Using smart farming technologies to improve the sustainability of livestock grazing systems

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Summary

Semi-natural grasslands, which contribute to a wide range of ecosystem services, such as soil erosion control, water storage and carbon storage, are severely threatened by both intensification and complete extensification, i.e. the abandonment of agricultural management. The genesis of these open landscapes is inextricably linked to grazing livestock, which in Europe was mainly herded on common pastureland by herders until the 19th century. The immense demand for labour for this form of pastoralism was met, among other things, at 'Hütekindermärkte'. With the simplification of fencing due to the invention of barbed wire in 1873 and electric fencing in 1937, the loss of common pastureland, the improved possibilities for preserving forage and the increasing lack of herdsmen, more and more pastureland was fenced. However, the main task of the herder, the continuous monitoring of the grazing livestock and the sward could not be taken over by fences. For successful grazing, the optimal spatial and temporal allocation of the grazing animal to the sward is crucial. The grazing animal influences the sward just as the sward influences the grazing animal. The lack of consideration of this relationship in grazing management leads to semi-natural grasslands being damaged by overgrazing, especially in arid areas, while on the other hand the abandonment of grazing (mainly in Europe) leads to natural succession and thus to scrub encroachment of the formerly open landscape.

Since the invention of the electric fence, there have been no major developments in the field of pasture management that could have led to improved spatial and temporal allocation of grazing animals on the sward.

The development of GPS-based virtual fencing, where each adult animal wears a special GPS collar, and grazing allocation takes place via a mobile device, seems to offer great possibilities for a more precise and flexible grazing system. Fencing could evolve again more towards holistic herding.

In this context, the aim of this work is to generate a knowledge gain in the existing system, as well as a test and evaluation of options for an innovative herding fencing system by using virtual fences. A kind of herdsman 4.0, a system which should be able to take into account the needs of the animals and the sward, as well as to enable a spatially and temporally continuous monitoring of the animals.

In order to analyse possible correlations between forage availability and movement behaviour and thus pasture use, **chapter one** of this thesis uses the long-term grazing trial 'FORBIOBEN' with its three different grazing intensities to investigate hourly and daily walking distances as well as the spatial distribution of Fleckvieh cows by the use of commonly used GPS collars for animal monitoring. The activity of the cows increased with lower forage availability and the spatial distribution during the active time of the animals, identified by daily hydrographs, is more even at the highest grazing intensity. It

should be noted, however, that in this study the animals increased their activity in both the highest and the lowest grazing intensity. There are indications that heterogeneity and, generally speaking, the distribution of forage resources on the pasture must be taken into account in order to be able to make reliable statements about animal movement as a reflection of forage availability on the pasture.

Chapter two addresses the use of virtual fences for the first time and investigates whether there is a negative effect of virtual fencing technology on Fleckvieh heifers. Continuous animal observations, faecal samples, grassland measurements and steps walked provided no evidence of deterioration compared to the control group. Furthermore, it could be shown that despite individual differences, all animals were able to adjust to the virtual fencing system. The exclusion of the virtually fenced animals was successful. No animal crossed the virtual boundary during the experiment.

Animal monitoring in the pasture may be a possible additional benefit of the GPS collars used for virtual fencing. **Chapter three** investigates in a model way correlations between spatiotemporally explicit animal behaviour and data from drone-based remote sensing of the grazing area in the context of a rotational grazing trial. This information, which has been validated as part of the study, may in combination provide information on hotspots of cattle residence time, biomass changes and animal behaviour. The comparison of the lying times identified by the collars with animal observation data provides accurate results. The relationship between RGBVI from UAV and biomass was significant with a moderate amount of explained variability. Overall, it appears that animal monitoring on pasture is possible through virtual fence collars and can provide highly valuable data presenting the GPS location of behavioural events. Distances walked by animals, their distribution over the area and lying times per day show an effect of decreasing forage availability. In this context, lying times decreased, walking distances increased and the distribution of animals on the area became more even. The drone data of the grazing areas before and after grazing showed a clear correlation to the local stays of the animals associated with grazing on the studied, predefined 2.5 × 2.5 m polygon grid. Further research is needed to be able to use the whole relationship between animal behaviour on pasture and drone data to define, among other things, thresholds for forage scarcity.

Overall, the results of the individual chapters build on each other and could provide basic requirements for the development of an innovative virtual herding system. An effect of forage availability on the movement behaviour of the animals could be demonstrated. The welfare of the virtually fenced animals, compared to the conventionally fenced animals is not affected in our trial, although further research over longer periods is needed to further validate the results. Drone-based remote sensing to record an actual condition of the pastures can provide a baseline that could lead to better detection of available biomass through continuous monitoring of animal residency. An appropriately adapted virtual herding system (Herder 4.0) can help to simplify and improve the di-

verse challenges of pasture management in a sustainable way. An example of this is soil degradation. With the help of the animal residence times from the collar data, which are verified with the UAV images and give an indication of the change in the sward, it may be possible to stop such processes or to react quickly with virtual out fencing of the heavily used areas. This high flexibility allows to rethink the previously known grassland systems, which are strongly oriented towards the possibilities of fencing. The technology of virtual fencing in combination with remote sensing enables fine-scale ‘grid grazing’ that effectively and sustainably considers grazing animals and swards.

Zusammenfassung

Naturnahe Grünlandflächen, die zu einer weiten Bandbreite an Ökosystemleistungen beitragen, wie zum Beispiel Bodenerosionsschutz, Wasserrückhalt und Kohlenstoffspeicherung sind sowohl durch die Intensivierung als auch die komplette Extensivierung stark bedroht. Die Entstehung dieser offenen Landschaften ist untrennbar mit Weidevieh verbunden, welches in Europa noch bis ins 19. Jahrhundert größtenteils auf Allmenden von Hirten gehütet wurde. Der immense Bedarf an Arbeitskräften für diese Form der Weidehaltung wurde unter anderem auf Hütekindermärkten gedeckt. Durch die Vereinfachung des Zaunbaus mit Erfindung des Stacheldrahts 1873 und des Elektrozauns 1937, dem Wegfall der Allmende, den verbesserten Möglichkeiten zur Futterkonservierung und dem zunehmenden Mangel an Hirten, wurde immer mehr Weideland umzäunt. Die Hauptaufgabe des Hirten - die kontinuierliche Überwachung des Weideviehs und der Grasnarbe - konnte jedoch nicht von Zäunen übernommen werden. Um erfolgreiche Weidehaltung betreiben zu können, kommt es in hohem Maße auf die optimale räumliche und zeitliche Allokation der Weidetiere bei gleichzeitiger Berücksichtigung der Grasnarbe an. Das weidende Tier beeinflusst die Grasnarbe genauso wie die Grasnarbe das weidende Tier. Die fehlende Berücksichtigung dieser Beziehung in der Weidehaltung führt dazu, dass vor allem in ariden Gebieten durch Überweidung naturnahe Grünlandflächen geschädigt werden, während auf der anderen Seite die Aufgabe der Weidehaltung zu natürlicher Sukzession und damit zur Verbuschung der vormals offenen Landschaft führt.

Im Bereich der Weidehaltung ist es seit der Erfindung des Elektrozauns nicht wirklich zu Neuentwicklungen gekommen, die eine bessere räumliche und zeitliche Allokation der Weidetiere auf der Grasnarbe, hätten lösen können.

Die Entwicklung des GPS-basierten virtuellen Zäunens, bei dem jedes erwachsene Tier ein spezielles GPS-Halsband trägt und die Weidezuteilung über ein mobiles Endgerät stattfindet, scheint große Möglichkeiten für ein präziseres und flexibleres Weidesystem zu bieten. Das Zäunen könnte sich dadurch wieder mehr in Richtung eines ganzheitlichen Hütens entwickeln.

Das Ziel dieser Arbeit ist es in diesem Kontext einen Erkenntnisgewinn im bestehenden System, sowie eine Testung und Evaluierung von Möglichkeiten für ein innovatives Hüteweidezaunsystem durch Nutzung von virtuellen Zäunen zu generieren. Eine Art Hirte 4.0, ein System, welches in der Lage sein sollte, die Bedürfnisse der Tiere und der Grasnarbe zu berücksichtigen, sowie ein räumlich und zeitlich kontinuierliches Monitoring der Tiere zu ermöglichen.

Um mögliche Zusammenhänge zwischen Futterverfügbarkeit und Bewegungsverhalten und damit der Weidenutzung analysieren zu können, wird in **Kapitel eins** dieser Arbeit der Langzeitweideversuch ‘FORBIOBEN’ mit seinen drei unterschiedlichen Beweidungsintensitäten genutzt um stündliche und tägliche Laufdistanzen sowie die räumliche

Verteilung von Fleckvieh Kühen mit herkömmlichen GPS Halsbändern zum Tiermonitoring zu untersuchen. Die Aktivität der Kühe nahm mit geringerer Futtermenge zu und die räumliche Verteilung während der, über Tagesganglinien identifizierten, aktiven Zeit der Tiere ist in der höchsten Weideintensität gleichmäßiger. Es ist jedoch zu bedenken, dass in dieser Studie die Tiere sowohl in der höchsten als auch in der niedrigsten Weideintensität ihre Aktivität steigerten. Es gibt Indizien dafür, dass die Heterogenität sowie allgemein gesprochen die Verteilung der Futterressourcen auf der Weide mit in Betracht gezogen werden müssen, um belastbare Aussagen zur Tierbewegung als Spiegel der Futterverfügbarkeit auf der Weide treffen zu können.

Kapitel zwei beschäftigt sich erstmals mit der Nutzung von virtuellen Zäunen und untersucht, ob ein negativer Effekt von virtueller Zäunungstechnologie auf Verhaltensweisen und Tierwohlparameter von Fleckvieh-Färsen nachgewiesen werden kann. Kontinuierliche Tierbeobachtungen, Kotproben, Graslandmessungen und Schrittzählungen lieferten keine Anhaltspunkte für eine Verschlechterung gegenüber der Kontrollgruppe. Ferner konnte gezeigt werden, dass trotz tierindividueller Unterschiede alle Tiere in der Lage waren, das virtuelle Zäunungssystem zu erlernen. Die Exklusion der virtuell gezäunten Tiere war erfolgreich. Kein Tier hat während des Versuchs die virtuelle Grenze überschritten.

Tiermonitoring auf der Weide kann ein möglicher Zusatznutzen der für das virtuelle Zäunen verwendeten GPS-Halsbänder sein. **Kapitel drei** untersucht im Rahmen eines Rotationsweideversuchs modellhaft Zusammenhänge zwischen raumzeitlich explizitem Tierverhalten und Daten aus der drohnenbasierten Fernerkundung der Weidefläche. Diese Informationen, die im Rahmen der Studie validiert werden, können in Kombination Aufschluss über Aufenthaltsschwerpunkte, Veränderungen der Biomasse und Tierwohlkriterien geben. Der Abgleich der durch die Halsbänder identifizierten Liegezeiten mit Tierbeobachtungsdaten lieferte präzise Ergebnisse. Der Zusammenhang zwischen RGBVI aus UAV und Biomasse war signifikant mit einem moderaten Anteil erklärter Variabilität. Es zeigt sich, dass Tierüberwachung auf der Weide durch virtuelle Zäunungshalsbänder möglich ist und durch die genaue GPS-Verortung der Ereignisse höchst wertvolle Daten liefern kann. Die gelaufenen Distanzen der Tiere, ihre Verteilung auf der Fläche und die Liegezeiten je Tag zeigen einen Effekt der abnehmenden Futterverfügbarkeit. In diesem Zusammenhang verringerten sich Liegezeiten, verlängerten sich Laufdistanzen und die Verteilung der Tiere auf der Fläche wurde gleichmäßiger. Die Drohnendaten der Weideflächen vor und nach der Beweidung zeigten einen deutlichen Zusammenhang zu den mit dem Grasen assoziierten lokalen Aufenthalten der Tiere auf dem untersuchten, vordefinierten 2.5×2.5 m Polygon Grid. Weitere Forschung ist erforderlich, um die gesamte Beziehung zwischen dem Verhalten der Tiere auf der Weide und den Drohnendaten zur Definition von u.a. Schwellenwerten für Futterknappheit nutzen zu können.

Insgesamt bauen die Ergebnisse der einzelnen Kapitel aufeinander auf und könnten die ersten Grundvoraussetzungen zur Entwicklung eines innovativen Hüteweidezaunsystems liefern. Ein Effekt der Futterverfügbarkeit auf das Bewegungsverhalten der Tiere konnte nachgewiesen werden. Das Wohlbefinden der virtuell gezäunten Tiere, verglichen mit den konventionell gezäunten Tieren, ist in unserem Versuch nicht beeinträchtigt, auch wenn weitere Forschung über längere Perioden notwendig ist, um die Ergebnisse weiter zu validieren. Dronenbasierte Fernerkundung zur Aufnahme eines Ist-Zustandes der Flächen kann eine Ausgangsbasis schaffen, die durch die kontinuierliche Überwachung der Tieraufenthalte zu einem besseren Erkennen der verfügbaren Biomasse führen könnte. Ein entsprechend angepasstes Hüteweidezaunsystem (Hirte 4.0) könnte helfen die vielfältigen Herausforderungen der Weidewirtschaft nachhaltig zu vereinfachen und zu verbessern. Mit Hilfe der Tieraufenthalte aus den Halsbanddaten, die verifiziert mit den UAV Bildern einen Anhaltspunkt zur Veränderung der Grasnarbe liefern, gibt es die Möglichkeit mit virtueller Auszäunung der stark genutzten Bereiche schnell zu reagieren und so beispielsweise Bodendegradation zu verhindern. Diese hohe Flexibilität erlaubt es, die bisherigen Graslandsysteme, die stark an den Möglichkeiten des Zaunbaus orientiert sind, neu zu denken. Die Technologie des virtuellen Zäunens in Kombination mit Remote Sensing könnte ein feinskaliertes Grid Grazing ermöglichen, welches effektiv und nachhaltig, sowohl das grasende Tier als auch die Grasnarbe berücksichtigt.

Part I

General Introduction

General relevance and history of livestock grazing

With about 3.2 billion ha worldwide (FAO 2022), grassland is the largest terrestrial biome, contributing to a wide range of ecosystem services (Bengtsson et al., 2019). A large part of this area is used by grazing herbivores, which support the livelihood of about 700 million people. Pastoralism is more than an agricultural production system. It is also a livestock based livelihood strategy and a way of live (Ayatunde et al., 2011). In the 8,000 years of genesis of anthropogenic grassland over 98 % of this time span was inseparably connected with grazing (mainly cattle) partially unregulated, later as controlled pasture.

As a product of human management, semi-natural grassland needs livestock grazing for their maintenance, otherwise it will generally be encroached by shrubs and trees (Queiroz et al., 2014). Pasturing all livestock of a village with a herder on commons was an essential part up to the 19th century (Ellenberg, 1988; Kapfer, 2010) in Europe. The required workers were found for the grazing season, among other places, at so-called ‚Hütekindermärkte‘ in German-speaking countries until approximately 1921 (Stepanek, 2009). Fence building was cost- and labour intensive and mostly used to protect cultivated fields and property in general. Barbed wire fences reduced the labour required for fencing (invented 1873 by Joseph Gidden (Netz, 2009)). Electric wire fences (invented 1937 by Bill Gallagher (Goldsmith, 2013)) reduced the risk of injury compared to barbed wire fences.

With the end of common pasture land use, the lack of herders, less labour-intensive fencing methods and the better possibilities for forage conservation, more and more livestock was kept indoors as observed in a clear trend of reduced grazing in Europe (van den Pol-van Dasselaar et al., 2020). Major technological innovations are missing for decades. On the other hand, precision livestock management technology has been continuously developed to improve housing.

Challenges in relation to grassland and livestock grazing

Although semi-natural grasslands are one of the most diverse and valuable habitats in Europe (Wilson et al., 2012), they are equally under severe pressure from both, intensification and abandonment of agricultural management (Porschlod and WallisDeVries, 2002; Uematsu et al., 2010). Species richness has drastically declined in European agricultural grasslands (Peeters, 2009). Since the mid-20th century, 50 % of the plant species at plot level in northern Germany have been lost due to intensified management, as described in a resurvey from the 1950/60s (Wesche et al., 2012). This decline is temporally related to the decline in grazing. Cattle is considered to be, in terms of grassland plant species richness, the most favourable livestock (Pykälä, 2000).

Grazing livestock, especially ruminants are able to mitigate the conflict with human nutrition and create heterogeneous landscapes (Adler et al., 2001), which are valuable habitats and are drivers for heterogeneity-related biodiversity (Jerrentrup et al., 2014 ; Tälle et al., 2016) in semi-natural grasslands. But it is important to precisely monitor the disturbances caused by the grazing animal to the sward. Ensuring the sustainable and efficient use of land resources is essential, especially in the face of climate change (Sustainable Food and Agriculture, FAO 2022). Small-scale and diversified agriculture embedded in at least 20 % seminatural habitats are necessary to build and maintain biodiversity (Tscharntke et al., 2021).

Proper forage utilisation without over- or undergrazing was less of a challenge before fencing replaced herding (Anderson, 2007) as monitoring of the animal and its interaction with the sward was continuously provided by the herders. With standard fencing technologies, optimal pasture utilisation is difficult, as spatial and temporal control of the animal is limited (Stevens et al., 2021). As one result, severe pasture degradation in arid areas, caused by overgrazing leads to soil erosion, decreased carbon input and increased soil organic carbon mineralisation (Breidenbach et al., 2022).

The main challenge of pasture management is to determine the availability of forage on the pasture as accurately as possible and to distribute the animals precisely on these resources. The determination of the biomass of a pasture with the help of a rising plate meter (Castle, 1976), has no spatial continuity. Unmanned-Aerial-Vehicles (UAV) are a promising remote sensing platform (Bindelle et al., 2021) and offer a continuous spatial high resolution monitoring. Lussem et al. (2022) recently demonstrated the applicability in grassland cutting systems for accurate estimating aboveground dry matter yield. Alvarez-Hess et al. (2021) have shown that UAV-based multispectral imaging helped to measure pasture depletion in paddocks while cows are grazing.

Continuous animal monitoring due to precision livestock technologies, which can be provided in indoor husbandry systems is known to enhance productivity levels, reproduction traits, and maintaining a good health (Tullo et al., 2019). The lack of appropriate technologies for animal monitoring on pasture is an obstacle for more animals on pasture. Monitoring the health and fertility of grazing livestock used to be done by the herders. Fences are not able to monitor the animals, therefore, manual control by the practise is required. Especially in remote areas, more than one check a day, is often not possible due to labour and time constraints.

GPS based animal monitoring and virtual fencing systems

In the 1990s, GPS collars to track the spatial behaviour of grazing cattle had been a well-established method to integrate spatial information into cattle management procedures (Agouridis et al., 2004). The use of GPS collars has many benefits: individuals can be tracked over a long-term period with automatically recorded geographical positions using predefined time intervals (Polojärvi et al., 2011), which provides helpful information on large pastures (Venter et al., 2018) and additional, efficient and accurate information on cattle behaviour and social structure (Meckbach et al., 2021) in relation to grazing intensity and forage availability (Hejcmanová et al., 2009). Information obtained from GPS-Collars has the potential to improve the productivity, profitability, sustainability and animal welfare of cattle on pasture as this information is temporarily and spatially continuous (Rivero et al., 2021).

Virtual fencing is a system to control and enclose livestock without traditional (ground-based) fencing (Anderson, 2007). In general, for virtual fencing systems to work, each animal must be equipped with a GPS collar and the virtual boundaries must be defined via an user platform (such as a smartphone, laptop computer or similar device). The collar emits an acoustic signal when the animal approaches the boundary. If the animal does not react to the acoustic signal and continues moving towards the virtual boundary, the acoustic signal is followed by a short-duration electric pulse. Virtual fencing can be seen as the next obvious step in fencing technology and aims to remotely map and control livestock without the use of fixed fences (Umstatter, 2011).

Commercial systems are used in the UK (140 virtual fencing users), New Zealand, Australia, Norway and the United States of America (Animal Welfare Committee, 2022).

The four virtual fencing systems, currently under development and in the early steps of commercialisation have common elements but vary in technology, capabilities and addressed species (Animal Welfare Committee, 2022). Available evidence from the literature shows that three different systems are used in the published research since 2018 on virtual fences analysed in grazing trials. Currently, there are two studies (Boyd et al., 2022a; Boyd et al., 2022b) conducted in the United States using a virtual fencing system designed and manufactured by Vence Corp ((San Diego, CA) which is now part of Merck Animal Health, a division of Merck & Co., Inc., Rahway, N.J., USA). Three studies are using Nofence virtual fencing collars (Nofence AS, Batnfjordsør, Norway), conducted in Denmark (Aaser et al., 2022), Italy (Confessore et al., 2022) and Germany (Hamidi et al., 2022). Currently, most of the grazing trials were conducted in Australia which use the virtual fencing technology eShepherd developed by Agersens (eShepherd®, Agersens, Melbourne, VIC, Australia), which is now part of Gallagher (Gallagher, Melbourne, Vic., Australia) (e.g. Campbell et al., 2018; Campbell et al., 2019; Lomax et al., 2019; Campbell et al., 2020; Colusso et al., 2021; Verdon et al., 2021). To the best knowledge available at present, there are no published studies using the virtual fence system manufactured and designed by Halter (Halter's Smart collar,Auckland CBD, New Zealand) although it is announced to be independently reviewed and approved by the AgResearch Animal Ethics Committee (Halter, 2022) and already used in practise in New Zealand.

