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Application of Virtual Fencing for the management of Limousin cows at pasture

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HIGHLIGHTS

- A sustainable use of pasture-based systems requires efficient grazing management.
- Virtual Fencing uses acoustic and electric cues to replace physical fences.
- Cow's learning ability was tested by setting three different virtual grazing areas.
- Results show a significant decrease of sounds and electrical pulses among trials.
- Hair cortisol content was not affected by Virtual Fencing management.

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ABSTRACT

A potential use of pasture-based systems requires an efficient grazing management strategy. Thanks to the Virtual Fencing (VF) physical fences are replaced by virtual ones and, when the animals approach the boundaries, they receive a paired stimulus: an audio cue followed by a low electrical pulse if animals cross over the fences. This study aims to i) to evaluate the animal's ability to learn, and then respond positively, to VF ii) VFs' efficiency to manage the herd within grazing areas virtually delimitated; iii) to assess the chronic stress related to the VF, evaluating the hair cortisol concentration (HCC), during the experiment. Twenty Limousine cows were fitted with a commercial VF-GPS collars (Nofence AS, Batnfjordsør, Norway). The experiment was divided into four trials: Trial zero (T0) with inactive collars to let the animals get acquainted with them; Trial one (T1) where three of the four virtual boundaries coincided with the physical ones, while the virtual one was set across the pasture to restrict the grazing area; Trial two (T2) in which the grazing area was further extended moving forwards the virtual board; Trial three (T3) in which the virtual line was set longways to the pasture. Results show a significant decrease of stimuli delivered (i.e., sounds and electrical pulses) ($p < 0.001$), among trials. Moreover, a reduction ($p < 0.0250$) in the ratio between sounds and electrical pulses was observed between T1 and T3, with T2 being like both. Regarding the cows' learning capacity, the events in which the sounds were followed by electrical pulses were significantly less in T3 ($p < 0.001$). Furthermore, in T3 the duration of the audio tones was lower than T1 and T2 ($p < 0.0005$). Animals were increasingly kept inside the inclusion zones during the trials, with the lowest number of escape events from the inclusion zone registered in T3 ($p < 0.001$). No differences were observed in the HCC before and after the VF treatment. The progressive reduction of the studied parameters between following sessions, indicates an increase in associative learning through time. VF virtual fencing has proven to be an effective tool in managing Limousin cows at pasture However, future research is needed to evaluate the animals' performances in terms of grazing activities and on the assessment of chronic stress conditions as well.

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Fig. 1. A NoFence Virtual Fencing collars used in the experiment.

1. Introduction

Pasture-based systems represent an important resource, especially for beef production. Despite the outcomes, in terms of animal performances coupled with ruminant methane emission, are lower than those obtained from intensive ones, pasture-based systems provide large amounts of ecosystem services (i.e., preservation and enhancement of biodiversity, conservation of landscapes, enhancement of meat quality and animal welfare perceived by consumers) (Bragaglio et al., 2018). So, exploiting the potential of pasture-based systems requires the adoption of grazing management strategies that allow a high use efficiency of forages (i.e., rotational grazing, strip-grazing), but, compared to the traditional ones, these strategies require high amounts of labour input to manage the herd and build fencing infrastructures as well. Therefore, in most cases, it is considered not cost-effective for the farmers. A valuable solution could be represented by Fencing system (VF). Umstatter (2011) defined VF as a structure serving as an enclosure, a barrier, or a boundary without a physical barrier. Thanks to VF, the traditional physical fences are replaced by virtual ones, and when the animals are close to the boundaries, they receive a paired stimulus: an audio cue followed by a low electrical pulse if the animal continues to walk forward. Usually, VF is led by wearable devices (e.g., collar). A VF-based management system can contribute to reduce labour and material costs associated with moving and maintaining physical fences, it allows more efficient pasture management, better protection of environmentally sensitive areas (Lee et al., 2018). Moreover, VF has the potential to support farmers towards the adoption of more efficient and flexible grazing management solutions (i.e., rotational grazing) that increase forage utilizations (Anderson et al., 2014). Several studies have been conducted so far in various contexts and in different species, mainly sheep and cattle, but also on goats (Muminov et al., 2019), to evaluate both the efficiency of the system and the animal's learning capacity. For instance, VF was tested in a comparison between naïve animals with ones experienced with physical electric fences. As results, the latter seems to be more rapid in the associative pairing of the audio and electric pulse during an individual feed attractant trial (Verdon et al., 2020). Moreover, has been demonstrated that the VF system successfully restricted animals within virtual inclusion zones both if they were tested in a small group (Campbell et al., 2017a; Lomax et al., 2019; Marini et al., 2018b, 2018a) or individually (Campbell et al., 2018), with an overall decrease of stimuli delivered over time, especially for the electrical pulses.

