Use of vocalizations to detect pain and discomfort in dairy cows at dry-off

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Abstract

Identifying and characterizing the acoustic taxonomy of cows' vocalizations might be useful to detect welfare problems such as pain. Drying-off is recognized as a painful and stressful event due to abrupt cessation of milking and consequently udder engorgement, change of pen and re-grouping, and change of diet. The aim of the study was to analyze vocalizations of dairy cows to determine their acoustic characteristics at dry-off and contrast them with pain-related behaviors and mechanical nociceptive thresholds (MNTs). An environmental microphone was placed above a pen with six cows at the beginning of the dry-off process. The cows had milk production of 13.24±7.35 liters^{-day} at dry-off (day 0). The audio recording lasted five days uninterruptedly (101.5 hours recorded). A behavioral pain score, built with direct observations of pain-related behaviors (cow's attention, ear position, facial expression, back position, head position, tail position, limb posture, and lying position; from a score of 0 or painless to a score 13 or severe pain), was used/observed twice daily for 5 days. Additionally, MNTs were measured in newtons (N) using a hand-held algometer to assess the pain due to udder engorgement. Statistical analyses were performed with a Glimmix model. Two kinds of vocalizations were identified based on listening and spectrogram analyses using the Audacity® software:

1. "*High* vocalization", short and with an ascendant fundamental frequency (f_0) with repetitions; and
2. "*Low* vocalization", longer and with a lower f_0.

Other spectral characteristics such as spectral bandwidth, centroid, flatness, and roll-off were computed for the two types of vocalizations. Significant differences were found for the duration, f_0, and spectral bandwidth between *High* and *Low* vocalizations. *High* vocalizations average count per cow was higher on the day+1 (6.41 ± 10.81) and day+3 (4.41 ± 6.62) after dry-off compared to other days studied (day 0: 0 ± 0.00; day+3: 1.00 ± 2.37; day+4: 1.08 ± 2.35; P<0.05). The total number of vocalizations decreased over the five days (P<0.05). The pain score was higher on day+2 (1.91± 1.31) and day+3 (1.58 ± 1.16) compared to other days studied (day0: 0.50 ± 0.54; day+1: 0.75 ± 0.62; day+4: 0.66 ± 0.77; P<0.05). The MNTs values were different across the five days assessed (P<0.05). Day+2 had the lowest MNTs measurement (23.35 ± 0.18 N), and day+4 presented the highest MNTs measurement (24.60 ± 0.18 N). In the current study, the contemporaneity of *High* vocalizations, pain scores, and MNTs might elucidate the possibility that vocalizations are related to the expression of pain and/or discomfort produced by the dry-off. These results may help understand dairy cows' welfare based on their vocalizations. Vocalizations show a big potential to assist farmers in detecting welfare problems and facilitate rapid interventions to mitigate them.

Keywords: dairy cattle, vocalizations, pain, animal welfare monitoring.

Introduction

The implementation of technologies to monitor animal production conditions is called Precision Livestock Farming (PLF). PLF aims to provide the farmer with information about the animal gathered in a continuous fashion to facilitate the decision-making process, increasing the efficiency of the production system (Guarino *et al.,* 2017). Additionally, cattle stakeholders and society have demanded the use of monitoring methodologies that do not affect the physical integrity of the animals (Gołębiewska *et al.,* 2018). In this context, animal vocalizations provide the opportunity to detect reliable information about the animals' welfare without animal manipulation.

In dairy cow production, some husbandry practices might produce discomfort, one of them is the dry-off. During the dry-off, cows are ceased to be milked, moved to the dried cows' pen, and their diet changes to a lower-calorie diet. All these changes might alter animal welfare status, as cows need to get adapted to their new management routine and environment. In addition, the irruption of the milking routine causes udder engorgement, as its milk production continues for some days, causing discomfort and udder pain (Bertulat *et al.,* 2013; Silanikove *et al.,* 2013, Mainau *et al.,* 2015).