Virtual fencing may be understood as a shift towards animal herding, as these virtual fences are not as static as conventional fences (Anderson, 2007). Cattle is able to learn the virtual fencing system via the audio cues and not the location of the boundary (e.g. Campbell et al., 2017). Therefore, multiple grassland management practises, normally related with high labour input for fencing, are easily possible, such as strip grazing (e.g. Lomax et al., 2019; Langworthy et al., 2021), cell-grazing (Verdon et al., 2021) and fencing out sensitive areas (e.g. Campbell et al., 2020). Although virtual fencing systems have already been developed, there is the need for further improvement to enable the complete control over the animal - sward interaction. Moreover, real-time GPS data for animal monitoring on pasture might permit a cost-effective solution as there is only one sensor necessary for health and fertility monitoring as well as decision support for moving the boundary.

The project GreenGrass

GreenGrass (https://www.greengrass-project.de/) is a supra-regional and interdisciplinary collaborative project which is investigating new ways of pasture management. Project partners are the Humboldt University of Berlin (agricultural policy, remote sensing), Cottbus University (environmental economics), Justus Liebig University of Giessen (animal ecology), Hohenheim University (agricultural management), Kassel/Witzenhausen University (food marketing), Cologne University (remote sensing), the companies Horizont and Texas Trading (pasture technology) and the Grassland Centre Lower Saxony/Bremen for agricultural practice.

The aim of GreenGrass is bringing grazing animals back into the landscape by using innovative herding technologies (e.g. virtual fencing) and various satellite- and drone-based remote sensing technologies to reduce the immense amount of labour involved in pasture management and to enable sustainable pasture use. With information from remote sensing, the actual forage supply on the pasture for the cattle may be calculated precisely. An innovative multi-level information system combines existing information with the remote sensing data, analyses it in real time and develops the routines for the spatio-temporal control of the animals. For precise grazing, constant temporal and spatial allocation of the animal is necessary. The large amount of work and high cost of fencing required for such well-controlled grazing often has a deterrent effect in practice. This effort is to be reduced by the planned automated technologies. In addition to reducing labour and material costs, the use of this technology will also provide the opportunity to protect important habitats within the pastureland for nature conservation. Plant growth and the distribution of excrement can be improved. By recording the movement data of the animals, a direct success can be made measurable.

The University of Goettingen is leading the project. It is in charge of project coordination of analyses of the virtual fencing trials.

Thesis objectives and chapter outline

In the framework of the interdisciplinary research project ‚GreenGrass’ the overarching objective of this thesis is testing and developing the options of virtual fencing as a contribution to future grazing management.

The thesis objectives are:

(a) Generating knowledge about the movement behaviour of cattle as a function of forage availability.

(b) Investigating animal well-being as a basic prerequisite for the use of new technologies such as virtual fencing in livestock farming.

(c) Evaluating and testing the options for animal monitoring and pasture utilisation supervision via virtual fencing and UAV-based remote sensing.

In **part II** of this thesis, the research results are presented in three chapters that refer to three scientific articles. The first and second scientific articles have already been published in peer-reviewed international journals. The third scientific article is about to be submitted.

The following topics are covered:

The **first chapter** examines the movement behaviour of suckler cows on semi-natural grassland and focuses on the effects of sward structure and grazing intensity on spatial distribution and walking distances, measured via conventional GPS collars. The following hypotheses have been formulated:

1. Cattle activity increased with lower herbage allowance.
2. The spatial distribution of cattle during active time (grazing peaks) is more even under moderate compared to lenient grazing intensity.

Chapter two investigates the potential impact of virtual fencing technology on the welfare of growing heifers. For this purpose, three groups of four Fleckvieh heifers with a virtually fenced exclusion zone were compared in three time replications for 12 days, to three control groups with a conventional fenced exclusion zone. We evaluate a range of behavioural and physiological characteristics to comprehensively answer the research question. The general hypothesis is:

Virtual fencing has a negative effect on grazing heifers compared to physical fencing, which can be measured by a range of behavioural characteristics and physiological responses.

In the **third chapter**, new options for animal monitoring through virtual fencing technology and pasture utilisation are analysed using the link to UAV-based remote sensing of the sward. Data of a larger rotational grazing trial in 2021 was used. The overall objective of this study was to test whether it is possible to derive decision support information in order to facilitate grazing systems using virtual fencing collars and UAV data. For this purpose, the following specific objectives have been defined:

1. to verify the suitability of the VF collars for animal monitoring and the ability of UAV based imagery to estimate dry biomass changes, as preconditions for the overall objective of the study,
2. to investigate whether cattle spent active time monitored using GPS could be related to high resolution UAV based observations of the grass sward on a polygon grid, and
3. to interpret the implications of the outcomes for monitoring and decision support in grazing system.

The thesis concludes with a **general discussion** in **part III**, and summarises the main findings of the three chapters in **part II**.

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Part II

Chapter 1

The Effect of Grazing Intensity and Sward Heterogeneity on the Movement Behavior of Suckler Cows on Semi-natural Grassland[1]

Abstract

Extensively grazed semi-natural grasslands contribute to a wide range of ecosystem services, including the preservation of biodiversity and provision of livestock feed. Depending on the grazing intensity, cattle are set in motion to fulfill their nutritional needs. In this way, they influence the vegetation composition, while at the same time the foraging behavior is affected by the vegetation. A better understanding of the relationship between grazing intensity and animal behavior is an essential component for strategies to improve the value of semi-natural grasslands and for gaining insights for the development of smart farming technologies. The long-term cattle grazing experiment "FORBIOBEN" with its replicated three paddock-scale (1 ha) grazing intensities [moderate (M), lenient (L), very lenient (VL)] was used to investigate the movement behavior of suckler cows during four grazing periods between 2017 and 2020. For this, pregnant suckler cows (Fleckvieh) were equipped with Vectronics GPS Plus (VECTRONIC Aerospace GmbH, Berlin) collars, which recorded the position of the animals at defined time intervals. The main outcomes were that with an increase in the grazing intensity, the herbage on offer declined and, consequently the herbage allowance. However, the spatial heterogeneity of the herbage on offer decreased with increasing grazing intensity (M $<$ VL) which means that the amount of available herbage was lower but more evenly distributed under mod-

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erate grazing. Further, there was a tendency that the moderate grazing intensity was associated with the highest effort of walking compared to lenient and very lenient grazing in three out of four grazing periods. We found a strong ($p < 0.001$) negative correlation among walking distance vs. herbage variability across all treatments × periods. Consequently, the grazing intensity itself was not a good predictor of walking distances which were mainly a result of the available herbage, its distribution or heterogeneity. Future smart farming livestock management systems will, therefore, likely require interfaces with the grassland growth rates and heterogeneity benchmarks if decisions based on livestock movement should be reliable.

Keywords: herbage allowance, GPS tracking, precision livestock farming, walking distance, spatial distribution

Introduction

Grassland is the largest terrestrial biome, covering $\sim$3.2 billion ha worldwide (1) and a large part of this area is used by grazing herbivores. Depending on the environmental conditions, the animal species and grazing method, these grazing herbivores influence the sward while their performance, on the other hand, is influenced by the sward properties (2, 3). Extensification of grassland leads to a shift toward a more diverse botanical composition and increased plant species richness (4). For instance, extensively grazed semi-natural grasslands host a great number of plant species, which is why they essentially contribute to the biodiversity of agricultural landscapes (5, 6). The vegetation often develops into a heterogenous pattern of different sward height classes of tall and short patches (7) which results from the so-called patch grazing (8). Patch grazing is characterized by a pronounced spatial heterogeneity in forage intake (9) with intensive and extensive grassland utilization occurring in close proximity within the same pasture. Several studies in semi-natural grassland found that the productivity (10), soil nutrient contents (10, 11), and the vegetation composition (4, 12) are driven by these temporally stable patches (7) rather than by the pasture-scale grazing intensity. The extent of patch grazing is controlled by the pasture stocking rate, i.e., the herbage allowance per grazing animal. It has been shown that under low stocking rates, animals tended to graze only on short grass patches even at the end of the grazing season (13). This indicates that the cattle regularly return to the same spots of high-quality herbage. Assuming that the productivity of these patches is maintained, the effort for foraging is low and the walking distances should mainly depend on the spatial distribution of these patches. With a more restrictive herbage allowance, i.e., higher grazing pressure, the animal has to visit more places every day to fulfill its energy demand because less herbage is available per patch so that more movement is required. On the other hand, a higher herbage allowance per animal does not always result in less movement since in a patchy grassland the foraging areas are spatially distributed (7). Hejcmanová et al. (14) investigated behavioral patterns under extensive and intensive continuous grazing (fewer vs. more cattle per pasture) and found a clear trend toward longer grazing time under intensive grazing. However, in a study of Dumont et al. (13), the walking distances per grazing event were not affected by the stocking rate and group size. Thus, it remains an open question to what extent the grazing intensity and, hence, the availability or distribution of herbage control the activity of grazing cattle in semi-natural grassland ecosystems. Such information is needed if any decision support tools in future smart farming systems will be based on the spatial animal movement. Using GPS (Global Positioning System) collars to track the spatial behavior of grazing cattle is a well-established method to investigate the drivers of animal behavior. Since 1978 GPS is operational and since 1984 civilian use is allowed. The University of Kentucky began to use GPS collars for cattle tracking in the 1990s to be able to integrate spatial information into cattle management procedures (15). Using GPS collars in studies of animal movement has many benefits: individuals can be tracked over a long-term period with predefined time intervals and automatically recorded geographical positions (16), which is very helpful information on large pastures and rangelands (17). In addition, accurate and efficient information on grazing behavior can be provided by the use of GPS for monitoring of grazing animals (18). Animal-related GPS recordings in combination with a geographic information system (GIS) can provide information on spatial interrelations of animal behavior and the vegetation (19). In recent years, several studies have investigated the potential of GPS tracking data to deduce behavioral patterns of grazing cattle. Homburger et al. (20, 21), both based on investigations in heterogeneous subalpine pastures, recommended to differentiate only grazing and resting when using GPS tracking. Walking is mainly correlated with grazing because cattle always walk several steps between bites while walking without grazing is a relatively rare activity (22). In the study by Homburger et al. (20), only 6.7% of movement was accounted for by walking without grazing as assessed by visual observations. In another study (17) it was shown that the time budgets of the main cattle behavior (grazing, resting, walking) were not influenced by the grazing management. However, the walking distances were affected in that study and also in that by Baudracco et al. (23), where cows on a pasture with lower herbage allowance spent more time walking. Consequently, assessing movement patterns in terms of walking distances will provide a reliable indicator for the effort of the grazing cattle to fulfill dietetic demands under conditions of varying herbage allowances. Moreover, such assessments can help to identify the driving forces of livestock movement, including the role of sward characteristics. The study presented here was conducted in a multi-year grazing experiment with livestock cattle on semi-natural grasslands under three different grazing intensities, defined by different target sward heights (moderate: 6 cm, lenient: 12 cm, very lenient: 18 cm) resulting in decreasing stocking rates (moderate to very lenient). The grazing experiment was established in 2002 under the EU framework 5 research project “FORBIOBEN” (3).

The aim of “FORBIOBEN” with its three paddock scale grazing intensities is to represent the entire gradient of grassland extensification. Over three seasons (2017, 2019, 2020), cattle were equipped with GPS collars with the aim to disentangle interactions between the grazing intensity and cattle movement by taking into account both herbage allowance and the spatial variability of the herbage on offer. We hypothesized that (i) cattle activity increased with lower herbage allowance because the area, size and stability of tall patches increase with decreasing grazing intensity (7), and foraging resources are the most obvious drivers of grazer distribution at pasture (8), we further hypothesized that (ii) the spatial distribution of cattle during activity (grazing) peaks is more even under moderate compared to lenient grazing intensity.

Material and Methods

Experimental site, setup and weather conditions

The present study investigated the movement behavior of suckler cows in response to three different grazing intensities. It was carried out over four periods between spring 2017 and spring 2020 as part of the grassland experiment ‘FORBIOBEN’, which is located at the experimental farm of the University of Göttingen in Relliehausen, Solling Uplands, Lower Saxony, Germany (51°46’55.9"N, 9°42’11.9"E), 250 m above sea level. The vegetation is a moderately species-rich semi-natural grassland classified as Lolio-Cynosuretum. The three most important grasses in 2017 were Festuca rubra, Lolium perenne and Cynosurus cristatus, while the three most important dicot species were Taraxacum officinale, Trifolium pratense and Galium mollugo. In 2020 this changed slightly towards F. rubra, Dactylis glomerata and L. perenne and for the dicots to T. officinale, Lotus corniculatus and Galium mollugo. The longtime climatic averages of the German weather service ‘Deutscher Wetterdienst’ reference period (1981-2010), measured approximately 21 km apart, were: annual precipitation sum: 805 mm, temperature: 9,4 °C, sunshine hours: 1412 (24). Weather conditions in the investigated periods are summarized in Table 1.

Table 1: Weather conditions (TM: mean daily temperature, °C, radiation (W m2), and precipitation sum (mm)) during the four investigated periods recorded by the meteorological station in Bevern 51°51’10.8"N 9°29’42.0"E coordinated by the German Weather Service ‘Deutscher Wetterdienst’ (DWD), 21 km from the experimental site.

Period	TM (°C)	Radiation (W m2)	Precipitation sum (mm)
2017	16.2	19950.6	52.1
2019 spring	16.1	19722.2	77.0
2019 autumn	12.6	13004.0	16.0
2020	17.3	17875.0	79.6

The grazing experiment 'FORBIOBEN' was established in 2002 (3) and is maintained in its current state since 2005. It compares three intensities of cattle grazing described by different target vegetation heights, hereafter M: moderate grazing (6 cm), L: lenient grazing (12 cm) and VL: very lenient grazing (18 cm target vegetation height). The three grazing intensities are replicated in a randomized block design of three paddocks (1 ha each) per grazing intensity. The general framework of the 'FORBIOBEN' experiment is extensive grassland management as no fertilizer, pesticide or any sward improvement measure is applied. Within this framework, the different grazing intensities represent the following strategies. Moderate grazing is aiming at reasonable agronomic performance; lenient grazing does not make full use of the herbage, leaving remaining herbage for biodiversity targets, and very lenient grazing is representing the minimum grazing intensity that is required to keep the grazing land open, i.e. maintain the open character of the grassland. The management is a continuous grazing system with a put-and-take approach. In this system, cattle are added to the paddocks when the target vegetation height is exceeded and removed when the vegetation height falls below the target.

Animals

During each stocking season (April/May – September/October), up to 27 pregnant, non-lactating Fleckvieh suckler cows grazed in all three grazing intensities. Usually, the target sward height of 6 cm in M is reached faster in spring, so that this treatment can be stocked earlier. The VL treatment was stocked when the target height of the L treatment was reached, to prevent natural succession of the grassland. Outside the grazing period, from November to April, the animals are in winter housing. Calving takes place in November and December; mating is in February and March. Cows return to pasture in mid-April, after weaning. Animals that were removed from the experimental paddocks because sward heights fell below the target values grazed an area adjacent to the experimental paddocks. During the investigated periods, the cows were randomly assigned to groups and distributed among the paddocks. Average stocking densities of the different grazing intensities during the investigation were, moderate grazing: 4.6 LU ha-1, lenient grazing: 3.8 LU ha-1, very lenient grazing: 2.7 LU ha-1 (LU: livestock unit, 500 kg live weight). A detailed overview is given in Table 2. The respective stocking rates under moderate, lenient and very lenient grazing, calculated as (LU × days on pasture) per year and pasture area, were 1.4, 0.5, and 0.4 LU ha-1a-1 in 2017; 0.9, 0.6 and 0.4 LU ha-1a-1 in 2019; and 0.7, 0.4 and 0.2 LU ha-1a-1 in 2020.

Table 2: Overview of the grazing management and treatments during the investigated periods and annual stocking rates. GI: grazing intensity, LW: live weight, SD: stocking density, LU: livestock unit, SR: stocking rates

Period (duration)	GI	Age years ± sd	LW kg ± sd	SD (LU ha^{-1})	SR (LU ha^{-1} a^{-1})
2017 (18.05.-14.06.)	M	4.8 ± 1.6	666.3 ± 73.3	5.3	1.4
	L	5.6 ± 2.8	638.7 ± 96.2	3.8	0.5
	VL	5.4 ± 1.2	658.3 ± 86.0	2.6	0.4
2019 spring (24.05.-27.06.)	M	6.0 ± 2.5	684.8 ± 97.0	5.5	0.9
	L	5.7 ± 2.4	667.0 ± 101.0	4.0	0.6
	VL	5.2 ± 2.2	638.0 ± 57.4	2.6	0.4
2019 autumn (06.09.-22.09.)	M	5.1 ± 2.7	749.3 ± 105.0	4.5	0.9
	L	6.2 ± 2.6	795.1 ± 60.8	4.8	0.6
	VL	5.5 ± 2.1	748.7 ± 91.2	3.0	0.4
2020 (11.06.-12.07.)	M	3.4 ± 1.3	620.0 ± 69.7	2.5	0.7
	L	6.0 ± 2.9	626.7 ± 80.6	2.5	0.4
	VL	7.8 ± 1.2	673.5 ± 44.6	2.7	0.2

Collecting data

The duration of the investigated periods differed in response to the weather conditions and, hence, the herbage growth (Table 2). Each period lasted for 28, 35, 17 and 32 days in 2017, 2019 spring, 2019 autumn and 2020, respectively. To avoid bias from acclimatization to the collars and increased movement associated with paddock changes, the data collected on the first and last day of each period were excluded. The dates shown in Table 2 omit these days and correspond to the actual daily data used.

At the beginning of each period, one cow per grazing intensity and replicate was equipped with a Vectronics GPS Plus (VECTRONIC Aerospace GmbH, Berlin) collar (weight: 1.36 kg), attached to the neck of a randomly chosen cow per paddock, corresponding to a total of nine GPS collars. In the periods 2019 spring and 2020 two collars, and in 2019 autumn one collar were found not to have recorded data when the collars were removed. The collars are equipped with internal devices for GPS localization and an activity sensor (three-way accelerometer). Every 128 seconds (2017 and 2019 spring), or every 60 seconds (2019 autumn and 2020), the GPS sensors in the collar recorded a signal about the location of the animal within the pasture. Each GPS data point was recorded with date, time, distance, speed, absolute and relative angle between two successive path segments. In addition, the activity sensor in the collar recorded data in 64-second intervals. For each interval, it measured the proportion of time that the head tilt angle of the animal exceeded 15°, i.e. the time that the head was not lowered. At the end of the respective grazing period, the collars were removed to retrieve data and analyzed to measure the activity in terms of walking distance.

Walking distance (m) per animal was measured at two temporal scales, per day and also per hour within day. Geographic coordinates were available in the Universal Transverse Mercator coordinate system (UTM) format. To calculate the distance between two sequential positions, the Pythagorean theorem was used. The results were summed for hourly and daily (24-h) periods.

Data obtained from the activity sensor of the collar in spring 2019 were used to assess the relationship between walking distance per hour and the duration of grazing in minutes per hour, following Homburger et al. (21). Measurement intervals during which the activity sensor reported a lowered head at least half of the time were classified as grazing. This classification was validated by visual observations during 2016.

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Sward herbage measurements and sward characteristics

To determine the grassland herbage on offer, a double sampling approach was conducted from early April to October. For this, the compressed sward height (CSH) was measured every two weeks using a rising plate meter of 30 cm diameter and 200 g plate weight (25) at 50 places randomly distributed in each paddock. Approximately every four to eight weeks, the standing herbage dry matter was determined at six to eight random points per paddock. Biomass was cut manually at 1 cm above the soil surface in a 30-cm diameter ring after first measuring CSH at this location. This procedure was conducted in order to calibrate the relationship between CSH and grassland herbage mass based on linear regression models (26,27). The herbage biomass samples were oven-dried at 60°C for 48 hours to obtain the dry matter weight. Based on the relationship between CSH and standing herbage dry matter, the available herbage on offer (herbage mass) was modelled for every other date and CSH measurement without calibration sampling so that 50 herbage values were available per paddock on each date of CSH measurements. Herbage biomass prediction from CSH was reasonable (RMSE = 70.4 g m^{-2} and mean R^2adj = 0.63 averaged over all periods). The derived herbage on offer per CSH sampling point was used to calculate the spatial heterogeneity of the herbage on offer by calculating the standard deviation within paddock (SD herbage).

Botanical composition in ten 1-m2 quadrats was assessed in accordance with the method of Scimone et al. (28) with average proportions between 2017 and 2020 of 59.7 ± 9.6, 59.2 ± 13.5 and 53.7 ± 10.9 % grasses and of dicotyledonous species of 26.1 ± 5.7, 27.8 ± 6.8 and 25.7 ± 6.2 (± SD) in M, L and VL, respectively. Further studies showed that within grazing intensities, the botanical composition differed between short and tall patches as a consequence of modified resource availability for light and soil nutrients (4). Tonn et al. (11) observed larger phytodiversity in short patches compared to tall ones, and Perotti et al. (29) found that species in tall patches had higher competitivity and the ones in short patches higher stress tolerance according to the competitor, stress tolerator, ruderal (CSR) theory after Grime (30).

The in vitro organic matter digestibility as assessed using near-infrared reflectance spectroscopy in ten continuous observation plots of 1 m2 size per paddock were 78.5 ± 7.4, 76.2 ± 6.2 and 74.6 ± 6.0 % (mean ± SD) on average over 2017 to 2020 in M, L and VL, respectively. No patch-specific forage quality data was assessed in the present study. We know, however, from the beginning of the grazing experiment, that tall and short patches differ in the stem-to-leaf ratio towards the end of the growing season (27) with consequences for forage quality (3). Pavlů et al (31) indicated differences in patch-specific forage quality and a recent study by Ebeling et al. (10) on the same site 12 years after extensive grazing revealed that the short patches were less productive and likely remained in a vegetative state as a consequence of selective grazing.

