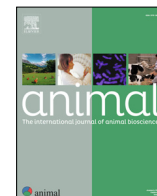




Animal

The international journal of animal biosciences



Supporting rotational grazing systems with virtual fencing: paddock transitions, beef heifer performance, and stress response



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ARTICLE INFO

Article history:

Received 21 April 2024

Revised 18 December 2024

Accepted 20 December 2024

Available online 28 December 2024

Keywords:

Cattle welfare

Glucocorticoids

Grazing management

Precision grazing

Sustainable grazing

ABSTRACT

Animal welfare is integral to sustainable livestock production, and pasture access for cattle is known to enhance welfare. Despite positive welfare impacts, high labour requirements hinder the adoption of sustainable grazing practices such as rotational stocking management. Virtual fencing (VF) is an innovative technology for simplified, less laborious grazing management and remote animal monitoring, potentially facilitating the expansion of sustainable livestock production. VF uses Global Navigation Satellite System technology, wireless communication, and stimuli (auditory and electrical) to manage livestock movements and contain animals without physical barriers. Training animals to associate the auditory cue with the subsequent aversive stimulus enables effective livestock containment without physical barriers. While previous studies have largely dispelled concerns about adverse effects on cattle behaviour associated with the use of VF collars, there is limited knowledge regarding the impacts on animal physiology, particularly in rotational stocking systems. Addressing this knowledge gap, this study investigated differences in diet digestibility, livestock performance, and stress response of beef heifers on pastures using a VF compared to a physical electric fence. The study was conducted over 8 weeks, subdivided into two grazing cycles, with 32 heifers in four groups. Each experimental pasture was subdivided into four paddocks. The study monitored the interaction with the VF by analysing the temporal development of the ratio of auditory and electrical cues (success ratio and confidence ratio) emitted by the collars. Additionally, the grassland herbage quality, BW gain, and concentrations of faecal cortisol metabolites (FCMs) were assessed, as well as the time required for animals to cross into a new paddock. VF success ratios increased in the second grazing cycle, reflecting enhanced adaptation over time. Similarly, the reduction in time taken to cross into new paddocks in the VF groups indicated that animals learned to interact with the VF and rely on the auditory cues for directing movements. The absence of a significant effect of the fencing system on FCMs suggested that stress was unrelated to the VF technology. Further, animal performance was not affected as indicated by similar BW gains under both fencing systems. This study also attempts to establish a benchmark threshold for successful responses to the auditory cues, allowing comparative evaluation of VF systems. Overall, under rotational grazing, VF did not adversely impact animal welfare or performance compared to physical fencing, opening avenues for further exploration of VF technology in diverse grazing conditions.

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Implications

This study evaluated virtual fencing as an alternative to electric fencing in rotational grazing systems, addressing the research

question of whether virtual fencing can effectively manage cattle while ensuring animal welfare. The research focused on cattle performance and stress response as key animal welfare indicators. The results indicate that virtual fencing effectively contained and managed cattle without negatively affecting stress levels or productivity. Cattle adapted to the system quickly, demonstrating improved interaction with auditory cues over time. These findings suggest that virtual fencing can support sustainable grazing practices while

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upholding welfare standards, providing an innovative tool for modern livestock production.

Introduction

Animal welfare is considered an essential component of sustainable livestock production (Buller et al., 2018; Dwyer, 2020; Keeling, 2005). In trials with dairy cattle, access to pasture has been found to significantly improve animal welfare compared to confined housing systems (Arnott et al., 2017; Crump et al., 2019). Additionally, studies have outlined the potential for effective grazing management practices, such as rotational stocking, to mitigate greenhouse gas emissions from pasture-based livestock production systems and contribute to improved animal welfare through the provision of higher-quality forage (Zubieta et al., 2021a; Zubieta et al., 2021b). Thus, by promoting livestock welfare and reducing the environmental impacts of livestock production, grassland-based systems play a crucial role in progressing towards the sustainable development goals (United Nations, 2015).

Although a positive welfare balance can lead to improved animal wellbeing and benefit production efficiency (McInerney, 2004; Rault et al., 2020; United Nations, 2016), the adoption of rotational grazing management practices is often restricted by the increased labour requirements. By providing a tool for simplified adjustment of pasture boundaries and remote animal monitoring, virtual fencing (VF) reduces labour requirements for fencing (Anderson et al., 2014). Virtual fencing technologies utilise Global Navigation Satellite System technology, wireless communication, and stimuli (auditory and electrical) to contain animals without physical barriers. Animals are typically fitted with smart collars that emit auditory cues when animals approach a predefined boundary. If the animal continues towards the boundary, the collar then emits an electrical cue.

Training animals to associate the auditory cue with the aversive stimuli enables the containment and control of livestock without the need for traditional electric fences (Campbell et al., 2019; Confessore et al., 2022; Nyamuryekung'e et al., 2023). The technology thus holds the potential to facilitate pasture-based production systems (Umstatter et al., 2015) and the expansion of other sustainable livestock production practices, such as the integration of cattle in oil palm cultivation (Grinnell et al., 2022). Additionally, the implementation of a more dynamic grazing management approach with frequent changes in pasture boundaries could contribute to the stabilisation of long-term pasture productivity within extensive grazing systems through mitigation of the formation of grazing patches and subsequent nutrient redistribution (Grinnell et al., 2023).

Despite the current implementation of VF in farming practices in various countries including Norway, the UK, the USA, New Zealand, and Australia along with a recent report (AWC, 2022) acknowledging the potential welfare benefits of VF, there is a notable absence of established legal regulations governing its utilisation and the effect of VF on animal welfare is a topic of ongoing public debate (Wilms et al., 2024). As consumer influence grows in agriculture (Agnew et al., 2023), the successful adoption of VF hinges on prioritising and effectively communicating beneficial animal welfare outcomes to build trust and ensure consumer acceptance of VF-produced products (Stampa et al., 2020). Similarly, the adoption of VF in practice is inherently linked to its effects on animal performance. This is because revenue from milk production and BW gain (BWG) directly correlate with the overall profitability of the production system. While Hamidi et al. (2022) reported no discernible impact of the fencing system on BWG of cattle on continuously stocked pastures, many studies investigating VF in rotational stocking systems thus far – e.g. Campbell

et al., 2017; Confessore et al., 2022; Hamidi et al., 2023; Marini et al., 2022; Ranches et al., 2021 – did not address animal performance, a crucial indicator of the economic profitability of beef production. Knowledge is particularly limited on the effects of VF on animal physiology and performance in systems with rotational stocking management.

To address this gap in knowledge, the present study aimed to assess the suitability of VF for sustainable livestock production. Previous publications from the same trial did not compare VF with traditional physical fencing using an electric wire. Instead, they focused on combining VF with remote sensing (Hamidi et al., 2023) and on training cattle to use VF (Hamidi et al., 2024). This study, however, aimed to compare the effects of VF on cattle performance and stress indicators with those of a traditional physical fence with electric wires in rotational stocking management. The general hypotheses of this study were that (i) virtual fencing can effectively contain cattle in a rotational stocking system, (ii) virtual fencing has no adverse effect on cattle weight gain, organic matter digestibility of ingested herbage, and faecal cortisol metabolite concentrations (FCMs), and (iii) that cattle learn to successfully interact with the virtual fencing system within short-term grazing cycles of several days.

Material and methods

The study was conducted from July to September 2021 at the experimental farm of the University of Göttingen in Relliehausen, Lower Saxony, Germany (51°46'55.9"N, 9°42'11.9"E). The long-term climatic averages of the German Weather Service reference period (1991–2020) show an annual average temperature of 9.8 °C, annual precipitation sum of 764 mm, and radiation of 1500 h. For July, August, and September 2021, monthly weather data showed an average temperature of 18.6, 16.9, and 15.6 °C, precipitation sum of 68.8, 85.7, and 23.8 mm, and radiation of 9.5, 7.2, and 7.3 h, respectively. Measurements originate from a climate station in Bevern (51°51'10"N 9°29'42"E), 21 km from the experimental site (Deutscher Wetterdienst, 2022). This article utilises the VF terminology outlined by Ehlert et al. (2024), maintaining consistency and coherence within the scientific discourse.

The grassland on the experimental site represents the association of *Lolio-Cynosuretum*. The pregrazing botanical composition comprised 91.5% grasses, 7.4% dicotyledonous non-legumes, and 0.7% legumes (estimated yield proportions) with *Lolium perenne*, *Dactylis glomerata*, and *Elymus repens* as the most abundant species.

Measurements of standing herbage height, mass, and quality

Table 1 displays information on the herbage quality of the standing herbage on offer and the compressed sward height in the experimental pasture. The compressed sward height was measured using a rising plate meter (Castle, 1976), at 30 locations within each paddock, before and after grazing. Additionally, the standing herbage mass and quality were assessed prior to grazing in each paddock from samples taken from four locations used for compressed sward height measurement, by cutting near the soil surface within a steel frame of 30 cm diameter. Samples were cooled immediately after cutting and frozen within 8 h of sampling. Subsequent analysis employed Near-Infrared-Reflectance Spectroscopy (NIRS, Phoenix 5000, Bluesun Sci, USA), involving two scans of each sample after drying at 60 °C for 48 h and milling to 1 mm, for assessment of *in vitro* organic matter digestibility, CP, ADF, NDF, and metabolisable energy concentrations. Concentrations were then processed and predicted using the large calibration data set stored on a central server (VDLUFA Qualitätssicherung,

Table 1

Pre- and postgrazing compressed sward height (CSH) and pregrazing herbage on offer (HM) and herbage quality (ADF, NDF, *in vitro* digestible organic matter [DOM]) in the two grazing cycles (C1, C2) of Fleckvieh heifers. Displayed are means $\pm$ SD across paddocks and replications for two fencing treatments.

