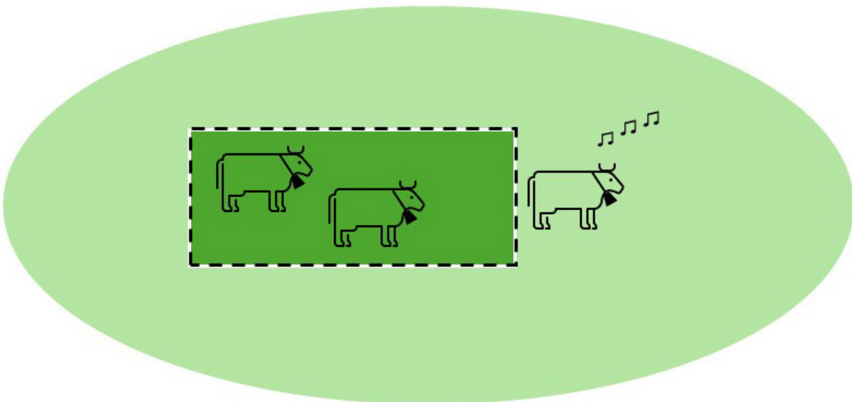
Cattle adaption to virtual fences in semi-natural pastures with multiple virtual borders

Impact on behaviour and level of cortisol in faeces and hair in comparison to physical electric fences

Lotten Wahlund



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Swedish University of Agricultural Sciences
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Errata for
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All pages	Location:	-
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Abstract	Location:	Titel
	Is now:	"pasture"
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Page 11	Location:	Third paragraph, row 2-3
	Is now:	"Virtual or physical electrical fencing of naïve cattle on pasture – effects on cortisol in faeces and hair"
	Should be:	"Virtual or physical electrical fencing of naïve cattle on pasture – effects on behaviour, body weight and cortisol in faeces and hair"
Page 13	Location:	Row 7
	Is now:	"SCAW Swedish Centre for Animal Welfare"
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Page 21	Location:	First paragraph, row 7
	Is now:	"cortisol is metabolised,"
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Cattle adaption to virtual fence in semi-natural pasture with multiple virtual borders

Abstract

The interest in GPS-based virtual fencing for grazing management of cattle, using audio cues combined with electric pulses, has increased in recent years. This thesis aimed to explore cattle adaptation to virtual fencing in semi-natural pastures with the relocation of multiple borders and to investigate the physiological and behavioural effects compared to traditional electric fencing. It presents results from two simultaneous studies (A and B) conducted from May to July 2022 in a semi-natural pasture in Sweden. Study A examined the learning curve and adaptation of seven heifers to a virtual fence with one to four virtual borders and two relocations over six weeks. Study B compared FCM (faecal corticoid metabolite) and HCC (hair cortisol concentration) levels, behaviour, and activity between heifers exposed to either a virtual fence (VFG) or a physical electric fence (EFG) over seven weeks. Results showed that the heifers, within seven days, learned to turn at the audio cue to avoid the electric pulse and improved their adaption and management skills over time, regardless of the number and relocation of the virtual borders. The results revealed higher FCM levels in VFG at the beginning of the pasture period compared to EFG. However, this difference was evident even before the virtual fence was introduced, suggesting that the stress response might be due to unknown factors rather than the fencing system itself. This was also supported by similar HCC levels and weight changes between groups, indicating no long-term stress. Additionally, no differences in behaviour were observed that could be attributed to stress. In conclusion, the findings suggest that virtual fences can be a promising alternative for grazing management in semi-natural pastures, with a similar impact on cortisol response and behaviour compared to traditional electric fences.

Keywords: (Virtual fence, Cattle, Learning curve, Stress, Cortisol, Behaviour, Precision livestock farming, Semi-natural pasture)

Nötkreaturs anpassning till virtuellt stängsel i naturbetesmark med flera virtuella gränser

Sammanfattning

Intresset för GPS-baserade virtuella stängsel för betesdjur, som kombinerar ljudsignaler med elstötar, har ökat markant de senaste åren. Denna avhandling syftar till att undersöka hur nötkreatur lär sig och anpassar sig till tekniken i en naturbetesmark, med olika antal och placeringar av de virtuella gränserna, samt att jämföra de fysiologiska och beteendemässiga effekterna med traditionella elstängsel. Avhandlingen baseras på två studier (A och B) som genomfördes i en svensk naturbetesmark mellan maj och juli 2022. Studie A fokuserade på sju kvigors inlärning och anpassning till ett virtuellt stängsel med en till fyra gränser, vilka flyttades två gånger under en sexveckorsperiod. Studie B undersökte kortisolnivåer i träck (FCM) och hår (HCC), samt beteende och aktivitet hos kvigor som antingen använde ett virtuellt stängsel (VFG) eller ett fysiskt elstängsel (EFG) under en sjuveckorsperiod. Resultaten visade att kvigorna inom sju dagar lärde sig att vända på ljudsignalerna för att undvika elstötar, och deras förmåga att hantera systemet förbättrades över tid, oavsett antal och placering av de virtuella gränserna. Inledningsvis var FCM-nivåerna högre hos VFG än EGF. Skillnaden observerades dock redan innan det virtuella stängslet introducerades, vilket tyder på att andra faktorer än själva stängselsystemet kan ha orsakat stressresponsen. Detta stöds även av liknande nivåer av HCC och viktförändringar mellan grupperna, vilket indikerar en avsaknad av långvarig stress. Dessutom kunde inga beteendeskillnader kopplade till stress påvisas mellan grupperna. Sammanfattningsvis pekar resultaten på att virtuella stängsel kan vara ett lovande alternativ för att hägna in nötkreatur på naturbetesmarker, med en jämförbar påverkan på både beteende och kortisolnivåer som vid användning av traditionella elstängsel.

Nyckelord: (Virtuellt stängsel, Nötkreatur, Inlärningskurva, Stress, Kortisol, Beteende, Precisionsdjurhållning, Naturbetesmark)

Dedication

To my wonderful husband and amazing children.

Have faith in your intuition and listen to your gut feeling.
Ann Cotton

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Wahlund, L., Nielsen, P., Jansson, A., Rönnegård, L. 2024. Cattle adaption to virtual fence with relocation of multiple borders over several weeks (manuscript)

II. Wahlund, L., Nielsen, P., Rönnegård, L., Jansson, A. 2024. Virtual or physical electrical fencing of naïve cattle on pasture – effects on cortisol in faeces and hair (manuscript)

The contribution of Lotten Wahlund to the papers included in this thesis was as follows:

I. Description of contribution to paper I: Conceptualisation, Data collection, Methodology, Data management, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualisation

II. Description of contribution to paper II: Conceptualisation, Data collection, Methodology, Data management, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualisation

Abbreviations

GPS	Global positioning system
SCAW	Swedish Centre for Animal Welfare
VFG	Virtual fence group
EFG	Electric fence group
FCM	Faecal corticoid metabolite
HCC	Hair cortisol concentration
SCAW	Swedish Centre for Animal Welfare
VF	Virtual fence
EF	Electric fence
VFB	Virtual fence border

1. Introduction

1.1 The functionality and applications of GPS-based virtual fences for cattle

The interest in using virtual fencing with GPS-based collars to manage cattle grazing within designated areas has increased in recent years. Unlike traditional electric fences that rely on visual stimuli, virtual fencing technology uses audible cues. When cattle reach a predefined GPS boundary (virtual border), the collar emits an audio cue at the position where the virtual border is crossed. The audio cue is deactivated once the animal turns around and returns to the virtual pasture. If the animal does not turn around, an electric pulse follows. The system is managed through a mobile application, allowing for the quick and easy creation of virtual pastures and adjustment of the virtual borders within seconds (Figure 1). This approach may reduce the amount of labour required and enhance efficiency and flexibility in pasture management (Umstätter, 2011; Lee et al., 2018), both in intensive grazing systems, such as strip grazing, but also in more extensive systems with challenging terrains where traditional fencing is impractical (Campbell et al., 2019; Hamidi et al., 2023). This allows for grazing ruminants to access previously inaccessible areas, including environmentally sensitive regions that might otherwise remain unused, which promotes diverse landscapes and habitat heterogeneity (Adler et al. 2001). Such areas support a wide variety of plant and animal species, thereby enhancing biodiversity and ecosystem services (Jerrentrup et al., 2014; Tälle et al., 2016). Both intensive and extensive pasture management systems may require the regular relocation of one or several virtual borders. Applying this type of management with physical electric fences would involve a significant increase in labour and might not be feasible in many situations. Consequently, virtual fence technology has the potential to enhance intensive grazing practices, such as those used in dairy farming, and also increase the number of grazing animals utilising areas that are currently inaccessible.