Data analysis

Statistical analyses were carried out with the software R (32). Linear mixed effects models were calculated for each target variable using the package 'nlme' (33). For this, every period was analyzed separately. Outliers were eliminated if present by considering values ranging 1.5-fold above the 75th or below the 25th percentile of the interquartile range (34). For all analyses, approximately <5% of the data were excluded as outliers. Normality of the residuals was checked by visual inspection of quantile–quantile plots. Variance homogeneity was evaluated by plots of residuals vs. fitted values and residuals vs. predictor values (35). Multiple contrast tests according to Tukey's test for significant influencing factor levels were followed using the 'emmeans' package (36) after analysis of variances.

The daily distance was regressed on the fixed effects of grazing intensity and date as well as their interaction. The cow nested in block was modelled as a random effect in order to account for correlation between measurements on the same object. Then model reduction was performed from the global model using the MuMIn package (37). The model with the lowest AICc was chosen as the final model.

To assess the diurnal patterns within days, models with fixed effects of grazing intensity, hour per day and their interaction and the random effect of the block and cow nested in block were generated. The dates per period were treated as replicates and the interaction between hour and date was consequently not considered. The hourly walking distance was log-transformed before analysis in order to improve normality of residuals.

The average period-wise herbage allowance was determined in order to assess the strength of competition for forage resources which may drive the walking distances in pastures (23). For this, the herbage allowance was regressed on the fixed effect of grazing intensity and the random effect of block. The herbage allowance was square-root transformed before analysis.

To quantify the extent of spatial clustering within period and grazing intensity treatment, each paddock was rasterized into 400 5 × 5 m squares. GPS locations were split into two groups: 'active time' included all animal locations during the activity peaks in the morning and afternoon, as determined from the analysis of walking distance per hour. 'Other time' included all other animal locations. For each of these sets, the duration (min) spent within each grid cell was calculated. These values were then used to determine the Camargo Index of Evenness across all cells within paddock and period (38) for both groups. The Camargo index allows to assess spatial patterns and the relative distribution of GPS locations within each paddock. Values near zero indicate a patchy distribution and values near one a homogenous distribution (38). This index is, thus, a metric for the requirement of searching to fulfill the herbage intake in relation to the grazing intensity. The Camargo Index was then analyzed in models with the grazing intensity as fixed and block as random effect separately for each period. For other time, the approach was similar.

The relationship between the activity of time spent grazing, (grazing time, min hour^{-1}, based on the activity sensor measurements) and the hourly walked distance was analyzed in an analysis of covariance with the walking distance per hour as covariate, the grazing intensity and the interaction of both as fixed and the block as random effect. Variance adjustments were allowed per date in that model. A significance level of $p \leq 0.05$ was chosen throughout.

All spatial maps were plotted with QGIS (3.10.12 'A Coruña').

Results

Average daily walking distances within each grazing period

Differences of the daily walking distances between grazing intensities were mostly significant but depended on the grazing period (Table 3, 4).

Table 3: Output of linear mixed effects models for the analyzed parameters of interest during each grazing period. Shown are F- and p-values.

Period	Variable	Fixed and interaction effects	F-value	P-value
2017	daily walking distance	Grazing intensity	30.6	P<0.01
	hourly walking distance	Grazing intensity	0.2	n.s.
		Hour	104.7	P<0.001
		Grazing intensity × hour	3.7	P<0.001
	herbage on offer	Grazing intensity	10.3	P<0.001
	sd herbage	Grazing intensity	4.8	P<0.1
	herbage allowance	Grazing intensity	118.8	P<0.001
	camargo active time	Grazing intensity	7.8	<0.05
	camargo other time	Grazing intensity	22.5	<0.01
2019 spring	daily walking distance	Grazing intensity	5.6	P<0.1
	hourly walking distance	Grazing intensity	1.7	n.s.
		Hour	71.6	P<0.001
		Grazing intensity × hour	5.0	P<0.001
	herbage on offer	Grazing intensity	75.8	P<0.001
	sd herbage	Grazing intensity	39.2	P<0.01
	herbage allowance	Grazing intensity	493.7	P<0.001
	camargo active time	Grazing intensity	5.1	n.s.
	camargo other time	Grazing intensity	7	n.s.
	grazing time	Distance	4064	P<0.001
		Grazing intensity	7.5	P<0.001
		Distance × Grazing intensity	38.7	P<0.001
2019 autumn	daily walking distance	Grazing intensity	58	P<0.01
	hourly walking distance	Grazing intensity	1.7	n.s.
		Hour	60.7	P<0.001
		Grazing intensity × hour	2.5	P<0.001
	herbage on offer	Grazing intensity	74.8	P<0.001
	sd herbage	Grazing intensity	3.4	n.s.
	herbage allowance	Grazing intensity	8.4	P<0.05
	camargo active time	Grazing intensity	18.3	<0.05
	camargo other time	Grazing intensity	17.5	<0.05
2020	daily walking distance	Grazing intensity	n.s.	n.s.
	hourly walking distance	Grazing intensity	6.3	n.s.
		Hour	107.1	P<0.001
		Grazing intensity × hour	5.5	P<0.001
	herbage on offer	Grazing intensity	29.6	P<0.001
	sd herbage	Grazing intensity	11.1	P<0.05
	herbage allowance	Grazing intensity	15.6	P<0.01
	camargo active	Grazing intensity	1.7	n.s.
	camargo other time	Grazing intensity	24.7	<0.05

While in 2017 and autumn of 2019, the daily walking distances were affected by the grazing intensity (Table 3), no effects were found in 2020 and spring of 2019 (although p<0.1). In most periods, walking distances were largest for grazing intensity M (not in 2020), while they were lowest for grazing intensity L in most periods (not in 2017) and those of VL tended to range between them (Table 4). The daily distances varied between 2592 m (2017 grazing intensity L) and 3929 m (2020 grazing intensity VL).

Average hourly walking distances within each grazing period

The interaction between hour per day and the grazing intensity affected the hourly walking distance in all periods (Table 3). A strong diurnal pattern became evident with a shift in the activity peaks during the autumn 2019 period compared with the other periods (Figure 1).

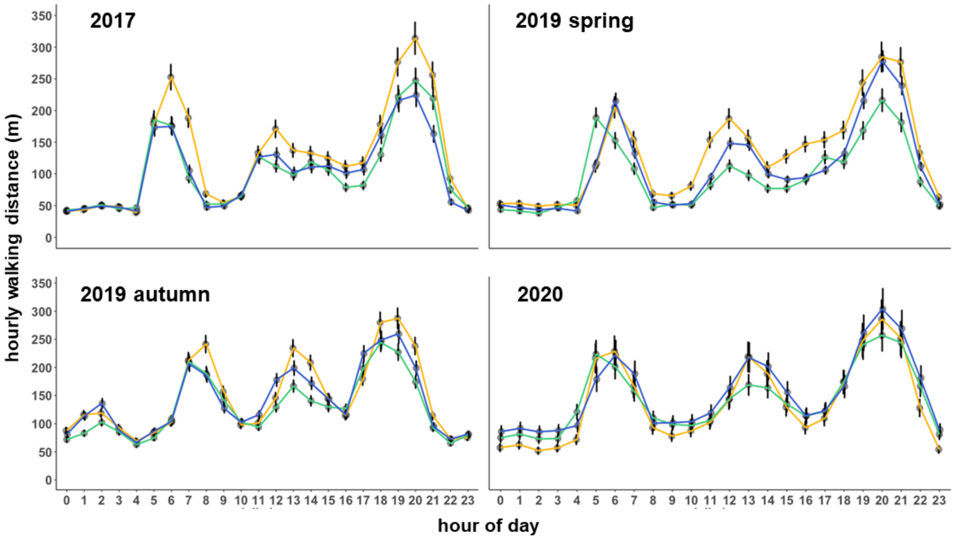

Figure 1: Estimated means (±SE) of the average hourly walking distance (m) as influenced by the grazing period, grazing intensity and hour per day. M: moderate, L: lenient, VL: very lenient grazing intensity.

The main activity was recorded in the hours 5, 6, 7 a.m. and 7, 8, 9 p.m. (spring and summer periods). In autumn, the activity peaks were narrower, comprising the hours 7, 8 a.m. and 5, 6, 7, 8 p.m. (Figure 1). These time periods were considered as 'active time' when the Camargo Index was calculated. On average, they encompassed 40% (M), 39% (L) and 39% (VL) of daily walking distances. The main periods of inactivity occurred during night time and between the activity peaks (Figure 1).

The hourly walking distance and the grazing time (spring 2019) were positively related, with the slope depending on the grazing intensity treatment (Figure 2) and significantly affected by distance and the interaction of distance × grazing intensity (Table 3).

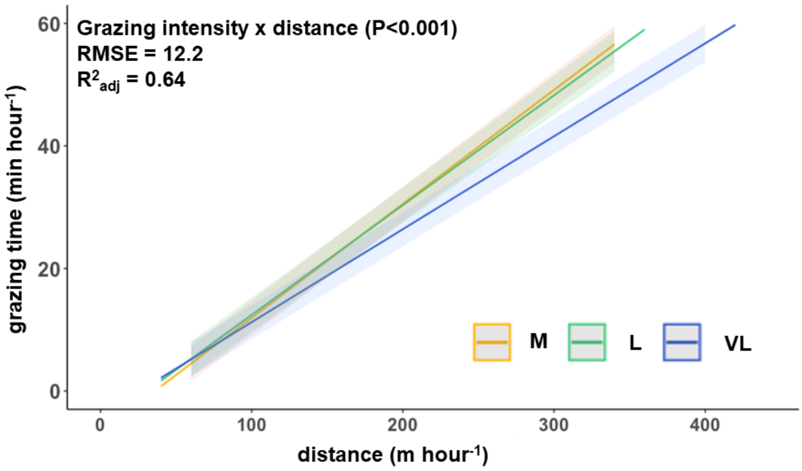

Figure 2: Functional relationship between the hourly walking distance and grazing time per hour (spring 2019) for the three grazing intensities (model prediction), M: moderate, L: lenient and VL: very lenient in spring 2019.

Herbage on offer, spatial heterogeneity of herbage on offer and herbage allowance

The average herbage on offer during each period was affected by the grazing intensity (Table 3, 4) with a general increase of available herbage from grazing intensity M, over L to VL, but also a visual decline in the available herbage from 2017 until 2020 (Table 4). The values for each measured date are provided in the supplements (Figure S1). The herbage allowance was affected by the grazing intensity in all periods (Table 3) and generally increased in the order M < L < VL (Table 4).

There were only significant effects of the grazing intensity on the SD herbage mass in spring of 2019 and 2020 (Table 3) with a clearly lower variability within grazing intensity treatment M compared with L and VL in that period (Table 4). A general trend for increases in SD herbage mass in the rank order M < L $\leq$ VL, however, became clear for all periods.

Table 4: Estimated means ± se (standard error) of linear mixed effect models for every period. Lowercase letters: means with different letters are significantly different between GI within year ($p<0.05$). GI: grazing intensity, HO: herbage on offer, SD Herbage: standard deviation of herbage on offer, HA: herbage allowance

Period	GI	Individual daily distance (m)	HO (g DM m^{-2} ± se)	SD Herbage (g DM m^{-2} ± se)	HA (kg DM LU^{-1} ± se)
2017	M	3642 ± 173 b	235 ± 19.1 a	81.3 ± 8.41	455 ± 51.6 a
	L	2958 ± 173 a	319 ± 19.1 ab	108.8 ± 8.41	854 ± 70.7 b
	VL	2901 ± 173 a	355 ± 19.1 b	119.2 ± 8.41	1421 ± 93.4 c
2019 spring	M	3542 ± 201	107 ± 7.1 a	56.6 ± 4.31 a	178 ± 14.9 a
	L	2592 ± 201	203 ± 7.1 b	89.6 ± 4.31 b	539 ± 25.8 b
	VL	3108 ± 142	219 ± 7.1 b	96.4 ± 4.31 b	902 ± 27.9 c
2019 autumn	M	3773 ± 92.7 b	99.5 ± 7.64 a	95.2 ± 5.5	265 ± 59.8 a
	L	3329 ± 91.4 a	196.8 ± 7.64 b	106.4 ± 5.5	339 ± 67.6 ab
	VL	3653 ± 91.4 b	215.1 ± 7.64 b	115.4 ± 5.5	695 ± 96.9 b
2020	M	3680 ± 448	80.9 ± 8.96 a	48.9 ± 3.5 a	358 ± 38.4 a
	L	3701 ± 402	156.1 ± 8.96 b	63.7 ± 3.5 ab	621 ± 50.7 b
	VL	3929 ± 448	172.2 ± 8.96 b	72.0 ± 3.5 b	670 ± 43.0 b

Spatial distribution in relation to grazing intensity and period

The Camargo Index was determined for the 'active time', identified as the hours of peak activity according to Figure 1, and for the remaining time (other time) within each period. The Camargo index for the active time was affected by the grazing intensity only in 2017 and 2019 autumn (Table 3), and declined from M to L and VL, indicating a more even distribution within the paddock in grazing intensity M during these periods (Figure 3).

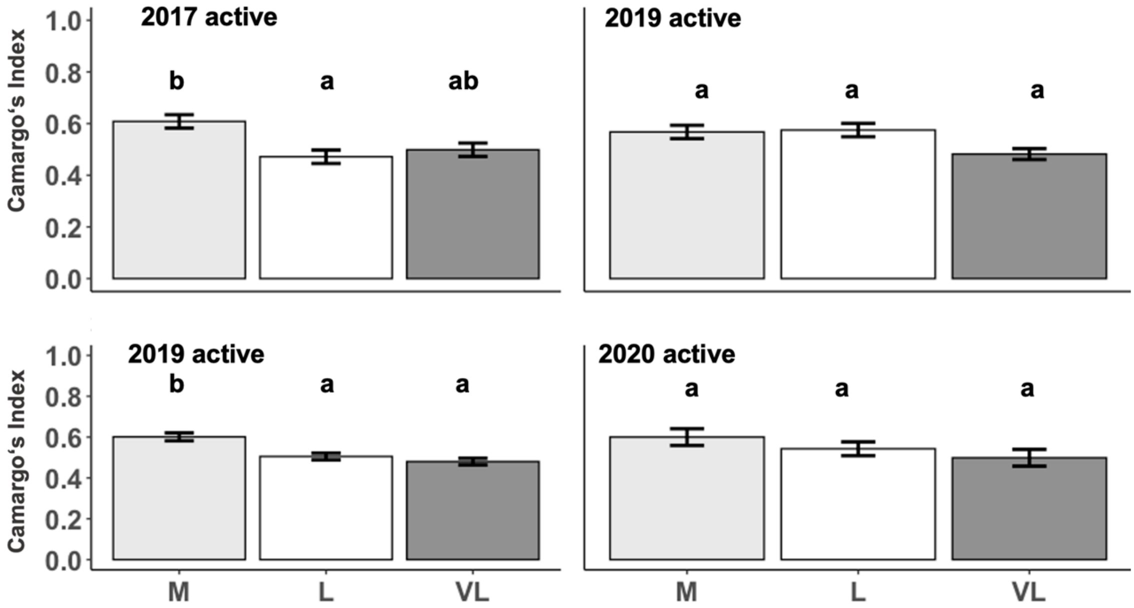

Figure 3: Estimated means (±SE) of the Camargo Index during active time as influenced by the grazing period and grazing intensity. M: moderate, L: lenient, VL: very lenient stocking rate. Identical lowercase letters indicate that means are not different at $p<0.05$.

This was also confirmed for the Camargo index of the other time periods (Figure 4) which were affected by the grazing intensity in all periods except of spring 2019 (Table 3).

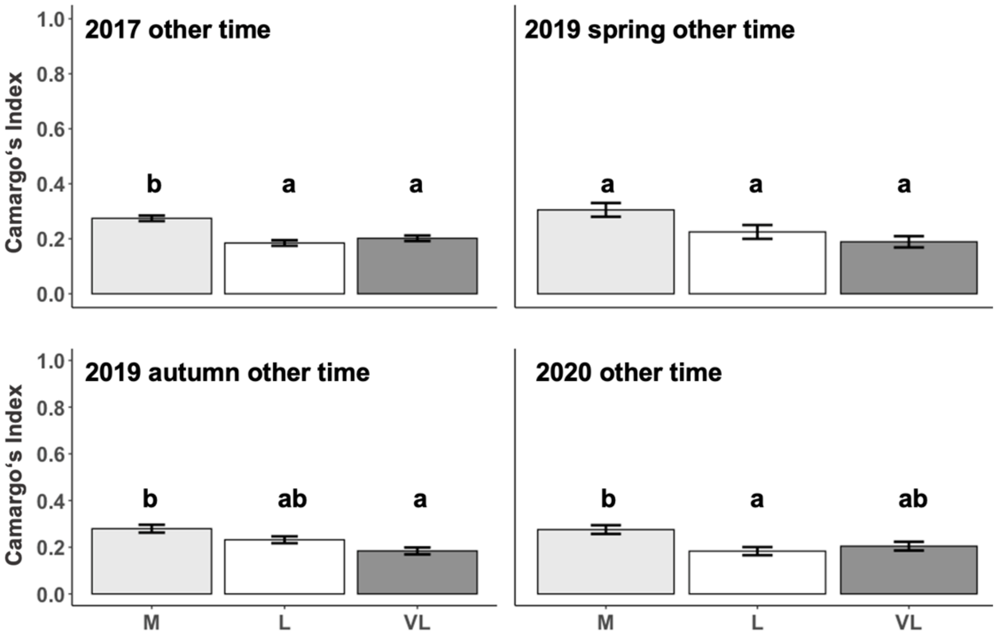

Figure 4: Estimated means (±SE) of the Camargo Index during other time as influenced by the grazing period and grazing intensity. M: moderate, L: lenient, VL: very lenient stocking rate. Identical lowercase letters indicate that means are not different at $p<0.05$.

The distribution of spatial points between pastures within each period is given in Figure 5. Time (s d^{-1}) spent in each 5 × 5m grid cell was categorized into five percentiles, visualized as density maps.

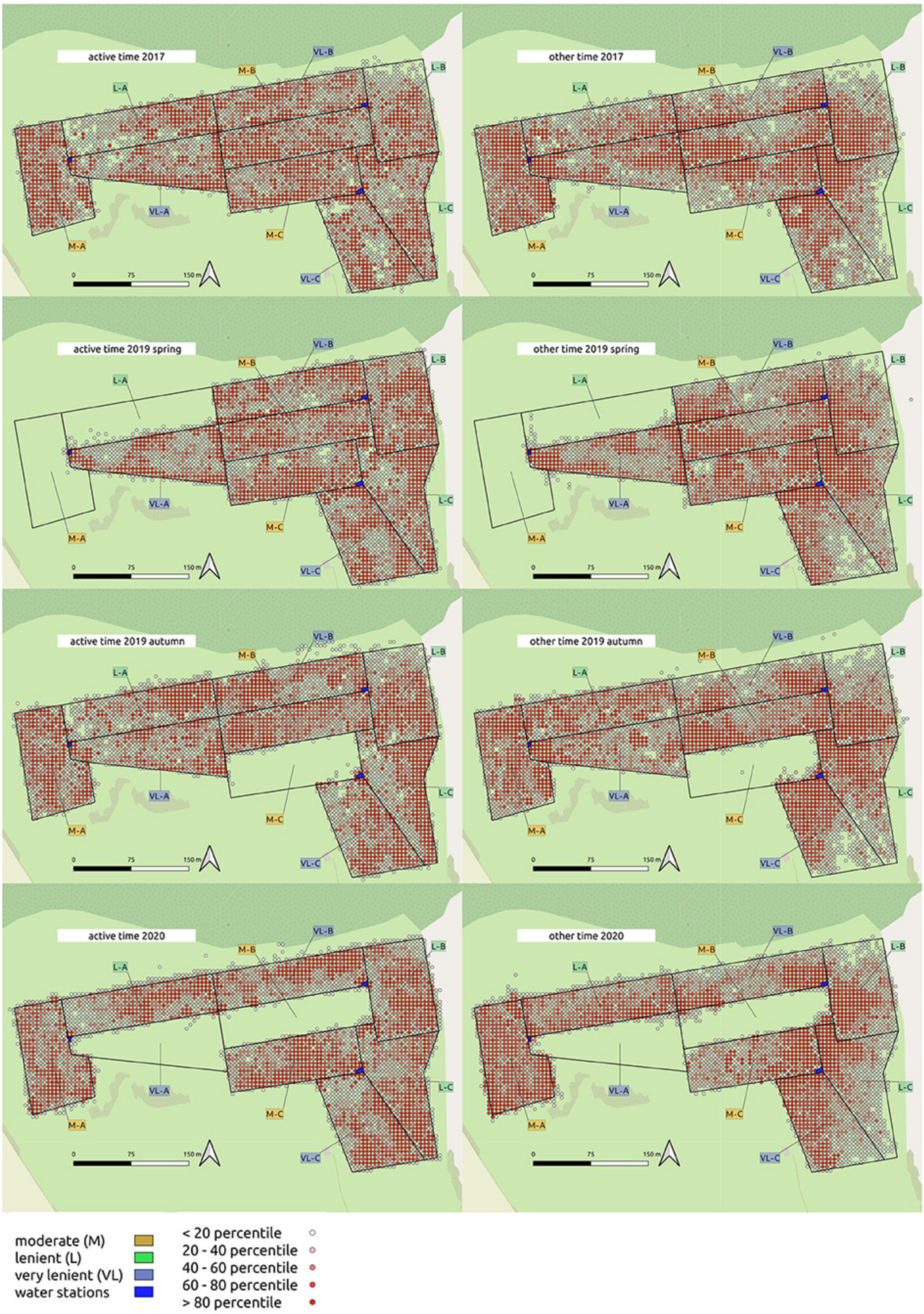

Figure 5: Density maps of cattle location during active time / other time within 5 × 5 m gridcells on the experimental site.

