49	0.0003***
Standing	Fencing system	1	24	0.003	0.95
	Day on pasture	11	238	5.48	<0.0001***
	Fencing system × Day on pasture	11	238	1.95	0.03*
Comfort behaviour	Fencing system	1	24	0.003	0.96
	Day on pasture	11	235	6.15	<0.0001***
	Fencing system × Day on pasture	11	235	1.88	0.04*
Walked steps per hour (IceTag)	Fencing system	1	20	0.1	0.7
	Day on pasture	11	241	18.6	<0.0001***
	Fencing system × Day on pasture	11	241	2.5	0.0050**

Abbreviations: numDF = degrees of freedom in the numerator; denDF = degrees of freedom in the denominator.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

($P < 0.001$). Herbage availability was significantly greater at the beginning of each time replicate than at mid and postgrazing. The average herbage availability across time replicates in the VF group was 340, 255, and 211 g DM m^{-2} at the start, middle, and end of the time replicates, respectively. In the PF group, the corresponding average herbage availabilities were 326, 213, and 160 g DM m^{-2}, respectively. In the PF group, the average herbage intake was 3.4 ± 0.97 and 1.28 ± 0.57 kg DM $animal^{-1}$ day^{-1} in the first

Table 3
Estimated means ± SE (standard error) of linear mixed effect models for daily time budgets (given as proportions per hour) of cattle (Fleckvieh) behaviour on pasture (proportions of time, missing values to 1 were other observed behaviour which was not analysed in this study) and walked steps per hour (measured with IceTag accelerometers). Main effects were shown for walked steps per hour and grazing. Lowercase letters indicate significant differences between days for the target variable grazing and walked steps and between fencing systems within day for all other variables.