Furthermore, the technology enables farmers to monitor their livestock around the clock as well as their previous position, as each collar is displayed with the real-time position in the app and through a heat map function. These features provide the potential for improved monitoring of grazing animals, with the ability to detect anomalies at an early stage. Additionally, some brands on the market offer functions within the system to alert farmers to the absence of collar movement or changes in animal activity levels, providing an early warning system when an animal may be unwell. The app also displays the number and position of audio cues, electric pulses, or escapes per individual. This allows the farmer to react to a specific occurrence for either total herd behaviour or that of a specific individual.

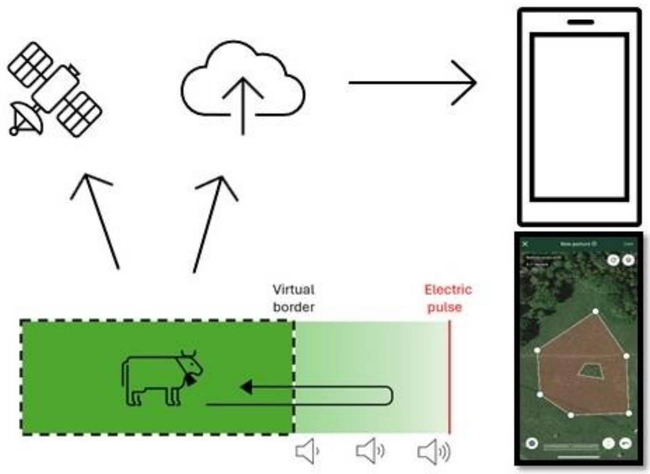

Figure 1. Functional overview of a GPS-based virtual fencing system. The system utilises GPS borders (dotted lines) and communicates via both satellite and cellular networks. Management is facilitated through a mobile app, where virtual pastures are created, and borders can be adjusted (see a screenshot from an app of the Nofence system, in the right corner).

1.2 Cattle´s initial learning curve and adaption over time to virtual fences

Studies have demonstrated that cattle can learn to turn around on audio cues to avoid electric pulses in virtual fence systems (e.g. Fuchs et al., 2024, Hamidi et al., 2024; Confessore et al., 2024). This was also supported by the result from Wilms et al. (2024) in a comprehensive analysis of results from virtual fence studies involving cattle between 2015 and 2022. They found that the ratio of electric pulses to audio cues decreased over time, indicating a learning process. Additionally, they concluded that virtual fence technology shows promise for animal welfare, particularly in terms of behaviour and cortisol levels, compared to physical fences. However, variations in individual learning curves have been observed (e.g. Aaser et al., 2022; Fuchs et al., 2024; Staahltoft et al., 2023), and several studies (e.g. Aaser et al., 2022; Campbell et al., 2019; Hamidi et al., 2022) have discussed individual temperament traits as an important factor in exploring these individual variations, highlighting the need for future research in this area.

Due to the system's use of electric pulses as an aversive stimulus and the individual differences, concerns have been raised by authorities and discussed among researchers regarding the impact of the system on animal welfare (e.g. Wilms et al., 2024; Waterhouse et al., 2023; SLU, 2019). At present, the use of virtual fencing on livestock is prohibited in many European countries including Sweden, but has been allowed in countries such as Norway, Spain, and the United Kingdom for several years. In the spring of 2019, the Swedish Centre for Animal Welfare (SCAW), on behalf of the Swedish Board of Agriculture, published a literature review of existing knowledge on how virtual fencing technology affects animal welfare, based on peer-reviewed scientific articles up to the end of 2018 (SLU, 2019). The conclusion was that the understanding of the causes of individual variation in learning curves and its impact on the welfare of individual animals, as well as the long-term effects, was limited, as other researchers (mentioned above) also have highlighted. This resulted in the Swedish Board of Agriculture's decision to prohibit the use of virtual fencing technology in Sweden at that time. However, Helena Elofsson (personal communication, 2024), the Animal Welfare Director at the Swedish Board of Agriculture has stated that if future research demonstrates that virtual fencing provides animal welfare comparable to electric fencing, this decision may be reconsidered. To build on the SCAWs report (SLU, 2019), Tables 1-3 present studies conducted from January 2019 to May 2024, illustrating the progress and findings in the field since then. Most of the previous studies within this time period have included various animal categories and breeds but have mainly focused on the first two to three weeks using one to two virtual borders (Tables 1, 2). Across these studies, the available area per animal in the different virtual fence enclosures varied between 0.05 and 5.42 hectares (Table 2). While many studies have explored the relocation of virtual borders, only one, Aaser et al. (2022), has tested a four-sided virtual pasture. This study was unique as it was conducted in a semi-natural pasture, whereas all other previous studies have been conducted on ley pastures. Consequently, knowledge about the use of virtual pastures in semi-natural environments, as well as the application of four-sided virtual fenced pastures, remains limited.

As described above, before virtual fencing can be approved in Sweden, the Swedish Board of Agriculture requires that the welfare of grazing animals managed with virtual fences must be equivalent to that with conventional electric fences. However, knowledge regarding the learning curve and the number of electric pulses received during the training phase for cattle using conventional electric fences is very limited. To the best of my knowledge, only two previous studies have aimed to explore this (Martiskainen et al., 2008; McDonald et al., 1981). Both these studies lasted for seven days and included 19 dairy bull calves and 38 cows, heifers, and steers, respectively, focusing solely on the number of electric pulses received. Consequently, there is a significant knowledge gap in understanding cattle's learning curve and physiological stress responses during the training phase with electric fences. As well as the knowledge of the subsequent adaptation and stress response over time where to the best of my knowledge, only two studies have recorded electric pulses from a physical electrical fence beyond the initial training phase. In Langworthy et al. (2021) involving dairy cows, the number of electric pulses from an electric front fence during strip grazing was recorded over a 3-day period, with only one electric pulse recorded in total. The animal in this study was accustomed to electric fences before the trial began. Hamidi et al. (2022) recorded the number of electric pulses from both virtual and traditional electric fences within the same experimental context. The number of electric pulses received from the electric fence by heifers (already accustomed to electric fences at the start of the experiment) was, in total, 93 pulses over a period of 12 days (three groups of four heifers each). In comparison, the heifers with the virtual fences received 156 pulses during the same time span, which also included the training period of the virtual fence. However, only the total number of electric pulses from the electric fence was presented; no individual data were provided. As previous studies with cattle and virtual fences have rarely included control groups with electric fences (n=6 in Table 3), our understanding of the comparative animal welfare impacts between electric and virtual fences is scarce.

Table 1. Overview of studies and their experimental designs (January 2019 - May 2024) on cattle using commercially available virtual fences (VF). The table provides details on the technology used (brand), the type of pasture, the total number of animals using virtual fences (VF), the total number of days with VF, the number of virtual fence borders (VFB), animal type, age, and breed for the different studies.

Publication*	Brand	Type of pasture	Total no. of animals with VF	No. of days with VF	No. of VFB	Animal type, age	Breed
Aaser et al. (2022) & Sonne et.al., (2022)	Nofence	Semi-natural	12	139[a]	1 to 4	Cows 4-9 years	Angus
Hamidi et al. (2022)	Nofence	Ley	12	12	1	Heifers 14-16 month	Fleickvieh
Confessore et al. (2022)	Nofence	Ley	20	8	1	Pregnant cows -	Limousine
McSweeney et al. (2020)	No brand presented	ley	12	9	1	Cows -	Holstein-Friesan
Staahltoft et al. (2023)	Nofence	Ley	17	89	1 to 2	Bull calves 9-12 month	Angus
Campbell et al. (2019)	eShepherd	Ley	32	27	1	Steers 12-14 months	Angus
Verdon et al. (2021b) & Langworthy et al. (2021)	eShepherd	Ley	30	13	1	Dairy cows Parity 2-7	Friesan x Jersey
Boyd et al. (2022)	Vence	Ley	20	20	1 to 3	Mature cows	Angus
Hamidi et al. (2024)	Nofence	Ley	16	12	3	Heifers 12-15 month	Fleickvieh
Hamidi et al. (2023)	Nofence	Ley	32	28[b]	1 to 2	Heifers 15 months	Fleickvieh
Confessore et al. (2024)	Nofence	Ley	20	31	1	Dairy ows 3-8 years	Holstein-Friesan
Lomax et al. (2019)	eShepherd	Ley	12	6	1	Cows 5 years	Holstein-Friesan
Fuchs et al. (2024)	Nofence	Ley	10	56	1	Dairy cows 2-7 lactation	Holstein-Friesan
Keshavarzi et al. (2022)	eShepherd	Ley	64	3	1	Steers 14 months	Angus
Colusso et al. (2020)	eShepherd	Ley	23	Na[c]	1	Dairy cows 4-9 years	Holstein-Friesan
Verdon et al. (2021a)	eShepherd	Ley	20	12	1-2	Heifers	Angus
Verdon et al. (2024)	Halter	Ley	80	38	1	Dairy cows Parity 1-8	Holstein-Friesian, Jersey, Swedish Red, Friesian-Jersey

* Rows with two publications refer to studies simultaneously using the same animals but with different study aims. [a]The study of Sonne et al. (2022) ended after 18 days. [b] The number includes a period of 12 days of training, but the result from this period was not presented within the publication. [c]Not applicable since animals were regrouped within the same day.

