Characterization and assessment of vocalization responses of cows to different physiological states

Yu Yoshihara and Kosei Oya
Graduate School of Bioresources, Mie University, Tsu, Japan

ABSTRACT
The objective of our study was to characterize and assess the vocalization responses of cows in four different physiological states using different measurements and relate those to salivary cortisol concentrations as a possible indicator of stress. We characterized and assessed the vocalization responses of cows in four different physiological states: feed anticipation, estrus, communication between or among individuals, and parturition. We used video camera recordings of the calls made by the cattle during these four states. We measured the duration of the calls, intensity and pitch of vocalizations, and the formant, using vocalization analysis software. Two hundred and ninety calls were recorded from 32 cows during the study. Cortisol increased from the least values in communications and feed anticipation, to two-fold concentrations in estrus, and three-fold concentrations in parturition. All the formants tended to increase in the same order with the stress concentrations, whereas low intensity and lower pitched vocalizations were associated with greater cortisol concentrations during parturition. These results indicate that acoustic parameters of calls in each of the physiological states may reflect the stress level in cattle, and thus, the vocalization of animals could be an important behavioural indicator of their stress level.

Introduction
Farm animals experience various physiological states and are exposed to different conditions of stress during handling, transportation, and heat, which may induce varying emotional responses and lead to decreased production (Grandin 1997, 2015). For example, heat stress markedly reduces milk production that can be attributed to the diminished dry matter intake (Garner et al. 2017). Therefore, it is of interest to animal researchers and to farmers to understand the animals’ behavioural responses to different physiological states and stressors, which are the biological responses elicited when an animal perceives a threat to its homeostasis.

Studies on the effects of painful or otherwise stressful protocols involving cattle frequently report that vocalizations are associated with these procedures as indicators of distress in the animals (Watts and Stookey 1999; Bourguet et al. 2011; Hemsworth et al. 2011). In contrast, cattle also vocalize under during other perhaps non- or less stressful events or physiological states such as conditions during mother-offspring interactions, inter-male rivalry, and anticipation of feeding (Kiley 1972; de la Torre et al. 2015). Vocalizations by cattle may not always be indicative of a negative or stressful experience (Watts and Stookey 1999). This contradiction affects several common measures often used as indices of stress. However, if we can correctly interpret the characteristics of cattle calls under stressful and less stressful conditions, we could better evaluate the welfare level of animals in farms without the use of invasive methods. Nevertheless, using vocalization to analyse stress levels or different physiological states in cattle remains poorly understood (Watts and Stookey 2000).

Briefer (2012) summarized that the emotional state of the caller causes changes in the physical structure and action of the vocal apparatus, which in turn, impacts the parameters of vocalization. Physiological arousal is mainly reflected in parameters linked to respiration and phonation, such as formant, amplitude, and timing, whereas emotional valence seems to be more reflected in intonation patterns and voice quality. Furthermore, vocalization produced in positive situations could be shorter in duration among mammals (Briefer 2012). An animal’s vocalizations thus may provide an important source of information about its physical and psychological condition (Weary et al. 1997; Watts and Stookey 2000; Yeon et al. 2006; Röttgen et al. 2020).

The objective of our study was to characterize and assess the vocalization measurements of cows to four different physiological states such as anticipation of feeding and intraspecies communication, and with some being potentially stressful negative conditions such as parturition and estrus, and relate those with salivary cortisol concentrations.

Materials and methods
Animals and data collection
Our study site was the Mie Prefectural Livestock Research Institute, Japan, where 80 Japanese Black and 33 Holstein cows were kept. The cows were maintained in 5 × 3 m pens (3–5
cows per pen) with a roughened concrete floor. Cows received a totally mixed ration of grain and artificially dried hay separately in 2 feedings per day. All experimental procedures were approved by the Mie Prefectural Livestock Research Institute as acceptable standard care of and experimentation with animals.

The vocalizations of the cows were recorded on 12 different days from August to December in 2017. All vocalizations were recorded using a camcorder (SONY, HDR-PJ675, Japan) set at a distance of approximately 1 m from the pen fence. The cows were identified by ear tag number visually associated with the vocalizations on the recordings. We targeted adult cows (aged 2.5 and 4.0 yr) to avoid the influence of age on sound (Deshmukh et al. 2012). Two hundred and ninety calls were recorded from 32 cows (21 Japanese Black and 11 Holstein cows) during the study. Other cows did not make any vocalizations. Although the significance of genetics is unknown, we believe the breed difference of characteristic vocalization is negligible due to their similar vocal apparatus within a species. The vocalizations were characterized during four physiological states exhibited by the cows: feed anticipation, estrus, communication among cattle, and parturition. Anticipatory vocal responses to feeding were recorded when feed was delivered as per routine schedule in the morning and the evening. Estrus and parturition were detected based on visual observation of the mounting behaviour and appearance of the foetal membranes. Communication was defined as an immediate return of vocalization (within 1 sec) by an animal devoid of other states such as estrus or hunger, solely in response to vocalization from other cows.