Target variable	Fencing system	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Grazing		$0.638 \pm 0.024_{a}$	$0.765 \pm 0.019_{bc}$	$0.760 \pm 0.019_{bc}$	$0.776 \pm 0.018_{bc}$	$0.806 \pm 0.016_{c}$	$0.749 \pm 0.020_{bc}$	$0.728 \pm 0.021_{b}$	$0.738 \pm 0.020_{bc}$	$0.764 \pm 0.019_{bc}$	$0.748 \pm 0.020_{bc}$	$0.734 \pm 0.020_{bc}$	$0.709 \pm 0.022_{ab}$
Social behaviour	PF	$0.075 \pm 0.022_{a}$	$0.046 \pm 0.014_{a}$	$0.049 \pm 0.015_{a}$	$0.058 \pm 0.017_{a}$	$0.060 \pm 0.018_{a}$	$0.053 \pm 0.016_{a}$	$0.063 \pm 0.019_{a}$	$0.037 \pm 0.011_{a}$	$0.061 \pm 0.018_{a}$	$0.063 \pm 0.019_{a}$	$0.073 \pm 0.021_{a}$	$0.044 \pm 0.014_{a}$
	VF	$0.060 \pm 0.018_{a}$	$0.065 \pm 0.019_{a}$	$0.091 \pm 0.026_{a}$	$0.041 \pm 0.012_{a}$	$0.046 \pm 0.014_{a}$	$0.058 \pm 0.017_{a}$	$0.078 \pm 0.023_{a}$	$0.081 \pm 0.024_{a}$	$0.062 \pm 0.018_{a}$	$0.080 \pm 0.023_{b}$	$0.049 \pm 0.015_{a}$	$0.030 \pm 0.009_{a}$
Locomotion	PF	$0.049 \pm 0.008_{a}$	$0.048 \pm 0.008_{b}$	$0.047 \pm 0.008_{b}$	$0.036 \pm 0.006_{a}$	$0.051 \pm 0.009_{b}$	$0.032 \pm 0.006_{a}$	$0.041 \pm 0.007_{a}$	$0.045 \pm 0.008_{a}$	$0.049 \pm 0.008_{a}$	$0.052 \pm 0.009_{b}$	$0.041 \pm 0.007_{b}$	$0.046 \pm 0.008_{a}$
	VF	$0.036 \pm 0.006_{a}$	$0.027 \pm 0.005_{a}$	$0.028 \pm 0.005_{a}$	$0.026 \pm 0.004_{a}$	$0.024 \pm 0.004_{a}$	$0.037 \pm 0.006_{a}$	$0.029 \pm 0.005_{a}$	$0.041 \pm 0.007_{a}$	$0.043 \pm 0.007_{a}$	$0.035 \pm 0.006_{a}$	$0.028 \pm 0.005_{a}$	$0.046 \pm 0.008_{a}$
Lying	PF	$0.083 \pm 0.052_{a}$	$0.020 \pm 0.013_{a}$	$0.006 \pm 0.005_{a}$	$0.024 \pm 0.016_{a}$	$0.006 \pm 0.005_{a}$	$0.087 \pm 0.064_{a}$	$0.075 \pm 0.051_{a}$	$0.004 \pm 0.003_{a}$	$0.043 \pm 0.030_{b}$	$0.015 \pm 0.014_{a}$	$0.005 \pm 0.004_{a}$	$0.007 \pm 0.008_{a}$
	VF	$0.050 \pm 0.031_{a}$	$0.063 \pm 0.042_{a}$	$0.022 \pm 0.017_{a}$	$0.081 \pm 0.053_{a}$	$0.031 \pm 0.022_{b}$	$0.023 \pm 0.017_{a}$	$0.053 \pm 0.034_{a}$	$0.022 \pm 0.016_{a}$	$0.003 \pm 0.002_{a}$	$0.006 \pm 0.004_{a}$	$0.032 \pm 0.022_{b}$	$0.009 \pm 0.008_{a}$
Standing	PF	$0.099 \pm 0.024_{a}$	$0.065 \pm 0.016_{a}$	$0.045 \pm 0.012_{a}$	$0.028 \pm 0.007_{a}$	$0.027 \pm 0.007_{a}$	$0.037 \pm 0.010_{a}$	$0.044 \pm 0.011_{a}$	$0.039 \pm 0.010_{a}$	$0.047 \pm 0.012_{a}$	$0.068 \pm 0.017_{a}$	$0.084 \pm 0.021_{b}$	$0.072 \pm 0.018_{a}$
	VF	$0.101 \pm 0.024_{a}$	$0.047 \pm 0.012_{a}$	$0.047 \pm 0.012_{a}$	$0.023 \pm 0.006_{a}$	$0.020 \pm 0.005_{a}$	$0.062 \pm 0.016_{a}$	$0.041 \pm 0.010_{a}$	$0.079 \pm 0.021_{b}$	$0.039 \pm 0.010_{a}$	$0.057 \pm 0.015_{a}$	$0.047 \pm 0.012_{a}$	$0.084 \pm 0.021_{a}$
Comfort behaviour	PF	$0.015 \pm 0.004_{a}$	$0.030 \pm 0.008_{a}$	$0.004 \pm 0.001_{a}$	$0.009 \pm 0.003_{a}$	$0.010 \pm 0.003_{a}$	$0.007 \pm 0.002_{a}$	$0.010 \pm 0.003_{a}$	$0.008 \pm 0.002_{a}$	$0.011 \pm 0.033_{a}$	$0.007 \pm 0.002_{a}$	$0.010 \pm 0.003_{a}$	$0.015 \pm 0.004_{b}$
	VF	$0.004 \pm 0.001_{a}$	$0.004 \pm 0.001_{a}$	$0.009 \pm 0.003_{a}$	$0.006 \pm 0.002_{a}$	$0.009 \pm 0.002_{a}$	$0.009 \pm 0.002_{a}$	$0.011 \pm 0.003_{a}$	$0.009 \pm 0.002_{a}$	$0.012 \pm 0.003_{a}$	$0.005 \pm 0.001_{a}$	$0.012 \pm 0.003_{a}$	$0.038 \pm 0.010_{a}$
IceTag Walked steps per hour	PF	$408 \pm 64_{a}$	$393 \pm 63_{a}$	$427 \pm 63_{a}$	$391 \pm 63_{a}$	$424 \pm 63_{a}$	$324 \pm 63_{a}$	$315 \pm 63_{a}$	$389 \pm 64_{a}$	$425 \pm 63_{a}$	$381 \pm 65_{a}$	$378 \pm 64_{a}$	$349 \pm 65_{a}$
	VF	$397 \pm 65_{a}$	$364 \pm 63_{a}$	$407 \pm 63_{a}$	$346 \pm 64_{a}$	$374 \pm 64_{a}$	$355 \pm 64_{a}$	$339 \pm 64_{a}$	$352 \pm 65_{a}$	$407 \pm 64_{a}$	$384 \pm 66_{a}$	$353 \pm 64_{a}$	$344 \pm 67_{a}$