Discussion

While there are many studies on the effects of cattle grazing in different grazing intensities on outcomes for herbage quality (13,39), biodiversity (3,40–42), sward botanical composition (43) or productivity (3,40,41), the current study is the first to quantify the relationship between cattle movement and grazing intensity, taking into account herbage availability. We hypothesized that (i) cattle activity increased with lower herbage allowance. We further hypothesized that (ii) the spatial distribution of cattle during activity (grazing) peaks is more even under moderate compared to lenient grazing intensity.

Variation in herbage availability and patterns of walking distances in relation to grazing intensity

With an increase in the grazing intensity, the herbage on offer and consequently also the herbage allowance declined (rank order: M < L < VL). However, the spatial heterogeneity of the herbage on offer decreased with increasing grazing intensity (M < VL) which means that the amount of available herbage was lower but more evenly distributed under the moderate grazing treatment M. Increases in the stocking rate and a decline in herbage allowance per individual will cause an increase in the effort of walking on pastures of similar botanical composition (22) – especially under low-input conditions when grassland growth rates are low. Except for the last period, moderate grazing intensity tended to be associated with the greatest effort in walking compared with the other grazing intensities, an effect which became clearly significant in 2017 (Table 4). Hejcmanová et al. (14) investigated behavioral patterns under extensive and intensive continuous grazing and found a clear trend towards longer grazing durations under intensive management. Generally, this larger effort in walking under moderate than under lenient grazing arose from longer durations of the two or three main peak activity phases per 24-hour period (Figure 3). However, walking distances were also higher under very lenient than under lenient grazing in some periods (Table 4). Based on the flatter slope between grazing time and walking (Figure 2), this could be attributed to an increased effort in searching of foraging sites.

The mean daily walked distances in the present study ranged between 2592 and 3929 m. These values are in accordance with Baker (44), who described a minimum daily activity of 3000 m on pasture. In a study by Draganova et al. (45), pregnant suckler cows walked between 2700 and 3300 m daily on pastures of 8 to 12 ha in size. Earlier reports state that the daily walking effort of cattle ranges between 2000 and 6000 m (22). Consequently, the daily effort in walking is in line with previously reported values (Table 4).

Spatial patterns of movement

In order to differentiate between potential reasons for differences in movement between grazing intensities, we investigated the spatial patterns of movement. As the Camargo index during the active time tended to decrease from M towards VL (Figure 3), we suggest that the larger variability of distribution of the short patch foraging sites is responsible for a stronger clustering in VL. The more even distribution of the animals across the paddocks in M was likely caused by the lower herbage on offer in that treatment and the resulting need to enlarge the grazing area to fulfil the dietetic demand. As described by Perotti et al. (29), in a study on the same experimental site in 2017, the botanical composition differed between short and tall patches. As indicated by Tonn et al. (7), the distribution of the patch classes is mediated by the grazing intensity with larger proportions of short areas under the moderate grazing intensity.

Heterogeneity/Homogeneity based on the standard deviation of herbage mass

It is well established that cattle prefer leafy and digestible vegetation (46) and search actively for it. Cattle are known to develop a spatial memory of the grazing land (47). The pattern of patches seems to be the landmap of the cattle to find preferred forage spots which are repeatedly visited (48). This behavior maximizes the foraging efficiency in terms of forage intake per unit of walking distance (49). However, we found a significantly negative relationship when regressing the walking distance on the standard deviation (SD) of herbage mass as indicator of spatial heterogeneity ($P<0.001$) (not shown). One has to take into account that the standard deviation of the herbage on offer may be misleading in terms of the actual variability in the spatial distribution of herbage as it is sensitive to the range of values (SD herbage will increase with greater herbage on offer values). Under very lenient grazing, tall avoided areas with large herbage on offer are close to shortly grazed patches with little herbage on offer (7). In contrast to this, under moderate grazing the overall amount of herbage on offer is lower and so is the SD herbage. The very lenient grazing intensity has, thus, a larger amount of unpreferred tall herbage while the moderate treatment has more valuable herbage sources at a lower amount, which both lead to a homogeneous distribution. However, both treatments have the same coefficient of variation (CV) in terms of herbage on offer (not shown). According to Pavlů et al. (31), patches differ in their forage quality and we found a decline of the paddock-scale in vitro digestibility from M to VL. When a pasture is stocked with less cattle (as in most cases during our study in VL compared with M) one grazing patch will provide forage resources for a longer duration. Visual cues associated with disparate feed qualities are used by cattle for more efficient forage intake (50), providing evidence for the spatial memory of the grazing livestock. On the contrary, more effort in walking in the moderate grazing treatment is likely a cause of the lower productivity of short patches (10) which requires to enlarge particular grazing areas per individual under higher stocking density in line with Gibb et al. (51). The negative relationship between SD Herbage and walking effort, however, supports our assumption of two different reasons for increased movement. In M, the grazing stations (short patches) provide forage and were evenly distributed but triggered the cattle to enlarge the grazing area during grazing to fulfil the dietetic demand. In VL, the homogeneously distributed tall and mature herbage drove the movement of the cattle to find palatable forage spots.

Limitations of the current study and variations among the periods

In the present study, only one cow per paddock was equipped with a GPS collar which might not fully reflect the potential effect of the group of grazing animals and individual differences on the grazing behavior. Yet, there is indication for the validity of the findings for the following reasons: the experimental setup provides true replication of the grazing intensity treatments at the paddock-level. Among years, the individuals changed between the grazing treatments. In addition, members of a group of animals usually graze simultaneously (52) while only for the resting time and the time spent for ruminating there is a higher variability among different animals within a group (53). However, we suggest that future studies should look into herd dynamics in greater detail to understand effects of the stocking density on the effort for walking.

The put-and-take systems aims at maintaining sward heights close to the target values by adapting stocking densities to current herbage growth rates, resulting in a gradient of stocking rates across the whole grazing system. The precision with which these aims can be achieved at a given moment strongly depends on the variability of paddock-specific dynamics in grass growth. Sward measurements during the periods showed that the mean measured CSH in grazing intensity M was mainly close to the intended sward height of 6 cm. Measured sward heights under L and VL were close to each other despite different target sward heights (Figure 6). The target sward height of VL of 18 cm was not achieved during our investigation period, which means that the grazing intensities L and VL differ mainly in their herbage allowance but not in the total herbage on offer.

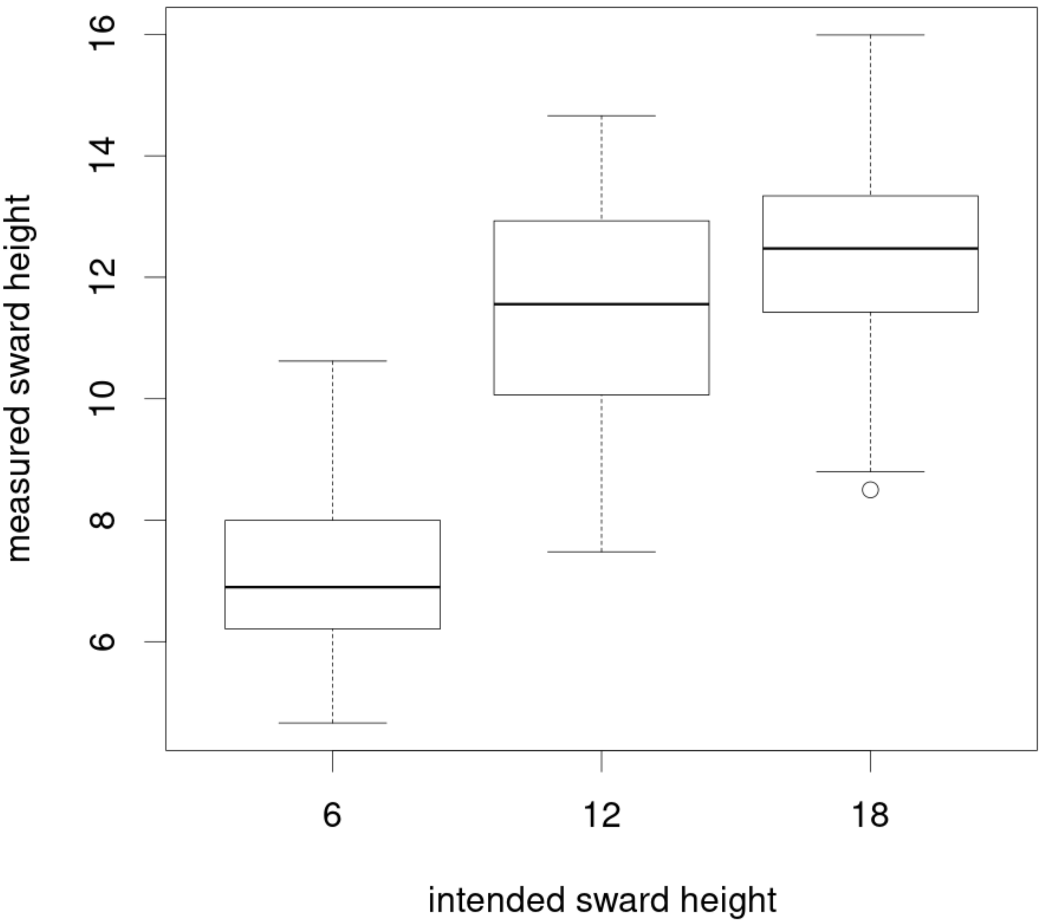

Figure 6: Measured (mean of 50 measurements per paddock taken every two weeks) against realized sward heights per grazing intensity across all study periods.

In the periods of 2019 autumn and 2020, the stocking densities between the grazing intensity treatments were nearly the same. Comparable stocking densities result when the actual herbage on offer requires some adjustment in the number of cows stocked per paddock in order to allow for at least 14 days of grazing, which is the rhythm of sward height measurements in the experiment. However, the treatment M is usually stocked earlier in the season so that the annual stocking rates differ clearly between treatments. Nevertheless, it cannot be excluded that the lack of differences in the walking effort between the grazing intensity treatments in 2020 resulted from the fact that the stocking densities among the treatments were the same during that period, even though herbage allowance differed. However, in a study by Dumont et al. (13), the group sizes did not affect the walking distances of individuals. Further research is necessary to prove this point.

Spatial patterns are usually analyzed in larger scale paddocks which give the livestock a higher probability of performing distinct behavioral patterns at specific places (54). Preliminary work had shown that the mean deviation of the GPS signals of the cattle collars used in the present study, were in a range between 0.6 – 1.9 m. As the collars were set to record values every 128 seconds in 2017 and 2019 spring, or every 60 seconds in 2019 autumn and 2020, this noise adds up to the hourly distances of c. 40 m recorded for the nighttime hours.

Conclusion

Our hypotheses could be confirmed with the present study: (i) cattle activity increased with lower herbage allowance and (ii) the spatial distribution of cattle during active time (grazing peaks) is more even under moderate compared to lenient grazing intensity. However, in our study, cows increased their walking efforts under both the most intensive and also the least intensive grazing treatment. Thus, the herbage availability in terms of herbage allowance and also the spatial distribution (i.e., heterogeneity) of the sward have to be taken into account since all these are drivers for cattle motion. This is relevant information in order to design decision support tools in future precision livestock farming, aiming at a better balance of biodiversity and production targets of grazing systems.

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Ethics Statement

The study is in accordance with the German legal and ethical requirements of appropriate animal procedures. The consultation of the Institutional Animal Welfare Body is documented under no. E5/20 by the Animal Welfare Officer of the University of Goettingen.

Author Contribution

Initiation and supervision of research JI, BT and MK. Conceptualization DH, MK, JI. Funding acquisition JI and BT. Data acquisition incl. field measurements JH, MK, BT. Data analysis DH, MK, BT, JH. Visualization and writing DH (lead), MK, NG. Manuscript revision NG, MK, JI, BT. All authors approved the submission.

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Supplementary Material

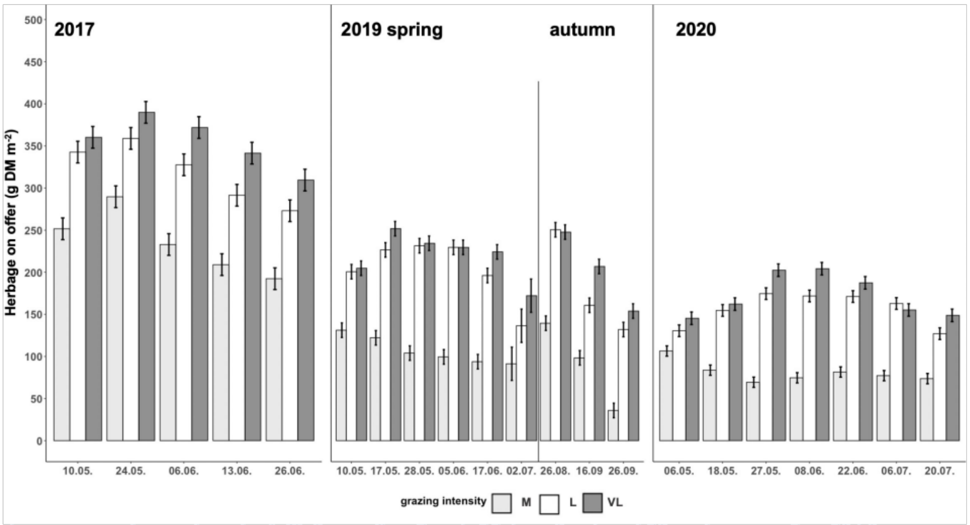

Figure S 1. Mean estimates estimates (±SE) of herbage on offer as determined during each grazing period by regular compressed sward height measurements (50 per date and treatment). Values are calculated from linear-mixed effects models with the grazing intensity and date as well as their interaction as fixed and block and random effects.

Chapter 2

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[1]

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

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[1] Published in: Hamidi, D., Grinnell, N.A., Komainda, Riesch, F., Horn, J. and Isselstein, J., 2022. Heifers don't care: no evidence of negative impact on animal welfare of growing heifers when using virtual fences compared to physical fences for grazing. Animal, 16, 100614 https://doi.org/10.1016/j.animal.2022.100614

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.

Keywords: Precision livestock farming, Europe, smart farming technology, stress hormones, Fleckvieh heifers

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 physical fenced heifers.

Introduction

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

Precision livestock farming (PLF), 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; Campbell et al. 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; Campbell et al., 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; Campbell et al. 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 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 post-grazing herbage mass, walked steps and individual live weight gain per time replicate was 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 in 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: 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 replicate, all sensors were removed and the heifers returned to a neighbouring pasture after final weighing.

Figure 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.

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 (GPS) 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 (82dB at 1m) is followed by a short-duration electric pulse (0.2J at 3kV Duration=1.0s) (source: 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 modi, 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.

IceTag accelerometers

IceTag accelerometers were attached on 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 seperated into paddocks each 1000 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 area 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 m2 (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 (Figure 2). On day eight the accessible area of the PF-group was increased as well for providing new forage by moving the PF-line. Both fence lines were shifted for 3 m.

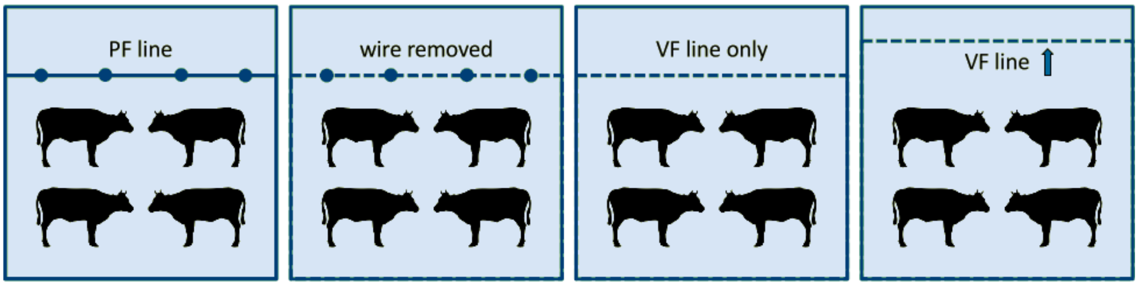

Figure 2: Sections of the 12-day time replicates with grazing Fleckvieh heifers (virtual fencing (VF) group): day one complete physical fence (PF) + VF line, day two only the fence posts of the PF + VF line, day three to seven only VF line, day eight to twelve, the VF line was shifted for 3 m.

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.

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

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.

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 R2 of 0.97 between CSH and herbage dry matter. Herbage dry matter (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 (pre-grazing), day 8 (mid-grazing), and day 12 (post-grazing) 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 cm2 per sampling location. Based on the difference between pre- and post-grazing 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 (FCMs)

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 post-grazing, respectively. Samples were first cooled after collection and then frozen (-18°C) within eight hours after sampling. Before analysis, 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 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 lab 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 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 effects models were calculated using the package 'nlme' (Pinheiro et al., 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 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 random factor (n = 12 replicate animals per fencing treatment in total with four animals per fencing treatment x 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 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 generalized least squares 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 was 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 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.

FCMs

Concentrations (ng g^{-1}) 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 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 Table 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 Figure 3 for GPS positions of the VF group.

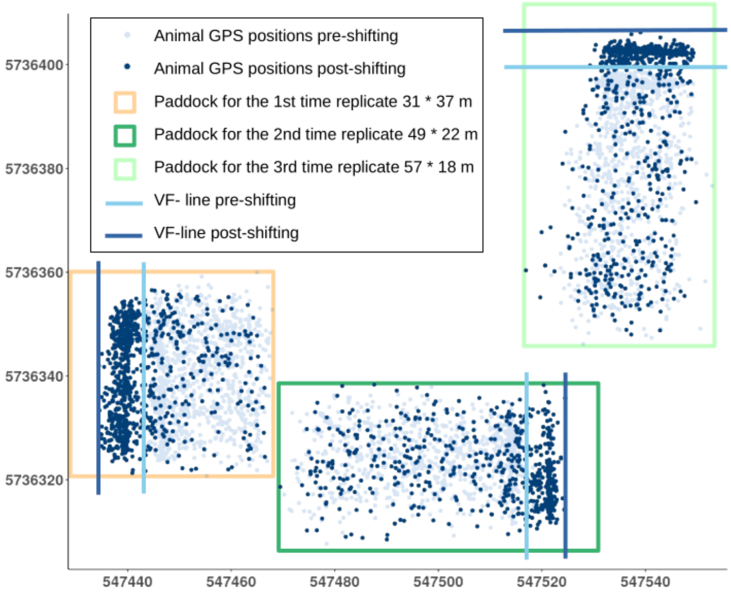

Figure 3: GPS locations of the virtual fencing (VF) groups of Fleckvieh heifers near the VF line were recorded at four signals per second. Positions apart from the VF line were reported in 15-minute intervals in order to save battery. Shown are the days preshifting the VF line and postshifting the VF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

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 day 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 day 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 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.

Table 2: Output of linear mixed effects models for the analyzed 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.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

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

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

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).

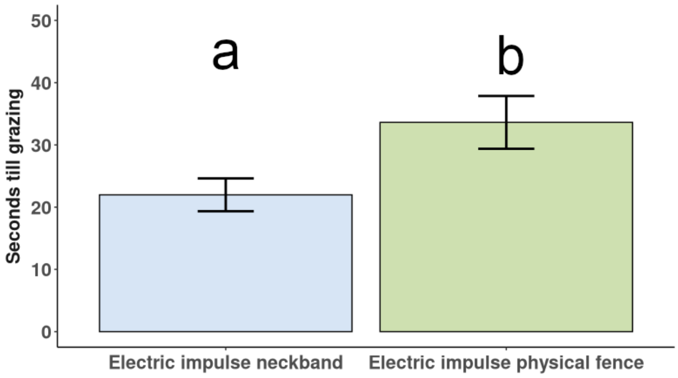

Figure 4: Estimated means ± SE of seconds until grazing after Fleckvieh heifers having received an electric pulse from the Nofence® Collar (only virtual fencing (VF)-group) or an electric pulse from the physical fence (PF) (includes both the PF groups and the VF groups). Lowercase letters indicate significant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

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).

Table 4: Output of linear mixed effects models for the effects of fencing system, day of sample and their interaction with faecal cortisol metabolites (FCMs), estimated herbage consumption and live weight gain 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 twelve) in FCM measurements and three levels for herbage consumption (day 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.* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.

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 ($P<0.001$). Herbage availability was significantly greater at the beginning of each time replicate than at mid and post-grazing. 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 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 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 minimize 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 VF 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 (Figure 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 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 of (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, these proportions decreased to 29.7 to 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 to 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 mostly 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 (Figure 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 point. If it is possible for cattle to learn to avoid a suitable level of electrical stimulus it is likely not harmful for 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 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 Figure 3 showed GPS-positions of the VF-group pre- and post-shifting 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 at 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, 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 post-grazing. This was in accordance with weekly measured FCMs reported in the study by Campbell et al. (2019), where endpoint 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 (3 time replicates, in total 36 days) which can serve as 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.

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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).

Author Contribution

JI, JH, FR, SA, IT and MK: initiation and supervision of research. DH, MK, and JI: conceptualization. JI, JH and IT: funding acquisition. NG, DH and MK: data acquisition incl. field measurements. DH, NG, MH, RP and MK: data analysis. DH (lead), MK, and NG: visualization and writing. NG, MK, JI, FR, JH, SA, RP and IT: manuscript revision. All authors contributed to the article and approved the submitted version.