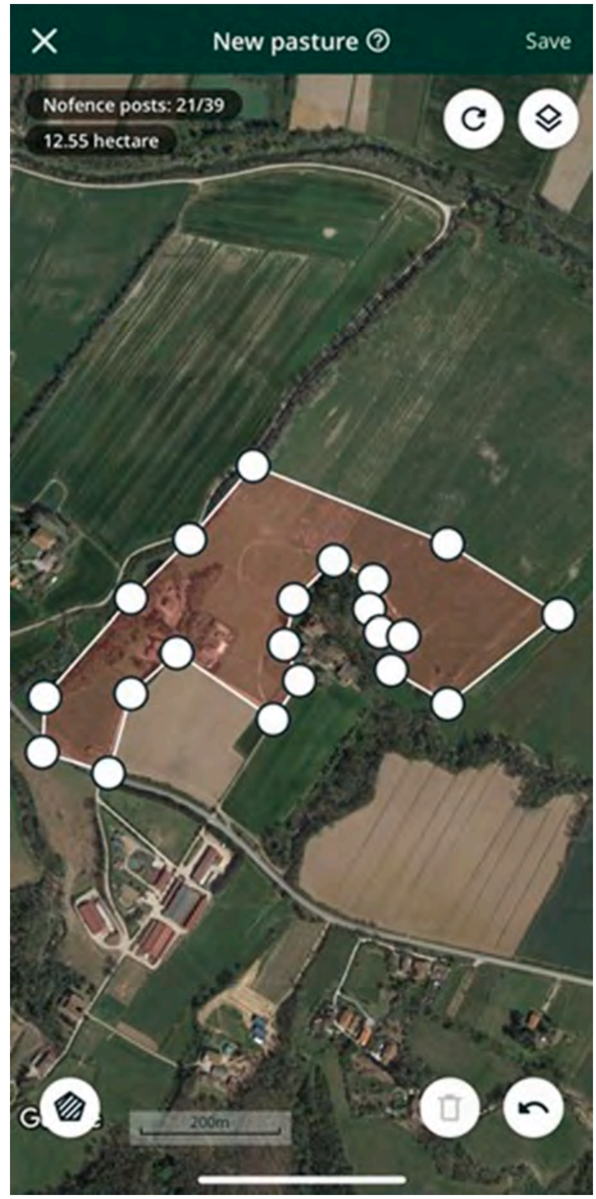

Fig. 2. Image show the procedure on the UI to design the virtual borders.

To become marketable, a new technology involving livestock, must be safe for the animals' welfare. Thus, important research outcomes have regarded the behavioural responses related to the VF system, as well as the welfare implication, and the consumer's constraints (Stampa et al., 2020) associated to it. A previous experiment conducted on cattle has demonstrated that animals did not show any substantial changes in behavioural patterns (Campbell et al., 2019b), in a comparison between VF system and electric fencing. While other studies showed changes in the normal behaviour of sheep managed with VF (Marini et al., 2018a) and few effects on cattle's overall activities (Campbell et al., 2017b).