Grazing cycle	Variable	EF		VF	
C1	pregrazing CSH (cm)	20.5	$\pm$ 5.0	19.6	$\pm$ 4.4
	pregrazing HM (kg/ha)	1 335	$\pm$ 550	1 198	$\pm$ 515
	pregrazing CP (g/kg DM)	147.5	$\pm$ 17.6	142.4	$\pm$ 22.9
	pregrazing ADF (g/kg DM)	319.7	$\pm$ 18.9	318.4	$\pm$ 27.6
	pregrazing NDF (g/kg DM)	535.0	$\pm$ 34.3	521.0	$\pm$ 56.8
	pregrazing DOM (%)	68.5	$\pm$ 3.1	69.3	$\pm$ 4.1
	pregrazing ME (MJ/kg DM)	10.1	$\pm$ 0.4	10.2	$\pm$ 0.6
	postgrazing CSH (cm)	12.0	$\pm$ 4.2	11.8	$\pm$ 4.3
C2	pregrazing CSH (cm)	15.9	$\pm$ 4.1	15.1	$\pm$ 4.4
	pregrazing HM (kg/ha)	1 340	$\pm$ 466	1 349	$\pm$ 454
	pregrazing CP (g/kg DM)	122.9	$\pm$ 22.1	125.2	$\pm$ 23.1
	pregrazing ADF (g/kg DM)	348.6	$\pm$ 33.4	341.4	$\pm$ 20.0
	pregrazing NDF (g/kg DM)	573.1	$\pm$ 55.9	570.1	$\pm$ 33.9
	pregrazing DOM (%)	65.2	$\pm$ 5.0	66.0	$\pm$ 2.9
	pregrazing ME (MJ/kg DM)	9.6	$\pm$ 0.7	9.7	$\pm$ 0.4
	postgrazing CSH (cm)	10.9	$\pm$ 3.7	11.3	$\pm$ 4.4

EF = physical electric fence.

VF = virtual fence.

ME = metabolisable energy.

NIRS GmbH, Kassel, Germany, [Tillmann, 2010](#)). The data sets for NIRS of the quality parameters CP, NDF, and ADF contained 3 169, 866, and 1 088 calibration samples, respectively. SE of calibration for CP, NDF, and ADF were 0.76, 1.73, and 1.32%, and the corresponding SE of cross-validation were 0.77, 1.79, and 1.35%.

Virtual fencing technology

All heifers were equipped with VF collars (Nofence®, Batnfjordsøra, Norway). The Nofence® VF technology consists of a 1 446 g battery- and solar-powered collar, a smartphone application, and an online web portal, both connected to a back-end database. The collar is fastened around the animal's neck by two metal chains that are held together by a rubber neck strap. It communicates with the app via the cellular network (built-in SIM card) and has an integrated Global Navigation Satellite System receiver for location tracking.

The VF system relies on associative learning, where animals develop a connection between an auditory cue (82 dB) emitted from the collar and an upcoming aversive stimulus. When the GNSS positions the animal at the virtual boundary, the collar emits the auditory cue. The auditory cue consists of a tone scale that increases in pitch. If the animal shows the desired response, namely turning away from the virtual boundary and back into the pasture, the auditory cue declines in pitch and stops. If the animal continues to approach or passes the virtual boundary, the auditory cue increases in pitch until, at the highest note, an electrical cue is triggered (0.2 J at 3 kV for 1 s). Upon passing the virtual boundary, a maximum of three consecutive pulses, each preceded by an auditory cue, is emitted. After that, the animal is considered escaped, an escape notification is sent via the smartphone app and the collar remains inactive until the animal returns to the virtual enclosure.

Auditory cues and electrical cues emitted from the collars, escape notifications, routine status updates, GNSS locations, and other data are recorded in a.csv file and made available for download for scientific purposes.

Experimental design and animals

The trial consisted of two consecutive grazing cycles (**C1**, **C2**) of 15 days each, with a 20-day intermission. Based on initial BW and age, a total of 32 Fleckvieh heifers were randomly assigned to one of four groups of eight heifers each (see [Hamidi et al., 2023](#) for

more detailed information on the animals). The groups were then randomly allocated to one of two fencing treatments, VF or traditional physical fencing with electric wire (**EF**). Both fencing systems were, thus, replicated twice across the groups. Average age and BW ($\pm$ SD) at the start of the experiment were 13.9 ± 1 months, 397 ± 47 kg for the EF groups and 13.5 ± 1 months, 409 ± 51 kg for the VF groups. Prior to the trial, animals of the VF groups were trained to the VF technology and all animals were released to a pasture with a physical electric fence near the experimental farm for grazing adaptation. The experimental design included four rectangular experimental plots of circa 2 ha each, with each plot subdivided into four paddocks (A, B, C, D) of roughly 0.5 ha each ([Fig. 1](#)). Two plots were located on either side of a road, and on both sides, one group was subjected to VF and the other to EF. Allocation of one group per paddock resulted in an average stocking density per paddock of 14.4 ± 1.9 livestock units (1 livestock unit = 500 kg BW)/ha. For proper distinction during observations, each of the animals was marked individually on the side and back with animal colour spray (Raiderx®, Dettingen/Erms, Germany).

The perimeter of each experimental plot was fenced with electric wire with two strands of cord connected to a power device (Siepmann®, Herdecke, Germany) with a pulse energy of up to 4.1 J. Metal posts marked the paddock corners, while plastic posts were used on the straights. A gate of approximately 5 m width was included for entry into the plots (paddocks A). The subdivision of the EF plots was done with the same two-strand electric wire as used for the perimeter fence. Additional gates of approximately 3 m width were installed between neighbouring EF paddocks for the transitions ([Fig. 1](#)).

For the VF groups, the subdivision into paddocks was done using only the virtual fence. Consequently, each VF paddock had four virtual boundaries, with the two boundaries along the plot's perimeter supported by the surrounding EF. Metal posts placed in the paddock corners served as orientation markers for the researchers when establishing the virtual boundaries. The virtual boundary along the perimeter of the VF paddocks was placed approximately 5 m inside the physical electric fence, to enable the emission of cues from the VF collars. The boundary placement was defined by the point at which the auditory cue is activated, allowing sufficient space for the animals to respond to the signal before reaching the physical perimeter fence. The animals were familiar with this setting from the training ([Hamidi et al., 2024](#)). Consequently, each VF paddock had two borders with both physical and virtual fences and two borders with only a virtual fence. On