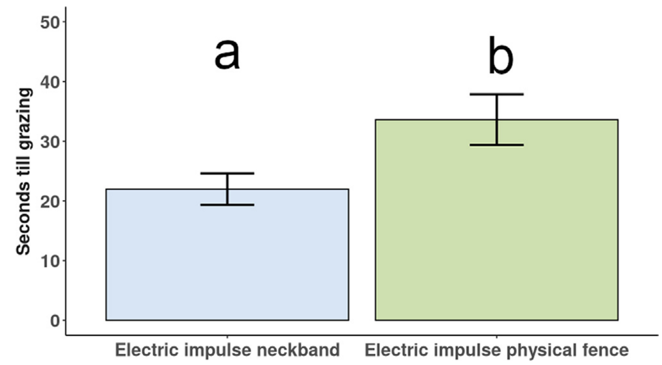

Fig. 4. Estimated means ± SE of seconds until grazing after Fleckvieh heifers having received an electric pulse from the Nofence® Collar (only virtual fencing (VF)-group) or an electric pulse from the physical fence (PF) (includes both the PF groups and the VF groups). Lowercase letters indicate significant differences between impulses at $P < 0.05$.

eight days and last four days, respectively. In the VF treatment, the average herbage intake was 3.02 ± 2.41 and 0.94 ± 1.01 kg DM animal^{-1} day^{-1} (arithmetic mean ± SD) in the first eight and last four days, respectively.

Faecal sampling

Concentrations (arithmetic mean ± SD) of FCMs for the PF group were 16.4 ± 12.6 ng g^{-1} faeces and for the VF group 14.3 ± 7.11 ng g^{-1} faeces, respectively, and no significant effects of the fencing system were found.

Discussion

While there are many studies on virtual fencing in Australia e.g. (Lomax et al., 2019; Campbell et al., 2019; Verdon et al., 2021) mainly using the eShepherd® technology (Agersens), studies using alternative virtual fencing technology are rare. The current study is the first to evaluate Nofence® virtual fencing compared to a control group using growing Fleckvieh heifers under continuous animal monitoring on small pastures in Europe. In a recent study, Boyd et al. (2022) documented the effectiveness of the virtual fencing system "vence" for the successful exclusion of sensible areas in rangeland of the USA. We have tested the feasibility of VF systems (Nofence®) to exclude grazing cattle from a virtually set exclusion zone using a 12-d schedule. Successful application of virtual fencing technology needs to meet the cognitive capacity and natural behaviours of cattle (Verdon et al., 2021). One of the 'five freedoms' (UK Farm Animal Welfare Council, 1993) is the freedom to express normal behaviour (by ensuring conditions which avoid mental suffering). The animals should be able to minimise receiving electric pulses of the VF system by reacting to the acoustic signals and, therefore predict and control their situation (Lee et al., 2018). Animal's quality of life is reflected by the net balance between positive and negative experiences (Mellor, 2016). Possible negative experiences associated with the VF system should be reflected in any of the measured behaviours compared to the PF group. Normal behaviour is defined in our trial as the behaviour of cattle in common pasture systems (PF). Deviations from these 'normal' behaviour patterns may be an indication for non-optimal animal welfare (Lee and Campbell, 2021). In addition to the analysis of the behavioural time budgets per day, we have done a separate analysis where we blocked days into periods before and after fence shifting. This analysis did not show differences to the daily data analysis as presented in this study. We hypothesised that (i) VF has a negative effect on grazing heifers compared to PF, which can be measured by a range of behavioural characteristics and physiological responses.