Table 2. Overview of experimental designs in studies (January 2019 - May 2024) on cattle using commercially available virtual fence systems for virtual fence pastures (VFP). This table details the training phase and the frequency of virtual fence border (VFB) relocations, specifying the number of VFB, duration (days), and accessible areas (ha) for each phase (training or relocation event).

				Relocation of VFB					
Publication*	**No. of animals within one VFP**	**No. of VF groups**	**Training phase**	**1**	**2**	**3**	**4**	**5**	**6**
Aaser et al. (2022) & Sonne et. al. (2022)	12	1	1 VFB 7 days 6,5 ha	1 VFB 3 days 6,5 ha	1 VFB 5 days 6,5 ha	4 VFB 48 days 35-49 ha[a]	4 VFB 12 days 28 ha	4 VFB 14 days 15 ha	4 VFB 53 days 65 ha
Hamidi et al. (2022)	4	3	1 VFB 7 days 0.87 ha	1 VFB 5 days 0.87 ha	-	-	-	-	-
Confessore et al. (2022)	12	1	1 VFB 2 days 16 ha	1 VFB 4 days 25 ha	2 VFB 2 days Na	-	-	-	-
Staahltoft et al. (2023)	17	1	1 VFB 2 days Na	1 VFB 2 days Na	2 VFB 2 days Na	1 VFB 2 days Na	0 VFB 2 days Na	2 VFB 2 days Na	2 VFB 2 days Na[b]
Campbell et. al. 2019	8	4	1 VFB 27 days 6 ha	-	-	-	-	-	-
Verdon et al. (2021b) & Langworthy et al. (2021)	30	1	1VFB 3 days 2.2 ha	1 VFB 1 day 0.3 ha	1 VFB 1 day 0.3 ha	1 VFB 1 day 0.3 ha	1 VFB 1 day 0.3 ha	1 VFB 1 day 0.3 ha	1 VFB 1 day 0.3 ha[c]
Boyd et al. (2022)	20[d]	3	1 VFB 3 days Na	1 VFB 3 days Na	1 VFB 14 days Na	-	-	-	-
Hamidi et al. (2024)	8	2	3 VFB[e] 9 days 3 ha	3 VFB 3 days 3 ha			-	-	-
Hamidi et al. (2023)[f]	8	2	1 VFB[f] 7 days Na	1 VFB[f] 5 days Na	2 VFB 3-4 days 0.5ha	2 VFB 3-4 days 0.5ha	2 VFB 3-4 days 0.5ha	2 VFB 3-4 days 0.5ha	-
Confessore et al. (2024)	5	4	1VFB 7 days 0.4 ha	1VFB 7 days 0.6 ha	1VFB 7 days 0.8 ha	1VFB 7 days 0.6 ha	1VFB 7 days 0.6 ha	-	-
Lomax et al. (2019)	12	1	1 VFB 4 days 2.8 ha	1 VFB 3 days 2.8 ha	-	-	-	-	-
Fuchs et al. (2024)	5	4	1 VFB 21 days 1 ha	1 VFB 14 days 1 ha	1VFB 14 days 1 ha	1VFB 7 days 1 ha	-	-	-
Keshavarzi et al. (2022)	8	1	1 VFB 3 days 6 ha	-	-	-	-	-	-
Verdon et al. (2021a)	10	2	1 VFB[g] 2 days 1 ha	2 VFB 1 day 1.2 ha	2 VFB 3 days 2-3 ha	2 VFB 3 days 1-3 ha	2 VFB 3 days 1-3 ha		

* Rows with two publications refer to studies simultaneously using the same animals but with different study aims. Na means that the VFP area was not stated. Colusso et al. (2020), was excluded from the table as the number of relocations did not fit the table design due to the regrouping of animals several times a day. Also, Verdon et al. (2024) were excluded as the animals were herded twice a day with the help of the VFB, training phase of 10 days. [a]Sonne et al. (2022) ended at relocation 3 after 3 days. [b]Continued up to 52 relocations with 1-2 VFB, with the physically fenced pasture totalling 8.2 ha. [c]Continued up to 10 relocations with daily movement and one VFB. [d]Only 18 animals were present during relocation 2. [e]The VFB was supported by physical electric fence lines, occasionally removed during the first six days. [f]The training phase and relocation 1 covers a 12-day period, similar to Hamidi et al. (2022), but was not detailed in the study. [g]During the training phase and relocation 1, the herd consisted of 40 animals.

1.3 Animal welfare assessment in virtual fence trials

Good animal welfare cannot be achieved under stress (Viessier & Boissy, 2006), and stress responses occur when animals are unable to predict and control their environment (Del Guidance et al., 2018; Lee et al., 2018). Animals’ stress responses are managed by the hypothalamic-pituitary-adrenal axis, and cortisol is produced by the adrenal glands. Once active, cortisol is metabolised, and the metabolites are excreted in urine and faeces. Baseline cortisol levels and stress responses differ across species, generally following a circadian rhythm (Möstl & Palme, 2002). Additionally, factors such as feed deprivation, temperature, humidity, and physical conditions can also influence cortisol levels (Chen et. al., 2015). In livestock production systems, stressful situations can also occur during handling and transportation due to environmental conditions and social interactions. Minimizing these stressors is crucial to enhance the overall animal welfare. According to Lee & Campbell (2021), the stress response related to the usage of virtual fencing is influenced by the livestock's interactions with virtual fence borders to avoid the electric pulses, which vary with the stage of learning. The variation stems from changes in the level of predictability and controllability over time, influenced by the animals' expectations of the response to audio cues to avoid aversive electric stimuli (Lee et al., 2018), where acute stress measures are relevant during the initial training phase and chronic stress measures are applicable at later stages. To ensure animal welfare and minimise stress, it is vital for animals to respond effectively to audio cues in controllable situations (Lee et al., 2018). Methods used to assess animal welfare in previous virtual fence studies have primarily involved behavioural observations and measures of cortisol levels in faeces, hair, and milk (Table 3).

1.3.1 Cortisol as a measure of stress response

Measuring the levels of cortisol in the blood, saliva, milk, faeces, or hair is a well-known method for assessing levels of stress in animals. Analysing cortisol in blood plasma is known as a good and reliable way to record the direct effect of a specific stress response. It could, therefore, be used to compare the direct reaction of an electric pulse from the physical electric fence or a virtual fence for comparison. However, the difficulty with this method is that the measure itself may have an impact on the result as the method is invasive, requiring fixation and sampling true needle sticks. This is further complicated in trials where the aim is for the animals to move freely, as in pastures. For this method to be used in virtual fence trials, there is a need for development where the blood sample could be taken without the involvement of handling and fixating the animals. Consequently today, other non-invasive sampling procedures are preferable in settings where animals move freely, such as on pasture.

Table 3. Overview of experimental designs in studies (January 2019 - May 2024) on cattle using commercially available virtual fences (VF). The table provides details of the number of groups with VF and groups with physical electric fences (EF). It specifies an overview of the assessed parameters behaviour, cortisol and personality traits, and the method used.

				Assessed parameters and methods used		
Publication	**No. of VF groups**	**No. of EF groups**	**No. of animals per group**	**Behavoiur**	**Cortisol**	**Personality traits**
Aaser et al. (2022)	1	-	12	-	-	-
Hamidi et al. (2022)	3	3	4	IceTag Direct observations	Faecal 2 occasions	Restraint test
Confessore et al. (2022)	1	-	20	-	Hair - front region	-
McSweeney et al. (2020)	2	-	6	Direct observation	-	-
Staahltoft et al. (2023)	1	-	17	-	-	-
Campbell et al. (2019)	4	4	8	IceCube	Faecal 5 occasions	-
Verdon et al. (2021b)	1	-	30	RumiWatch IceTag	Milk	-
Langworthy et al. (2021)	1	-[a]	30	-	-	-
Boyd et al. (2022)	3	3	3	-	-	-
Hamidi et al. (2024)	2	-	8	Direct observation	-	Restraint test
Sonne et al. (2022)	1	-	12	-	Faecal 5 occasions[b]	-
Hamidi et al. (2023)	2	2	8	Direct observation	-	Restraint test
Confessore et al. (2024)	4	-	5	Pedometer	Hair - head	-
Lomax et al. (2019)	1	-	12	-	-	-
Fuchs et al. (2024)	2	2	5	IceQubes Direct observations	Milk	-
Keshavarzi et al. (2022)	8	-	8	Direct observations	-	Social facilitation
Colusso et al. (2020)	Not applicable	-	-	Direct observations	-	Dominance rank
Verdon et al. (2021a)	2	2	10	-	-	-
Verdon et al. (2024)	2	-	40	-	-	-

[a]The animal in the VF group was exposed to strip grazing with physical electric fences prior to the introduction to VF. [b]Five out of 12 animals in the group were sampled.