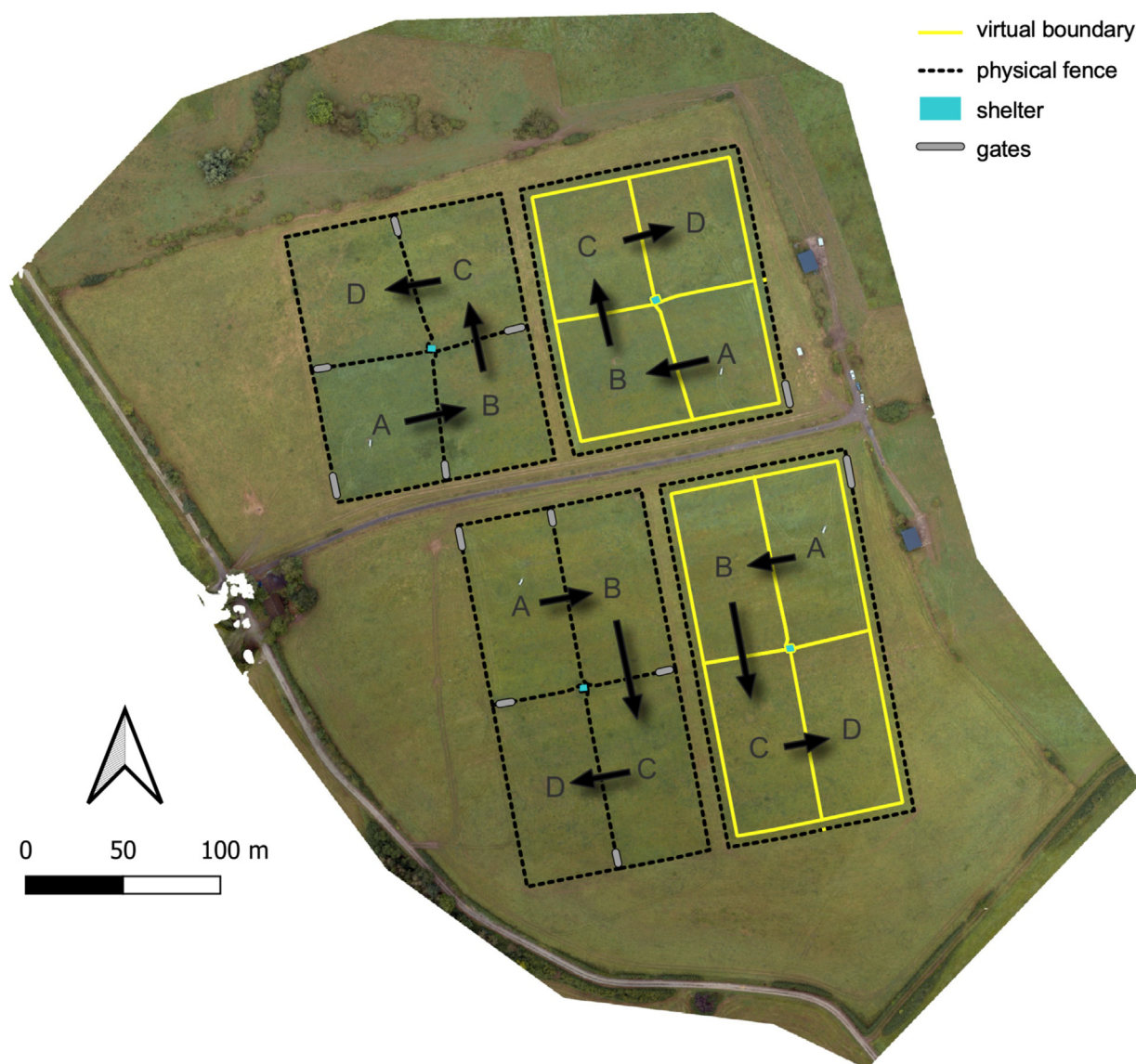


Fig. 1. Satellite view of the experimental area with four experimental plots for Fleckvieh heifers. Virtual fence lines (yellow) and physical electric fence lines (black dashed) mark the perimeter and paddock subdivisions of all plots. Gates were installed between paddocks (grey rectangles). Shelters (blue squares) in the centre of each plot were always accessible to the animals. The arrows mark the transition direction from paddocks A to D in each experimental plot. (Image credit: Christoph Hütt).

the first day of both grazing cycles, the inner division of the first VF paddock A was further supported by a two-strand electric wire fence which was placed approximately 5 m outside the VF and successively removed (first the wire, then the posts) on day two on pasture.

The heifers assigned to the VF groups were trained to the VF technology in a 12-day training period preceding the trial, as described by Hamidi et al. (2024), similar to the training protocol described by Hamidi et al. (2022). All animals were naïve to the VF technology prior to the training but were familiar with physical electric fencing. During the training of the VF groups, the EF animals were kept on a physically fenced pasture for adaptation. Throughout the trial, the collars for the EF groups were powered on but not assigned to a virtual pasture, thus allowing reporting of GNSS positions while the fence function remained inactivated. Consequently, no sensory cues were delivered from the VF collars to the animals in the EF groups at any time throughout the trial. Animals in the EF groups were not trained with the VF.

In C1, the animals of the VF groups escaped when the supportive EF was removed on day two. Consequently, the supportive EF was reinstalled and successively removed on day three. The heightened agitation of the cattle resulting in the escape from the experimental pasture upon removal of the EF on day two can be attributed to their unfamiliarity with the surroundings and the general commotion of the trial.

Rotational grazing management: paddock transitions

All groups moved from one paddock to the next after 3–4 days of grazing, resulting in four paddock transitions within one grazing cycle of 15 days in length. The day of paddock transition was denoted as day 0, while day 1 to day 3 refer to the subsequent days on the same paddock up to the next day of paddock transition (day 0). Because some paddock transitions occurred after 3 days of grazing on day 2, data from day 3 were excluded from the analyses of the success probability and confidence ratio. Paddock transitions were usually initiated around 1100 h on day 0, with minor devia-

tions occurring for organisational reasons. For the EF groups, the gate to the new paddock was opened and closed immediately after the last animal had passed through. For the VF groups, the virtual boundary was expanded to encompass both the current and the new paddock. After all heifers had passed into the new paddock, the virtual pasture was adjusted to encompass only the new paddock. In all groups and treatments, animals were allowed to transition at their own pace. The metal fence posts on the perimeter of each experimental pasture served as visual orientation for the researchers in setting the virtual boundaries. Thus, for the VF group, the border of each paddock was marked by these metal fence posts. The virtual boundaries were set and adjusted by the same researcher, who would physically move towards the marker posts while setting the boundary.

After completing the first grazing cycle (C1), animals grazed continuously on an area surrounding the experimental pasture within a physical electric wire fence for a 20-day intermission without paddock transitions. For the second grazing cycle (C2), the same procedure of a 15-day grazing period was replicated, with each group being assigned to the same experimental plot and fencing treatment as in C1.

The available grassland herbage on offer was sufficient to always meet animal requirements. The animals always had *ad libitum* access to water via water tanks placed in the centre of the paddocks. On the days of paddock transition, the water tanks were moved to the new paddock by tractor. The timing of the move on day 0 varied for organisational reasons. Animals were provided constant access to a roofed shelter located in the centre of each experimental pasture.

For each paddock transition, the paddock transition time was measured in seconds for each animal, representing the total time it took each animal to move to the new paddock, starting at the opening of the border and concluding with the crossing into the new paddock. Time was recorded using stopwatches by the two researchers responsible for the animal behavioural observations and, on one occasion, a student assistant who had been practising the procedure on previous days of paddock transition. The recorded paddock transition time for each animal further enabled the calculation of the time difference between the first and last animal (paddock transition time span) of a group to cross the border. Thus, while the paddock transition time indicates the time individuals took to discover the open border and make the transition, the paddock transition time span indicates the time between the first and last individual.

Generally, animals were left to transition in their own pace without human intervention. However, on the first paddock transition in C1, after the animals of both VF groups and one EF group had not approached the new paddocks more than 3 h after opening the gate, three researchers slowly moved into the paddock and herded the animals calmly towards the gate (EF) or the new VF paddock (VF). Thus, the first paddock transition in C1 was excluded from the analysis of paddock transition time and paddock transition time span.

Cattle response to the virtual fence

The desired, i.e. successful response to the VF was defined as an animal responding to the auditory cue by returning into the virtual enclosure without triggering an electrical cue. Conversely, instances where collars emitted an electrical cue were considered unsuccessful responses, because the animals failed to exhibit the desired response to the auditory cue.

In this study, the understanding of and interaction with the VF is expressed in the success ratio, calculated per animal and day from the VF collar data output, following Eftang et al. (2022), as:

$\text{Success ratio} = (\text{No. Audio Cues}$

$- \text{No. Electric Pulses}) / \text{No. Audio Cues}$

where No. denotes the number of and, consequently, at a success ratio of 1 no electrical cues were triggered, indicating a correct response to every auditory cue emitted by the collar. Consequently, the success ratio decreases with every electrical cue emitted by a collar.

Due to failure to meet model assumptions, analysis of temporal effects on the success ratio was not possible. Consequently, a success threshold was defined based on the success ratio as the lower fence of a boxplot:

$\text{Success threshold} = Q1 - 1.5 \times IQR$

Q1 is the first quartile representing the 25th percentile. IQR is the interquartile range between the first and third quartile, measuring the spread of the middle 50% of the success ratio dataset, calculated as the difference between the third quartile (Q3) and Q1.

The success threshold was implemented to categorise animal responses to the VF in a binary variable, to investigate the probability of a successful response to the VF. Success ratios above the success threshold were categorised as successes (1), whereas success ratios below the success threshold were defined as unsuccessful (0) in the binary variable. The success threshold thus provides a single value that serves as a reference point for a standardised definition of a successful interaction with the fence. This unified measure can then be used to compare the success ratios of individual animals across different groups.

The confidence ratio was calculated per animal per day, following the method outlined by Hamidi et al. (2024), using the following formula:

$\text{Confidence ratio} = \text{success ratio} \times \frac{S'}{20}$

S' represents the number of successful interactions capped at a maximum of 20 successful interactions. The confidence ratio functions as an adjustment to the success ratio, accounting for the proportion of successful auditory cues while excluding those followed by an electrical cue. Thus, while the success ratio reflects the animals' responses to the electrical cue, the confidence ratio weighs the success ratio to reflect the animals' interaction with the auditory cue.

Faecal nitrogen and faecal cortisol metabolites

Faecal samples were collected at two sampling times, the first day and the last day of grazing, in each grazing cycle to determine the FCMs, a non-invasive measure of adrenocortical activity (Palme, 2019). Up to three samples per animal were obtained directly from the pasture immediately after defaecation, ensuring prompt cooling and subsequent freezing (-18°C) within 8 h of collection.

Further, faecal samples collected at the end of each grazing cycle were used for the analysis of faecal nitrogen content, following the methodology outlined by Schmidt et al. (1999), which serves as an internal marker to determine the organic matter digestibility of ingested herbage (fOMD). For this analysis, faecal samples were defrosted, dried at 60°C for 48 h, and weighed. Subsequently, the samples were milled to pass through a 1 mm screen, dried at 105°C for 24 h, and weighed to determine DM content. Finally, for the determination of total organic matter, the samples were incinerated in a muffle furnace (Nabertherm, Lilienthal) at 550°C for 3 h. The samples were milled to pass through a 0.2 mm screen and subjected to elemental analysis (vario EL cube, Elementar, Langenselbold) for total C and total N analysis.

Subsequently, fOMD was calculated from the faecal N concentration according to Schmidt et al. (1999) as:

$$fOMD[\%] = 95.90 - 460/N[g/kg \text{ OM}] - 0.1582 \text{ GD} + 0.00062 \text{ GD}^2$$

GD refers to the number of growing season days after April 30 to consider seasonal fluctuation in the estimation for increased accuracy, and OM refers to organic matter content of the faeces.