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Supplementary Material

Tab S1: Total number of acoustic signals / electric pulses per animal and day on pasture

Animal ID	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
First time replicate (17-28 August)												
35083	13 / 6	14 / 1	14 /1	10 / 0	22 / 2	11 / 1	23 / 3	9 / 1	18 / 3	33 / 3	22 / 1	2 / 0
35072	5 / 3	4 / 1	2 / 1	1 / 0	12 / 0	16 / 2	7 / 1	14 / 0	19 / 2	22 / 2	18 / 1	5 / 0
35050	7 /2	4 / 1	0 / 0	1 / 0	6 / 0	3 / 1	7 / 1	4 / 0	20 / 3	6 / 1	1 / 0	0 / 0
35033	9 / 4	3 / 1	4 / 1	10 / 1	6 / 0	22 / 3	3 / 0	6 / 2	28 / 5	25 / 3	34 / 4	32 / 4
Second time replicate (31 August-11 September)												
35087	1 / 1	1 / 0	0 / 0	13 / 2	15 / 2	2 / 1	1 / 0	16 / 2	11 / 0	4 / 0	14 / 2	3 / 0
35062	3 / 1	2 / 0	7 / 3	2 / 0	6 / 0	16 / 1	18 / 2	15 / 2	23 / 2	2 / 0	0 / 0	3 / 0
35043	3 / 1	4 / 0	2 / 1	0 / 0	4 / 1	4 / 0	0 / 0	15 / 1	3 / 0	1 / 0	0 / 0	2 / 0
35021	0 / 0	8 / 1	7 / 3	8 / 1	2 / 0	9 / 0	11 / 1	17 / 2	9 / 1	1 / 0	4 / 0	17 / 0
Third time replicate (14-25 September)												
35074	0 / 0	5 / 2	9 / 1	18 / 3	8 / 0	16 / 2	19 / 3	12 / 3	9 / 0	24 / 4	21 / 1	5 / 0
35060	6 / 0	7 / 1	8 / 1	5 / 2	2 / 0	13 / 1	8 / 1	7 / 0	20 / 2	0 / 0	0 / 0	1 / 0
35053	5 / 1	8 / 1	14 / 4	4 / 0	7 / 0	22 / 4	0 / 0	19 / 4	21 / 3	13 / 2	7 / 0	13 / 3
35022	2 / 1	5 / 0	5 / 2	1 / 0	8 / 0	13 / 2	4 / 1	20 / 2	21 / 0	31 / 3	17 / 1	26 / 1

Tab S2: Proportion of acoustic signals (%) in the total number of cues (acoustic signals + electric pulses) per animal and day on pasture. If an animal did not receive any cues on a given day, we used the symbol “-“.

Animal ID	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
First time replicate (17-28 August)												
35083	68.4	93.3	93.3	100	91.7	91.7	88.5	90.0	85.7	91.7	95.7	100
35072	62.5	80.0	66.7	100	100	88.9	87.5	100	90.5	91.7	94.7	100
35050	77.8	80.0	-	100	100	75.0	87.5	100	87.0	85.7	100	-
35033	69.2	75.0	80.0	90.9	100	88.0	100	75.0	84.8	89.3	89.5	88.9
Second time replicate (31 August-11 September)												
35087	50.0	100	-	86.7	88.2	66.7	100	88.9	100	100	87.5	100
35062	75.0	100	70.0	100	100	94.1	90.0	88.2	92.0	100	-	100
35043	75.0	100	66.7	-	80.0	100	-	93.8	100	100	-	100
35021	-	88.9	70.0	88.9	100	100	91.7	89.5	90.0	100	100	100
Third time replicate (14-25 September)												
35074	-	71.4	90.0	85.7	100	88.9	86.4	80.0	100	85.7	95.5	100
35060	100	87.5	88.9	71.4	100	92.9	88.9	100	90.9	-	-	100
35053	83.3	88.9	77.8	100	100	84.6	-	82.6	87.5	86.7	100	81.3
35022	66.7	100	71.4	100	100	86.7	80.0	90.9	100	91.2	94.4	96.3

Chapter 3

Grid grazing: Virtual fencing and UAV for innovative grazing management[1]

Abstract

Sustainable utilisation of the available grazing area acts to increase the profitability and productivity of livestock grazing and should consider animals and sward. The labour-intensive and time-consuming tasks of fencing, animal monitoring, and controlling forage availability on pasture are general obstacles to the wider implementation of grazing. Virtual fencing (VF) opens up new opportunities, as it acts to reduce fencing labour input and increase flexibility. In this study, we investigated and validated the potential of animal monitoring via VF collars and combined this with Unmanned-Aerial-Vehicle (UAV) data to monitor grazing animals and pasture continuously. 32 Fleckvieh heifers were equipped with VF collars (Nofence®, Batnfjordsøra, Norway) and divided equally into four groups. Each group was assigned to a rotation, consisting of a 2-ha pasture divided into four paddocks, each grazed for three to four days. For all heifers, GPS data were logged by the VF collars and used to evaluate walking distance, lying time, and spatial pattern of movement. Lying time measured via the VF collars was validated via a confusion matrix by using observational data as a reference. Our results suggest that valid animal monitoring using VF collars is feasable. UAV campaigns were carried out pre-and post-grazing each paddock. 3D reconstructions, which allowed the calculation of digital orthomosaics and digital surface models were created from UAV imagery. On that basis, the Red-Green-Blue Vegetation Index (RGBVI), the change of the RGBVI, which was used to determine dry biomass changes validated by using ground truthing

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data (R^2=0.51) and the height change between surveys were calculated and analysed on a polygon grid (2.5 × 2.5 m). A random forest model to analyse animal active time (lying time excluded) spent per polygon grid cell out of RGBVI and height change provided a mean R^2 of 0.43. We used generalised linear mixed effect models to evaluate the impact of day on pasture on cattle behaviour. Lying time decreased, walking distance increased, while the distribution of cattle became more even by day on pasture. These results appeared to reflect the decrease of grassland forage from pre- to post-grazing. Behavioural and UAV-based pasture utilisation analyses on a polygon grid have the capacity for sustainable, fine-scale decision support in grassland management. Virtual fences offer the possibility of flexibly adapting the boundaries to the results of the grid-based analyses. As demonstrated in this case study, grid grazing has the potential to improve grazing systems in a comprehensive way.

Keywords: Precision livestock farming, Nofence® Collars, animal monitoring

Introduction

Grazing is considered a worthwhile form of grassland management because of its potential low costs (Isselstein et al., 2005) and biodiversity benefits (Rook and Tallowin, 2003; Tälle et al., 2016). General obstacles to realise more grazing, are the labour and cost-intensive tasks of fencing and animal monitoring. Building and maintaining fences to contain livestock is time-consuming and costly (Bishop-Hurley et al., 2007). Furthermore, the profitability and productivity of grazing livestock depend on the optimum utilisation of the available grazing area. Under the premise of standard fencing technologies, optimal pasture utilisation is difficult (Stevens et al., 2021), as spatial and temporal control of the animal is limited. The development of virtual fences opens up new possibilities to improve the flexibility and efficiency of grazing systems (Umstatter 2011). The technology uses GPS data to create virtual boundaries, which can be easily shifted whenever it is desired. Animals were able to learn the system via the acoustic signals and not the location of the invisible boundaries (Campbell et al., 2017). Each adult animal has to wear a virtual fencing (VF) collar that emits an acoustic warning signal when the animal moves towards the border, followed by a short-term electric pulse when it ignores the acoustic warning signal. The warning signal stops immediately (and no electric pulse is emitted) when the animal stops and turns around. Concerns about negative impacts on cattle behaviour when using VF collars seem to have been largely refuted so far by previous studies, e.g. (Umstatter et al., 2015; Campbell et al., 2019; Lee and Campbell, 2021; Ranches et al., 2021; Hamidi et al., 2022; Confessore et al., 2022). Several studies indicated that a proper training period and a good preparation are necessary for a successful use of the technology e.g. (Umstatter et al., 2011; McSweeny et al., 2020; Verdon et al., 2021) and long-term studies are missing so far. However, VF is thus a promising future option for improved grazing management and is already used to some extent in the farming practice in Europe (e.g., Nofence® in UK, Norway) or overseas (e.g., Halter® in New Zealand). The advantages of VF for reducing the labour inherent to controlled grazing management are obvious.

Potential additional animal monitoring opportunities arising from a system that uses real-time GPS data have been highlighted much less in previous studies. Monitoring the behaviour of grazing animals can support innovations in grassland management (Carvalho et al., 2013). Detecting frequently used sites via GPS, for example, could be an indication of possible overgrazing. Protecting overgrazed areas by forcing the animals to avoid these locations (Laca, 2009; Riaboff et al., 2020) should be easily possible by using VF technology because virtual fence lines can be easily and flexibly set up and moved. However, the appropriate identification of the necessity to shift virtual boundaries might pose a challenge to the management, particularly when grazing areas are large or in distant regions. Remote sensing provides an opportunity for efficient grassland monitoring (Wachendorf et al., 2018). Optical satellite-based monitoring has already been established for observations at a larger scale to evaluate the impact of drought (Kowalski et al., 2022). However, precision grazing applications are time-critical and demand a very high spatial resolution. Therefore, Unmanned-Aerial-Vehicles (UAVs), which can be used flexibly and offer high spatial resolutions, are a promising remote sensing platform (Bindelle et al., 2021). Wijesingha et al. (2019) demonstrate that the structure-from-motion (SfM)-based 3D reconstruction could be used to estimate grassland biomass. Alvarez-Hess et al. (2021) show that UAV-based multispectral imaging helped to measure pasture depletion in paddocks during grazing. Lussem et al. (2022) demonstrate the applicability of UAV-based structural and spectral features for estimating aboveground dry matter yield. The combination of VF and UAV-based remote sensing appears to be promising for developing innovative grazing concepts (Horn and Isselstein, 2022). VF, real-time animal monitoring and remote sensing on pasture might be combined in a comprehensive system, which could be used to optimise the interaction of the grazing animal with the grass sward. The advantage is that the interaction between animal and grass sward is reflected continuously which can help to decide when to allow new pasture access or to detect overgrazing or soil degradation. To the best of our knowledge, studies testing the combination of these technologies to improve grazing systems are missing so far.

In this study we apply VF technology and link different data sources in order to provide a case example for a future grazing system on a polygon grid base (grid grazing) with facilitated animal monitoring and remote decision support. We used data from a larger rotational grazing trial with cattle, which was conducted from July to September 2021 (32 animals in total) on an experimental farm in Germany. All animals were equipped with VF collars (® Nofence, AS, Batnfjordsøra Norway). Pre- and post-grazing UAV-borne images were made to create digital orthophotos and digital elevation models. The overall objective of this study was to test whether it is possible to facilitate grazing systems and provide decision support in grazing systems using VF collars and UAV data. To achieve this, we have formulated the following specific objectives:

(i) to verify the suitability of the VF collars for animal monitoring and the ability of UAV based imagery to estimate dry biomass changes, as preconditions for the overall objective of the study,

(ii) to investigate whether cattle spent active time monitored by GPS could be related to high resolution UAV based biomass changes of the grass sward on a polygon grid, and

(iii) to interpret the implications of the outcomes for monitoring and decision support in grazing systems

Material and Methods

Experimental site, animals, study design and climatic conditions

The study was conducted at the experimental farm of the University of Göttingen in Relliehausen, Solling Uplands, Lower Saxony, Germany (51°46'48"N 9°42'15"E) on grass-dominated permanent grassland belonging to the association of Lolio-Cynosuretum and has been a part of a larger rotational grazing trial. The botanical composition of the grass sward consisted of 91.5% grasses, 7.4% dicotyledonous non-legumes, and 0.7% legumes (estimated yield proportions) with Lolium perenne, Dactylis glomerata and Elymus repens as most abundant species. The longtime climatic averages of the German Weather Service reference period (1991-2020) were: temperature: 9.8°C, annual precipitation sum: 764 mm, radiation: 1500 hours. Monthly weather data during the study period were: temperature, 16.9 and 15.6°C; precipitation sum, 85.7 and 23.8 mm; radiation, 7.2 and 7.3 hours for August and September 2021, respectively. All data measured at a climate station in Bevern (51°51'10"N 9°29'42"E), 21 km from the experimental site (Deutscher Wetterdienst (DWD), 2022).

The current study utilised data from one grazing rotation between August 23rd, 2021 and September 6th, 2021. In this grazing experiment, 32 non pregnant heifers (Fleckvieh) were used, which had no experience with virtual fences or rotational grazing and were therefore naive before the larger rotational grazing trial started. Four groups of eight Fleckvieh heifers each were assigned to 2-ha pastures and each pasture was divided into four paddocks. Treatments consisted of two fencing systems (virtual (VF) vs. physical (PF)). Cattle individuals were randomly assigned to either a VF or PF treatment according to age and life weight after a covariate measurement period. The animals were additionally scored according to body condition score (BCS) and temperament (adapted from Hoppe et al., (2009)) (Table 1).

Table 1: Summary of heifer characteristics at the start of the rotation as assessed on the day of pasture release (August 23rd, 2021). VF, virtual fencing; PF, physical fencing; BCS, body condition score; LW, live weight; Mean ± SD refers to the eight heifers per group. Body condition and temperament were scored nine weeks before the day of pasture release.

	VF-1	VF-2	PF-3	PF-4
Age months	15.1 ± 1.2	15.3 ± 1.2	15.6 ± 1.0	15.6 ± 0.9
BCS	4.3 ± 0.7	3.8 ± 0.5	3.9 ± 0.6	3.9 ± 0.8
Temperament score	2.0 ± 0.5	1.9 ± 0.8	2.6 ± 1.2	2.0 ± 1.4
LW kg	423.3 ± 47.3	423.1 ± 56.4	414.8 ± 49.5	418.0 ± 58.0

For spatial analyses the whole study site was divided in 2.5 × 2.5 m gridcells (Figure 1).

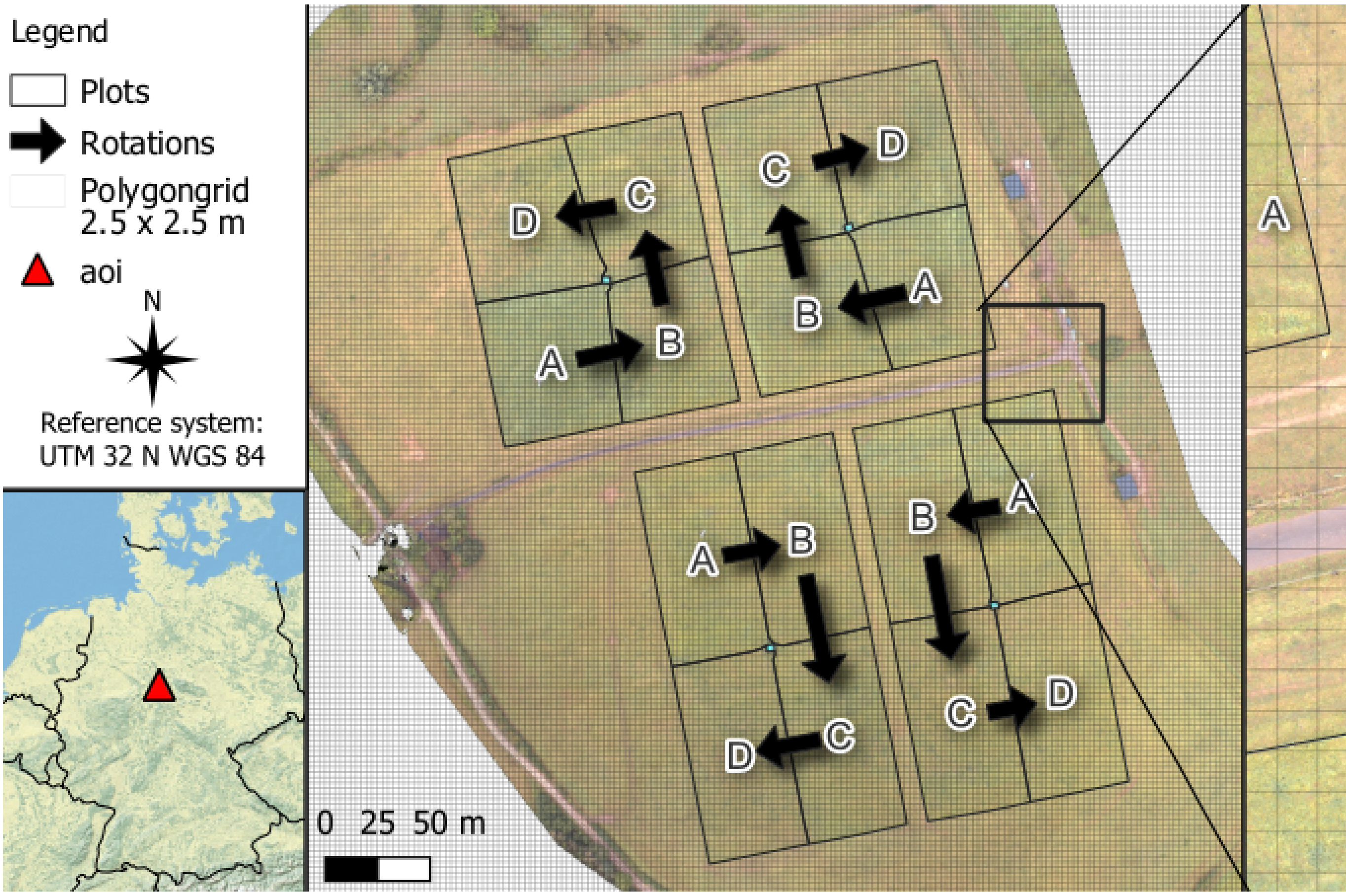


Figure 1: Representation of the study site and the grazing management during the rotation (23.08.2021 – 06.09.2021). Paddocks were grazed in alphabetical order, grazing duration per paddock were 3-4 days; Polygongrids were used for spatial analyses.

The heifers grazed for 3-4 days per paddock, resulting in a rotation length of 15 days. A first full rotation of the paddocks during July and early August 2021 served as an adaptation period to the environment and fencing techniques.

Animal Sensors

A battery/solar-powered VF collar (® Nofence, AS, Batnfjordsøra Norway, weight: 1465 g) was attached to the neck of each animal. An internal device for GPS localisation, sound generators, and electric pulse generators and an accelerometer (data could not be used to evaluate animal behaviour so far due to high energy costs) are the functional components of the technology. Virtual boundaries can be set via an app for smartphones. If an animal approaches a virtual boundary, a melody is played as an acoustic warning signal (82 dB at 1 m). It stops immediately when the animal turns away from the virtual boundary. If the animal continues moving towards the boundary, a short-duration electric impulse (0.2 J at 3 kV, 1.0 s duration, www.nofence.no/en/product/cattle; January 25, 2022) is emitted. If an animal does not draw back, this combination of acoustic warning and electric pulse can only be repeated three times before the animal is considered to have escaped. The system then automatically switches off and only turns on again when the animal returns to the virtual pasture. The animal owner is continuously informed about the signals on the client, e.g. a smartphone or computer. The VF collars of the PF groups were activated to collect GPS positions, but assigned to a pasture far away not including the animals, which prevents them from receiving signals. The 'normal' data interval without proximity to the fence was 15 minutes. This setting was changed by the company to minute-wise data as we used the GPS positions of the VF collars to analyse cattle movement behaviour. Since 2022, as an end user, you can also set the VF collars to minute-wise GPS position data.

Pasture acclimatisation, virtual-fence training, and grazing management

Before the larger rotational grazing trial started, all heifers were released to a pasture near the experimental farm for grazing adaptation.

In early July, the heifers of the VF groups were fitted with VF collars and underwent a 12-day training period to ensure that they were able to pair the audio cue with the electric pulse of the collar. This conditional learning is necessary for the heifers so that the VF system becomes predictable for the animals. For the training, animals were assigned to their VF groups, which were allocated to two physically fenced pasture areas side by side. Within these pastures, virtual fences were successively set up on three sides to allow for gentle learning of the VF system as reported by Hamidi et al. (2022).

After this conditioning, all heifers were equipped and weighed before being assigned to the first paddock (A) of the rotation grazing trial (Fig. 1). The VF groups were completely physically fenced on the first two days to allow for habituation to the new environment. On the third day, the physical fence was removed on two sides (inner sides). On the days of paddock change, PF animals entered the new paddocks through an open gate, while for VF animals, the virtual pasture was first enlarged and then reduced to the new size after all heifers had entered the new paddock. Days on paddock A and the days of paddock changes were considered as habituation periods and not included in the analyses because we assumed that the animal behaviour was strongly influenced by the new environment.

Data collection

As mentioned above, the time spent on the first paddock (A) as well as the first day on the paddocks B – D (days of paddock change) were regarded as habituation periods for the animals and therefore, excluded from the analyses. Data from three paddocks per pasture were analysed (paddocks B, C, and D, see Figure 1) for all analyses, which include animal behaviour.

All sources and the intention of the respective analysis were presented in Table 2.