1.3.2 Faecal corticoid metabolite (FCM)

The use of faecal cortisol metabolite (FCM) as a non-invasive method to measure stress in various animal species is widely used (Keay et al., 2006), and Möstl & Palme (2002) further highlighted its effectiveness for assessing stress responses in cattle in various research settings, such as handling, housing, and transportation, where animals move freely. However, the level of FCM in a given sample does not provide an immediate response. In cattle, the physiological response reflects events occurring approximately 10-12 hours prior to sample collection, exposing the intestinal passage time Möstl & Palme (2002). Therefore, a precise sampling strategy is crucial when FCM is used as an indicator of stress to avoid missing out on a specific stress response.

As presented in Table 3, FCM has been used in virtual fence studies with cattle by Hamidi et al. (2022), Sonne et al. (2022), and Campbell et al. (2019). Neither Hamidi et al. (2022) nor Campbell et al. (2019) found any differences in levels of FCM compared to control groups using physical electric fences, nor, in the study by Sonne et al. (2022), any change was found over time. However, the sampling regime differed between the studies. In Hamidi et al. (2022), the first sampling of two took place eight days after the activation of the virtual fence, which was the same day as the first movement of the virtual border. Sampling number two was taken on day 12, the final day of the experiment. In the study by Campbell et al. (2019), one constant virtual border was used throughout the trial, and faecal samples were taken on the last day of each study week during the four weeks of the experiment. In Sonne et al. (2022), the sampling took place every other day during the 18 days of the experiment. However, the sampling strategy in previous studies does not include the potential stress response to the relocation of virtual borders as there was no clear strategy for sampling before and after the virtual borders were relocated. Therefore, additional research is needed within this area to understand how the relocation of the virtual borders potentially affects the level of FCM in cattle.

1.3.3 Hair cortisol concentration (HCC)

The use of hair cortisol concentration (HCC) has proven to be a promising non-invasive method for evaluating repeated stress responses over extended periods (Heimbürge et al., 2019; Confessore et al., 2024), with minimal impact from circadian rhythms, seasonal changes, and animal handling. To the best of my knowledge, Confessore et al. (2022, 2024) conducted the first studies on virtual fencing in cattle using HCC to assess long-term stress responses. However, neither study found significant differences over time; Confessore et al. (2022) observed no significant changes over a 16-day period for one group of cattle using virtual fences, and Confessore et al. (2024) found no significant differences when comparing older lactating dairy cows (>4 lactations) and younger cows (1 lactation) during a 35.5-day study with virtual fences. The study designs are detailed in Tables 1, 2, and 3.

2. Aims of the thesis

The aim of this thesis was to explore cattle's initial learning curve and adaptation to virtual fencing in semi-natural pasture over time, with the relocations of multiple virtual borders. Additionally, the study sought to investigate the physiological and behavioural effects of virtual fencing compared to traditional electric fencing.

Specific questions were:

- How does the interaction with the virtual border change in terms of the number of audio cues, electric pulses, and audio cue duration from the initial training phase to the period beyond with the relocation of multiple virtual borders?
- What individual differences exist in the learning curve, number of audio cues, electric pulses, turnaround success, and audio cue duration during the initial training phase and the period beyond?
- Are there any differences in the level of faecal corticoid metabolism (FCM) between cattle using virtual fences compared to physical electric fences during the initial training phase and the period beyond with the relocation of multiple virtual borders?
- How do the long-term effects of hair cortisol concentration (HCC) compare between heifers using virtual fences and those using physical electric fences?
- What differences in behaviour and activity levels are observed between heifers using virtual fences and those using physical electric fences?
- Does animal personality (behaviour test reaction) influence physiological responses?
- Are there any differences in weight gain between heifers using virtual fences and those using physical electric fences?

3. Material and methods

This thesis presents results from two studies (Study A and B) conducted simultaneously in a semi-natural pasture at a commercial farm in Uppland, Sweden, from May to July 2022. Both studies partly used the same animals and pastures.

Study A (Paper I) focuses on the learning curve and adaptation of cattle to a virtual fence with the relocation of multiple virtual borders, involving one group of seven heifers over a six-week period.

Study B (Paper II) compares the concentration of FCM (faecal corticoid metabolite) and HCC (hair cortisol concentration), as well as behaviour and activity, between two groups of seven heifers each. Heifers were first exposed to pasture release and physical electric fences for the first time in their lives for five days. Subsequently to either a virtual fence with the relocation of multiple virtual borders (VFG group, the same animals as in Study A) or a physical electric fence (control group, EFG) over a six-week period.

3.1 Virtual fence technology used

In both Studies, Nofence virtual fence collars (® Nofence, AS, Batnfjordsøra, Norway, cattle model year 2020) were used. When the virtual pasture is activated and an animal crosses a virtual border, the collar emits an audio cue. The desired behaviour is for the animal to turn around upon hearing the audio cue and return to the virtual pasture, thus avoiding an electric pulse (Figure 2). If the animal remains outside the virtual border, the audio cue continues for a minimum of 5 seconds and up to 20 seconds, depending on the animal's movement speed. Subsequently, the collar administers an electrical pulse through a metal chain around the animal's neck, with a strength of 0.2 Joules and a maximum of 3 kV over 0.5 seconds (Nofence Manual, 2024). If the animal continues to stay outside the virtual border, a new audio cue will start. This sequence of audio cues followed by an electric pulse can be repeated up to three times. After that, the system switches off, and the animal is considered to have escaped (Paper I). For both Studies A and B, data from the collars (including time and position of individual audio cues and electrical pulses, audio cue duration, escapes, and pasture ID) were stored within the system and transferred from each collar to the Nofence server (Paper I). To evaluate the learning process by examining how effectively the animals responded to the audio cue, "turning around" to avoid the electric pulse, a parameter called turnaround success was calculated per day per individual using the formula: $\sum$ (audio cue – electric pulse)/$\sum$audio cue (Paper I). Also, for the audio cue duration, a daily mean per individual was calculated before data was used in statistical analyses.

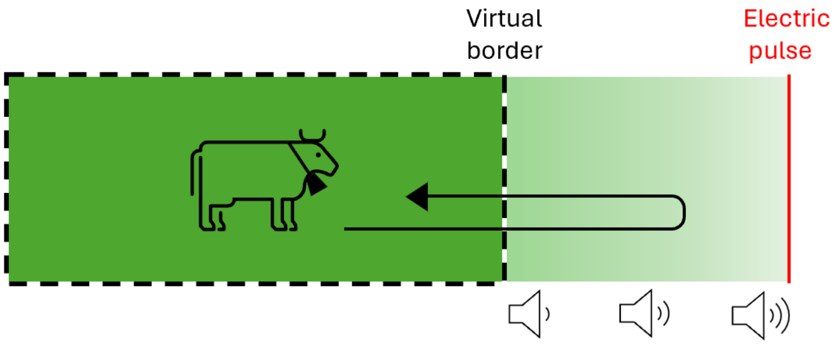

Figure 2. Illustration of the functionality of the Nofence virtual fence system used in Studies A and B, exploring heifers' adaptation and behavioural and physiological responses to the system. The system emits an audio cue from a collar when an animal crosses the virtual border, followed by an electric pulse if the animal does not return to the virtual pasture (dark green area).

3.2 The experimental design

Studies A and B were conducted simultaneously, partly using the same animals. Study A included the VFG group, while Study B included both the VFG and EFG groups. Study A ran from May 24 to July 4, 2022, and Study

B from May 19 to July 5, 2022. The VFG and EFG groups grazed in two semi-natural pastures located approximately 400 meters apart, covering a total area of 5.5 hectares each. Both pastures consisted of older production grassland, areas with shrubs, and mixed forest (Figure 3), and were enclosed by a two-wire physical electric fence (Paper II).