The FCMs were extracted from the defrosted faecal samples according to Palme and Möstl (1997). For this, a portion of the wet faeces (i.e. 0.5 g), suspended in 5 mL of 80% methanol, was shaken and centrifuged, and FCMs were measured in an aliquot of the supernatant via an 11-oxo-aetiocholanolone enzyme immunoassay (EIA, Palme and Möstl, 1997). This EIA has been successfully validated for cattle and FCMs were found to reflect the cortisol secretion in the body approximately 12 h earlier (Palme et al., 1999).

Organic matter digestibility

Hand-plucked biomass samples were collected pregrazing in each paddock, 3–4 days before each collection of faecal samples. For each paddock, three samples were taken, consisting of five to ten manual hand pickings of the upper third to half of the standing herbage. The samples were cooled immediately and processed with NIRS (Phoenix 5000, Bluesun Sci, USA) for the assessment of *in vitro* organic matter digestibility of the herbage on offer (hOMD, Schmidt, 2004). Hand-plucking biomass samples from the top layer of vegetation mimics the grazing behaviour of cattle and thus better depicts the actually consumed biomass compared to cut samples. hOMD was calculated from parameters estimated using NIRS according to Schmidt (2004):

$$hOMD (\%) = 100 \times (940 - CA - 0.62 \text{ EULOS} - 0.000221 \text{ EULOS}^2 / (1000 - CA))$$

CA indicates crude ash and EULOS indicates enzymatic insoluble organic matter. The ratio of fOMD to hOMD was used to assess the extent of selectivity (Hodgson, 1990) of foraging cattle, to evaluate potential differences in herbage intake patterns between the fencing treatments. Therefore, pregrazing hOMD values were averaged over the grazing cycle and fencing treatment and compared to the respective animal-individual fOMD values at the end of each grazing cycle.

BW gain

BW measurements were done for all heifers prior to and after each grazing cycle for the calculation of average daily BWG. For weighing, a scale (Model FX 31) with weigh beams (Model WB 6A, both Texas Trading GmbH, Windach, Germany) was used and weighing was performed in the mornings. All animals were weighed prior to the start of the trial, at the time of collar fitting, and before and after each grazing cycle.

Statistical data analysis

All data analyses were performed in R version 2022.07.2 (R Core Team, 2021), using the statistical package ‘glmmTMB’ (Brooks et al., 2017) for modelling. Normality of the residuals was checked using the packages ‘DHARMa’ (Hartig, 2022) and ‘car’ (Fox and Weisberg, 2019), and posthoc comparisons were done using Tukey’s HSD test in the package ‘emmeans’ (Lenth, 2023). Cook’s distance (Cook, 1977) was used as a diagnostic tool for identifying and removing outliers. In the analysis of FCMs, 5.5% of the dataset

was removed to improve the model fit, while no outlier removal was performed for other variables.

For the target variables paddock transition time and paddock transition time span, data analysis was performed using generalised linear mixed effects models (GLMM) with a gamma distribution and a logarithmic link function to account for the positively skewed nature of the data. The models included the fixed effects and interaction of fencing system and grazing cycle and the random effects of the paddock transition nested in group.

To analyse the probability of a successful response to the VF (success probability) from the binary success variable, data analysis was performed using a GLMM with binomial distribution and the fixed and interaction effects of paddock and days since paddock transition (day 0–day 3), the additive effect of grazing cycle, and the crossed random effect of the individual animal and the group. One animal was excluded from the analysis of the success probability as well as the auditory and electrical cues because the data were not transmitted by the VF collar in C1 due to connectivity issues. Further, day 3 was excluded from the dataset for the analysis of the success probability and the confidence ratio, as some rotations had no day 3.

The confidence ratio was analysed using a GLMM with beta regression. The model included the fixed effects of grazing cycle, paddock, and days since paddock transition, along with their interactions, as well as the random effects of the individual animal nested in the group.

For the target variable FCMs (ng FCM/g faeces), data analysis was performed using a GLMM with Gaussian distribution and the fixed effects and interaction of fencing system, grazing cycle, and sampling time (beginning or end of grazing cycle), and the random effects of the individual animal nested in the group. Additionally, the target variable FCMs was analysed for the VF groups only, with the aim to examine the effect of the number of pulses applied to an animal on its FCMs at the end of the relevant grazing cycle. For this, a GLMM with a Gaussian distribution and the fixed and interaction effects of electrical cues (total number of pulses per animal per grazing cycle) and grazing cycle, and the random effect of the individual animal nested in the group were used.

For the target variables fOMD (%), and hOMD (%), data analysis was performed using a GLMM with Gaussian distribution and the fixed effects and interaction of fencing system and grazing cycle, and the random effect of the individual animal (fOMD) or the group and paddock (hOMD). The ratio of hOMD to fOMD, was analysed using a GLMM with Gaussian distribution and the fixed effects and interaction of fencing system and grazing cycle, and the random effect of the individual animal nested in the group.

For the target variable BWG (kg per animal per day), data analysis was performed using a GLMM with Gaussian distribution and the fixed and interaction effects of fencing system and grazing cycle, and the random effect of the individual animal nested in group. Additionally, the target variable BWG was analysed for the VF groups only, with the aim to examine the effect of the number of pulses applied to an animal on its BWG within the designated grazing cycle. For this, a GLMM with Gaussian distribution and the fixed and interaction effects of electrical cues (total number of pulses per animal per grazing cycle) and grazing cycle, and the random effect of the individual animal nested in group was used.

A significance level (α) of 0.05 was used throughout the analyses, corresponding to a 95% confidence level.

Results

No technical issues interfered with paddock transitions of physically and virtually fenced animals. In the following section,

we display the results of multiple analyses. For detailed information on the respective test statistics, please refer to the supplementary material (Tables S1–S11).

Paddock transitions

The analysis revealed a significant effect of the fencing system ($\chi^2 = 20.7$, $df = 1$, $P < 0.001$) and grazing cycle ($\chi^2 = 8.0$, $df = 1$, $P < 0.001$), but not their interaction on the paddock transition time ($\chi^2 = 2.3$, $df = 1$, $P > 0.05$). Specifically, multiple comparisons indicated a significantly longer paddock transition time for VF compared to EF in C1 ($P < 0.001$). The observed longer paddock transition time for VF in C2 was, however, not statistically significant ($P > 0.05$, Fig. 2).

The analysis of the paddock transition time span from first to last animal revealed a significant effect of the fencing system ($\chi^2 = 21.3$, $df = 1$, $P < 0.001$) but not the grazing cycle ($\chi^2 = 1.2$, $df = 1$, $P > 0.05$) or their interaction ($\chi^2 = 1.6$, $df = 1$, $P > 0.05$). Further examination of the data through multiple comparisons revealed a significantly higher paddock transition time span ($P < 0.05$) for VF with 456.2 ± 217.4 s compared to 24.0 ± 11.4 s for EF in C1 (Fig. 3). However, in C2, the difference was not significant ($P > 0.05$), with 413.7 ± 241.5 s and 82.8 ± 48.3 s for VF and EF, respectively (Fig. 3).

Cattle response to the virtual fence

Fewer escapes (animals receiving three consecutive pulses) were recorded in C2 than in C1, and no escapes occurred on day 3 in either grazing cycle (Table 2).

Success ratios ranged from 40 to 100% between animals and across different days, with an average success ratio ($\pm$ SD) of $94.6 \pm 9.2\%$ in C1 and $97.9 \pm 4.8\%$ in C2. The daily success ratios of all animals are given in Supplementary Fig. S1 and Supplementary Fig. S2. Quantile analysis conducted on the success ratio variable revealed a first quartile (Q1) of 0.96 and an interquartile range (IQR) of 0.04, defining the success threshold at a success ratio of 90%. Consequently, 50 observations, representing 10.7% of the initial dataset, were identified as unsuccessful, while 89.3% of the observations were classified as successes. One observation refers to the success of one animal in 1 day, based on all the signals emitted from the respective collar throughout the respective day. A total of 18 animals (58.1%) consistently maintained success above the 90% threshold throughout the trial, while 13 animals (42.0%) were unsuccessful each on a maximum of 6 out of 30 days. Only 9 observations (2.0%) showed a success ratio below 75% and the minimum success ratio was 40% for only one observation, while all other observations (99.8%) had a success ratio of above 50%. The nine observations with lowest success ratio are given in Supplementary Table S3.