Functionality of the virtual fencing system

Excluding heifers from the exclusion zone via the VF line was effective with a rate of 100% in our trial. No heifer crossed the VF line during our trial as measured via collar data and as confirmed by visual observation. Therefore, our formulated objective was achieved. Other studies showed similar values of effectiveness for the eShepherd® technology, ranging from 87% (Campbell et al., 2017) to >98% (Lomax et al., 2019; Campbell et al., 2019; 2020; Keshavarzi et al., 2020; Langworthy et al., 2021; Verdon et al., 2021).

The shift of the VF line on the eighth day simulated a first approach (using the Nofence technology) to economically interesting grazing systems such as rotational grazing, but also the temporary fencing of ecologically sensitive areas. From our observations, we can conclude that the discovery of new grazing access depends on how high the grazing pressure is, which is in line with (Langworthy et al., 2021) who, however, found a small effect of pasture depletion reducing the efficacy of the virtual fence in their study. In addition, we were able to ensure that there was a complete understanding of the invisible boundaries as a logical consequence of the acoustic signal and not an environmental marker that the animals used to orient themselves to remember the boundary as they easily adapted to the new grazing area and the changed position of the VF line on day 8. All groups were able to understand the increased pasture access. The use of the acoustic signal to locate the boundary appeared to be more common for the animals during the trial. They obviously learned to interact with the signal in order to make full use of the area (Fig. 3), which is in line with comparable studies analysing the shift of virtual boundaries through the use of eShepherd® technology (Campbell et al., 2017; Langworthy et al., 2021). Campbell et al. (2017) showed that animals learned about the acoustic signals, not the location at which the signals were given.

Deviation from common cattle behaviour when using virtual fences

Main cattle behaviour

Grazing represents the major behaviour on pasture (Kilgour, 2012), and the average proportions (arithmetic means ± SD) spent grazing in the present study were 74.7 ± 9.06% and 72.0 ± 9.6% for the PF and VF groups, respectively. These values were on the upper end compared to the ones previously reported for day grazing of cows sheltered at night in an investigation by (Homburger et al., 2015), who found that grazing accounted for 55–75% of the time on pasture. Cattle observed in 24 h periods on pasture had lower values of grazing, and these proportions decreased to 29.7–43.9% as reported in a review by (Kilgour, 2012) where the overall average of 13 studies is 37.7%.

When accounting for ruminating and resting (including lying and standing) in addition to grazing, usually 90–95% of the behaviour is covered. These main behaviours, recorded continuously on female cattle on 134 ha pastures in North-Western England during two summer seasons, represented 84.4% of the total time (Hall, 1989). Similarly, grazing, lying and standing together accounted for 86.6 (PF) and 86.0% (VF) in the current study.

Lying is seen as an indicator for assessing comfort, restlessness or even fear for cattle (Haley et al., 2000). Contrary to a study by Campbell et al. (2019), who have found that cattle from the VF groups were lying less than cattle from PF groups, the VF group in the current study had greater proportions of lying time compared to the PF group on a few days (Table 4). However, it needs to be considered that in the study by Campbell et al. (2019), the animals were 24 hours on pasture and lying tended to occur mainly at night and less during the day.

Although the compared time budgets of animal behaviour on pasture were most significantly affected by the interaction of fencing system × day (Table 2), there was no evidence that heifers in the VF group were systematically restricted in their behaviour.