Table 2: Overview of the data sources used for analyses

Analyses	Data type and unit of measurement	Data Sources				
		Virtual fencing collars	UAV		Manual determination of dry biomass changes	Observational animal monitoring
			RGBVI	Height		
Sensor validation						
Lying time	categorised as yes/no (minute wise)	x				x
Biomass changes	Dry biomass changes between pre- and post-grazing on paddock level (t)		x		x	
Relationship UAV data – GPS positions						
Spatial patterns of active time	Summed active time per grid cell and paddock of all animals compared to UAV biomass changes pre- and post-grazing	x	x	x		
Analyses for decision support						
Daily walking distance	aggregated distance per paddock and day and animal (m d-1)	x				
Lying time for analysis of animal behaviour	aggregated lying time per paddock and day and animal (min d-1)	x				
Camargo's index of Eveness	Summed active time per grid cell, paddock and day of all animals (min d-1)	x				

Data collection by VF collars

The VF collars logged GPS data every minute and, additionally, when the animal received an acoustic warning signal or electric pulse. The GPS device recorded the time and date for each location. Sometimes the interval of the data was higher than minute-wise: "The reason is that the 1 minute resolution is achieved by increasing the frequency by which we send updates to the server. So, if for some reason we're having problems connecting over the mobile network we will get delays or even lost updates.There is a proposal to fix this by decoupling the GPS data collection from the sending" (personal communication with Natascha Grinnell (Nofence) on the 7th of September 2022). We used an interval of 60-120 s to normalise the measurements. Therefore, 0.1 % of the datapoints (>120 s) were excluded before analyses due to this issue. In the current study, one collar of the PF group was not used for data analysis because it was defective.

Geographic coordinates were available by the VF collars in the World Geodetic System 1984 (WGS 84) and then transformed in the Universal Transverse Mercator coordinate system (UTM) format before calculating the distance between two sequential positions by using the Pythagorean theorem. Results were summed for daily periods as walking distances (m). The time associated with lying (at least two consecutive minutes without movement) was summed for daily lying time (Table 2). The hours of darkness (22:00-5:59h) were excluded, as this time is known to be spent mostly inactive by cattle (Chilibroste et al., 2017; Hamidi et al. 2021) and therefore, not associated with forage consumption, which was confirmed by visual inspection of the hourly walking distance in the present study.

Data from animal observations

On the 25th, 26th, 27th, 30th, 31st of August and the 1st of September approximately two hours of continuous animal observation per day and VF group was performed. One observer per VF group continuously recorded the behaviour of four animals per VF group using the app 'Observasjonslogger' by Morten Sickel.

Manual determination of dry biomass changes

In each paddock standing herbage on offer was determined pre and post grazing by manual cutting within a defined steel frame (30 cm diameter) at four random points using electric hand shears. After weighing to determine the fresh weight, each sample was dried (60°C) in a forced air oven until constant weight and weighed to determine dry matter (DM) and dry biomass changes between pre- and post-grazing.

Additionally, to consider forage losses for calculating individual daily herbage intake a sward stick was used to assess the trampling area post-grazing in each paddock by conducting transect walks across the paddock area at 25 positions. At each contact with the stick the class trampled or not trampled was given. In total 839 measurements were performed in total across paddocks.

UAV survey and structure-from-motion processing

UAV-based monitoring was performed using a Phantom 4 Obsidian (weight: 1.4 kg, maximum flight time ~ 30 min) equipped with a 1 inch 20 Megapixel RGB sensor. Flight planning was performed with UgCS (photogrammetry tool for UAV land surveying missions) with the following parameters: Flight speed of 3.5 m/s, forward and side overlap of 75 %, and a ground sampling distance of two cm, which resulted in an altitude of about 75 m. Four flights were carried out: T1: August 23rd, 2021, T2: August 30th, 2021, T3: September 2nd, 2021, T4: September 6th, 2021. Two flights, each lasting approximately 20 minutes, were carried out per date. The first flight was over the northern pastures, covering an area of about 5 ha. The second flight was carried out over the southern pastures and covered 5.9 ha. Approximately 400 images were taken per survey, acquired in auto mode, and saved as raw (.dng) image files.

3D reconstruction was performed using the Agisoft Metashape SfM software (Version 1.8.1., St.Petersburg, Russia). Image alignment and camera optimisation were carried out with standard settings. Subsequently, a digital elevation model was generated using the depth maps approach. The models had a pixel spacing of about four cm. On the basis of the elevation models, digital orthomosaics were computed with a pixel spacing of two cm. 21 ground control points (GCP) were deployed, evenly distributed throughout the study site, for precise geolocation. Twelve GCPs were painted on concrete surfaces, and nine GCPs were elevated, wooden plates, installed on wooden stakes, and driven into the ground. The precise positions of the GCPs were measured with a DGPS GR-5 (Topcon, USA) in real-time kinematic positioning (RTK), in a base-rover constellation. The spatial reference system was UTM32N, WGS 84. The GCPs were either used as control points during the SfM process or as independent checkpoints to evaluate the spatial accuracy of the derived products (see Table 3). The processing time for each survey was about 4 hours on a higher-grade computer (Intel Xeon E5 v4, 128 GB RAM).

Table 3: Accuracy obtained from the structure from motion image matching.

Date	Control points (cm)	Independent checkpoints (cm)
23.08.2021	1.4	4.5
30.08.2021	1.9	3.9
02.09.2021	2.4	4.5
06.09.2021	1.9	3.3

Spatial patterns of active time and Camargo's Index of Evenness

The whole study site was rasterised into a 2.5 × 2.5 m polygongrid (Figure 1) to quantify the extent of spatial clustering of the heifers (Bareth et al., 2016). The polygon grid provides a basis to analyse and combine the different spatial datasets on fixed polygon locations. The summed active time (lying time and hours of darkness (22:00-5:59) were excluded) spent by all animals within one grid cell, which refers to one polygon of the polygon grid, was calculated per paddock and used to determine spatial patterns, which were compared with UAV based changes in the sward (see Fig. 2 for an example cell).

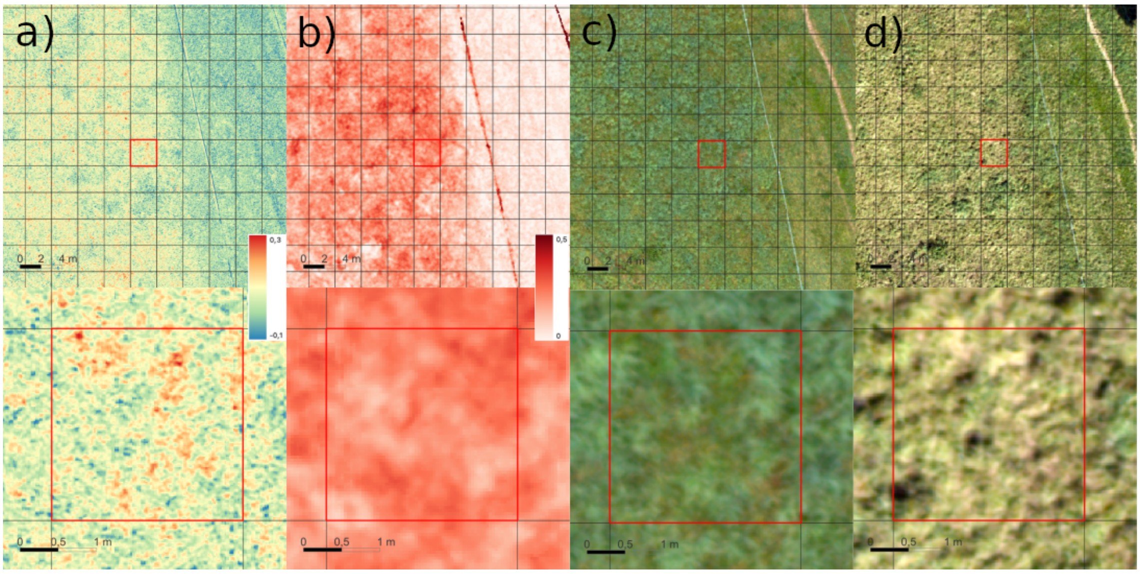


Figure 2: Example cell (ID 8841): virtual fencing, left side, paddock D, 4.34 m distance to the fence line. Cattle spent active time 28 min.
a) RGBVI change, mean rgbvi decrease: 0.09
b) Height change, mean height decrease: 0.21 m
c) Digital orthophoto 02.09.2021
d) Digital orthophoto 06.09.2021

Summed active time of all animals per day on paddock was used to calculate the Camargo's Index of Evenness (Table 2). The index allows to assess the relative distribution of GPS locations and therefore, detects differences in spatial distribution (Payne et al., 2005). Values near one indicate a homogeneous distribution and values near zero a patchy distribution (Payne et al., 2005). In this study, the Camargo's Index of Evenness is used as a metric for the distribution of animals related to decreasing forage per day.

Data analyses

The software environment R (R Core Team, 2022) was used to carry out statistical analyses of the collected animal data.

Sensor validation

For sensor validation analyses, data from paddock A was included.

Observational data – VF collars

We used the observational lying time data to validate the lying time data obtained from the VF collars. Vf collar lying time is identified as the same GPS position for at least two consecutive minutes in this study. The details of the algorithm of the accelerometer included in the VF collar, which detected low movement and then sent the same GPS position in order to save battery life are proprietary but "roughly speaking it uses a moving average of the last 3 minutes of activity" (personal mail communication with Andre Naess (Nofence) April 26th, 2022). The data of the two sources was categorised beforehand into two classes of lying (yes/no) on a minute basis. Sensor validation was carried out using a confusion matrix (also known as error matrix) (package 'caret' (Kuhn, 2021)). A confusion matrix is a special kind of contingency table that allows visualisation of the performance of an algorithm with two dimensions ('true' and 'predicted'). In the contingency table, each combination of dimension and class is a variable. Ruuska et al. (2018), support the use of a confusion matrix as a robust and stringent evaluation of validity for measuring feeding behaviour in their study. We used the confusion matrix' quality measures accuracy which indicates the percentage of correctly made predictions of a model (TP+TN) / (TP+TN+FP+FN), precision which indicates the ratio of true positives correctly predicted by the model TP/(TP+FP), recall TP/(TP+FN) which is a metric that tells us how well the model is able to identify positive outcomes, with T = true, P = positive, N = negative and F = false.

Manual determined dry biomass changes - UAV detected dry biomass changes

An average value of standing herbage on offer (g DM m-2) pre- and post-grazing was generated per paddock and regressed on an average paddock-wise RGBVI detected dry biomass change from the UAV observation. The replicate constituted the random effect. This linear model was used to predict the dry biomass changes within paddocks between pre- and post-grazing.

Analyses of animal behaviour

Generalised linear mixed effect models were calculated for each target variable using the package 'glmmTMB' (Brooks et al., 2017) and the gaussian error distribution. For all analyses, ~ <5% of the data were excluded by considering values ranging 1.5-fold above the 75th or below the 25th percentile of the interquartile range (Pham, 2006). The normality of the residuals was checked by visual inspection and by using the package 'DHARMAa'.

Homogeneity of variance was assessed by examining plots of residuals versus fitted values and residuals versus predictor values and additional, by using the package 'DHARMAa'.

Comparisons of means were performed post hoc using Tukey's HSD test ('emmeans' package, Bartón, 2018) with Sidak's method of confidence level adjustment for significant influencing factor levels.

The target variables walking distance (m) and lying time (min) per animal and day were evaluated on the fixed effects of day on pasture (2-3 levels) and treatment (2 levels) and the interaction. The individual animal nested in paddock nested in pasture served as crossed random effect.

Results of the Camargo's Index of evenness per paddock were analyzed in models with day on pasture and fencing treatment as fixed effects and paddock nested in pasture as a random effect.

Information extraction from UAV imagery

The four UAV surveys used for this study, carried out the day the heifers changed paddocks, allowed surveying all 16 paddocks repeatedly. Thus, an RGB orthomosaic and a digital elevation model were available for each survey using the SfM workflow described above. The elevation models were combined to calculate the elevation change between dates using the digital surface modeling approach (Hoffmeister et al., 2010). This means that the change in height was computed pixel-wise by subtracting the absolute height of the following date from each height model (e.g. T1-T2) (see Figure 3).

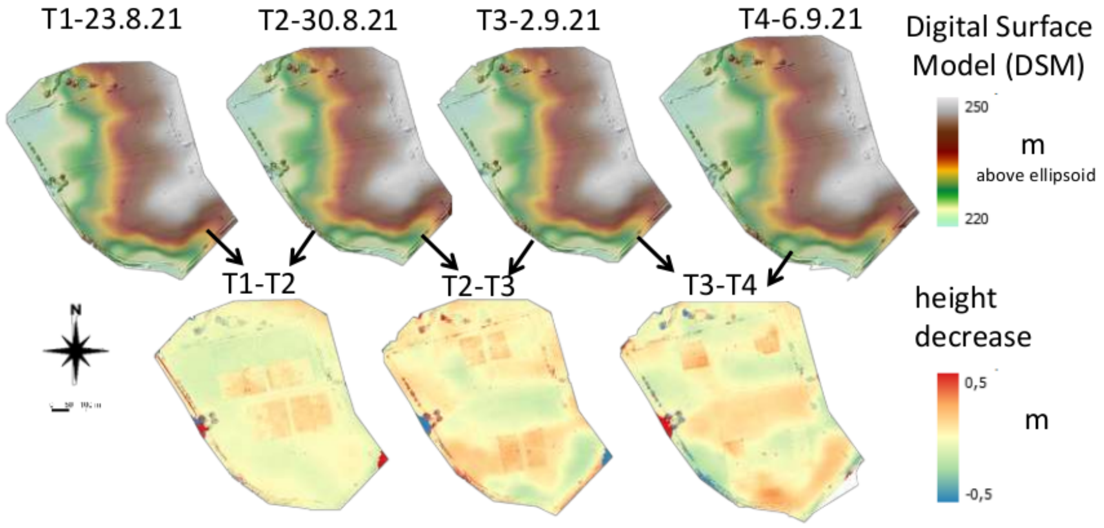


Figure 3: Elevation model to calculate the elevation change between dates of pre- and post- grazing per paddock. Please note that high values indicate a large height decrease

Based on the RGB information of the digital orthomosaics, the RGBVI was calculated. The RGBVI was introduced by Bendig et al., (2015) and already applied to grasslands by Bareth et al. (2015). The idea of the RGBVI is based on the reflectance differences due to chlorophyll-a absorption and chlorophyll-b absorption (Bendig et al., 2015). It is computed pixel-wise using Formula 1 (Bendig et al., 2015): RGBVI = $\frac{G^2+(B*R)}{G^2-(B*R)}$; R=Red, G=Green, B=Blue are the digital numbers of the respective channel of the orthomosaic. The change of RGBVI is computed by pixel-wise subtracting the RGBVI of the following date from each RGBVI (Figure 4).

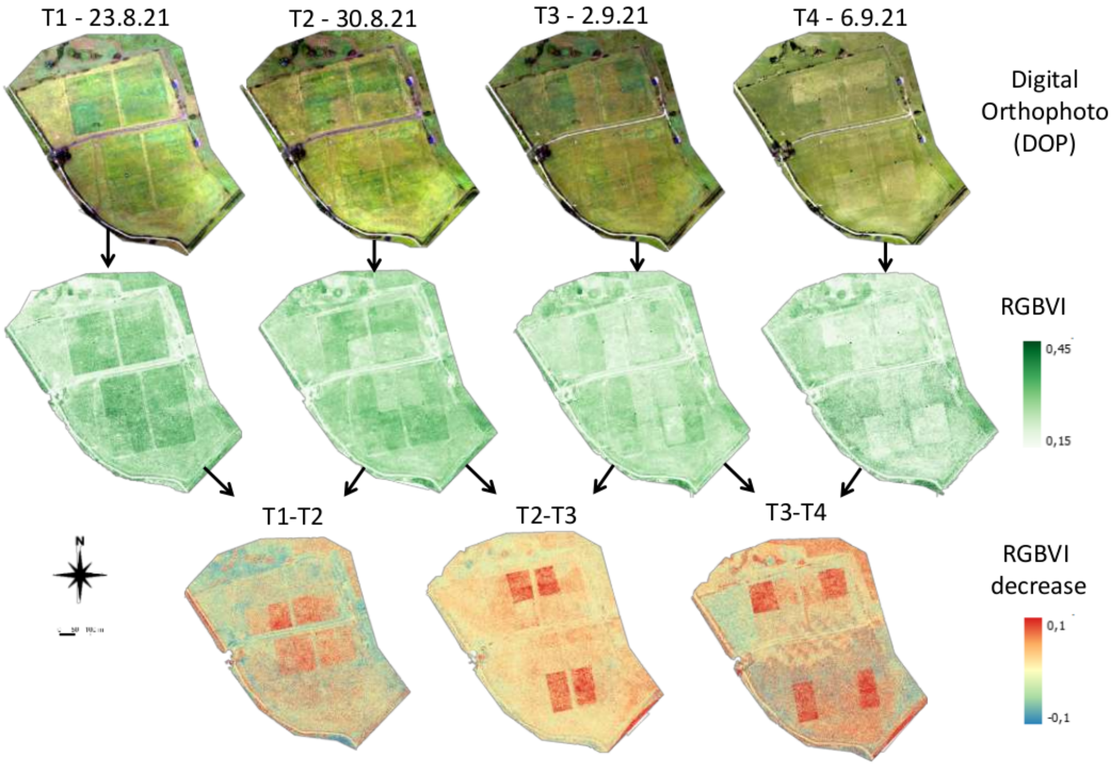


Figure 4: RGBVI calculation of changes between pre- and post-grazing per paddock based on the orthophotos. Please note that high values indicate a large decrease in RGBVI.

To combine the UAV-based data with the animal data, all information was aggregated on the same 2.5 m × 2.5 m polygon grid (Bareth, 2016).

Modelling was performed using all available UAV remote sensing data, each summed up per polygon of the polygon grid: the digital numbers of the individual color channels (R, G, B) of the acquisition before and after the grazing, RGBVI change between the acquisitions, surface height change during grazing and the active time as the dependent variable. Only polygon grids having an active time were used for the model building. The dataset with all grazed polygons from all three splits (n=8998) was split into 75% training data (n=6746) and 25% (n=2252) validation data.

A random forest (RF) regression was established, and model performance and parameter tuning of the parameters "trees" and "mtry" were performed with 10-fold cross validation using maximized r2 as tuning aim. The tuning revealed that “trees” was set to 200 and “mtry” to 2. Furthermore, the cross validation showed a mean and std for rmse and r2.

We chose modelling at an increased spatial dimension of 0.5 m to demonstrate the ability of the approach to identify grazing induced patterns of the grazed sward.

All raster-based pixel-wise calculations, geospatial analyses, and the regression analyses were carried out using R 4.1.3 with the packages: 'raster', 'rgdal', 'sf', 'exactextract', and 'tidymodels'.

Results

Sensor validation

Observational data – VF collars

The validation of lying time via using a confusion matrix showed a precision of 92%, a recall of 78%, and an accuracy of 91% (in total 5,270 data points).

Manual determined dry biomass changes - UAV detected dry biomass changes

We found a significant effect of the RGBVI detected dry biomass changes (Figure 5) on the manual detected dry biomass changes (F1,29=21.3, p=0.0001). The model had a RMSE of 47.1 and R2c = 0.51. On average there is a dry biomass change of 0.40 ± 0.09 t on all paddocks (Figure 5) calculated from UAV analyses.

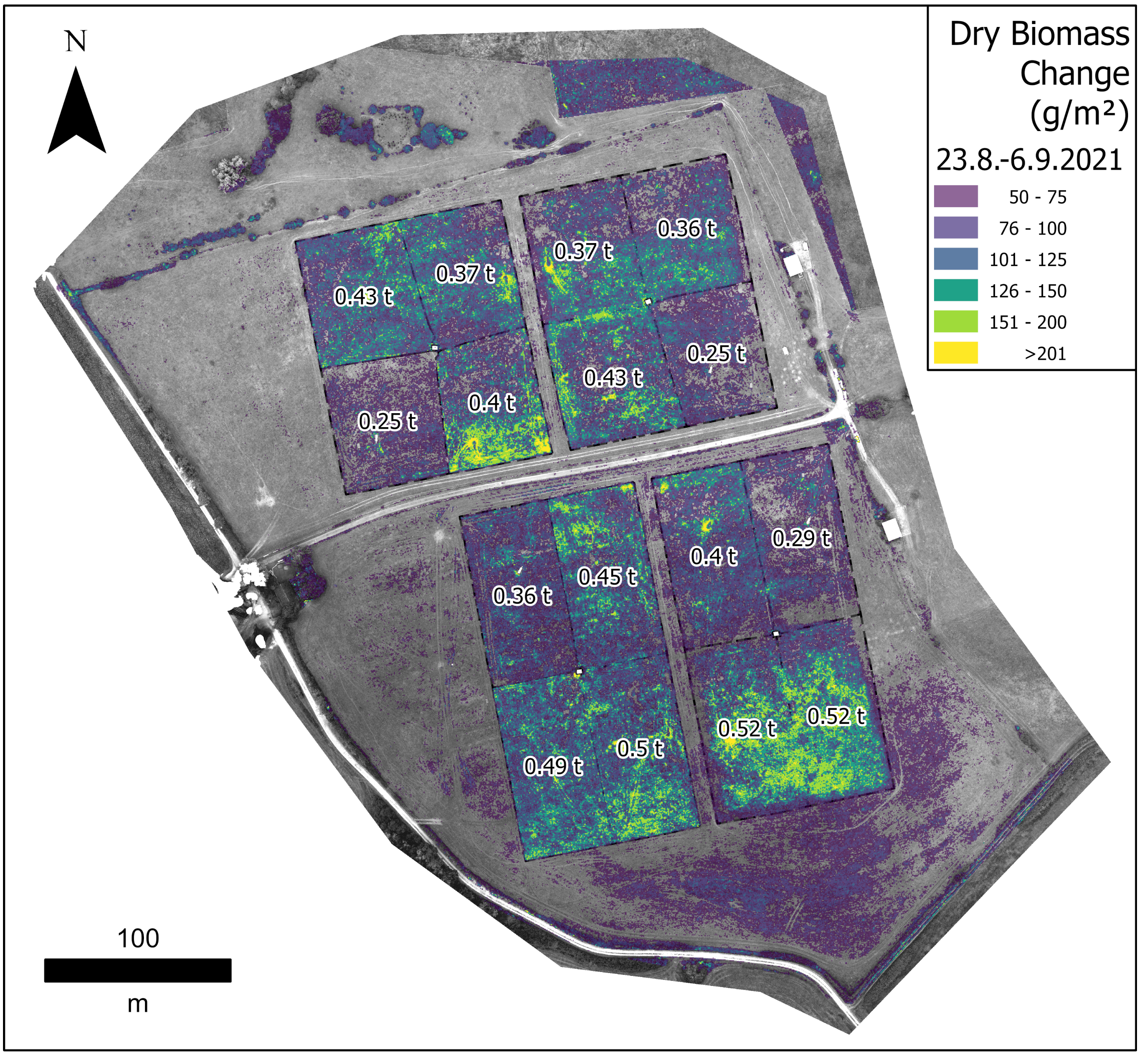


Figure 5: Summed changes of dry biomass per 0.5×0.5m polygon grid cell (g/m2) and total dry biomass per paddock detected via RGBVI changes. Background image in grey scale depict on the 23 August 2021.