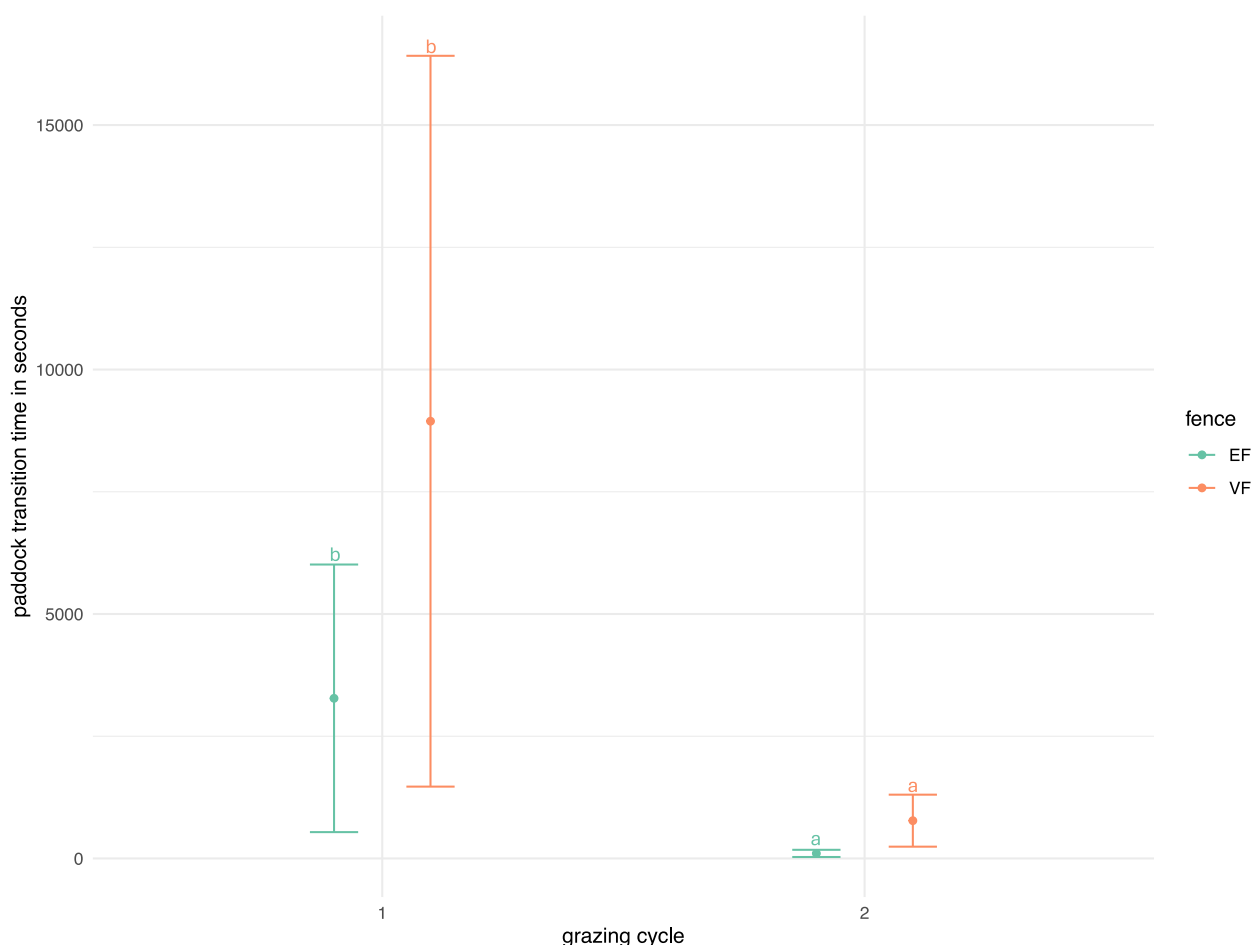


Fig. 2. Comparison of paddock transition time in seconds for cattle groups transitioning into new paddocks, measured from the opening of the paddock border until the last animal in a group completed the crossing. Virtual (VF) and physical electric (EF) fencing systems are contrasted across two 15-day grazing cycles. Points show estimated means, and error bars indicate the SE. Lowercase letters denote significant differences within the fencing systems between the grazing cycles ($P < 0.05$). Bars with the same lowercase letter are not significantly different.

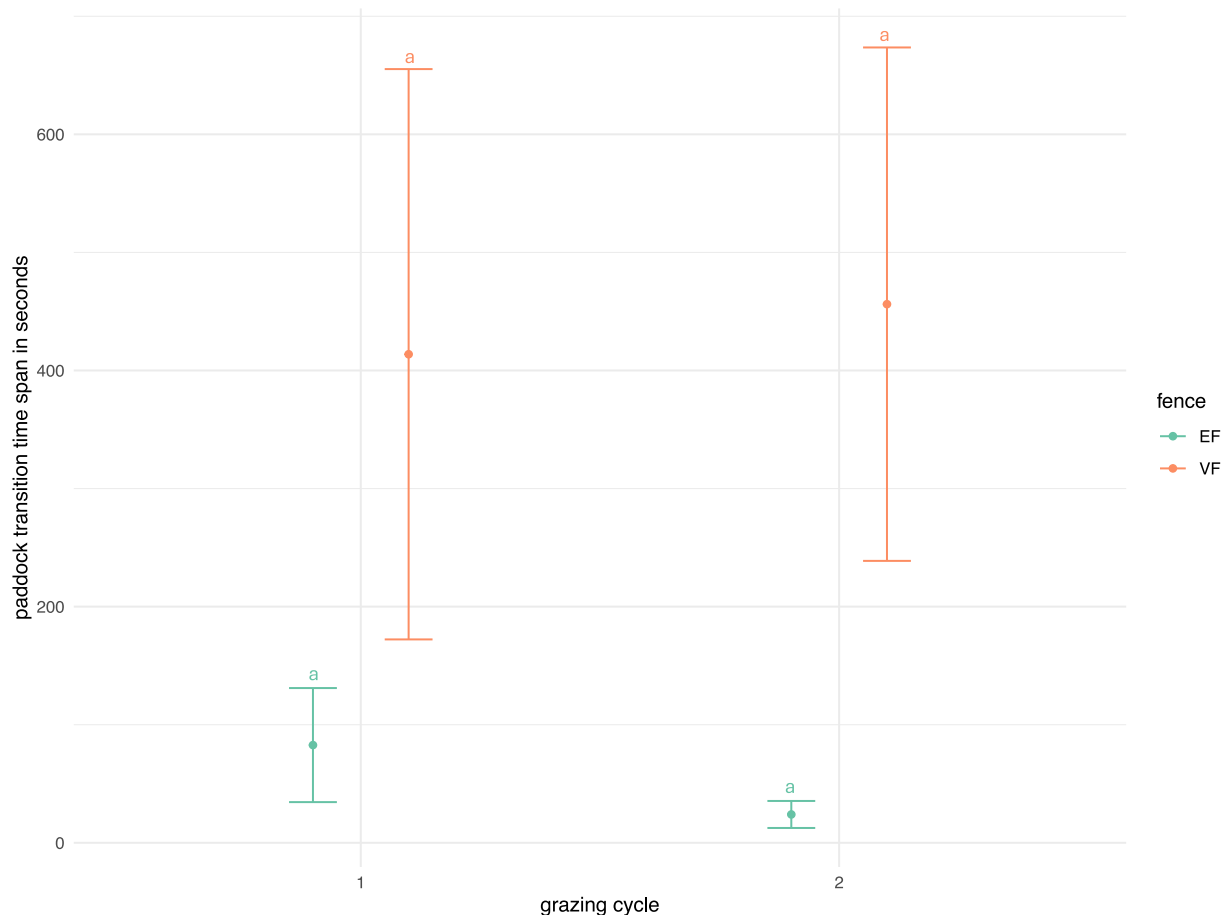


Fig. 3. Comparison of time span in seconds for cattle groups transitioning into new paddocks. Timing commenced when the first animal crossed into the new paddock and concluded when the last animal in the group crossed. Virtual (VF) and physical electric (EF) fencing systems are contrasted across the two grazing cycles. Points show estimated means, and error bars indicate the SE. Lowercase letters denote significant differences within the fencing systems between the grazing cycles ($P < 0.05$). Bars with the same lowercase letter are not significantly different.

Table 2
Days with escapes from the virtually fenced pasture, total number of escapes per day, and number of individual animals that escaped in two groups of eight Fleckvieh heifers across two 15-day grazing cycles (C1, C2).

Grazing cycle	Day	Group	Days since paddock transition	Number of escapes	Number of escaped animals
1	2	A	1	9	8
1	4	A	0	4	4
1	8	B	0	1	1
1	10	B	2	4	4
1	11	A	0	1	1
1	12	A	1	3	1
2	3	A	2	1	1
2	4	B	0	2	2

The analysis of the success probability indicated a significant effect of both grazing cycle ($\chi^2 = 17.9$, $df = 1$, $P < 0.001$), and days since paddock transition ($\chi^2 = 14.3$, $df = 5$, $P < 0.05$), but no significant effect of paddock ($\chi^2 = 11.1$, $df = 6$, $P > 0.05$) or the interaction between paddock and days since paddock transition ($\chi^2 = 6.5$, $df = 6$, $P > 0.05$). Multiple comparisons within grazing cycles between the days since paddock transition across the paddocks revealed a significantly higher success probability on day 2 compared to day 0 for paddock B in both grazing cycles ($P < 0.05$). No significant differences were observed for other combinations of days since paddock transition and paddock ($P > 0.05$, Fig. 4).

Fig. 4 shows an increase in success probability across grazing cycles, with overall higher values in C2 compared to C1. Thereby, the success probability was consistently above 55% in C1 and 87% in C2.

The analysis of the confidence ratio indicated significant effects of paddock ($P < 0.001$), days since paddock transition ($P < 0.01$), and their interaction ($P < 0.05$), while the grazing cycle showed no significant effect. Multiple comparisons within paddocks and days since paddock transition revealed a significant increase in the estimated confidence ratio, starting at a very low 0.0000013 for day 0 in paddock A, and progressively increasing to 0.75 for day 2 in paddock C (Fig. 5, Table 3). The confidence ratios showed clear grouping patterns, with the lowest values in paddock A and the highest in paddock C, indicating distinct and significant differences across days since paddock transition and paddocks (Fig. 5, Table 3).