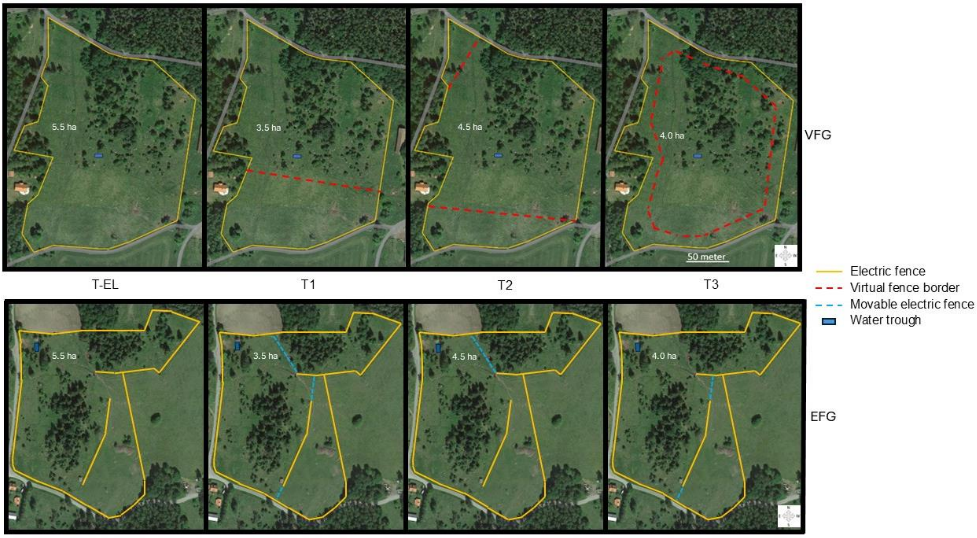

Figure 3. Overview of the two experimental pastures used in Studies A and B, which investigate heifers exposed to either physical electric fencing (EFG) or virtual fencing (VFG). The top section shows the pasture layout for VFG, while the bottom section depicts the pasture for EFG. This figure illustrates the different fence borders and the total available pasture area for each time period during the experiment.

3.2.1 Study periods and treatments

Study A was divided into three time periods, T1-T3 (referred to as Treatments in Paper I). These time periods were the same in both Study A and B. However, Study B also included three additional time periods: T0, T-EL, and T-H (Table 4). Each period had different durations, types of sampling, and handling of the animals (Paper II). Time period 0 (T0): An 11-day period at the stable during which faecal and hair samples were collected, and collars and ear tags were applied. Time period EL (T-EL): A five-day period that included transportation to pasture and pasture release with only physical electric fences (training phase electric fence). Time period 1 (T1): Lasted seven days, during which VFG was introduced to one virtual border (training phase), and EFG was introduced to three new electric fence borders.

Time period 2 (T2): A 14-day period where VFG was introduced to two new virtual borders, while in EFG, two of the new electric fence borders were removed. Time period 3 (T3): A 21-day period during which VFG had a four-sided virtual fenced pasture. The two electric lines previously removed were reintroduced in EFG, while the last new electric fence borders were removed. Time period handling (T-H): The final day in Study B, where both VFG and EFG were transported from the pasture to the home stable and back again. At the stable, animals were individually fixated in a handling crush for weighing and the collection of a second hair sample. After receiving treatment in the barn, all individuals, except three, were transported back to the pasture and released as a joint group.

Throughout all time periods in the study, all animals in both studies and groups were handled identically by the same personnel. Conditions were standardised for both stable and pasture environments, including available pasture areas in T-EL and T1-T3, access to feed and water, climate, weather, and protection from rain and sun.

Table 4. The table outlines the experimental setup, time periods, animal group, and sampling procedures for two studies (A and B) investigating heifers exposed to either physical electric fencing (EFG) or virtual fencing (VFG). Animals in VFG were the same in both studies. The time periods were defined as follows. T0: Period in the stable before pasture release. T-EL: Pasture release with only physical electric fencing. T1: Period at pasture where EFG had a physical electric fence and VFG had a virtual fence with one virtual border. T2: Period at pasture where EFG had a physical electric fence and VFG had a virtual fence with two virtual borders. T3: Period at pasture where EFG had a physical electric fence and VFG had a virtual fence with a four-sided virtual fenced pasture. T-H: Occasion where both groups were transported from pasture to the stable, individually fixated in a handling crush for sample collection, and then transported back to pasture.

		Study A		**Study B**			
		Time period	**Animal group**	**Time period**	**Animal group**	**No. of occasions of faecal samples**	**Hair sampling**
No. of days in time period	11	-		T0	VFG + EFG	1	1
	5	-		T-EL	VFG + EFG	2	
	7	T1	VFG	T1	VFG + EFG	3	
	14	T2	VFG	T2	VFG + EFG	3	
	21	T3	VFG	T3	VFG + EFG	5	
	1	-		T-H	VFG + EFG	1	1

3.3 Animals

The animals in Studies A and B were all heifers with a mix of purebred Holstein and crossbreeds of Holstein x Hereford, Brown Swiss Cattle x Hereford and Swedish Red and White x Danish Red x Hereford. In May 2022, at the start of the experiment, they were between 12-14 months old, with body weights of 342 ± 12 kg in the VFG group and 347 ± 10 kg in the EFG group. All animals were then naïve to grazing, physical electric fences, and virtual fences. The two groups (VFG and EFG) were established with the objective of ensuring comparability in response to a behavioural test (see below) and breed. In both studies, all animals were fitted with Nofence collars in the barn nine days prior to being released into pasture. However, only the animals in the VFG interacted with the virtual borders in Studies A and B. During T1 (training phase), the virtual border was approximately 200 meters long, with the electric fence positioned about 150 meters behind the virtual border.

Due to the ethical approval limitations for both Study A and B, which allowed for a maximum of 15 electric pulses from the virtual fence collars during the initial five-day period post-activation, two individuals in the VFG (both classified as bold in the behaviour test) had to be removed from the experiment on the second day in T1 (Paper I). Additionally, in the EFG, one individual had to be removed during T3 due to illness. The removed animals were not replaced.

3.4 Behaviour and activity measurement – Study B

All animals were equipped with ear tags (SenseHub™, USA) before being released to pasture. These sensors recorded the number of minutes per hour each animal spent eating and ruminating, as well as two activity levels: “mid” and “high” (Paper II). During the study, data were transmitted via an antenna connected to a modem with access to an external SenseHub server. Missing data occurred when animals did not move close enough to the antenna, affecting all individuals in the EFG group on the first day of pasture release in T-EL and some individuals in the VFG group during the first hour. Additionally, data from certain hours during T2 were not recorded for two individuals.

3.5 Behaviour test

For both Studies A and B, a novel object test was conducted in the barn approximately one month before the animals were released to pasture to assess the animals' response to a potentially risky situation, categorising them as bold or shy (Papers II). The test took place in the same part of the stable where the animals had been housed throughout the prior winter period. Each animal was taken individually to a designated test arena (85 m^2). The novel object consisted of five orange plastic cones, each containing a plastic bag with metal bells. When a cone was touched, overturned, or moved, it produced a ringing sound. Each heifer was filmed for five minutes (300 sec) upon entering the test arena. Animals that interacted with the cones within 30 sec were classified as bold, while those that took longer were classified as shy. Six animals were classified as shy, and eight as bold. They were evenly distributed between the VFG and EFG to ensure comparability between groups (Paper II).

3.6 Sampling and analysis method of faecal corticoid metabolite (FCM) – Study B

Faecal samples were collected after natural defecation from all animals in both the VFG and EFG groups on 15 occasions: once in the stable (T0) and the rest at the pasture (Table 4). The sampling strategy aimed to capture changes influenced by various actions (e.g., introducing electric fences, virtual fences, moving virtual or electric borders, and transportation). Samples were collected before, during, and after these different time periods (detailed description in Paper II). Sampling was performed simultaneously in both groups between 10:00 a.m. and 2:00 p.m., with a few exceptions where individual samples were collected close to 5:00 p.m. due to missed defecation events. The samples were placed in clean plastic bags, stored with ice packs, and frozen at −18°C within five hours of collection. The analysis of FCM was performed approximately eight months later at the Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala. A detailed description of the analysis is provided in Paper II.

3.7 Sampling and analysis methods for hair cortisol concentration (HCC) – Study B

Hair samples were collected from the tail tassel of all animals in both groups on two occasions (Table 4): before pasture release (“before” samples) and on the last day of the study (“after” samples). The first sample was obtained by cutting the tassel just below the root of the tail. For the final sample, the newly grown tail tassel was cut. The samples were placed in clean plastic bags, stored with ice packs, and frozen at −18°C within five hours of collection. The analysis of HCC was performed approximately eight months later at the Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala. A detailed description of the analysis is provided in Paper II. Due to the exclusion of animals from the study explained above, three “after” samples were missing at the end.