VF is not a useful instrument to manage livestock at grazing, only. Thanks to the GPS tracking, and accelerometer module, it can provide information about the overall animals' activity, as well as secondary information for real-time health monitoring (i.e, detecting lameness and heat) (Aquilani et al., 2022).

So, on a global overview, investigating the potential use of the VF in different beef production systems (i.e., testing on different breeds), offers to the farmers a technology capable to enhance farms' productivity and efficiency, as well as obtain many environmental outcomes (Greenwood, 2021).

Fig. 3. Picture shows the four-consecutive phase of the experimental protocol (T0, T1, T2 and T3). Green lines indicate the virtual boundaries, while blue circle indicate the water troughs. In T0 virtual boundaries matched with physical ones. From T1 to T2 surface was gradually implemented. In T3 virtual lines restricted the access from one side of the pasture, in which one water trough was present, and a piece of sheltered zone.

To date, there is a lack of knowledge regarding the potential use of VF in European beef production systems. Thus, the novelty of this work is to evaluate, for the first time, the effectiveness of VF in managing Limousin beef cattle, in specialized cow-calf pasture-based system. As well as there are no studies in the scientific literature, in which this breed has been used to test this type of technology. Therefore, this experiment aimed to i) to assess the animal's ability to learn, and so, to avoid the adverse stimulus. Therefore, if cows are trained to interact with the system, then, through time, they will receive fewer stimuli. ii) to evaluate the efficiency of the system to manage the herd within grazing areas virtually delimitated. So, the cows, will either not escape or escape less from the virtual grazing area once they learn how to positively interact with the system. iii) to assess the chronic stress related to the VF, performing the analysis of the hair cortisol content (HCC), between the beginning and the end of the trial. If HHC shows no differences, VF will not negatively effect on the animals' welfare.

2. Materials and methods

2.1. Animals and location

The experiment took place in spring summer in a sown pasture of 30 ha located in Borgo San Lorenzo (Tuscany), on twenty pregnant Limousin beef cows. All cows had not experience neither with rotational grazing nor Virtual Fencing system, but they were usually kept at pasture in spring-summer using electrical fences as physical borders. Pastures' grazing capacity was enough to satisfy nutritional requirement of cows, and, during the whole experiment, no additional hay was given. Two water throughs were present, and *ad libitum* water was supplied. Two animals were eliminated from the experiment because of parturition events.

2.2. Virtual Fencing collars

Commercial Virtual Fencing collars (Nofence AS, Batnfjordsør, Norway) were used in this experiment (Fig. 1). Collars were fitted around the neck of the animals, with a slack of approximately 4 cm on the upper edge. A right fitting is crucial to avoid the risks of losing the collar or to give no cues (i.e, mainly the electric pulse). Collars were equipped with a 3.6 V 20.1Ah Li-ion batteries, and their continued recharge was guaranteed by the double solar panel positioned on the two sides of the collars. The average level of sunshine of the study area provided a full recharge during all the experiment.

Collars communicated by 2G mobile network with a user interface (UI), accessible by mobile phone app and web portal, in which animals' GPS position were constantly monitored. Moreover, an alert was sent to the mobile app every time an electrical stimulus was delivered. The study area had a good coverage of the GPS and 2G mobile network, so no problems have occurred.

Once the virtual grazing area was designed on the UI (Fig. 2), all the animals were remotely controlled and if they cross over the virtual boundaries they entered in a "warning zone": collars emitted a rise tone scale of sounds lasting from 5 s to 20 s, depending on the cow's response, that were stopped if the animals came back to the virtual grazing area. On the contrary, if they continued to go forward, they received a low electric pulse. The system had three consecutive warning zones and, if the animals chose to cross all three of these, they were considered "Escaped" and collars entered in the escaped mode. In this mode, collars emitted no warnings, only the GPS locations were logged. When the animals came back to the virtual grazing area the collars returned in the

Table 1
Summary of the studied parameter elaborated after the end of the trial.