Faecal cortisol metabolite concentrations

Analysis of FCMs revealed a significant effect of grazing cycle ($\chi^2 = 42.8$, $df = 1$, $P < 0.001$) and sampling time ($\chi^2 = 10.0$, $df = 1$, $P < 0.01$) and the interaction of sampling time and grazing cycle ($\chi^2 = 11.7$, $df = 1$, $P < 0.001$), but no effect of the fencing system ($\chi^2 = 1.1$, $df = 1$, $P > 0.5$) or its interactions with grazing cycle ($\chi^2 = 0.04$, $df = 1$, $P > 0.5$) or sampling time ($\chi^2 = 0.3$, $df = 1$, $P > 0.5$). Multiple comparisons between the sampling times within each grazing cycle revealed an increase in FCMs in C1 ($P < 0.05$), but no significant differences between sampling times in C2

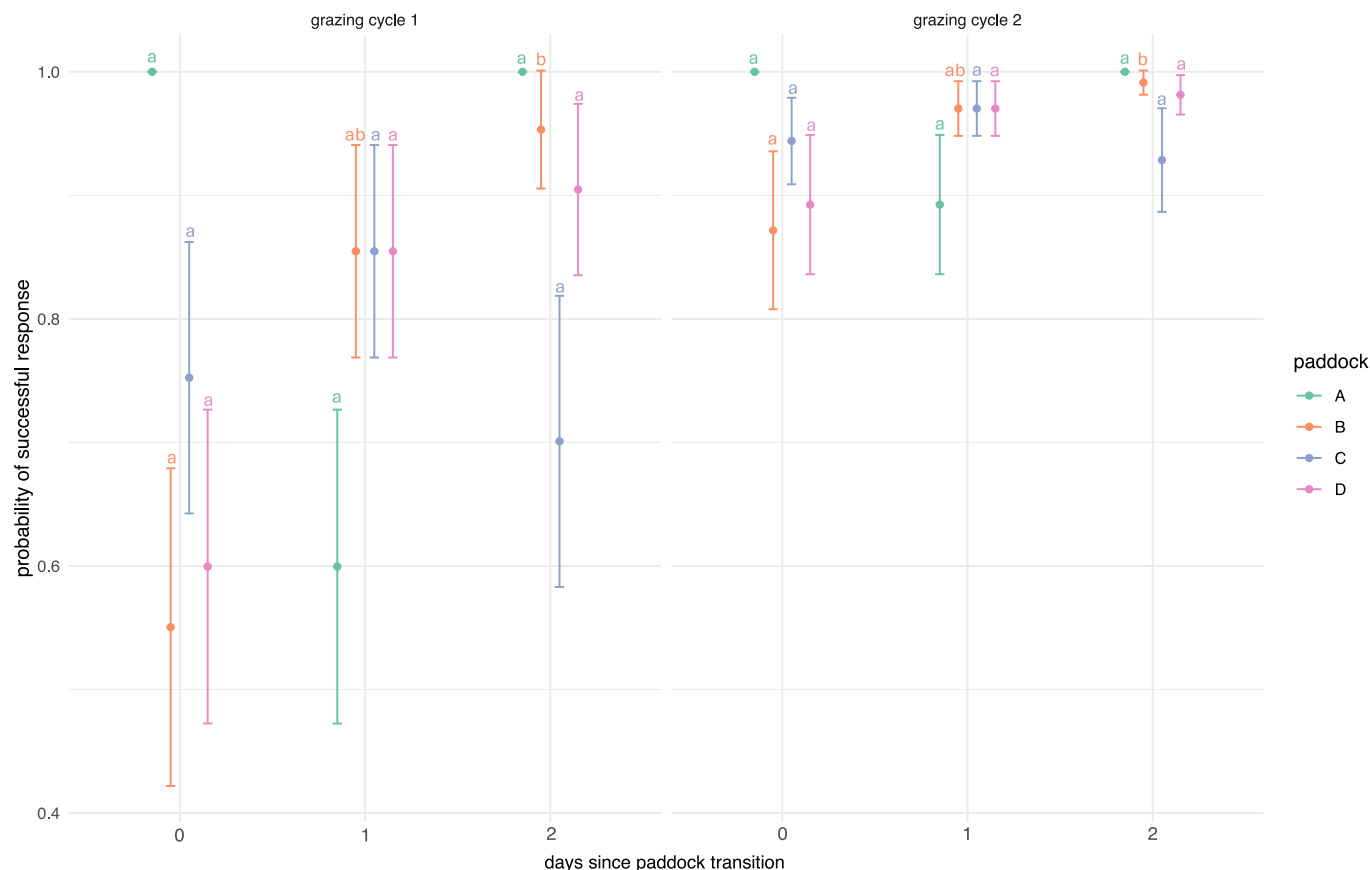


Fig. 4. Probability of successful cattle response to the virtual fence (returning to the virtual enclosure after hearing the auditory cue without triggering the electrical cue) as affected by the interaction between grazing cycle and days since paddock transition (0 = day of paddock transition) across all paddocks (A, B, C, D). Points show estimated means, and error bars represent the SE. Lowercase letters denote significant differences between days within grazing cycle ($P < 0.05$). Bars with the same lowercase letter are not significantly different.

($P > 0.05$, Table 4). FCMs at the end of C1 were significantly higher than in C2 ($P < 0.001$, Table 4). The average FCMs for the two sampling times in both grazing cycles are displayed in Table 4.

The separate analysis of the VF groups revealed no significant effect of the number of pulses ($\chi^2 = 0.3$, $df = 1$, $P > 0.05$) or their interaction with the grazing cycle on FCMs ($\chi^2 = 0.02$, $df = 1$, $P > 0.05$), but the effect of the grazing cycle remained significant ($\chi^2 = 15.0$, $df = 1$, $P < 0.001$).

Organic matter digestibility

The analysis of fOMD revealed a significant effect of the grazing cycle ($\chi^2 = 149.4$, $df = 1$, $P < 0.001$), but not the fencing system ($\chi^2 = 1.5$, $df = 1$, $P > 0.05$) or their interaction ($\chi^2 = 1.6$, $df = 1$, $P > 0.05$). Thereby, multiple comparisons revealed significantly higher ($P < 0.001$) fOMD in C1 compared to C2 (Table 5).

Similarly, pregrazing hOMD from hand-plucked samples was significantly affected by the grazing cycle ($\chi^2 = 42.7$, $df = 1$, $P < 0.001$), but not the fencing system ($\chi^2 = 0.6$, $df = 1$, $P > 0.05$) or their interaction ($\chi^2 = 1.6$, $df = 1$, $P > 0.05$). Thereby, hOMD was significantly higher in C1 compared to C2 (Table 5). Higher values for fOMD compared to hOMD resulted in ratios of fOMD to hOMD above 1 for both fencing systems and grazing cycles (Table 5). The analysis of the ratio between fOMD and hOMD (fOMD/hOMD) revealed a significant effect of the fencing system ($\chi^2 = 8.2$, $df = 1$, $P < 0.005$) and the interaction ($\chi^2 = 10.1$, $df = 1$, $P < 0.01$), but not the grazing cycle ($\chi^2 = 0.4$, $df = 1$, $P > 0.05$).

Multiple comparisons revealed a significantly higher fOMD/hOMD for EF compared to VF in C1 ($P < 0.05$), but not in C2 ($P > 0.05$, Table 5).

BW gain

BWG was significantly affected by the grazing cycle ($\chi^2 = 46.4$, $df = 1$, $P < 0.005$), with a higher BWG in C2 compared to C1. In C1, the mean BWG was -0.103 kg/day for the VF group and 0.281 kg/day for the EF group. In C2, the mean BWG was 1.134 kg/day for the VF group and 1.415 kg/day for the EF group. However, there was no significant effect of the fencing system ($\chi^2 = 3.7$, $df = 1$, $P > 0.05$) and the interaction of fencing system and grazing cycle ($\chi^2 = 0.1$, $df = 1$, $P > 0.05$) on BWG. In the VF groups, there was no significant impact of the number of electrical cues per animal on the daily BWG ($\chi^2 = 2.3$, $df = 1$, $P > 0.05$), but the effect of the grazing cycle on BWG remained significant ($\chi^2 = 23.8$, $df = 1$, $P < 0.005$).