Animal behaviour

For all parameters analysed, day on pasture was significant (Table 4). Fencing system and the interaction of day on pasture × fencing treatment were never significant.

Lying time

Lying time decreased significantly over time on paddock (Table 4). The rank order of lying time is Day 2 > Day 3 > Day 4 (Table 5).

Walking distance

Walking distance significantly increased by day on paddock (Table 4). However, comparisons of means revealed no significant difference between day two and three (Table

5).

Camargo's Index of Evenness

The Camargo's Index of Evenness (referring to the time animals spent active) showed a more even spatial distribution on day 3 and day 4 compared to day 2, despite a relatively small difference between days (Table 5). However, the estimated mean value for day 4 is in between the values from day 2 and day 3.

Table 4: Output of generalised linear mixed effects models for the parameters of interest to evaluate cattle behaviour and spatial distribution on pasture (*p<0.05 **p<0.01 ***p<0.001)

Target variable	Predictors	Estimates	Marginal R^2 / Conditional R^2
			(Method: Maximum likelihood)
Lying time	Day [3]	-19.54*	0.184 / 0.589
	Day [4]	-50.40***	
	Fencing treatment [VF]	-37.60	
	Day[3]×fencing treatment [VF]	-6.36	
	Day[4]×fencing treatment [VF]	-8.45	
Walking distance	Day [3]	102.78*	0.106 / 0.592
	Day [4]	237.37***	
	Fencing treatment [VF]	-159.42	
	Day[3]×fencing treatment [VF]	-33.77	
	Day[4]×fencing treatment [VF]	-51.62	
Camargo's Index of Evenness	Day [3]	0.01*	0.263 / NA
	Day [4]	0.01*	
	Fencing treatment [VF]	0.01	
	Day[3]×fencing treatment [VF]	-0.00	
	Day[4]×fencing treatment [VF]	-0.01	

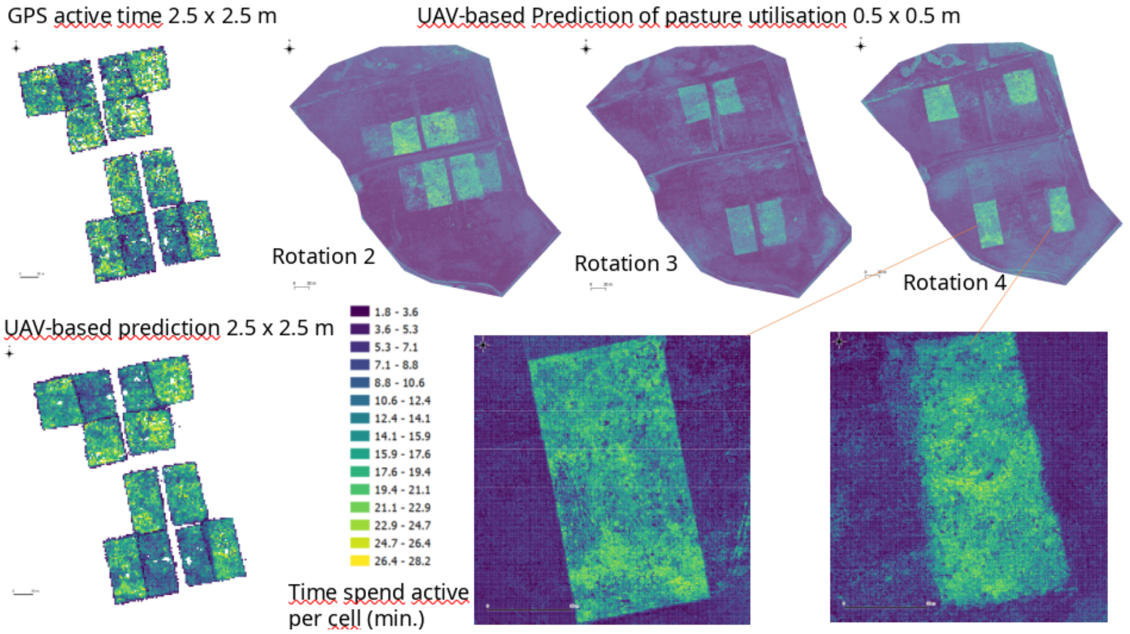
Table 5: Estimated means ± SE (standard error) for cattle behaviour and spatial distribution on pasture as presented for day on pasture. Lowercase letters indicate significant differences between days within the target variable ($p < 0.05$).

Target variable	Day 2	Day 3	Day 4
Lying time (min animal^{-1} day^{-1})	255.0 ± 9.67 c	232.0 ± 9.67 b	200.0 ± 10.40 a
Walking distance (m animal^{-1} day^{-1})	2547.0 ± 62.8 a	2633.0 ± 62.7 a	2758.0 ± 66.8 b
Camargo's index of eveness	0.679 ± 0.003 a	0.692 ± 0.003 b	0.690 ± 0.004 ab

UAV – animal spent active time per grid cell

The evaluation of the Random Forest model with the test data showed a rmse of 5.59 min and an r^2 of 0.43 based on the cross validation the standard deviations of rmse and r^2 were 0.19 min and 0.3, respectively. See Figure 5 for further information. The established model was then applied to a finer polygon grid resolution of 0.5 m to create the map shown in Figure 6. The derived geoinformation shows patterns where the cows spent time and induced changes identified by the UAV data. Frequently used feeding stations and avoided areas could be clearly identified via upscaling on the finer polygon grid (Figure 6).

Figure 6: Outcome of the Random Forest model on the 2.5× 2.5m grid – up scaled on a finer polygon grid (0.5 × 0.5 m) to increase the accuracy of pasture utilisation data.



Discussion

Although VF has already been analysed in grazing trials (e.g. Campbell et al., 2019; Lomax et al., 2019; Campbell et al., 2020; Verdon et al., 2021; Aaser et al., 2022; Hamidi et al., 2022; Confessore et al., 2022) and also used in commercial systems in Norway (®Nofence), the United States, New Zealand, Australia and the United Kingdom (Animal Welfare Committee, 2022), the immense opportunities of this technology, especially when combined with UAV-based remote sensing have yet not fully be explored. The present study is the first which tested the applicability of VF collars for animal monitoring with promising results and evaluated the impact of the grazing animal on the sward via GPS and UAV data on a polygon grid base (Bareth, 2016).

Sensor validation and animal behaviour related to day on pasture

For scientific purposes, position data retrieved from conventional GPS collars have previously been used to detect and to analyse cattle behaviour on pasture (Schlecht et al., 2004; González et al., 2014; Riaboff et al., 2020; Hamidi et al., 2021; Meckbach et al., 2021) inter alia in relation to the effect of grazing intensity or forage availability (Scarnecchia et al., 1985; Gibb et al., 1997; Hejcmanová et al., 2009). However, data from such collars are known as inadequate solution to distinguish standing from lying (Ungar et al., 2011). Confusion between standing and lying (Ungar et al., 2011) should result in an overestimation of lying. With regard to the results of the sensor validation of the present study, the high precision (92%) indicating the proportion of correctly predicted positive values suggests that this argument can be neglected. The lower recall (78%) showed, that false negative deteceted minutes of lying were much more likely than false positive and should be taken into account. However, the battery saving function could provide valuable services in determining lying time by transmitting the same GPS position, which helps to enable cost-saving animal monitoring on pasture as an additional benefit of the VF collars. The likelihood of getting false positive values was low. Continuous observation could help to improve animal welfare on pasture, as for example lying time is seen as an indicator for assessing restlessness or comfort of cattle (Haley et al., 2000). A major advantage of monitoring lying via VF collars, compared to commonly used pedometers, is the knowledge of the location of the lying animal, which should be useful in confusing terrain, among other things. Furthermore, the GPS data enable monitoring of motion behaviour such as walking distance, walking time or speed, which could be used to detect lameness, heat or forage scarcity via deviation from the individual long-term mean.

Involving animals in the processes that determine their environment (concept of cooperative livestock management) could enable cost-effective and flexible solutions with the potential to improve animal welfare at the same time (Manteuffel et al., 2021). Fences are known to determine the environment (Xu et al., 2022). Therefore, using behavioural indications of the animals to adapt the forage allocation via VF involves them in decision making.

We tested the potential of monitoring walking distance, lying time and spatial distribution (as expressed by the Camargo's index of evenness) during active time of the animals to find indications for the necessity to shift VF boundaries as based on the spatial grazing impact. We found a significant increase of walking distance and a significant decrease of lying time from the first to the last day on paddock. O'Driscoll et al. (2019) found that dairy cows spent less time lying at lower than at higher forage availability on pasture, as they need more time to meet their daily requirements. For reliable decision support based on monitoring animal behaviour, it is necessary to consider not only the forage availability but also its spatial distribution (Hamidi et al., 2021), which is possible in the present study by combining the spatial distribution of the animals with UAV-based analyses of biomass changes.

Combining UAV and animal data for measuring and evaluating local impacts of grazing

The grazing animal is influenced by the grass sward and vice versa (Opitz von Boberfeld, 1994). We combined UAV data with VF collar location data on a grid basis to link the data on the active time of the animals with information based on UAV imagery, so that both sides of the relationship can be considered in the current study.

As a first step, we evaluated the suitability of UAV based RGBVI calculations to estimate dry biomass change with a moderate amount of explained variability. Considering these promising results, we calculated RGBVI dry biomass changes on different scales: paddock level, 2.5 × 2.5 m and 0.5 × 0.5 m grid base (Figure 5), as up scaling UAV data is known to work well (Bareth and Hütt, 2021) and provides interesting spatial patterns (Figure 5 and 6). Mean dry biomass changes appear to be high with a total average of 14.26 kg per animal and day on pasture. Considering a total average trampling rate of 50 %, manually determined in this trial, the value was reduced to an average of 7.13 kg per animal and day. Considering the uncertainty, whether trampling also took place on previously grazed sites, we assume the middle between both values, 10.70 kg herbage intake per animal and day, which is in line with Berngruber (1977) who also investigated Fleckvieh Heifers on pasture.

Remote animal tracking via GPS technology combined with accelerometer data for studying livestock use patterns on the landscape showed promising results in a study by Brennan et al. (2021) but commercially available tracking systems are often prohibitively expensive for the practise (Brennan et al., 2021). The use of VF collar location data, which can also be used for a range of grazing systems, such as strip grazing (e.g. Lomax et al., 2019; Langworthy et al., 2021), cell grazing (Verdon et al., 2021) and fencing out sensitive areas (e.g. Campbell et al., 2020), could be a cost-effective solution for future grazing systems, assuming wider commercialisation of the VF system. Umstatter (2011) noted that fencing has always been subject to an evolutionary process. Therefore, VF systems can be seen as the next obvious step (Umstatter, 2011).

In our study, predicting the active time of cattle within a grid cell by RGBVI and height changes using Random Forest proved promising. Considering this, and the potential of UAV data to determine aboveground dry matter as also shown by (e.g., Michez et al., 2019; Théau et al., 2021; Lussem et al., 2022), as "starting point" for an individual pasture, it appears to be possible to predict biomass losses by the spatial distribution of the animals per grid cell (ceteris paribus). In practice, due to changing weather and seasonal conditions, validation flights are necessary although the frequency might be reduced due to the relationship between cattle spent active time and biomass changes. Combining different sensor technologies permits a better understanding of the interactions between animals and landscapes (Handcock et al., 2009). For example Théau et al. (2021) categorised the level of vegetation cover into four classes by combining information. Overgrazing and possibly, pasture degradation which is a common threat under arid conditions, result in losses of ecosystem functions (Breidenbach et al., 2022). Grid grazing based on GPS and UAV data analysed on a grid base, appears to be a method to detect inter alia overgrazing and respond quickly, such as fencing out potentially affected areas. Furthermore, detecting overgrazed patches and fencing them out based on the same GPS device have the potential to reduce the bias caused by GPS, which seemed to be an additional reason for using VF collars for both animal monitoring and fencing.

VF can be used as a tool for rapid paddock relocation and spatial allocation in dynamic landscapes (Boyd et al., 2022b). Campbell et al. (2018) temporary excluded cattle from a riparian zone using VF technology (eShepherd). In a recent study, Boyd et al. (2022a) documented the successful exclusion of sensible areas in rangeland of the USA using the VF system "vence". Due to the possibilities to influence cattle distribution, even fire prevention is possible by creating fuel breaks using virtual fences (Boyd et al., 2022b). In addition, it appeared to be possible, out of the results of our study, to detect the necessity to shift boundaries for providing new forage resources, via the combination of VF collar animal monitoring data (Table 5) and UAV imagery (Figure 6). Grid grazing has the potential to improve the sustainability of grassland management by: (i) providing the information base (ii) increasing spatial and temporal precision (ii) facilitating implementation. It might protect, among others, the desirable function of the grass sward as a soil organic carbon and nitrogen storage (Breidenbach et al., 2022) and helps to decide when to shift the boundaries for an optimal use of the available grazing area.

Limitations and Implications

One limitation of the current study is that the entire study was designed as a case study, which limits the possibility of drawing generalisable conclusions about the specific use of VF and UAV for decision. Further research is needed to be able to use the whole relationship between animal behaviour on pasture and UAV data to define thresholds for forage scarcity. However, we have focused on the validation of the sensors and highlighted the possibilities of using the data from the VF system and UAV in terms of a comprehensive pasture management which could protect both, the grazing animal and the sward on a grid base, whereas most previous work has just focused on the different possibilities of (out) fencing through VF collars (e.g. Campbell et al., 2018; 2020; Aaser et. al., 2022; Hamidi et al., 2022). Monitoring specific behaviour could help understand levels of welfare and animals' responses to their environment (Schillings et al., 2021). In brief, our study has shown the potential to use the VF collars, for (i) animal (health) monitoring, (ii) behavioural indications for rotation decisions, (iii) evaluating correlations with UAV detected biomass changes, (iv) grid based analyses to enable precision grazing (grid grazing), and (v) fencing per se. From an economical and sustainable point of view, it is very interesting to have all these possibilities with just one sensor.

Conclusion

Regarding the objectives of the current study, the conclusions are:

(i) The validation of animals' lying times from the VF collars via confusion matrix showed promising results. Regarding the high precision, the use of VF collars for animal monitoring on pasture can be recommended. Likewise, estimating dry biomass changes via RGBVI provided a significant correlation to manual determind biomass changes.

(ii) Using active time per grid cell might be a future approach to predict biomass changes via animal data. Additionally, the combination of UAV data and spatial animal data offer significant potential to protect the sward and its desirable functions, especially via using fine-scale modelling data based on remote sensing data.

(iii) We demonstrated the potential of combining UAV-based remote sensing and GPS-based animal behaviour data from VF collars to improve pasture management by taking the forage distribution into account. Changes in animal behaviour and the sward can be immediately visualised and used as a basis for management decisions, e.g., moving animals, increasing grazing areas or fencing out vulnerable areas.

Grid grazing, whereby the unique grid cell is used for pasture utilisation analyses in addition to real time animal monitoring should be able to allow decision support in fine scaled grazing management. On this basis, it should be possible to rethink grazing systems, detached from groundbased fencing systems and forage measurements.

Further research is necessary to prove the applicability of the results reported here to practical grazing systems.

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

Data and model availability statement

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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Part III

General Discussion

This thesis has explored the underlying processes of cattle movement on pasture as well as the possibilities for animal monitoring through the use of virtual fencing technology, and has investigated possible welfare implications of virtual fenced Fleckvieh heifers using a wide range of observed behaviours and physiologically measured responses. All this is considered as a prerequisite to analyse, develop and improve new technologies.

In the following sections, the most important findings of this thesis are summarised and examples of their application are presented. Furthermore, some unpublished research related to the outcomes of the three chapters are presented and discussed. Finally, the practical implications and further possible research directions are outlined.

Basic requirements and possible options for the (further) development of comprehensive livestock grazing systems

The need to (further) develop, integrate and adapt new technologies to increase both, efficiency and sustainability of pasture-based grazing systems is widely recognised (French et al. 2015; Filazzola et al. 2020; Aquilani et al. 2022; Horn & Isselstein, 2022). Some key benefits associated with virtual fencing include improved forage allocation (Stevens et al. 2021), environmental protection (Campbel et al. 2018), access to previously unavailable grazing areas (Umstatter; 2011), reduced labour input (Bishop-Hurley et al. 2007; Anderson et al. 2014), real-time location data (French et al. 2015; Brier et al. 2020). In the case of more efficient and precise grazing management, a participant of a survey on virtual fencing in New Zealand mentioned "I agree that a technology that is aimed at providing more precise management is only useful if the underlying decision making of farmers is precise already." (Brier et al. 2020). Therefore, it is necessary to define the underlying processes of animal behaviour related to forage availability, as we have been aiming at in **chapter 1** *& 3* to evaluate possible indications for decision support.

Foraging resources are the most obvious drivers of grazer distribution at pasture (Adler et al., 2001). Therefore, it appears to be reasonable to use the distribution data as well as the daily walking distances and lying time to evaluate the options for decision support, when providing more forage by extending virtual boundaries. In **chapter 1** daily walking distances and the evenness of animal distributed on the pasture increased in the highest grazing intensity compared to lenient grazing intensity under continuous grazing. In **chapter 3** daily walking distance is increasing with decreasing forage availability by day on paddock in a rotational grazing trial. At the same time lying time decreased by day on pasture which is in line with O'Driscoll et al., (2019) who found less lying time in dairy cows with low than with high forage availability on pasture. Hart et al., (2022) indicate the importance of providing new forage once a day in order to identify behavioural changes related to forage scarcity. However, further research is recommended to validate the approaches of **chapter 3** with regard to identify critical thresholds of forage scarcity.

GPS position data for continuously monitoring the behaviour of cattle on the pasture

As recommended by the British Animal Welfare Commitee, no livestock animal should be double collared (Animal Welfare Committee, 2022). It also makes sense from a cost-efficiency point of view to have one collar for all needs. A first approach to detect lying time using the virtual fencing collars in **chapter 3** appears to be promising. Continous GPS data so far provides walking distances, speed and real-time animal positions. The potential of this data for analyses of health, fertility and social rank appears great. For example Halter's smart collar provides a motion index monitoring the fertility and health of cows (Halter, 2022). An open question regarding GPS tracked behaviour, might be the frequency of GPS positions used to evaluate, inter alia, daily walking distances. In order to quantify the information loss, using lower frequency data, we analysed five, ten and fifteen minute intervals (Figure 1) and compared them to the originally used minute-wise data analysed in **chapter 3**. McGavin et al., (2018) evaluated the effect of GPS interval and paddock size on walking distances and found an increase of walking distance related to larger paddock size. Nevertheless, the reduction in average apparent distance travelled with increasing GPS interval persisted across all paddock sizes in this study. There is an obvious trade-off between battery life and the accuracy of monitoring cattle behaviour. This is evidenced by the average reduction of 60.2 % of the walking distance from a 1 s interval to a 60 s interval (McGavin et al., 2018). Our data (Figure 1) visualised further information reduction when using larger data intervals as it is common for virtual fencing. It must be carefully determined whether it is more important to conserve battery life or to monitor cattle behaviour as accurately as possible.