Data group	Variable	Meaning
Stimuli delivered	Sounds (S)	Number (n) of total sounds emitted by the collars
	Shocks (Z)	Number (n) of total shocks emitted by the collars
	Rate (S/Z)	Rate between total sounds and total shocks delivered
	Sounds per day (SD)	Number (n) of total sounds emitted by the collars per day
	Shocks per day (ZD)	Number (n) of total shocks emitted by the collars per day
	Rate per day (SD/ZD)	Rate between total sounds and total shocks delivered per day
Learning capacity	Sounds with shocks (SZ)	Number (n) of total sounds followed by a shock
	Sounds without shocks (SnZ)	Number (n) of total sounds not followed by a shock per day
	Sounds plus shocks per day (SZD)	Number (n) of total sounds followed by a shock per day
	Sounds with shocks on total sounds (SZ/T)	Number (n) of total sounds followed by a shock on the total Number (n) of sounds emitted
	Sounds without shocks on total sounds (SnZ/T)	Number (n) of total sounds not followed by a shock on the total Number (n) of sounds emitted
	Total sounds' duration (Wd)	Length of all sounds (seconds) emitted by the collars
	Sounds duration with shocks (WdZ)	Length of sounds (seconds) emitted by the collars when animals receiving the shocks
	Sounds' duration no shocks (WdnZ)	Length of sounds (seconds) emitted by the collars without the animals receiving the shocks
VF ability to restrain cows	Escape (E)	Number (n) of times animals escaped from the virtual grazing area
	Escape per day (Ed)	Number (n) of times animals escaped from the virtual grazing area per day
	Percentage Escaped time (perE)	Percentage of time spent outside the virtual grazing area on the total time

normal mode.

2.3. Hair cortisol analysis

Hair samples were collected during the collars' wearing procedure (Ti), with a 5 mm blade up to the skin, from the frontal region of each cow. At the end of the experiment (Tf), hairs were resampled in the same region. A total of 32 hair samples was collected. HCC has been demonstrated to allow assessing the cortisol accumulation during a long period, in contrast with the short-term cortisol levels measured in other matrices (i.e., saliva and blood) (Heimbürge et al., 2019). So, this procedure was conducted to evaluate the HCC accumulated during the experiment. The samples were then stored in a dry and dark place. Both cortisol extraction and determination methodology were performed as illustrated in Accorsi et al. (2008). Two samples were removed due to the presence of contaminants which could had interfere with analysis.

2.4. Experimental design

The experiment was divided into four trials (Fig 3):

- Trial zero (T0): all cows were endowed with Virtual Fencing collars and, to let the animals get acquainted with them, for eight days, virtual boundaries were matched with the physical ones, giving animals free access to the entire pasture area. In T0 only the animal's location was recorded.
- Trial one (T1): at the end of T0, for two days, the pastures' surface was modified with virtual fences, where three of the four virtual boundaries coincided with the physical ones, while the virtual one was set across the pasture to restrict the grazing area. Indeed, from the initial 30 ha in T0, approximately 16 ha were available for the cows in T1.
- Trial two (T2): the grazing area was further extended moving forwards the virtual board thus giving animals access to 25 ha for four days.
- Trial three (T3): the virtual line was set longways to the pasture for two days. Also, in T3 one of the two water troughs was excluded, as well as a piece of a sheltered zone where animals usually went to repair from heat. That is because these zones have been largely exploited in the previous trials, especially in T1.

2.5. Data acquisition and processing

During their operation, the collars sent a status report every fifteen minutes. Further, this report messages, contained: the date and time-stamped GPS location including information about the total duration of the audio cues, steps taken by cows and the collar status (e.g., normal or escaped). Normally, these records are logged on server as raw data. In our case, they were provided, for scientific purposes, from the manufacturer. At the end of the experiment in total 33449 records were downloaded by the server in .csv format.

