Topic: Vocalizations as a behavioral indicator of pain in *Bos taurus.*

The following annotated bibliographies are organized based on the physiological causes of cattle vocalization, sound analysis of vocalizations, ways reduce vocalization, and suggestions for future research on vocalizations.

Physiological Causes

Bristow, D. J., & Holmes, D. S. (2007). Cortisol levels and anxiety-related behaviors in cattle. *Physiology & Behavior*, *90*(4), 626–628. https://doi.org/10.1016/j.physbeh.2006.11.015

Summary: The objective of this study was to determine if increased cortisol levels result in stress-related behavior in cattle. To determine this, cortisol levels and stress-related behaviors were measured from a sample of nine Angus/Hereford cattle. The cattle were initially observed in a non-stressful pasture setting for 17 days. During this time, researchers recorded any stress-related behavior such as if the cattle were ruminating, standing, and how close they were standing to other cattle. After this 17-day period, the cattle were subject to a stressful event and then their cortisol levels were compared through serum samples. The stressful event that the cattle were subject to was being isolated from their herd and calves. The cattle were isolated for 90 minutes before being herded into a squeeze chute in order to be restrained for serum sample collection. The vocalization of each cow was tallied during the isolation period. The results showed that there was a statistically significant difference in cortisol levels between cattle, and therefore they were grouped either into a high cortisol group, with an average cortisol concentration of 29.5 ng/mL, or a low cortisol group, with an average cortisol concentration of 7.0 ng/mL. Cattle in the low cortisol group had an average of 2.33 vocalizations each, whereas cattle in high cortisol group vocalized an average of 19 times during observation. From their results, the researchers determined that cows who had high levels of cortisol spent less time ruminating, vocalized more often, and stood more frequently, indicating these as stress-related behaviors. These results were not statistically significant, but authors believe that this was due to the small sample size used in the experiment. Ultimately, the results provide strong evidence that cattle with increased cortisol levels vocalized more often, indicating that cortisol concentration is associated with stress-related behaviors.

Contribution: Research associating cortisol levels to stress-related behavior is limited and therefore this study was a great contribution to this field. Previous research in this field has shown that cortisol is released in cattle as a response to stress but has not focused on how it affects stress-related behaviors. I included this article because it shows that increased rates of vocalizations are linked to an increase in cortisol levels. Using vocalizations as an indicator of stress in cattle is beneficial as it is far less invasive and inexpensive compared to taking serum samples.

Sound Analysis

Meen, G. H., Schellekens, M. A., Slegers, M. H. M., Leenders, N. L. G., van Erp-van der Kooij, E., & Noldus, L. P. J. J. (2015). Sound analysis in dairy cattle vocalisation as a potential welfare monitor. *Computers and Electronics in Agriculture, 118*(C), 111–115. https://doi.org/10.1016/j.compag.2015.08.028

Summary: The objective of this study was to determine if there was a correlation between specific vocalizations and cattle behavior. Previous studies have shown that stress causes specific vocalizations in pigs, and researchers in this study questioned whether sound analysis can be applied to cattle as well. In order to determine this, the researchers observed two groups of Holstein cattle, the first group consisted of 95 adult dairy cattle and the second group consisted of 46 heifers. Audio and video were recorded over the duration of the study for each group. Researchers were then able to associate each call to a specific cow and categorize it with the observed behavior. The vocalizations were categorized as being associated with either lying/ruminating, feeding related, social interaction, sexual behavior, stress-related behavior, or remaining behavior. The authors then used ultrasonic equipment to determine the maximum frequency and the average amplitude of these calls. From the results, the authors noted that vocalizations from heifers were most often associated with stress-related behaviors compared to any other behavior. The researchers stated that they were unable to determine a significant difference between the frequency of stress-related vocalizations compared to feeding, social, sexual, or other remaining vocalizations. However, the maximum frequency of cattle vocalizations during rumination/lying behavior was found to be significantly lower than all other vocalizations. Meen et al. (2015) suggested that the detection of murmuring should be further investigated as it occurs during lying and rumination, allowing it to potentially be an indication of good welfare. The authors explained that more research should investigate sound analysis to determine how vocalizations associated with stress-related behavior differ from other vocalizations produced by cattle. They believe that an experimental design with more video coverage would allow for more data collection and ultimately more conclusive results.