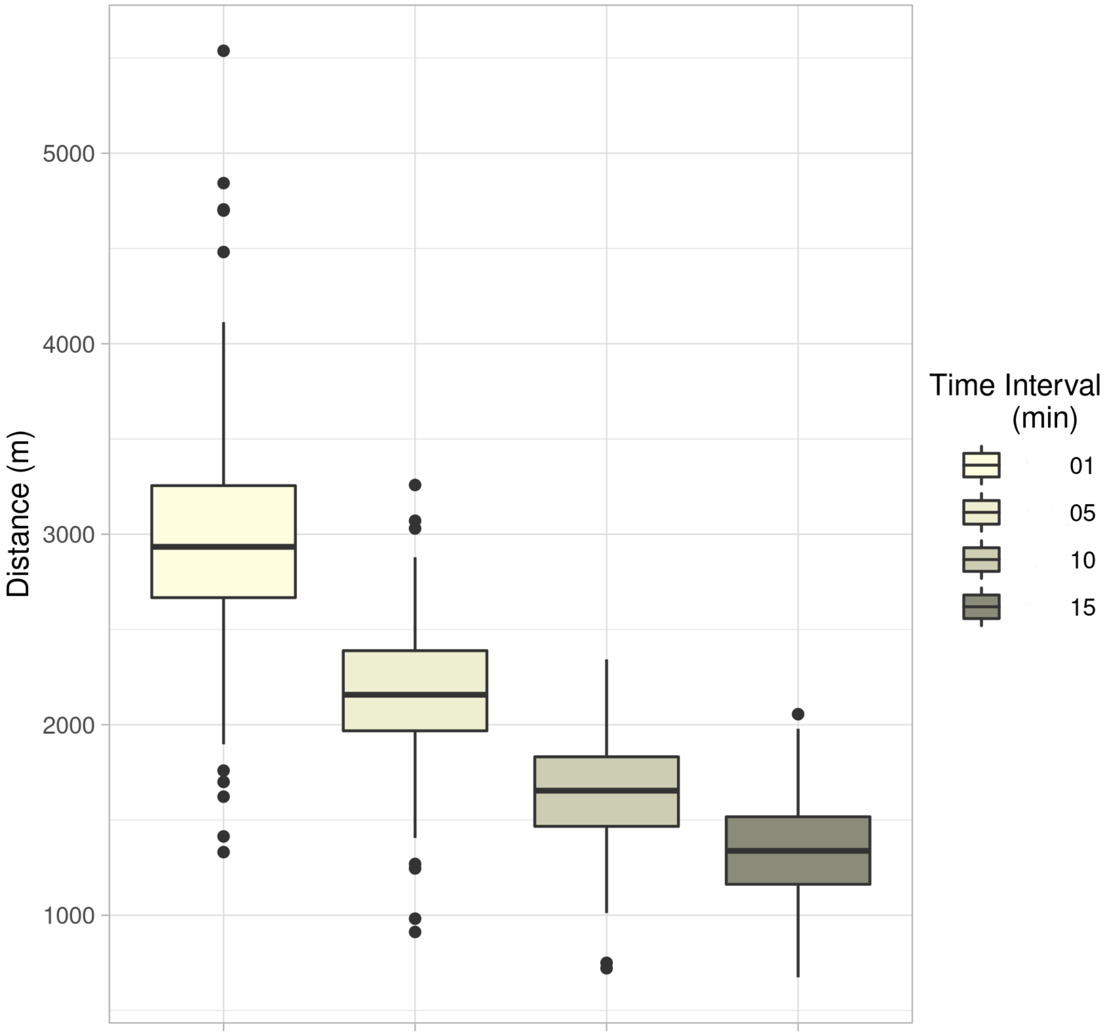

Figure 1: Testing different time intervals of GPS data points obtained from virtual fencing collars for calculating walking distances of Fleckvieh heifers. A time interval of 5 min provided 73 ± 5 %, 10 min 56 ± 7 %, 15 min 45 ± 7 % (mean ± sd) compared to minute wise measured walking distances.

Spatial analyses to measure and control the impact of grazing livestock on the landscape

Virtual fences have the potential to comprehensively rethink grazing management, beyond the options to improve traditional systems such as rotational grazing. The allocation of forage to the cattle follows the capability of controlling both, forage and animals. The fencing systems known so far are mostly very geometric, such as dividing a 2 ha plot into four 0.5 ha sub-plots for rotational grazing. These concepts are achieved by building groundbased fences. Herders have not adhered to geometric shapes to allow livestock access to forage resources, and virtual fences do not have to either. The first losses in straight lines can be seen in **chapter 3**, Figure 6. Sometimes heifers that had access to very palatable forage at the virtual fence line were observed grazing while listening to the melody of the acoustic signal and then quickly turning around before the last sound activated the electric pulse, and then immediately approaching again to graze at that spot again. The meandering boundaries appear to be a result of this behaviour discovered through animal observation. Regardless of this slightly inaccuracy, the potential of virtual fencing to control the impact of grazing livestock on the landscape is immense. Especially remote monitoring of cattle residence time on a polygon grid to identify hotspots of grazing as shown in chapter 3 appears to be a future perspective for a more finely scaled allocation of the animal on the sward. On this polygon grid base, taking into account pasture utilisation and forage availability, the decision on the forage allocation of the animals could possibly be made in the future. In chapter 1 a 5 × 5 m resolution was chosen. In **chapter 3** a 2.5 × 2.5 m polygon grid was used for a more fine scaling evaluation. With regard to the current GPS error of the virtual fencing collars, an increase in fine scaling can not be recommended, although UAV data can provide a more accurate monitoring of the grass sward as shown in the 0.5 × 0.5 m polygon grid (**chapter 3, Figure 5** and **6**). The use as a reference of remote sensing to validate the impact of grazing livestock on the sward (chapter 3) and to determine the available biomass to adjust the virtual boundaries sustainably appears to be very important to exploit the full potential of the technology especially in large landscapes (Jansen et al., 2022). The accurate and timely measurement of pasture is an integral part of effective grazing management (French et al. 2015), which could be provided by the use of remote sensing.

Thapa-Magar et al. (2022) suggest that (negative) effects of livestock grazing on pollinator communities may be driven by the impact on nesting habitats due to variables such as soil compaction. Soil compaction in pasture use is associated with highly frequently visited places. Regarding the analyses of pasture utilisation on the polygon grid in chapter 3, it should be possible to detect and protect vulnerable polygon grid cells. Increasing cattle residence in a polygon grid cell (lying time excluded) could lead to overgrazing, soil compaction or in general soil degradation, which is always linked to negative environmental impacts (Breidenbach et al. 2022). Boval and Dixon (2012) defined the role and responsibility of scientists as providing reliable information on the impact of livestock production systems on grassland ecosystems. Grid grazing as described in **chapter 3** appears to provide the base for this kind of information and allow the practice to respond accordingly. Likewise, the US governement emphasises the potential of virtual fencing for climate adaption strategy because of the potential of the technology to diminish soil erosion associated with overgrazing by outfencing vulnerable areas (USDA, 2022). Lower stocking densities and shorter stocking periods may also mitigate the effect of climate change and protect soil structure (Animal Welfare Committee, 2022).

Motion behaviour in virtual fencing systems

Although development for improved grazing systems is required, technology which disturbs ‚normal' behaviour should be avoided (van den Pol-van Dasselaar, 2020). Therefore, we analysed and evaluated in **chapter 2** the potential impact of virtual fencing compared to physical fencing on a wide range of behavioural and physiological responses on grazing groups of heifers. It turned out that none of the considered behavioural and physiological parameters were affected systematically (**chapter 2**). However, on five days (in total 36 days, divided equally into three time replicates) physically fenced heifers revealed more time for locomotion than virtually fenced heifers. Additional analyses of counted steps (IceTag© pedometer) comparisions of means between both treatments revealed no significant differences. Although not statistically relevant, more steps were counted for the physically fenced groups in each of the three time replicates. Verdon et al. (2021) found significant fewer steps on day 4-6 when comparing virtually and physically fenced dairy cows. In **chapter 3**, the effect of treatment (virtual fencing vs. physical fencing) was never significant in the analyses of movement behaviour, but again the virtually fenced animals always walked slightly less than the physically fenced animals (not statistically relevant). Possible underlying reasons for slightly less locomotion in the VF groups remain an open question. From the visual observation in the rotational grazing trial described in **chapter 3**, we only know that the paddock change is calmer, as the animals do not run through an open gate, but slowly (while grazing) find their way into the newly provided area. Considering that an effect of the virtual fence must be more visible near the virtual boundary than near the centroid of the respective paddock, we divided each paddock analysed in **chapter 3** into a center (at least ten metres apart from the fence) and a perimeter zone (see Figure 2 for details). Then we counted GPS positions of each heifer in the respectively group in both zones and calculated the percentage of GPS positions (Table 1) per zone. As also exhibited in Figure 2, there are more GPS positions for the virtually fenced Heifers in the center zone than for the physically fenced heifers.

Table 1: Mean ± sd and percent of GPS positions in the center and perimeter zone of three paddocks. VF-L: Virtual fence-left; PF-L: Physical fence-left; VF-R: Virtual fence-right; PF-R: Physical fence-right.

	VF-L		PF-L		VF-R		PF-R	
	n	%	n	%	n	%	n	%
Center zone	598 ± 201	48	381 ± 172	30	776 ± 149	62	499 ± 166	39
Perimeter zone	649 ± 189	52	909 ± 173	70	480 ± 158	38	777 ± 171	61

Further statistical analyses are necessary to fully cover the underlying reasons for slightly reduced movement of virtually fenced animals. Lomax et al., 2019 and McSweeney et al., 2020 both provide evidence in their research that dairy cattle avoid the area near where a virtual fence had been established for grazing. We also looked for the time associated with grazing by excluding lying time and the hours of darkness and found a decrease in the gap between virtually and physically fenced heifers. Consequently, considering solely the lying time, the gap increases to a maximum of 72 % of residence time in the center zone for the virtually fenced heifers on the right side (Figure 2). During animal observation time of virtually fenced heifers, we observed a few incorrect acoustic signals followed by electric pulses when the heifers were lying near the virtual boundary. These incorrect GPS positions could be a consequence of battery life function used for lying time detection (described in **chapter 3**). The reason appears to be that after the lying time associated with deactivation and sending unchanged GPS position, the initial GPS position has been sometimes incorrect. Perhaps shifting their lying time towards centroid is a behavioural response of the heifers to these unlogical signals and may need to be addressed in further research and/or further technological improvement. However, the increase in walking distances correlates positively with paddock size when analysing commonly fenced animals (McGavin et al. 2018). Therefore, it could be assumed that each kind of boundary affects normal movement pattern.

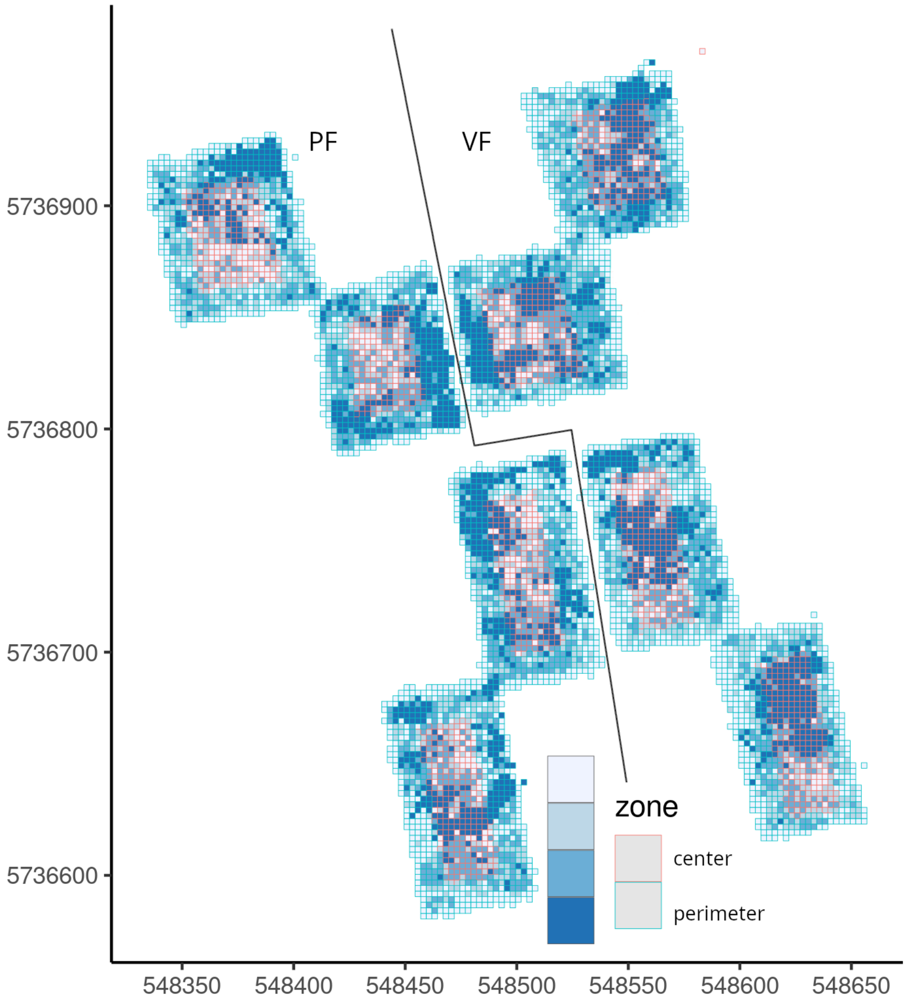

Figure 2: GPS positions per polygon grid cell of paddock B and D. Each paddock was divided in a center and a perimeter zone. PF: physical fence; VF: virtual fence. The number of GPS positions per polygon grid cell were categorised in quantiles identified by colour. Dark blue is associated with the highest frequency, light blue with the lowest frequency of heifers GPS positions per polygon grid cell.

Virtual fencing learning success visible through mode change

So far, there is no scientific evidence that a cow does not learn the connection between an acoustic signal and an electric pulse. Hence, learning to interact appropriately with the virtual fence was possible for the experimental cattle in various grazing trials and did not lead to any measurable deterioration in animal welfare (e.g. Langworthy et al. 2021; Verdon et al. 2021; **chapter 2**). Learning to avoid an appropriate level of electrical stimuli is a prerequisite for assessing the technology as non-harmful to the animals (Lee et al. 2008). However, it remains an open question how fast cattle are capable to learn the virtual fencing system and on what evidence the virtual fencing system is considered to be learned. Therefore, we used the data of the training period of the rotational grazing trial described in **chapter 3** adapted from the training schedule in **chapter 2** to analyse learning speed and the ability of learning to predict and as a result of this, to avoid the electric pulse.

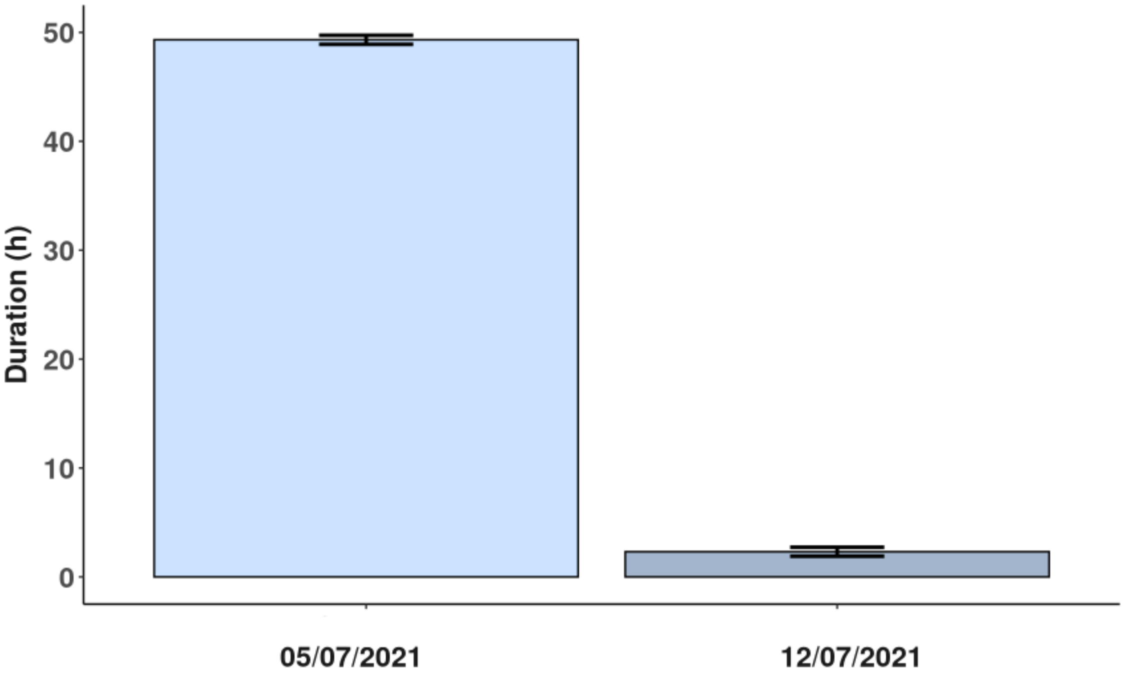

Figure 3: Analyses of mode change speed from teaching mode to operating mode (mean ± se) on two consecutive starting times for two groups of equal size (in total 16 animals) of virtually fenced Fleckvieh heifers.

A teaching and an operating mode are provided by the ®Nofence virtual fencing collars. Animals automatically change the mode after responding correctly to 20 consecutive acoustic signals, without receiving an electric pulse (see **chapter 2**). We used the time until mode change (from teaching mode to operating mode) to evaluate the learning ability and speed of 16 Fleckvieh heifers divided in two groups of eight heifers. All heifers were naive to virtual fencing prior to the training period (05.07.-16.07.2021). On the first day, the two groups were equipped with virtual fencing collars and assigned to two adjacent pastures. On day eight, the collars were deactivated for a short time and then activated to start in teaching mode again. We investigated the delta (Δ) of time to receive the operating mode for each consecutive starting time (day one and eight). Statistical analysis was carried out with the software environement R (R Core Team 2022). Linear mixed effect models were calculated by using the package nlme (Pinheiro et al. 2018). Normality of the residuals was checked by visual inspection of quantile–quantile plots (Zuur 2009). Variance homogeneity was evaluated by plots of residuals vs. fitted values and residuals vs. predictor values. For significant variable levels, multiple contrast tests according to Tukey's HSD test using Sidak's method of confidence level adjustment were conducted in the 'emmeans' package (Barton, 2018). The time interval for each animal and starting time (two level) to change mode was regressed on the fixed effects starting time and group. The animal was modelled as a random term. We found a significant effect of starting time ($p<0.0001$). Average Δ was 49.32 ± 0.41 h and 2.31± 0.41 h (mean ± se) for starting time one and two (see Figure 3), respectively. The faster mode change after the second starting time suggests successful learning. Our results suggest, the animal learned to predict (and avoid) the electric pulse. A manually calculated ratio has also been used previously to assess the learning success of the virtual fence system, e.g. Verdon et al. (2020). It appears, as if the ®Nofence virtual fencing collar integrated mode change automtically checked a kind of ratio of acoustic and electric signals. The approach used in the present study (restart to assess the speed of switching to operate mode) could be understood as a metric to assess the cognitive abilities of cattle. Further research is needed to increase our knowledge of learning speed and adapt this to optimise the appropriate training schedule.

In sum, successful learning of the virtual fencing technology appears to be easily possible for Fleckvieh heifers. All of the animals (in total 28 Fleckvieh heifers) used for the trials descriped in **chapter 2** and **3**, were able to learn the virtual fencing system, although there was temporal variation in associative learning of the audio signal and the electric pulse among individuals (also described by e.g. Lomax et al. 2019; Verdon et al. 2020; Langworthy et al. 2021).

Outlook on possible practical implications

The preconditions for the acceptance and (further) legalisation of a technology that replaces fences with collars on a certain scale are increasingly given and this work, too, may contribute to this.

The form in which the potential of the new technology might be used depends on environmental conditions. In heavily populated areas with a rather small-scale agricultural structure, it is more likely that a complete abandonment of fences surrounding a pasture is not possible. Nevertheless, an advantage by using the technology combined with conventional fencing can be achieved through the more precise capture of forage allocation, outfencing vulnerable areas, and real-time monitoring of animal behaviour on pasture. Grazing practices with high fencing efforts such as strip grazing, rotational grazing and mob grazing could be more easily realised and are therefore becoming increasingly attractive for practical farming. Moreover, grazing based on a polygon grid (grid grazing) should be possible and can allow for finely graded grazing taking into account UAV-based remote sensing and biodiversity markers that address both efficiency and sustainability.

In landscapes with limited options for fence construction and little contact with humans such as areas that are difficult to access or large scaled and also agroforestry systems, it should be possible to dispense with the fence completely. The possibilities of remote animal monitoring are particularly valuable in these cases and can help to identify and help sick animals as quickly as possible. Additional, unexpected increased movement can indicate the presence of predators, which permits to take timely protective action.

Conclusion

In this thesis, the effects of grazing intensity and forage availability on cattle movement behaviour are investigated (**chapter 1** & **3**). The results indicate that the movement behaviour of cattle is related to the amount and spatial distribution of forage availability, which can be used as a decision-making tool for grassland management. Furthermore, the thesis evaluates the possible impact of virtual fencing technology on growing heifers, using a wide range of behavioural and physiological measurements (**chapter 2**). None of the considered parameters were affected systematically by the virtual fencing system. Additional analyses to measure the learning success and possible differences in the spatial behaviour of virtually fenced heifers (General Discussion) could help to expand the existing knowledge about virtual fencing. The evaluation of the usability of the virtual fencing collars for animal monitoring revealed that valuable information such as lying behaviour, walking distances and spatial distribution of the animals can be provided and linked to UAV based monitoring of the sward on a polygon grid base (**chapter 3**).

The main outcome of this thesis, based on the described results, is the option of developing a herder 4.0 as a networked comprehensive IoT assistance system. This could permit monitoring of the sward and grazing animals, as well as the possibility of flexible forage allocation based on pasture utilisation data (combined remote sensing and animal residence time) by (out)fencing and adjusting virtual boundaries on a polygon grid. Grid Grazing appears to be a future perspective for a precise grazing management, for which the first basic requirements could possibly be clarified here.

As it was hard for our ancestors to imagine the herder disappearing from the landscape and being replaced by indoor husbandry and electric fences, it is difficult today to imagine animals in the landscape without visible boundaries.

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Picture Two Fleckvieh cows wearing Nofence collars in ‚FORBIOBEN' © 2022 Caroline Siede

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Thesis declaration

I confirm that I have written this thesis independently using no other sources or facilities than those stated. I have not used unauthorised assistance and have completed all parts of my dissertation on my own. Furthermore, I have not submitted this thesis previously in any form for another degree at any university or institution.

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Göttingen, November 2022

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Publications

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