4. Results

4.1 Learning curve and adaption to the virtual fence – Study A

The animals were contained within the virtual fenced pasture for the entire six-week study period, except on day 1 in T1. About 30 minutes after activating the virtual fence, all animals ran over the virtual border, resulting in several electric pulses and escape records for four of the heifers. Later that day, one of these individuals escaped again. However, all animals returned voluntary to the virtual pasture within 30 minutes each time. The running behaviour was repeated three times in the first two hours after activation of the virtual fence. Due to this, two individuals had to be removed from the experiment on day 2 in T1, as the ethical approval allowed a maximum of 15 electric pulses per animal during the initial five-day period.

The highest record of the mean number of audio cues (33.4±1.9) and electric pulses (9.9±1.3) per day was recorded on day 1 in T1 and the second highest on day 2 in T1 (12.4±2.3 and 3.0±0.4, respectively). After day 2 in T1, audio cues only occurred occasionally until day 8 (the first day in T2), and for electric pulses, there were no recordings between day 3-7 (in T1). They occurred firstly on day 8 (the first day in T2) when the new virtual borders were activated in T2. During T2 and T3, at least one electric pulse was recorded on 24 out of 35 days, where the highest number per day for one individual was three, which occurred twice. Audio cues were recorded on all days except one in T2 and T3 (Paper I).

In T1, no individual differences were found between individuals for any of the analysed parameters, electric pulses, audio cues, audio cue duration, and turnaround success. For the number of electric pulses, no individual differences were found in any of the time periods. However, for audio cues, individual differences were found for audio cues in T2 and T3. For audio cue duration, individual differences were found in T3, where one individual had a significantly longer audio cue duration than the rest. Due to the small sample size after the exclusion of two individuals on day 2 in T1, no statistical analysis was performed to assess the impact of temperament traits on individual differences.

4.2 Effects on learning and adaptation between and within time periods – Study A

As illustrated in Figure 4, the result for the number of electric pulses showed a decrease between time periods, where T1 significantly differed from T2 and T3 ($P<0.00001$) with 2.2, 0.6, and 0.2 electric pulses/day, respectively. However, the daily turnaround success increased between time periods, where T3 differed from T1 and T2. Comparing the daily audio cue duration between time periods, T2 differed from T1 and T3 with a longer duration time, whereas the number of audio cues per day did not differ between time periods.

Results for the estimated changes per day within a time period (further explained in Paper II) showed a significant decrease in the number of audio cues per day in T1 (-4.60, SE=0.50) and T2 (-0.41, SE=0.16), whereas no significant change was found in T3 (Figure 4). For the electric pulses, there was a significant decrease in the number per day in T1 (-1.24, SE=0.12) but not for T2 and T3. For the turnaround success, there was a significant increase per day in T1 (+0.05, SE=0.01), whereas no significant change was found in T2 and T3. The daily mean audio cue duration decreased significantly per day in T1 (-837, SE=191.0) and showed a significant increase per day in T2 (+190, SE=50.0) and T3 (+149, SE=26.5).

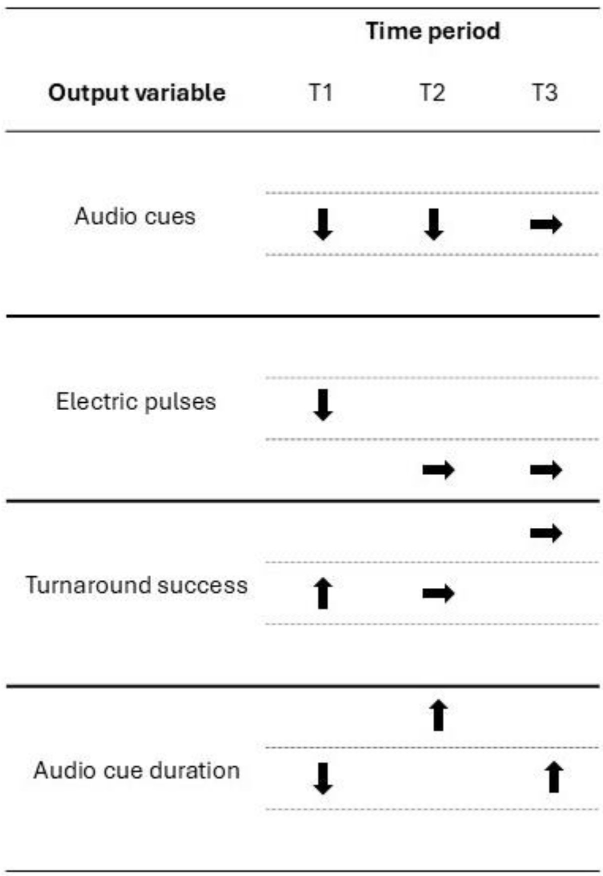

Figure 4. This figure shows the significant differences in audio cues, electric pulses, turnaround success, and daily mean audio cue duration between and within time periods in Study A, which aimed to explore seven heifers' adaptation to a virtual fence over a six-week exposure with relocations of 1-4 virtual borders. Time periods correspond to the following: T1 (one virtual border for seven days), T2 (two virtual borders for 14 days), and T3 (four-sided virtual fenced pasture for 21 days). Arrows on the same row level within an outcome variable indicate no significant difference (P > 0.05). Arrows above the row indicate a significant increase between time periods, while arrows below indicate a significant decrease. Right-pointing arrows show no significant change within a time period. Upward arrows indicate a significant increase within a time period, and downward arrows indicate a significant decrease (P < 0.05).

4.3 FCM and HCC – Study B

There was an increase in FCM levels for both VFG and EFG up to sample 5 in T1, where sample 5 significantly differed from the baseline value (sample 1) at stable (T0) for both groups (Figure 5). Additionally, for VFG, sample 3 (the last value in T-EL, the day before the activation of the virtual fence) and sample 4 (the day after the activation of the virtual fence in T1) significantly differed from sample 1. After sample 5, FCM levels gradually decreased for both groups, becoming significantly lower than in sample 1 for EFG in sample 12, and for VGF in sample 13. Additionally, significant differences in FCM levels between groups were found for sample 3 (T-EL), sample 4 (T1), and sample 5 (T1), with P-values of 0.0022, <0.0001, and 0.0003, respectively. In each case, VFG exhibited higher FCM values compared to EFG.

For the HCC, no significant differences were found between EFG and VFG (p-value 0.933) between the calculated difference of the samples before and after. However, there was a significant increase (p-value 0.0002) in the HCC value calculated for both groups between the samples before and after. No effects of bold or shy (animal personality, behavoiur test) were found for either FCM or HCC.

4.4 Behaviour, activity and body weight – Study B

Significant differences in time spent eating were observed between the two groups in T3 (p-value 0.004), with the VFG eating 4.45 minutes less per hour than the EFG group (Paper II). For rumination, significant differences were found between the groups for all treatments except T-H, with the VFG group consistently showing higher values than the EFG group. Additionally, a significant difference in ActivityMid was noted in T3, where the VFG group spent 1.78 minutes less per hour on ActivityMid than the EFG group (p-value 0.001). The body weight change (before and after pasture) showed no difference between VFG and EFG (mean ± SD, 4 ± 19 *vs* -8 ± 10 kg, p-value 0.161).