Contribution: This article explained that vocalizations associated with lying and ruminating behavior are significantly different than other vocalizations, and therefore can be used to indicate good welfare. This had not been previously researched and therefore it is a significant contribution to this field. I included this article because it shows that specific cattle vocalizations can be associated with behavior, and the authors believe that further investigation into sound analysis can eventually provide a tool for livestock management to assess welfare based on vocalizations.

Watts, J. M., & Stookey, J. M. (1999). Effects of restraint and branding on rates and acoustic parameters of vocalization in beef cattle. *Applied Animal Behaviour Science, 62*(2–3), 125–135. https://doi.org/10.1016/S0168-1591(98)00222-6

Summary: Watts and Stookey (1999) investigated vocalizations as a reflection of welfare in cattle by evaluating vocalizations as a result of hot-iron branding. 189 crossbred beef cattle were used in this experiment. Half of the cattle were hot-iron branded meaning an electric iron was applied for approximately 5-8 seconds, and half were sham branded meaning that an unheated iron was applied. Some of the cattle in both treatment groups were restrained, which involved the cattle being brought through a head gate and squeeze apparatus for the hot-iron or sham branding. However, the authors noted that restraint by the head gate had little impact on vocalization occurrence. The results of this experiment show a statistically significant difference between the number of cattle that vocalized during the hot-iron branding treatment compared to the sham branding treatment. The sound analysis also revealed that vocalizations from the hot-iron branded cattle had a greater frequency range, maximum frequency, and intensity compared to the sham branded cattle. However, the authors were unable to determine if the vocalizations due to the hot-iron branding were different in duration compared to vocalizations from the sham branding treatment. Watts and Stookey (1999) highlighted the fact that not all animals in the hot-iron treatment vocalized, and conversely some animals in the sham branded treatment did vocalize. Therefore, they concluded that using vocalization analysis on individual animals may not be a reliable indicator of welfare and suggested that using group averages of vocalizations would be a more accurate representation of welfare.

Contribution: This study contributes to previous findings that vocalizations provide important insight into cattle condition and welfare. Watts and Stookey (1999) add to this research by concluding that vocalization associated with distress have a higher frequency, amplitude, and frequency range. Another important result they add to the field is that vocalizations should be interpreted for the group treatment, not on an individual level, as not all cattle vocalize while experiencing distress. I included this article because it supports the idea that vocalization is reliable method of assessing welfare and it is not invasive for the cattle.

Reducing Vocalization

Grandin, T. (1998). The feasibility of using vocalization scoring as an indicator of poor welfare during cattle slaughter. *Applied Animal Behaviour Science*, *56*(2–4), 121–128. https://doi.org/10.1016/S0168-1591(97)00102-0

Summary: This article evaluated cattle vocalization scoring as a simple objective method to indicate welfare problems in slaughter plants. Grandin (1998) observed six federally inspected slaughter plants and scored 100-250 cattle per plant. In experiment 1, cattle at each plant were counted as they were moved through the forcing pen, leadup race, and stunning box, and were recorded as either a vocalizer or non-vocalizer. The vocalizations were associated with the aversive event that happened immediately prior to the vocalization, such as electric prodding, slipping on the stunning box floor, missed captive bolt stuns, or excessive pressure exerted on the animal's body by a restraining device. These results showed that 98.2% of vocalizations occurred immediately after an aversive event and 64% of the vocalizations were a result of electric prodding. The average vocalizations of cattle at plants 1-4 was 4.5%, and 22% at plants 5 and 6 which were considered to have "excessive prod usage". In experiment 2, plants 5 and 6 were instructed to tap the cattle on the rear and only use the electric prod if the cattle would not move after being tapped, the objective was to see if reducing electric prodding would reduce vocalization. The results of experiment 2 showed that in plant 5 average cattle vocalization decreased from 32% to 13%, and in plant 6 it decreased from 12% to 3%. It is important to note that using the more humane instructions did not impact plant efficiency, as the hourly line speed in plants 5 and 6 remained the same throughout both experiments. This study is significant as it showed that vocalization scoring can be used to objectively evaluate animal welfare. It shows that a high average percent of vocalizing cattle, and high numbers of vocalizations associated with aversive events, indicate welfare concerns.

Contribution: This article solidified previous findings that vocalizations can be used as an indicator of pain by using a simple objective method (vocalization scoring) to indicate specific welfare problems. Vocalization scoring does not require sophisticated equipment making it a practical method for evaluating welfare. Grandin (1998) provided new insight by showing that humane methods of handling significantly reduce vocalizations without decreasing efficiency of the plants. I included this article because it reveals that the most important component of animal welfare is the behavior and attitude of handlers, and therefore we have complete control over the ability to improve cattle welfare.