These data were further processed to obtain more detailed information. In doing so, the entire data set was divided into three different categories of data; 1) stimuli delivered; 2) learning capacity; 3) VF ability to restrain cows, whose description is provided in Table 1.

Coordinates of virtual boundaries and animals' GPS tracking were imported into QGIS software, to recreate the spatial distribution of the cows among trials. Moreover, distribution heat maps were generated as well (Fig. 4).

2.6. Statistical analysis

Data were normally distributed so, parametric test was applied. Data were analyzed by generalized linear model (GLM) procedure (SAS Institute., 2004). The fixed effects Animal (18 levels) and Trial (3 levels) were used. The three consecutive trials (i.e., T1, T2 and T3) were compared, to test the differences between them, in order to evaluate the animals' response among the studied parameters (Table 1). Then, the obtained means were compared using a Post-hoc Tukey-Kramer test. To perform hair cortisol statistical analysis also a generalized linear model function was used to assess the differences in HCC between Ti and Tf. Significance was determinate at $p<0.05$, while $0.05 < p < 0.1$ was considered as a statistical tendency.

3. Results

3.1. Stimuli delivered

Results are summarized in Table 2. The total number of sounds (S) delivered decreased significantly in T3 ($p< 0.001$). Also, the number of total shocks (Z) was constantly reduced from T1 to T3 ($p< 0.0001$). A positive response to the stimuli was confirmed by the increasing in the S/Z ratio between T1 and T3, with T2 being similar to both ($p<0.0250$). Similar trend was also showed in the daily stimuli delivering. Indeed, the number of SD and ZD reduced from T1 vs T2 and T3, as well as SD/ZD increased in T3.

3.2. Learning capacity

Animals' response to the paired stimuli differed between the trials (Table 3). Therefore, the events in which the sounds were followed by electrical pulses (SZ) were significantly less in T3 ($p< 0.001$). SnZ were also reduced ($p< 0.001$) in T2 and T3 in respect of T1, likewise the combined stimuli per day (SZD). There was no difference between trial in SZ/T and SnZ/T. There was a significant variation ($p< 0.001$) in the