The aim of the study was to analyze the vocalizations of dairy cows to determine their acoustic characteristics at dry-off and contrast them with pain-related behaviors and mechanical nociceptive thresholds (MNTs). The goal is to monitor the level of discomfort experienced by the cows during the dry-off process. If successful, the results of this study could validate the use of vocalizations as a non-invasive method for monitoring the welfare of dairy cows at drying off.

Material and methods

Animals and farm management

Six Holstein-Friesian cows (mean ± SD/SE; 44.9 ± 8.39 months old, 1.5 ± 0.54 parities) were housed in an open-sided barn (15 m x 23 m). The pen had straw bedding at the resting zone and concrete flooring for the feeder and drinker areas. The cows were fed a TMR diet (76% silage, 12% straw, 12% grain) three times a day at a feeder with headlocks, and *ad libitum* access to water. One day before dry-off, the cows had a mean milk production of 13.24 ± 7.35 liters/day. On the day of dry-off (day 0), the cows were milked for the last time at 11:30h, spray-marked for individual identification, and then moved to the dry-off pen along with thirteen other dry cows.

Experimental design

Vocalizations recording and acoustic analyses

Vocalizations were recorded using an environmental microphone (XM1800S, Behringer, Germany) placed above the pen and attached to a digital recorder (Zoom H5; Zoom, Madrid, Spain). The study started on the dry-off day (day 0) and lasted for five consecutive days. Two hours of direct observations were done twice a day (09:00h-11:00h and 15:00h-17:00h) to register the vocalizations from the cows. The observer was positioned at an elevated location on the side of the pen, approximately 1.50 meters above ground level. On day 0, only one observation was performed since the cows entered the dry-off pen until 11:30h. Overall, 101.5 hours of audio were recorded, but only the vocalizations registered during the observation hours were considered for acoustic analysis.

The detected vocalizations were processed through Audacity audio editing software (Audacity® 3.1 version, 2021). The recorded audio was segmented into 15-minute tracks and converted into the spectral domain including frequencies between 100 and 1200 hertz (Hz), allowing the vocalizations to be aurally identified. Additionally, vocalizations were analyzed to extract acoustic features such as duration, fundamental frequency (f_0), and spectral features such as bandwidth, centroid, flatness, and roll-off (McFee *et al.,* 2015, Yamamoto *et al.,* 2019). Vocalizations were categorized and clustered based on their acoustic taxonomy.

Pain score assessment

The same observer assessed individual pain scores twice daily (11:30h and 17:00h) during the recording period. The score was built from nine behaviors scored from 0 to 1-2, and integrated into a total pain score, from a score of 0 (painless) to a maximum of 13 (severe pain), calculated by summing up all behavioral scores. The behaviors assessed were attention toward the surroundings, head position, ear position, facial expression, back position, lying position, tail position, and limp posture (Gleerup *et al.,* 2015, de Boyer des Roches *et al.,* 2015).

Udder engorgement / Udder pain threshold

A pressure algometer (Digital Force Gauge ZMF-100; Boshi Electronic Instrument, Japan) equipped with a pointless pressure pad was used to measure the mechanical nociceptive thresholds (MNTs) three times per day (11:30h, 14:00h, and 17:00h). The algometer measured in newtons (N) the pressure applied to the cows' udders at a constant rate of 5 N/s while placed on the cauda-ventral side of the rear quarters with the cow standing and locked at the feeder. Two measurements per quarter were taken at 60 seconds intervals. The maximal peak force applicable was set at 24.6 N (Giovannini *et al.,* 2017), further than that, it was registered as a lack of reaction (Krug *et al.,* 2018). Whenever the algometer and pain score measures were scheduled at the same time, the pain score was performed first to avoid affecting the cows' behavior. Each cow's base level was the measurement taken on day 1 after the last milking at the beginning of the study.

Statistical analysis

All statistical analyses were performed with the statistical package SAS (version 9.4, SAS Institute Inc., Cary, NC). The number of cows' vocalizations was analyzed with a Proc Glimmix after a logarithmic transformation. The acoustic features (duration, f_0, and spectral features) were analyzed with a non-parametric test through a Proc Npar1way. The pain score and MNTs were analyzed using a Proc Genmod with day, hour and its interaction as the fixed effects of the model. Results are presented as the average values of the variables (mean ± SD/ES). Significant differences were declared at $P \leq 0.05$ whereas near-significant trends were considered at $0.05 < P \leq 0.10$.