Grandin, T. (2001). Cattle vocalizations are associated with handling and equipment problems at beef slaughter plants. *Applied Animal Behaviour Science, 71*(3), 191–201. https://doi.org/10.1016/S0168-1591(00)00179-9

Summary: Previous research has shown that vocalization scoring associated with aversive events can be used to identify handling and equipment problems that are negatively impacting animal welfare. The objective of this article was to determine total number of cattle that vocalized during handling procedures and reduce this percentage by improving the primary welfare concerns at each slaughter plant. Grandin (2001) collected data on 48 different slaughter plants and audited them based on the ability to move cattle with less than 3% of all cattle vocalizing; this threshold is based on the criterion presented in the American Meat Institute Guidelines. 100-500 cattle were scored at each plant with a variety of cattle being observed such as, Holstein, beef breed cows, fed steers heifers, and more. The results showed that 32 plants had less than 3% of cattle vocalizing during handling procedures. The majority of these plants had no observable equipment problems, no slipping occurred on the stunning box floor, and there was no excessive pressure applied to cattle by restraint equipment. In the 20 plants where vocalization only occurred in 0-1% of cattle, there were no distractions, lighting issues, or visual cliff problems that could make cattle baulk. 14 plants had 4% or more of cattle vocalizing, with 4 plants having over 10% of cattle vocalizing. Plants that failed the audit (had more than 3% of cattle vocalizing) had easily identifiable equipment problems including; repeated electric prodding, excessive pressure applied by a head restraint, visible moving equipment, no false floor in restrainers, and lighting issues. Based on these results, modifications to the previous noted equipment problems were made in 5 plants, and the average vocalizations for these 5 plants combined was reduced from 12.8 to 0.8%. Grandin (2001) indicates that vocalizations of 3% or less of cattle is an attainable standard for all plants.

Contribution: This article supports the criteria proposed by the American Meat Institute Guidelines that 3% or more of cattle vocalizing is a welfare concern. Grandin (2001) even suggests that this is minimum standard, and, in most cases, vocalization can be reduced further. I included this article because it shows how simple modifications to equipment and plant protocols are able to drastically improve cattle welfare. The results of this study further indicate the importance of vocalization when it comes to determining cattle welfare and encourages further investigation into this field.

Simon, G. E., Hoar, B. R., & Tucker, C. B. (2016b). Assessing cow–calf welfare. Part 2: Risk factors for beef cow health and behavior and stockperson handling. ***Journal of Animal Science****,* ***94*****(8), 3488–3500. https://doi.org/10.2527/jas.2016-0309**

Summary: Simon et al. (2016b) conducted an epidemiological study to identify welfare concerns for cattle. The authors observed 30 cattle operations in California that used a chute for cows or heifers during handling. The ranches were visited for 1 day and a total of 3065 cattle were observed between the 30 ranches. The authors used questionnaires and interviews to assess ranch characteristics and management, as well as the producers' perspectives toward their cattle. Individual cows were assessed for body condition, lameness, and any visible health concerns. Stockperson handling was observed, and the researchers recorded electric prod use, number of mis-catches, the occurrence tail-twists used in handling the cattle. For the purpose of this review, I focused on their results regarding cattle behaviour in the chute, where they focused on whether the cattle baulked, the number of vocalizations, and how often they stumbled or fell. The results of their study showed that when a hydraulic chute was used for handling vocalizations increased by 166%, they also noted that vocalization increased by 3% for every 1m increase in alleyway length. Another notable result was a decrease in vocalization by 42% for every additional handling event experienced by the cattle per year. From this study, the authors determined that in order to decrease cattle vocalizations, cattle should be handled 2 to 3 times to reduce agitation. They suggested decreasing alleyways to 12 to 15m to reduce stress behaviour such as vocalization. Since the use of hydraulic chutes decreased the rate of stumbling and falling, Simon et al. (2016b) recommended experimenting with adjusting the pressure applied by the hydraulic chute. The assessment of cattle behaviour in a chute allowed for researches to suggest key areas for future improvement to reduce cattle vocalization, resulting in an increase in welfare.

Contribution: This article contributed to knowledge on cattle behaviour and was able to identify welfare concerns and give recommendations for improvement based on observed situations that caused cattle vocalization. I included this article as it provided a new perspective on the relationship between facility design and the resulting response of the cattle. This study provided important results that any facility can use to improve the welfare of their cattle and allows adjustment to current regulations and recommendations for facilities.