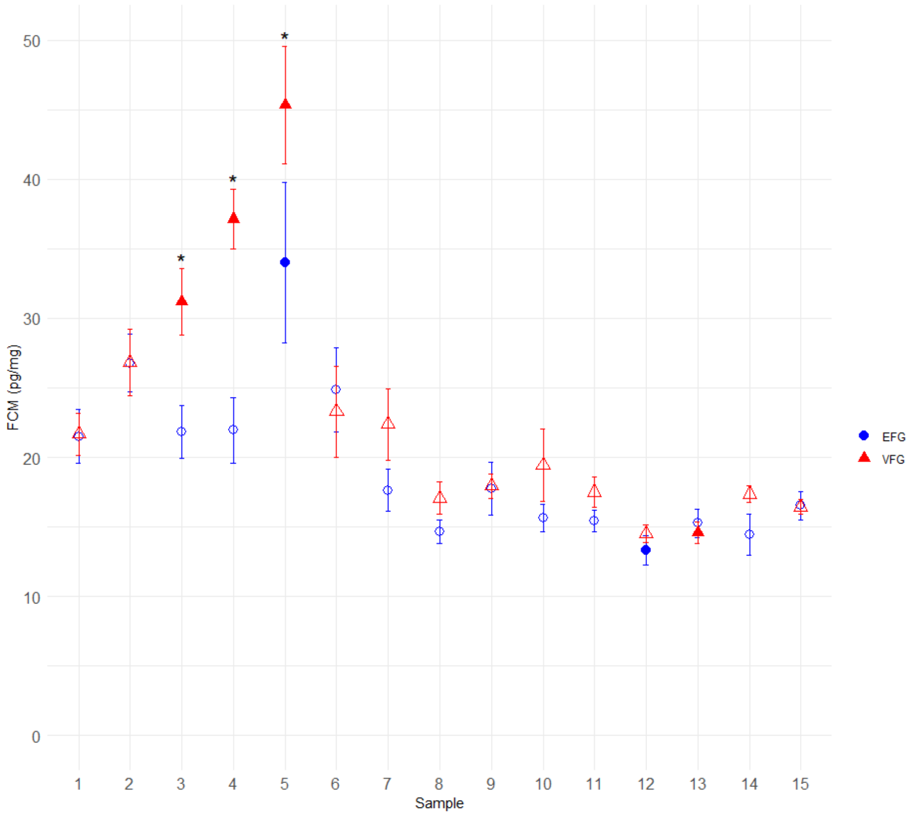

Figure 5. Mean faecal corticoid metabolite (FCM) levels (pg/mg freeze-dried faeces) per sampling occasion for heifers managed with either a virtual fence (VFG) or a physical electric fence (EFG). Error bars represent standard errors. Sample 1 (T0): stable conditions before pasture release. Samples 2-3 (T-EL): pasture with a physical electric fence. Samples 4-6 (T1): EFG with a physical electric fence, VFG with one virtual border. Samples 7-9 (T2): EFG with a physical electric fence, VFG with two virtual borders. Samples 10-14 (T3): EFG with a physical electric fence, VFG with a four-sided virtual fenced pasture. Sample 15 (T-H): transportation from pasture to the stable, including fixation in a handling crush, hair sampling, and return to the pasture. Samples marked with * indicate significant differences between groups. Filled red triangles and blue circles indicate significant differences ($P<0.05$) from sample 1 for VFG and EFG, respectively.

5. General discussion

This thesis aimed to explore how heifers adapt to virtual fencing with relocations of multiple virtual borders in a semi-natural pasture and to compare the physiological and behavioural effects with those of traditional physical electric fences. Study A focused on one group of heifers' adaptation to a virtual fence over a six-week period, with relocations of several virtual borders, examining learning curves and adaption over time through the success of responding to audio cues to avoid electric pulses. Study B compared the physiological effects, measured by cortisol (metabolite) levels in faeces and hair, as well as behaviour, between two groups of heifers. Both groups were first exposed to pasture release and physical electric fences for the first time in life, for five days. Subsequently, one group continued with physical electric fences (EFG) while the other group (same animals as in Study A) transitioned to virtual fences (VFG) with relocations of multiple virtual borders over a six-week period.

Study A demonstrates that the virtual fence effectively enclosed the heifers beyond day 1 in T1 (the first week of training with one virtual border), where they quickly learned to turn around at the audio cue to avoid the electric pulse within the first seven days. The heifers showed improved adaption and management skills throughout the trial, regardless of the number and relocation of the virtual borders. Individual differences were observed in T2 (two-week period with two virtual borders), for the number of audio cues and in T3 (three-week period with 4-sided virtual enclosure) for both the number of audio cues and the audio cue duration.

Study B indicates no long-term stress for VFG, as the results for HCC and weight change did not show any significant difference between the groups. However, there appeared to be occasional stress responses in both groups at certain points in the study compared to the baseline value (sample

1) at stable, where the levels of FCM in sample 5 in T1 (three days after activation of the virtual fence in VFG) significantly differed for both groups. Additionally, for VFG, samples 3 (one day before activation of the virtual fence) and 4 (one day after activation of the virtual fence in T1) showed higher levels of FCM compared to the baseline. For all these three, sampling occasions (samples 3, 4, 5), VFG showed significantly higher values of FCM compared to EFG, implying a higher stress response during this period. Interestingly, the difference between groups was evident even before the virtual fence was activated, suggesting that the stress response might have been due to unknown factors rather than the virtual fence itself. This was supported by the fact that the results did not show any differences in behaviour between the groups that could be attributed to stress-related factors and similar levels of HCC and weight change.

5.1 Influences on the number of electric pulses from the virtual fence during the training period (T1)

The results in Study A showed the highest number of electric pulses during day 1 and 2 in T1, which is believed to be attributable to several factors. Firstly, these were the initial days when the animals were exposed to the virtual fence. At this point, they did not comprehend that they needed to respond to the audio cue to avoid the electric pulse. With this type of learning procedure, the animals will receive electric pulses. Secondly, the next potential factor was attributed to the animals' motivation to access a previously familiar area. Upon activation of the virtual fence on day 1 in T1, approximately 10 min prior to the activation, the animals were herded into the virtual pasture, thereby excluding them from their usual resting area. At the time of initiating the movement, the animals were lying down and resting. Based on anecdotal observations, it appeared that the animals, or at least some of them, exhibited a strong drive to cross the virtual border to access the resting area again. When the animals received the initial electric pulses, the herd's immediate response was to run towards the resting area rather than retreat into the virtual pasture. This running behaviour was repeated several times in close succession to the activation of the virtual fence, resulting in the animals receiving multiple electric pulses within a short period. Consequently, several animals were recorded as having escaped. According to Keshavarzi et al. (2020), all members of a cattle group tend to behave similarly, and different group compositions can lead to varying behavioural reactions. During these instances of running over the border (data from video recordings), one individual cow was frequently observed leading the herd. Therefore, the repeated border-crossing behaviour on day 1 might have been influenced by a combination of group dynamics and the motivation to return to the prior resting area. If the excluded area had been less motivated to access the incidents of running might have been avoided, potentially resulting in fewer electric pulses for the animals and a calmer learning process. Thirdly, the positioning of the virtual border relative to the electric fence (the distance between the border and the electric fence), approx. 150 meters in Study A, may also have influenced the number of electric pulses, as the animals had an abundance of space to move outside the virtual border. This, Hamidi et al. (2024) also highlighted as an important factor for optimal learning. If the virtual border in Study A had been positioned closer to the electric fence, the animals might have returned to the virtual pasture more quickly, potentially also resulting in fewer electric pulses. The impact of virtual border placement relative to the physical fence on the number of electric pulses during the initial training phase, along with the motivation to enter areas beyond the virtual border, is a crucial area for future research due to the current limited knowledge.

Previous studies show a wide range of durations for the initial training phase (the first period before relocation of the virtual border), spanning 2-21 days (Table 2). However, most previous studies do not specify whether the animals should have interacted with the virtual border before the first relocation. This is likely due to pre-established research setups involving preplanned movements of the virtual borders, as also was the case in Study A. However, establishing clear criteria for determining when an animal has fully learned the system and is ready to be introduced to new virtual borders or the relocation of existing ones should be a priority in future research. This is crucial because the individual learning process may be disrupted if the virtual border is changed before the animal has fully adapted to the system, which is essential for ensuring animal welfare and minimising the number of electric pulses delivered by virtual fences.

5.2 Factors affecting the animal adaption to virtual fences over time

As described earlier, to utilise the system's full potential, achieving effective pasture and grazing management systems, frequent relocation of virtual borders is crucial. However, this strategy may encourage animals to graze near or cross the virtual border in search of more attractive feed. As a result, this behaviour could activate additional audio cues, longer audio cue durations, and potentially if the animals have not yet fully learned to respond to the audio cues, as described above, increase the number of electric pulses. This reasoning for grazing near the virtual border aligns with the findings presented in Study A, which demonstrated a significantly longer audio cue duration in T2 compared to T3 (Figure 4). Further, GPS data, not shown in Study A but presented by Wahlund et al. (2023) also indicates that the animals mainly stayed near the virtual border in the southern parts of the pasture during T2. These areas consisted of ley vegetation, presumed to be more attractive for grazing than the regions further inside the virtual fenced pasture, which were characterised by natural vegetation with trees and bushes (semi-natural pasture type). However, no differences in the number of audio cues or electric pulses were observed between T2 and T3. Also, in the study of Aaser et al. (2022), the cattle spent more time grazing in areas with fresh grass whenever the virtual border was moved, especially when the areas were small. The same was observed by Staahltoft et al. (2023), where calves also chose to graze near the virtual border when fresh grass was available outside the border. In neither of these studies any increase in the number of electric pulses was found.

As previously described, one potential future application of this technology is in semi-natural pastures, where the landscape is heterogeneous. In such environments, virtual borders may end up in inaccessible or "odd" positions. Little is known about whether placing virtual borders in areas where movement is restricted, such as dense forests or rocky terrain, poses challenges for the animals. Future research should, therefore, investigate the impact of the positioning and placement of the virtual border on cattle adaptation. Additionally, there is a lack of knowledge on how the placement of borders relative to each other, such as at angles or in narrow corridors, affects the animals. Considering these factors is also crucial in the implementation of virtual fencing systems to enhance animal welfare.