**Figure 1.** Spectrograms of vocalizations during the four different physiological state. The dotted lines represent formant contour; dark grey band represents large concentration of sound energy in a specific time and frequency; red, yellow, and blue lines indicate formant, intensity, and pitch of vocalization.
To quantitatively assess the degree of stress level of each animal in the four physiological states, we measured salivary cortisol concentrations (Negrao et al. 2004; Dzviti et al. 2019). Saliva samples were collected in the morning from three randomly selected cows that had vocalizations in each of the four physiological states for cortisol analysis. An observer was waiting for the focal cow to vocalize and then she was immediately restrained in a stanchion after calling, and the saliva sample was collected into a cotton swab. Saliva samples were frozen at −20°C for subsequent analysis. Salivary cortisol concentrations were measured using a commercial ELISA kit (Salimetrics, USA).

### Results and discussion

We obtained for the analysis 64 vocalizations or calls of communication among 8 cows, 114 calls in anticipation of feeding from 13 cows, 74 calls associated with estrus from 7 cows, and 38 calls associated with parturition from 4 cows.

We detected significant differences in salivary cortisol concentrations among cows in the four different physiological states (Figure 2, F = 32.12, d.f. = 3, 289, p < 0.001), with the greatest concentrations observed during parturition. The cortisol concentrations suggest that the stress levels of these cows increased from least to most for communications among cows, then anticipation of feed, then to estrus, and finally to parturition. The formants were significantly different among the four physiological states (Figure 3, p < 0.001, d.f. = 3, 289, F = 21.40, 21.26, 23.68, and 15.50 for the first, second, third, and fourth formants, respectively). The first and second formants of communication calls were lower than those of the other calls, but the other three kinds of calls were comparable in terms of these formants. The third and fourth formants of the parturition calls were higher than those of other calls (p < 0.001). The duration of estrus calls was the greatest among the four states (Figure 3, F = 32.12, d.f. = 3, 289, p < 0.001). The pitch and intensity of vocalizations show the opposite trend with the third and fourth formant (Figure 3, F = 27.41 for the pitch, F = 90.26 for the intensity of vocalizations, d.f. = 3, 289, p < 0.001). That is, the pitch and intensity of estrus calls were higher than those of parturition calls (p < 0.001); but, were lower than those of communication calls (p < 0.001). In summary, the communication, anticipation of feeding, estrus, and parturition calls were characterized by lower formants and higher pitch and intensity, middle range of all sound measurements (non-characteristic), long duration, and higher formants and lower pitch and intensity, respectively.

Vocalization parameters showed a clear response to stress levels, where formants 2, 3, and 4 increased, and the intensity of vocalization and pitch decreased with increasing stress level. Yeon et al. (2006) have also reported that formant analysis has an important role in discriminating between estrus and feed anticipation vocalizations, although the third and fourth formants of estrus calls were lower than those of feed anticipation. In another experiment, Cordeiro et al. (2018) showed that the pitch frequency, the maximum amplitude (Pa), and intensity increased in pain-free pigs exposed to the marking procedure, compared with both tail trimming and castration. On this basis, we can judge the stress levels of cattle in farms using vocalization analysis of formant, intensity, and pitch. These results further support that acoustic parameters of calls reflect the stress level in cattle, and thus, vocalizations could be an important behavioural indicator of the animal’s stress level.

However, there is a caveat that different or higher salivary cortisol concentrations are not always directly related to higher stress levels. Moreover, our results may simply support the theory that vocalizations in cattle will differ based upon context or physiological state, but not necessarily stress level. For example, horses responded to physical activity (equestrian competition) with a transiently increased cortisol release (Becker-Birck et al. 2013), albeit a lower one than in horses exposed to transport stress (Schmidt et al. 2010).

Figure 2. Salivary cortisol concentrations of cows in the four physiological states. Error bars are given as mean ± S.E. Different letters depict significant differences among the different physiological states.

**Sound measurement and data analysis**

The sound characteristics of each call were extracted from the recordings and edited using VideoPad (NCH Software, ver. 5.06) and WavePad (NCH Software, ver. 7.08) at a sampling rate of 44.1 kHz and 24-bit amplitude resolution. The sound files were analysed using Praat 4.1 (Boersma P. & Weenink D., University of Amsterdam, the Netherlands). Praat 4.1 uses a Fast Fourier Transform routine to display sound files as an audio spectrogram plot of frequency over time, with sound density represented as a variable-density grey scale plot. We measured the following variables for each vocalization: duration of the call (the recording time from start to end of the vocalization), intensity of vocalization (dB, or the degree of strength of vocalization), pitch (frequency (Hz) or auditory feature of the sound representing the high and the low points of the sound bite), and the first to the fourth formant (of a complex sound, a range of frequencies in which there is an absolute or relative maximum in the sound spectrum; see Figure 1).

We analysed differences statistically in the salivary cortisol concentrations among calls in the four physiological states using ANOVA (R version 3.3.2), after confirming the assumption of homogeneity of variance. We analysed differences in the acoustic characteristic among calls in the four physiological states as fixed effects using mixed ANOVA, with cow as the random effect. Tukey’s post-hoc test was performed to determine whether or not the characteristics of the calls differed among the four physiological states.
Figure 3. Characteristics of vocalizations of cows in four different physiological states from spectrogram analysis. Error bars are given as mean ± S.E. Different letters depict significant differences among different physiological states.
Applications
The potential applications of the results of this study are: (1) vocalization by cows could be an important behavioural indicator of their physiological state and associated stress level; and, (2) perhaps, cattle farmers could be able to judge the physiological state and stress levels of cattle using non-invasive method of vocalization analysis.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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