Results

Vocalizations detection and characterization

Two main groups of vocalizations were aurally identified from the detected vocalizations: "*High*" vocalizations and "*Low*" vocalizations (Figure 1). The comparison of the acoustic features of both vocalizations is reported in Table 1.

Number of vocalizations

High vocalization count was significantly different across the days (P=0.0455), being the day+1 the one with the highest count (6.41 ± 10.81). On the other hand, *Low* vocalization did not have differences across the days (P=0.1643).

Pain score assessment

There were significant differences among the total pain scores among the days (P<.0001). Higher pain scores were reported on day+2 and day+3 (1.91 ± 1.31 and 1.58 ± 1.16, respectively) compared to day0 (0.50 ± 0.54), day+1 (0.75 ± 0.62) and day+4 (0.66 ± 0.77).

Udder engorgement / Udder pain threshold

The engorgement of the udder of the cows was different among the five days (P<.0001). Higher engorgement was recorded on day+2 (23.35 ± 0.18 N). On day+1 the cows

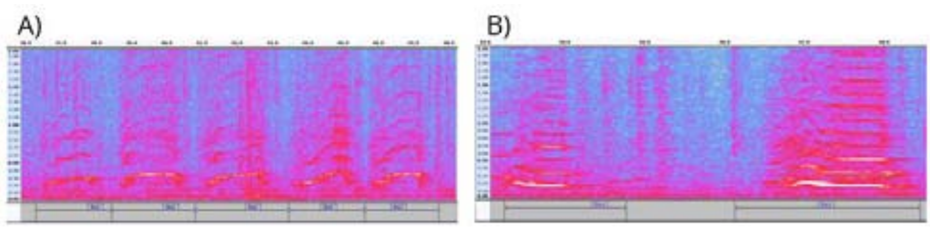

Figure 1. Spectral representation of the vocalizations detected during the study. A) "High vocalization", short and with an ascendant f0 with repetitions; and B) "Low vocalization", longer and with a lower f_0.

Table 1. Acoustic features measured from the "high" and "low" vocalizations recorded during the five consecutive days after dry-off.

	High		Low		
	Mean	SD	Mean	SD	P-value
Duration	1.72	0.538	2.60	1.012	<.0001
f_0	212.16	55.393	106.59	65.144	<.0001
Spectral centroid	2993.38	827.598	3536.51	975.151	<.0001
Spectral bandwidth	3888.41	654.359	4796.66	892.374	<.0001
Spectral flatness	0.009	0.009	0.014	0.013	<.0001
Spectral roll-off	5982.06	1832.040	7632.90	2979.760	<.0001

presented high sensibility to the MNTs measurement (23.91 ± 0.18 N). On days 0, +3, and +3 the MNTs measurements were 24.53 ± 0.22 N, 24.20 ± 0.18 N, and 24.60 ± 0.18 N, respectively.

Discussion

Vocalizations have the potential to provide information about animal welfare in a continuous and less invasive manner. *High* and *Low* vocalizations identified in the present study, have been previously reported in the literature as possible cues of cows to express discomfort (Briefer, 2012, de la Torre *et al.,* 2015). *High* vocalization count was higher on days+1 and +2, and pain behavioral score and udder pain threshold pointed out that the discomfort of the cows was higher on day+2. Thus, *High* vocalizations might be a way to express pain and discomfort in dairy cows at dry-off. Other authors have associated vocalizations with discomfort. For instance, Coetzee *et al.* (2010) counted the number of vocalizations during the castration in calves as a pain indicator.

Conclusions

Vocalizations have shown to be a feasible tool to monitor animals without disturbing their physical integrity or daily routine, particularly at dry-off., in the assessment of stressful husbandry practices such as the dry-off in the dairy systems. However, further research is needed to validate the preliminary results of this study and elucidate how the incidence can change in each animal over time.

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