5.2.1 Impact of GPS-drift

The number of electric pulses over time may also be influenced by factors beyond those present during the initial training phase or the motivation to stay close to the virtual border. GPS drift was occasionally anecdotally observed, causing the virtual borders to inadvertently move in and out of the pasture area. This poses a potential animal welfare issue, as it increases the risk of the animal's ability to navigate and deactivate the audio cue being compromised. Consequently, this could lead to a higher frequency of electric pulses, where the additional pulses are not indicative of the animals' misunderstanding of the system but rather a malfunction in its operation, potentially causing unnecessary stress due to the unpredictability of the situation. However, the present results of the long-term stress response, measured by HCC, body weight, and behaviour did not indicate any increased stress in the VFG compared to the EFG. Consequently, as this is an unexplored area of research, future studies should focus on the frequency of GPS drifts and differences in accuracy between various locations and brands.

5.3 Individual differences in adaption to virtual fence

As described in a previous section, studies on virtual fencing have reported individual variability in learning curves for cattle during the initial training phase with some animals requiring more interactions with the virtual border, resulting in a higher number of electric pulses compared to others. This contrasts with the findings of Study A, where no such differences were observed. However, the limited sample size of only seven animals may have precluded the detection of potential deviations in individual learning curves. Additionally, the exclusion of two animals on day 2 in T1 led to even more restricted data collection from day 3 onwards, further complicating the evaluation of its impact on the results. Still, there is limited information on the extent of differences in the learning curve reported in previous studies, including 1) the number of animals involved, 2) the ratio of electric pulses to audio cues for specific individuals, 3) whether these differences persist or diminish over time, and 4) how and if this difference did affect the animals' welfare. These are crucial aspects to consider in future research when evaluating these systems looking at individual differences.

5.4 Faecal sampling regime and effects on results

As described and discussed above, the results in Study B for FCM revealed differences between groups, with higher levels in VFG for the last sample in T-EL (the day before activation of the virtual fence) and the first two samples in T1 (the day after and three days after activation of the virtual fence) compared to EFG. Additionally, variations were observed from baseline sample 1 at stable over time for both groups, with an increase at the beginning of the study followed by a decrease over time. These results contrast with previous studies, as Campbell et al. (2019) and Hamidi et al. (2022) found no differences between groups of cattle using either virtual fences or physical electric fences for 27 and 12 days, respectively, with one virtual border each and zero or one relocation of the virtual border (Table 2). Neither Sonne et al. (2022) showed any differences over time for one group of cattle using virtual fences for 18 days with one to four virtual borders and three relocations. However, the sampling strategies for faeces in these studies varied, as previously described in the introduction. This may have impacted the results, as FCM levels in a sample do not represent the conditions at the time of collection but rather reflect the physiological response to events that occurred approximately 10-12 hours earlier (Möstl & Palme, 2002). Based on this understanding, Campbell et al. (2019) measured FCM levels on specific days of the week over time and Hamidi et al. (2022) on two separate days during the experiment but not in relation to the border change, while Sonne et al. (2022) did so over time every second day, although the measurements were not always related to the border change. In summary, these studies did not implement a sampling strategy capable of accurately evaluating the introduction of a new virtual border or the relocation of an existing one. This limitation arose because samples were not collected before and after a border change. In contrast, Study B employed a comprehensive faecal sampling strategy, collecting samples both before and after the introduction and relocations, as well as on a weekly basis. Additionally, the long-term effects were analysed by comparing HCC levels before and after the experiment, revealing no significant difference between VGF and EGF. However, using FCM to assess stress responses for certain events reveals several limitations. Firstly, FCM is not an ideal method for assessing changes in cortisol release, as levels can fluctuate based on individual excretion patterns and the diurnal rhythm (Möstl et al., 2002). Additionally, we may have missed FCM peaks due to the varying times when individuals interacted with the virtual fence borders. To address this, we chose to sample 24 hours after the border had been changed and conducted the remaining sampling at the same time each day. Lastly, conducting standardised, comparable studies in semi-natural pastures is inherently challenging. These pastures are highly variable, making it difficult to find two similar replicates. Variations in vegetation, shade, solar radiation, insect prevalence, and disturbances from roads, agriculture, people, and wild animals are all factors that can influence the results.

5.4.1 Electric pulses from the physical electric fence and the potential of stress response

In Study B, all animals in VFG received electric pulses, with the highest level during the first two days of exposure. However, the experiment did not measure to what extent the animals in EFG received electric pulses. This lack of information complicates the comparison of FCM levels between the groups, as it hinders a full understanding of the stress responses associated with each fencing system. Despite this, we can conclude that animals in the EFG group learned the system, as no escapes were recorded during the study (Paper II), suggesting they also received electric pulses to some extent. As previously described in the introduction, there is an overall lack of knowledge regarding the learning curve and to what extent animals receive electric pulses from physical electric fences. This represents a crucial area of study, particularly given the emphasis on the negative impact of electric pulses on animal welfare within virtual fencing systems. It is essential to compare these findings with those from physical electric fences, which also administer electric pulses, to provide a comprehensive understanding of their relative effects on animal welfare. However, conducting such studies over an entire grazing season is challenging. It would require equipment on the animals to record electric pulses within their bodies or equipment at the physical fence. In the latter case, this would need to be combined with positioning and timing equipment on the animals to identify which animal interacted with the fence and received the electric pulse at a specific moment. Alternatively, direct observations could be used, but these will be very time-consuming, especially if the animals need to be monitored around the clock for several weeks. Additionally, this approach is nearly impossible in large pastures, particularly semi-natural pastures with many animals, to accurately capture conditions in these types of areas. Future comparable research studies should aim to record instances of electric pulses uniformly across all groups to fully understand the stress responses associated with each fencing system. Using FCM, it is also crucial to ensure that the sampling strategy is well-designed to address the specific research questions of the study.

5.5 Hair cortisol concentration (HCC) as a measure of long-term stress

There were no observed differences in HCC between the VFG and EFG groups, indicating that type of fencing system did not influence long-term stress. This finding aligns with previous studies by Confessore et al. (2022 and 2024), which also highlighted the ease of using this method to assess long-term stress responses. Interestingly, both VFG and EFG showed significantly higher HCC levels after the pasture phase compared to before, likely due to increased physical activity in the pasture environment versus barn housing. However, this was not further analysed as activity data was only collected during the pasture phase. To fully understand the impact of virtual border relocation on animal welfare and other management practices associated with this technology, it is crucial to combine HCC with other measurement methods to capture potential short-term stress, for example, FCM as in Study B.

5.6 Behavioural responses to the fencing systems

The results from the behaviour data of SenceHub in Study B revealed no differences between groups, except during T3 (the last three weeks on pasture) when the VFG spent less time eating and were engaged in less low physical activity (ActivityMid) compared to the EFG. The reasons for these differences are not entirely clear, but they are likely not related to stress from the fencing systems, as the VFG also spent significantly more time ruminating throughout all pasture periods than the EFG. A decrease in rumination has been linked to the emergence of diseases and reduced animal welfare (Herskin et al., 2004; Fogsgaard et al., 2012). Additionally, no differences in weight change were observed, suggesting similar nutrient and energy intake among the groups.

6. Conclusion

This thesis concludes that:

- Heifers were able to adapt to a virtual fence system, learning to respond to audio cues to avoid electric pulses within the first week.
- Throughout a six-week period, heifers exhibited improved adaption and management skills of a virtual fence system, regardless of the number and relocation of virtual borders.
- No long-term stress, as measured by hair cortisol concentration (HCC), was observed between heifers using either a virtual fence or a physical electric fence.
- Higher levels of faecal corticoid metabolites (FCM) were detected in heifers using virtual fences compared to those using physical electric fences. The difference was evident even before the virtual fence was introduced, suggesting that other factors may have influenced the stress response rather than the fencing system itself.
- No difference in weight change or overall behaviour that could be related to stress was observed between two groups of heifers using either a virtual fence or a physical electric fence.

Overall, the findings suggest that virtual fences can be a promising alternative for grazing management in semi-natural pastures, with a similar impact on behaviour and cortisol responses as traditional electric fences.

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In this thesis, the adaptation of cattle to GPS-based virtual fencing in semi-natural pastures was investigated, comparing its physiological and behavioural effects with electric fencing. Results showed that cattle quickly adapted to the virtual fence, with improvement over time. The occasional higher cortisol levels observed for these cattle were not believed attributed to the virtual fence. The findings suggest that virtual fencing has the potential for grazing management in semi-natural pastures, with a similar stress response as electric fences.

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