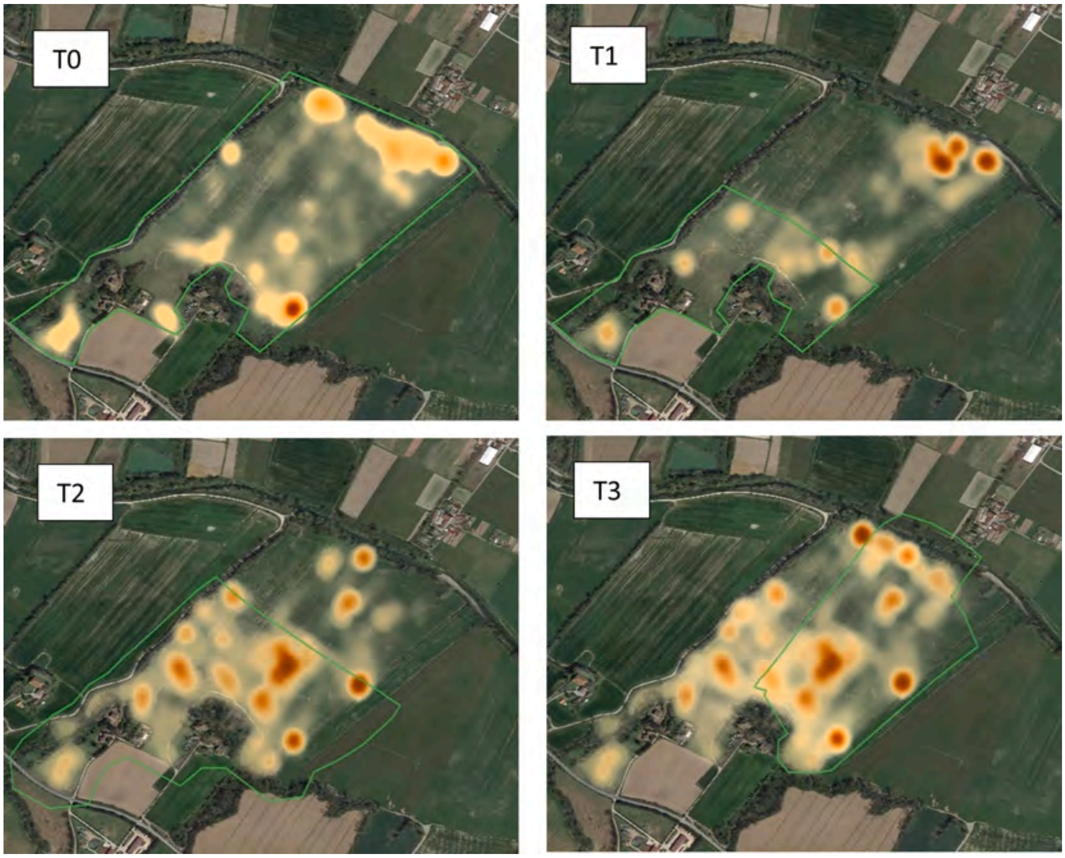

Fig. 4. Image displays the heat maps distributions of cows' geographic positions in each trial session. High dense areas are coloured in intense red; otherwise, low dense areas are coloured in yellow.

Table 2
Mean number of each studied parameters per animal per trial. a,b,c, represents significant difference in the mean numbers between trials. Significance was set at p<0.05.

Variable	T1	T2	T3	RMSE	*p*-value	
					Animal	Trial
S	30.773[a]	27.130[a]	10.204[b]	7.950	0.5902	<0.001
Z	16.038[a]	10.174[b]	4.303[c]	3.503	0.1242	<0.001
S/Z	1.178[b]	2.857[ab]	3.085[a]	1.179	0.4040	0.0250
SD	13.447[a]	5.586[b]	4.759[b]	2.583	0.5291	<0.001
ZD	6.923[a]	2.191[b]	1.986[b]	1.331	0.1855	<0.001
SD/ZD	0.040[b]	0.114[b]	0.425[a]	0.227	0.5225	0.0003

Table 3
Mean number of each studied parameters per animal per trial. a,b,c, represents significant difference in the mean numbers between trials. Significance was set at p<0.05.

Variable	T1	T2	T3	RMSE	*p*-value	
					Animal	Trial
SZ	15.472[a]	10.148[b]	4.294[c]	3.486	0.1247	<0.001
SnZ	3.872[a]	2.125[b]	1.600[b]	0.977	0.4066	<0.001
SZD	6.671[a]	2.180[b]	1.982[b]	1.319	0.1806	<0.001
SZ/T	50.935	40.429	36.380	17.134	0.3587	0.1123
SnZ/T	28.814	35.024	40.196	14.615	0.3678	0.1556
Wd	13.437[a]	12.876[a]	10.150[b]	9.022	<0.0001	0.0005
WdZ	15.902[b]	18.463[a]	13.894[b]	8.795	0.4454	0.0011
WdnZ	11.011	9.568	7.938	8.163	0.0081	0.1034

Table 4
Mean number of escape events per animal per trial. a,b,c, represents significant difference in the mean numbers between trials. Significance was set at p<0.05.

Variable	T1	T2	T3	RMSE	*p*-value	
					Animal	Trial
E	3.159[a]	1.637[b]	0.996[b]	0.982	0.4684	<0.001
Ed	1.392[a]	0.355[b]	0.456[b]	8.163	0.3931	<0.001
perE	0.353[a]	0.038[b]	0.106[b]	0.102	0.3408	<0.001

Table 5
Descriptive statistics and p-value for different time sampling. Ti, hairs sampled at beginning; Tf hairs sampled at the end of the experiment; n, number of samples; SEM, standard error of the mean; p-value, model p-value Ti-Tf.