Discussion

While VF is already in use in several countries, and multiple studies refute concerns about adverse effects on cattle behaviour – e.g. Campbell et al., 2017; Confessore et al., 2022; Hamidi et al., 2022; Lee et al., 2008; Marini et al., 2022; Ranches et al., 2021; Umstatter et al., 2015 – few countries have legal regulations in place, and debates are ongoing. The present study addresses some of the persisting knowledge gaps, evaluating the suitability of VF for rota-

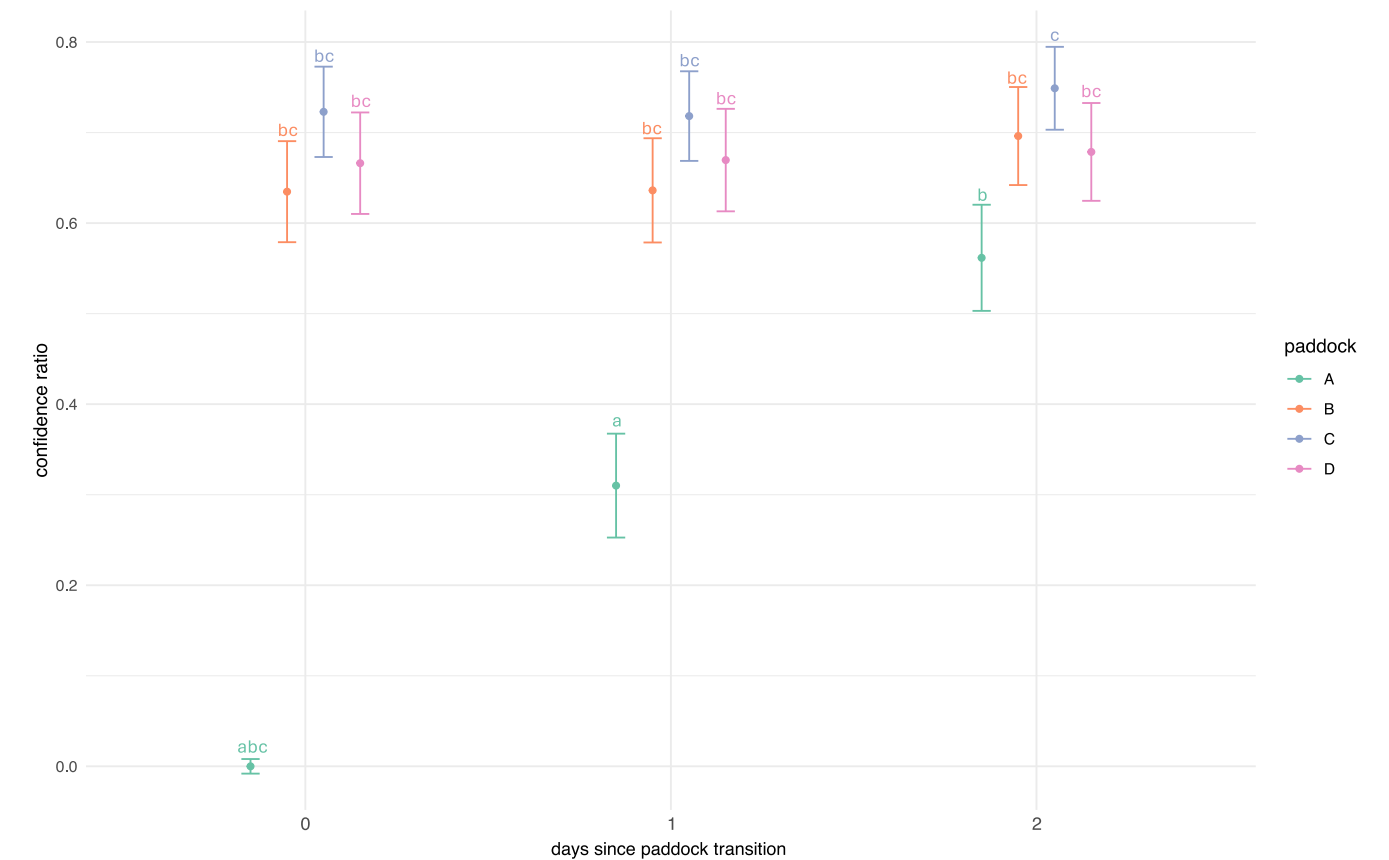


Fig. 5. Confidence ratio of cattle interacting with the virtual fence as affected by the interaction between days since paddock transition (0 = day of paddock transition) and paddocks (A, B, C, D). Points show estimated means, and error bars represent the SE. Lowercase letters show significant differences between days within paddocks ($P < 0.05$). Bars with the same lowercase letters are not significantly different.

Table 3
Estimated means (mean) and SE of the confidence ratio of grazing Fleckvieh heifers in four paddocks (A, B, C, and D) on days 0, 1, and 2 since paddock transition (PT). Significant differences in means for the interaction between days since PT and paddock are indicated by lowercase letters. Groups showing the same lowercase letters are not significantly different ($P < 0.05$).

Days since PT	Paddock	Confidence Ratio	SE	Significance
0	A	0.00	0.01	abc
1	A	0.31	0.06	a
2	A	0.56	0.06	b
0	B	0.64	0.06	bc
1	B	0.64	0.06	bc
2	B	0.70	0.05	bc
0	C	0.72	0.05	bc
1	C	0.72	0.05	bc
2	C	0.75	0.05	bc
0	D	0.67	0.06	bc
1	D	0.67	0.06	bc
2	D	0.68	0.05	c

PT = Paddock Transition

tional stocking management and its impacts on animal physiology and livestock performance compared to a physical electric fence.

Slower paddock transitions under virtual fencing

The observed significant effect of the fencing system on the paddock transition time corresponds to the differences in paddock transition time span between VF and EF and the paddock transition procedures that differed between the two fencing systems. The EF groups, transitioning through a gate of 5 m width, were observed

Table 4
Estimated means (mean) and SE of faecal cortisol metabolite concentrations (FCMs) in ng FCM per g faeces from virtually fenced Fleckvieh heifers at the start and end (Time) of two 15-day grazing cycles. Significant differences in means between the sampling times within each grazing cycle are indicated by lowercase letters. Groups showing the same lowercase letters are not significantly different ($P < 0.05$).

Grazing cycle	Time	Mean	SE	Significance
1	start	48.9	3.82	A
1	end	73.0	4.02	B
2	start	37.4	3.71	A
2	end	37.2	3.77	A

to disrupt their grazing and transition as a group. Thereby, one animal would typically detect the open gate and initiate the transition, while the other animals in the group would follow promptly. Thus, paddock transition time in the EF groups was greatly influenced by the time it took for the first animal to spot the open gate and move through it.

Contrastingly, the VF groups were able to transition along the full length of the paddock and lacked a visual cue indicating the accessibility of the new paddock. This resulted in an individualised, calm transition within the natural grazing movement for the VF groups, rather than a concerted paddock transition event. Consequently, VF groups exhibited prolonged crossing times (paddock transition time, Fig. 2) and a more extended total time span of paddock transition (Fig. 3) compared to EF groups throughout the study. A similar paddock transition time of 4 h 15 min before animals crossed over the old virtual boundary into a new paddock was reported by Campbell et al. (2017). Other studies employing VF with changes in the virtual boundary (Hamidi et al., 2022; Marini et al., 2018;

Table 5

Estimated means (Mean) and SE of *in vitro* digestible organic matter of the available herbage biomass (hOMD), faecal organic matter digestibility (fOMD) of Fleckvieh heifers, and the ratio fOMD/hOMD under two fencing systems, virtual (VF) and physical electric (EF), in two 15-day grazing cycles. Significant differences in means between the levels of the factor variables (fence and grazing cycle) are indicated by lowercase letters. Groups showing the same lowercase letters are not significantly different ($P < 0.05$).

Fence	Grazing Cycle	hOMD			fOMD			fOMD/hOMD		
		Mean	SE	group	Mean	SE	group	Mean	SE	group
EF	1	72.3	0.9	B	73.8	0.4	b	1.04	0.01	b
VF	1	74.2	1.1	B	74.7	0.4	b	1.01	0.01	a
EF	2	67.8	0.8	A	69.6	0.4	a	1.02	0.01	ab
VF	2	67.7	0.9	A	69.6	0.4	a	1.03	0.01	b

hOMD = *in vitro* digestibility of available organic matter.

fOMD = faecal organic matter digestibility.

Staahtoft et al., 2023) have not extensively reported on animal behaviour during transitions or in response to changes to the virtual boundary. In a trial investigating different cattle herding methods with a VF, Campbell et al. (2021) concluded that transition times varied based on the herding method employed. They found that a moving back fence, which prevents cattle from returning to the previous pasture can facilitate faster movement (Campbell et al., 2021).

Despite these differences, the decrease in paddock transition time for both fencing treatments in C2 (Fig. 2) suggests that animals in all groups gradually acclimated to the trial procedures. Anecdotally, animals were noted to recognise cues for the days of paddock transition, including the movement of water tanks, an increase in personnel numbers for faecal sampling, and the personnel entering the EF paddocks to open gates.

While calm and stress-free paddock transitions are desirable in terms of animal welfare (Barnes, 2015), the introduction of an additional auditory cue to signal a change in the virtual boundary could aid in facilitating fast transitions, which may be desirable from a management perspective. However, an additional cue would require further training of the animals and further research on possible implications.

Virtual fencing effective for grazing in rotational stocking management

The success threshold calculated in this study provides the first attempt to define a standardised benchmark for successful interactions with the VF. While the success ratio allows the continuous evaluation of an individual's interaction with the VF, comparison to the success threshold facilitates the interpretation of individual success ratios and allows the comparison across groups or different studies. Further, including one (or several) success thresholds based on experience with the VF in the user interface could help users to interpret the interaction of individual animals with the VF more effectively and at random intervals (Hamidi et al., 2024), facilitating an ongoing evaluation of VF interactions.

The success threshold of 90% established in this study aligns with success ratios reported in earlier studies with the Nofence system, such as those by Eftang et al. (2022) with goats and Staahtoft et al. (2023) with cattle. However, Confessore et al. (2022) reported a rate between auditory cues and electrical cues of 0.23, corresponding to a success ratio of approximately 56%, from a training trial with beef cattle using the Nofence technology.