However, the technology of virtual fencing raises animal welfare concerns among animal welfare advocates, members of the public and authorities because electrical pulses are emitted by a neckband. A visual barrier, as it is provided by common physical fence technology, is missing. As far as we can tell, there is no knowledge yet on i) how the reactions of animals to electric pulses from collar and to electric wire fences differ in growing heifers on continuous pastures using the Nofence system and ii) how intensely the (continuously observed) behaviour is affected after having received an electric pulse. We have approached the latter question by comparison of the time needed after an electric pulse from the Nofence collar against an electric pulse of the PF until returning to grazing. The time needed after receiving a pulse was significantly shorter after VF collar pulse than after physical fence contact (Fig. 4).

According to an early study, the same physical stressor produces different effects, depending on whether its occurrence is predictable or not (Weiss, 1970). The always constant pulse energy of the VF collar pulse, reliably announced by the acoustic signal, might be an advantage over physical fences although it is not possible to clarify this point with the current study. The pulse energy of the physical fence likely varies in intensity in relation to the contact duration, fence wire conductivity and distance to the device, which all determine the local charging of the fence at the contact

Table 4
Output of linear mixed effect models for the effects of fencing system, day of sample and their interaction with FCMs, estimated herbage consumption and live weight gain of the Fleckvieh heifers over three time replicates (n = 36 days). Shown are *F*-values, degrees of freedom and *P*-values. Day of sample has two levels (day eight and day twelve) in FCM measurements and three levels for herbage consumption (days one, eight and twelve).

Target variable	Fixed and interaction effects	numDF	denDF	*F*-value	*P*-value
FCMs	Fencing system	1	19	0.5	0.49
	Day of sample	1	52	0.5	0.46
	Fencing system × Day of sample	1	52	0.1	0.79
Estimated herbage consumption	Fencing system	1	36	0.2	0.649
	Day of sample	2	36	14.8	<0.0001***
	Fencing system × Day of sample	2	36	0.3	0.7285
Live weight gain	Fencing system	1	19	0.03	0.8594

Abbreviations: FCMs = faecal cortisol metabolites; numDF = degrees of freedom in the numerator; denDF = degrees of freedom in the denominator.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

point. If it is possible for cattle to learn to avoid a suitable level of electrical stimulus, it is likely not harmful to them (Lee et al., 2008). However, as reported in preliminary studies using eShepherd® e.g. Verdon et al. (2021); Langworthy et al. (2021), behavioural responses to electric pulses from VF technology resulted in no measurable deterioration in animal welfare, although the number of received VF electric pulses is higher than the number of pulses received from a common electric PF. Given the short-term nature of our study and the potential of longer-term accumulation of stress effects in animal body tissue (e.g. cortisol metabolites in milk), future studies over extended periods will help to exclude remaining doubts on animal behaviour.

An advantage of the Nofence VF technology is that recording the electric pulses in the stored collar data makes remote monitoring of the animals possible, which is not the case with physical fencing and the positioning data may overall be an advantageous step towards continuous animal welfare monitoring on pasture.

Motion behaviour

An increased stress level could be reflected by more locomotion as manifested in more steps walked. The average quantity of walked steps per hour increased from time replicate one to time replicate three, and there was a tendency of more steps in the PF group. In time replicate three, the herbage availability was less, furthermore, there was a tendency of lower herbage availability in the PF groups compared to the VF groups at all days of sample. A study by Hamidi et al. (2021) compared walking efforts retrieved from GPS collars of suckler cows as affected by grazing intensity in a long-term experiment. There, the herbage availability seemed to affect the daily walking distances which was reflected in the greatest effort of walking under conditions of lowest herbage mass. The average hourly walking distance per cow (arithmetic Mean ± SD) was 142.2 m ± 75.91 m in that latter study. When we use average step lengths of 0.28 m for grazing and 0.5 m for inter-bout step lengths (Rook et al., 2004) and classify the steps retrieved from the IceTag accelerometers according to observed cattle behaviour during observation, distances of 219.3 ± 81.30 m (mean ± SD) for the PF group and 204.5 m ± 96.87 m per hour for the VF group (mean ± SD) resulted. These values are much higher than for suckler cows, but close to the ones reported for young steers (216 m) measured with GPS collars over 11-day periods (Trotter et al., 2010). Therefore, it seems unlikely that the fencing system had a negative impact on the cattle motion behaviour. However, walking behaviour was highly variable in terms of duration and distance travelled in previous studies (Kilgour, 2012).