Sample	N	Mean	SEM	Min-max (pg/mg)
Ti	16	1.14	0.14	0.53-2.72
Tf	16	0.82	0.08	0.26-1.47
p-value	0.0677			

duration of the audio tones (Table 3). Hence, in T3 Wd was lower than T1 and T2. While its duration, in a complete paired warnings sequence, were longer in T2. No differences were observed in the lengths of sounds duration when animals did not take a shock.

3.3. VF ability to restrain cows

The GPS data locations indicated a regular occupation of the entire pastures' surface in T0. Then, cows were increasingly kept inside the inclusion zones during the trials (Fig. 4). Indeed, most of aversive reactions to the stimuli were observed in T1, where animals have escaped more than in the following trials ($p< 0.001$). The same was observed in the daily average of escape events and in the percentage of time spent outside the virtual grazing area escapes per trial ($p< 0.001$) (Table 4).

3.4. Hair Cortisol determination

Results are shown in Table 5. HCC values vary from a minimum of 0.53 pg/mg to a maximum of 2.72 pg/mg and from a minimum of 0.26 pc/mg to a maximum of 1.47 pc/mg for Ti and Tf respectively. The mean value for Ti was 1.14 pc/mg, while for Tf was 0.82 pc/mg. Also, no significant differences are shown between Ti and Tf.

4. Discussion

Our study demonstrated that cows gradually learn to stay inside the inclusion zones that are virtually delimitated. The differences observed in the studied parameters, suggested that animals were able to avoid the aversive stimuli between following sessions. Thus, in the last trial (T3), cows received the lowest number of sounds and electrical pulses than T1 and T2, and their rate as well. Similar results have been found in a previous study conducted by Lomax et al. (2019), where dairy cows were managed over two consecutive different grazing allocations set with VF. As result, the total number of cues delivered decreased among these. However, in contrast with our findings, cows' responses varied also among individuals.

To be easily understood and safe for the animals' welfare, VF system should be highly controllable and predictable (Lee et al., 2018). Thus, the recognizing of audio cues as a conditioning stimulus to a subsequent aversive stimulus (e.g., electrical pulse) (Marini et al., 2018a), facilitate the animals' learning process, rather than use an aversive stimulus alone (i.e., audio cue or electrical pulse) (Marini et al., 2019; Umstatter et al., 2013). Indeed, at the beginning (T1), cows did not easily recognize audio cues alone as an alert, and they needed more interactions to understand, and more paired stimuli as well. Subsequently, once cows pairing the audio cues to an incoming unpleasant event, the total number of sounds followed by a shock gradually decreased from T1 to T3. At the same time, in T2 and T3 cows received fewer sounds not followed by a shock. The latter considered only the positive interactions with the virtual lines, in which animals did not receive the electrical pulse, and so, did not escape from the virtual pastures. So, in the last trials, they needed a lower number of audio cues alone to understand when to stop and avoid the shock. The increase in the associative learning across time was shown in a previous similar study conducted on Angus heifers. Cattle, after a period of 48 h, minimize the number of electrical stimuli received and learn to respond to the audio cues alone (Campbell et al., 2017b). According to what shown by Aaser et al. (2022), a good indicator of the animals' learning ability is related to the ratio between audio warnings and electrical pulses, or vice versa. In our case, as higher will be the ratio, as higher will be the associative learning. Thus, our results have shown a significant increase of this ratio from T1 to T3, confirming an adaptive response trough time.