While it is important to note that this benchmark is specific to the Nofence system, a comparison to other VF systems may still be possible if differences in cue delivery and operations are considered. For example, a study with the Halter system (Halter®, Auckland, New Zealand) reported a success ratio of approximately 97.4% after a 10-day training period (Verdon et al., 2024), while a study with the eShepherd technology (eShepherd™, Agersens, Melbourne, Victoria, Australia) reported lower success ratios ranging from 67 to 87% (Campbell et al., 2017).

The success ratios in this study, with 89.3% above the success threshold, demonstrate a high level of understanding of the VF, with a significant number of animals consistently achieving success and being effectively contained by the VF throughout the trial (Fig. 4). The average success ratios of 94.6% in C1 and 97.9% in C2 were similar to the overall success ratio of 91.3% scored throughout the training preceding this trial (Hamidi et al., 2024), suggesting that the animals learned to understand the system within the course of several days during the training. Fuchs et al. (2024), Hamidi et al. (2024), and Lee et al. (2018) report similarly short time spans for learning.

Moreover, it is worth noting that for the 2% of observations with a success ratio below 75%, the animals received no more than five pulses on that respective day and only one animal scored a success ratio below 75% twice. Success ratios < 75% occurred predominantly in C1 and only on the day of paddock transition or day 1 after paddock transition. This, paired with the overall success ratios similar to the 91.3% achieved during training (Hamidi et al., 2024), suggests that the issue lay in the animal's familiarity with its surroundings, considering animals were transported to a previously unknown pasture after the training, as well as disturbance in the form of paddock transitions and increased handling, rather than poor understanding of the VF principle. Further, 55% of success ratios < 75% are associated with escapes, which were mainly caused by human error and disturbance, emphasising the need for low-stress cattle handling, as described by Barnes (2015), in VF systems and proper training of users. The need for proper user training to VF has been previously emphasised by the Animal Welfare Committee in the UK (AWC, 2022).

The confidence ratio reflects the animals' ability and willingness to successfully engage with virtual boundaries, by weighing the success ratio against successful interactions with the VF (Hamidi et al., 2024). The observed numerical increase in confidence ratio over time – from day 0 to day 2 and from paddock A to paddock C (Table 3) – may be attributed to increased familiarity with the fence location, indicating the desired understanding of the VF by the cattle and a lack of fear in interacting with it (Hamidi et al., 2024). It is important to note that on the first day on pasture in both grazing cycles, interaction with the VF may have been reduced due to the visual cue of the physical fence on all four sides. The low confidence ratio in paddock A was caused by few fence interactions, leading to success ratios of 1 with little to no successful interactions.

The increased testing of the VF boundary by heifers indicated by the increase in confidence ratio over time underlines their adaptability to the VF technology and their ability to use auditory cues to navigate environmental changes, which is consistent with findings by e.g. Aaser et al. (2022), Campbell et al. (2017), and Hamidi et al. (2022). From a 12-day training trial with the same animals prior to this study, Hamidi et al. (2024) reported an increase in the confidence ratio from 0.2 to 0.67 and a decline back to 0.51

after shifting the virtual boundary. This suggests that the confidence ratio depicts the habituation of cattle to new virtual boundaries. Further, higher confidence ratios in this trial (Table 3) compared to the values reported by Hamidi et al. (2024) are indicative of a learning process.

Additionally, the increased confidence in the fence location paired with the motivation to reach better quality forage may lead to animals being more daring to trigger auditory cues. In a VF trial with feed attractants, Colusso et al. (2021) found that cattle with restricted feed access were more likely to breach the virtual boundary than cattle with ample feed availability. However, while in this trial quality of ingested herbage was lower in C2 compared to C1 (Table 5), this experiment was conducted under conditions of abundant grassland herbage availability, with on average $2\,808 \pm 1\,150$ kg DM/ha in C1 and $2\,992 \pm 968$ kg DM/ha in C2 (i.e. 176 and 187 kg/animal). Further testing the system in scenarios with limited feed availability is imperative to investigate the effect of feed scarcity on the interaction with the VF and potential changes in cattle behaviour.

Readers should consider that in this study, the two outer sides of each paddock consisted of a double fence with the virtual boundary set 5 m away from the perimeter fence. This setup may have reduced the overall number of signals from the collars and escape incidents compared to studies where the virtual boundary is placed further from a physical fence. Generally, it is important to consider the varying conditions present in different studies when comparing results.

Virtual fencing had no impact on livestock stress or performance in rotational grazing

The absence of a significant effect of the fencing system on FCMs aligns with findings from Campbell et al. (2019) and Hamidi et al. (2022), who found no effect of the fencing system on FCMs of cattle in VF trials in continuously stocked pastures. Similarly, Confessore et al. (2022) found no effect of the fencing system on hair cortisol content in a VF trial in continuously stocked pastures. Hamidi et al. (2022) reported FCMs of 14.3 and 16.4 ng FCMs/g faeces for VF and EF, respectively. Higher FCMs in this study (Table 4) suggest that, under rotational grazing with frequent transitions between paddocks and more human interaction, e.g. for weighing, the animals experienced some stress. However, this stress was likely unrelated to the new VF technology. In a study with dairy cows in confined housing, Ebingerhaus et al. (2020) reported FCMs between 11.15 and 13.95 ng FCMs/g faeces (the latter two studies utilised exactly the same methodology to measure FCMs). Thereby, increased human-animal contact led to lower FCMs, presumably due to reduced fear towards humans as a result of positive experiences with human contact (Ebingerhaus et al., 2020). Similarly, the missing effect of the fencing system on FCMs and BWG and the significant influence of the grazing cycle on both factors in this study suggest that the cattle adapted over time to the circumstances of the trial, i.e. the unfamiliar environment and the higher exposure to the general handling and human interaction throughout the trial. Likewise, the notable increase in FCMs for cattle in both fencing treatments from the initial sampling at the beginning of C1 to the subsequent sampling at the end of C1 suggests that the handling and change in location had a more pronounced effect on the animals than the fencing system. This aligns with findings from Lee et al. (2008) who observed cattle exhibiting a stress response to low-energy electrical cues similar to that induced by restraint in a crush. Similarly, Lee et al. (2018) observed that establishing a clear association between the auditory cue and the electrical cue facilitated predictability and controllability of the VF, thereby improving the animals' agency, resulting in a more positive animal welfare state when exposed to the VF

technology. Therefore, the lack of heightened stress indicators in the VF groups in this study suggests that the auditory cue functions as intended, replacing the visual cue of traditional physical electric fencing, and enabling animals to predict aversive stimuli such as the electrical cue effectively.

The ratio of fOMD to hOMD above 100% (Table 5) indicates that cattle in all groups were able to select higher-quality herbage from the available forage. The lack of a significant effect of the fencing system shows that herbage selection and, consequently, grazing behaviour are not influenced by VF.

Furthermore, VF had no adverse effect on animal productivity, which became evident in the lack of influence of the fencing system and the number of electrical cues on BWG. Campbell et al. (2019) reported higher daily BWGs between 0.46 and 1.32 kg/animal per day compared to values reported in the present study, from a VF trial without paddock transitions, which may be attributed to the exposure to handling and personnel prior to the trial as well as the adaptation phase on the trial pasture in that study (Ebingerhaus et al., 2020).

In this study, under rotational grazing management with frequent transitions between paddocks, VF did not negatively affect livestock performance and animal welfare, as indicated by faecal cortisol metabolite concentrations, herbage selection, and BWG when compared to a physical electric fence. These findings confirm the initial hypothesis that VF is effective in rotational stocking systems, has no adverse effect on performance and stress indicators of cattle, and that cattle learn to interact with the system within a short time frame of several days. While further research is recommended to investigate the long-term effects of VF on cattle behaviour, welfare, and productivity, this study dispels concerns regarding animal welfare within VF systems, opening avenues for further exploration of the technology's potential to enhance grazing management across various conditions. Moreover, this study shows that rotational stocking with VF can meet the productivity expectations of farmers, demonstrating the potential of VF to facilitate the implementation and expansion of pasture-based cattle production.

Supplementary material

Supplementary Material for this article (<https://doi.org/10.1016/j.animal.2024.101416>) can be found at the foot of the online page, in the Appendix section.

Ethics 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: 33.19-42502-04-20 / 3388).

Data and model availability statement

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

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used OpenAI GPT-3.5 to improve language. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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Declaration of interest

The authors declare that there is no conflict of interest to declare.

Acknowledgements

We are grateful to Barbara Hohlmann for her support throughout the field experiment. We further thank Laura Ahlers, Nele Busse, Lara Guedes de Aquino, Magdalena Kalio, Franziska Kunz, Jonas Steinkuhl, and Sabine Macho-Maschler for their support in the field and lab. Thanks also to Marten Steinke for providing the grazing livestock and the experimental area, as well as for the general maintenance of the experiment. Special thanks go to Knut Salzmann and his team for supervising the livestock management and for competent and patient handling of the animals and to the technical team of Dirk Hunold for setting up the experimental area. We would also like to show our gratitude to Dr. Irina Kuzyakova, for her general review of the statistics. We acknowledge support by the Open Access Publication Funds of the Göttingen University.

Financial support statement

This study was supported by the German Federal Ministry of Education and Research under grant number [031B0734A] as part of the project “GreenGrass”.

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