For evaluating the motion behaviour of the VF group, Fig. 3 showed GPS positions of the VF group pre- and postshifting the virtual boundary which was used to evaluate whether there was a lack of understanding of the VF line position for the heifers (Lee and Campbell, 2021). The heifers used the whole available areas of the paddock, which was similar to (Campbell et al., 2019). Areas near the virtual boundary were not avoided by the animals which was confirmed by the visual observation. Consequently, heifers understood that the VF line represented a barrier even after shifting the line on day eight.

Physiological indicators of animal well-being

We found no indications of altered livestock performance in the present study. In addition, we have not observed significant differences in herbage availability between the VF and PF groups. Based on the herbage consumption and animal performance, it seems unlikely that the heifers were too stressed to perform usual behaviour when confined with virtual fencing. In the study by Campbell et al. (2019), a reduced animal performance (not in all cohorts and due to an initial higher starting weight in the control group) was recorded when using virtual fencing on Angus cattle. In our study, however, we found no differences in livestock performance as affected by the fencing system.

FCM concentrations, as a non-invasive indicator of adrenocortical activity (Palme, 2019), were (although not statistically significantly) higher in the PF group compared to the VF group on both days of sampling and they generally decreased in both groups from mid-grazing to postgrazing. This was in accordance with weekly measured FCMs reported in the study by Campbell et al. (2019), where end point values were also quite similar to each other. Values in both groups were in the lower region of the normal range (Ivemeyer et al., 2018). Thus, based on this parameter, there was also no evidence of increased physiological stress in either group during the trial.

Limitations of our study

When evaluating the acoustic signals and electrical pulses of the collars, it should be noted that the programmed transition from teach mode to operating mode did not take place as intended. The collars revert back to teach mode each day because the internal count of acoustic signals and electric pulses was reset by deactivation outside of pasture access. This might have increased the number of electric pulses as an animal will possibly receive more pulses in the moment the collars switch from teach mode to operating mode (max. once per day and animal). However, this does not affect the general learning of the system.

Conclusion

Our study provides an evaluation of cattle behaviour using the VF system of Nofence® on a group of Fleckvieh heifers over a duration of 12 days (three time replicates, 36 days in total) which can serve as an example for training schedules in future trials. Our schedule can be recommended for future studies as the visual support of the virtual fence ensures ‘gentle’ learning and the shifting of the VF line ensures that the animals understand the system without visual cues. Given the lack in response of animal behaviour to virtual fencing, we found compelling reasons for further utilisation and exploration of this technique in Europe. None of the considered behavioural and physiological parameters were affected systematically by the fencing system underlining the potential of this smart livestock farming technology. After electric pulses emitted from the Nofence® collar, cattle returned faster to grazing than after contact with the physical fence. We can draw the conclusion that animal welfare was not endangered by using VF when compared to conventionally (electric tape) fenced groups, which leads us to reject our hypothesis that VF has a negative effect on grazing heifers compared to PF.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2022.100614.

Ethical approval

The trial was approved by the animal welfare service of the LAVES (Lower Saxony State Office for Consumer Protection and Food Safety (Germany) – ref. Number: 20/3388).

Data and model availability statement

The data/models were not deposited in an official repository. The datasets generated for this study are available on request to the corresponding author.

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Declaration of interest

None.

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