To our knowledge. sounds' duration has not been completely explored in any studies before. Nevertheless, they could give relevant information on the individual animals' degree of learning, considering that it depends on the animals' reactions to the stimuli. Hence, independently by the shock or not, the lower duration registered in T3 indicates that cows were more receptive to the stimuli. Thus, thanks to their previous experiences on T1 and T2, they came back and switch off the sound more quickly. At the same time, the higher duration in T2 of the sounds followed by a shock indicates that cows spent more time interacting with the system, become more prudent in the overcoming of the boundaries. This is also confirmed by the high concentration close to the virtual line (Fig. 4). Differently, the lower duration in T1 indicates that, considering the high proportion of cues, animals probably ran forward to avoid the shock, and so the duration was short. In addition, comparing these results to the ones obtained for the total number of sounds followed by a shock, we assume that, despite the latter was lower than in T1, its duration was much longer in T2. Hence, sounds were lesser in T2 but last more. However, in contrast with the other results, the sounds' length without a shock showed a significant difference between individuals and not between trials. These results show that animals had different individual learning rates, that could be affected by the herd as well. Indeed, it has been demonstrated that when animals were trained in a group were more likely to receive stimuli rather than when were trained individually (Colusso et al., 2020). Otherwise, in another study, when treated as a group, some individuals didn't receive any stimuli thanks to only the observation of conspecifics (Keshavarzi et al., 2020) . However, this could be not enough to reduce the number of animals wearing the collars within the herd (Marini et al., 2020).

One of the potential applications of VF is to manage the herd in a rotational grazing system. Furthermore, VF also may provide selective use of various type of lands (i.e., rangeland) (Monod et al., 2008), protected areas (Boyd et al., 2022), or exclude livestock from some areas within the pastures, which may be either difficult or not convenient to enclose with traditional systems (Campbell et al., 2019a; Umstatter et al., 2015) (Stevens et al., 2021). All these aspects, may can contribute to a better environment, and achieve both short and long-term environmental goals (Pošiváková et al., 2018). In our experiment, we excluded access from a sheltered zone and one of the two water resources in T3. VF kept away cows from these points of interest, which were fully accessible before and where cows used to stay in the previous trial sessions (Fig. 4). Moreover, the escapes from the virtual pastures were more frequent in T1 when cows did not already learn the system. After that, cows were successfully restrained, and the escape events decrease in T1 and T2.

As proposed by Lee et al. (2021), in the last stage of learning (i.e., the stage in which the associative learning has occurred), changes in chronic stress indicators should not be found. In our case, no differences were observed in the HCC before and after the VF application. Similarly, minimal differences in fecal cortisol concentrations were also found when VF was compared with electric fencing (Campbell et al., 2019b). On the contrary, Verdon et al. (2021) found an increase in milk cortisol concentration and a reduction in some animals' behavioural activities.

5. Conclusions

This study has shown that cows learn how to positively interact with VF over three consecutive trials conducted in a pasture-based scenario. The progressive reduction of the stimuli delivered indicates an increase in associative learning through time. Moreover, VF kept the cows inside the inclusion zones, containing and reducing the number of escape events. In addition, preliminary results in the HCC, revealed no differences in cortisol content between the beginning and the end of the trial. So, the animals did not show any significant change in stress condition during the experiment.

The obtained results confirm the potential of VF as a helpful technology to manage beef Limousin herd in a pasture-based system. In addition, the use of a cosmopolitan breed, such as Limousin, in a Mediterranean area, that is very prevalent environment, makes this application of this system experiment easily replicable both locally and globally. However, working with adult animals represents a research limitation, as well as testing the system for a short period. So, future research is needed to evaluate the animals' performances and behavioural changes, using different breeds in an intensively grazing scenario. Likewise, investigate the efficacy of VF to improve the pasture consumption rate.

Author contributions

Andrea Confessore: Conceptualization, Methodology Investigation

Writing- Original draft preparation **Chiara Aquilani**: Conceptualization, Methodology, Visualization, Editing **Lapo Nannucci**: Methodology, Writing- Reviewing and Editing, Investigation **Maria Chiara Fabbri**: Data curation, Software. **Pier Attilio Accorsi**: Methodology, Resources **Camilla Dibari:** Supervision, Project administration, Funding acquisition **Giovanni Argenti:** Supervision, Editing **Carolina Pugliese**: Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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