Further Research

Simon, G. E., Hoar, B. R., & Tucker, C. B. (2016a). Assessing cow–calf welfare. Part 1: Benchmarking beef cow health and behavior, handling; and management, facilities, and producer perspectives. *Journal of Animal Science*, *94*(8), 3476–3487. https://doi.org/10.2527/jas.2016-0308

Summary: Simon et al. (2016a) measured cattle health and welfare outcomes in order to create a welfare assessment program. They conducted research at 30 Californian ranches and observed a total of 3065 cattle. At each ranch, a portion of the herd was used to measure health, behavior, and stockperson handling. They specifically measured the occurrence of cattle baulking, electric prod use, weaning age, vocalization and more. For the purpose of my review, I am going to focus on the results regarding vocalizations and management perspectives about their cattle. The authors assessed management and producers' perspectives on cattle pain, health, and handling protocols through the use of questionnaires and observations. An important result found by this study that a majority of stockperson handlers disagree with the statement "animals experience physical pain as humans due". This solidifies the idea that further scientific research must be done so that handlers can be educated on what cattle behaviors are indicative of pain and stress, such as increased vocalizations. The authors were able to identify issues with facilities and ranch procedures through the percent of total cattle vocalizing being higher than 5%. However, due to the lack of knowledge on identifying cattle pain, there were simple welfare concerns that had been missed by management. They noted that in one of the biggest welfare concerns observed was the use of an electric prod that was used on an average of 23.5% of cattle, resulting in a drastic increase in cattle vocalization. The authors concluded that many of the welfare concerns could be addressed by a regulation of handler and management practices, as these varied widely throughout the ranches and did not always align with scientific recommendations.

Contribution: This article provided new research into the field as it showed how a criterion for welfare assessment program can be determined. I included this article as the research on management and handlers' perspectives is not commonly done, and this provides new results on how animals are perceived and treated by their handlers. These results allow us to determine if welfare concerns of specific ranches is impacted most by equipment problems or the attitudes and education of management and handlers.

Green, A. C., Johnston, I. N., & Clark, C. E. F. (2018). Invited review: The evolution of cattle bioacoustics and application for advanced dairy systems. *Animal, 12*(6), 1250–1259. https://doi.org/10.1017/S1751731117002646

Summary: In this article, Green et al. (2018) reviews the lack of current reliable research regarding cattle vocalization analysis and highlights the importance of investigation into this field. The authors suggest that further research could greatly impact farming practices by indicating how cattle are responding to different farming practices such as; calf separation, slaughter practices, social ion, and husbandry procedures. The article reviews the source-filter theory which explains vocal production. Vocal parameters are influenced by larynx anatomy, which varies based on age, sex, breed, and more. The parameters are also influenced by the arousal states of the cattle, which impacts respiration and muscle tension. Therefore, cattle vocalizations can reveal a plethora of information such as; physiological state, age, breed, emotional state, and more. After discussing the type of information vocalizations can encode, the authors go on to review vocalizations associated with aversive events like calf separation and social isolation. In all cases of stress-related events, vocalizations increased, as well as other parameters of poor well were noted, these included; increased heart rate, salivatory cortisol, and changed in urination and defecation rates. Green et al. (2018) identifies that further research should focus on temporal and spectral characterization of cattle vocalizations associated with arousal. The authors also suggest research into determining the encoded information in vocalizations by determine heard responses to several different types of calls.

Contribution: This article contributes valuable information to the field by identifying the current gaps in knowledge surrounding cattle vocalizations, and how further research could allow us to encode the meaning behind specific calls. I included this article as the authors are focused on how individual cattle are responding to farming practices, and this is a new and different perspective on cattle welfare then previous articles. Green et al. (2018) state that further research on vocalizations will be able to give insight into current farming procedures and determine if welfare can be improved by pain alleviation or farm intervention.

Manteuffel, G., Puppe, B., & Schön, P. C. (2004). Vocalization of farm animals as a measure of welfare. *Applied Animal Behaviour Science*, *88*(1–2), 163–182. **https://doi.org/10.1016/j.applanim.2004.02.012**

Summary: In this article, Manteuffel et al. (2004) reviews why vocalization is linked to animal welfare, the modern techniques of sound analysis that can categorize vocalization, and future experiments necessary for vocalizations to be linked to correlated with welfare concerns. Different welfare concerns such as, emotionally relevant external events, thirst, hunger, separation/isolation, and pain, are all capable of producing behavior such as vocalization in cattle. In animals, a correlation between welfare and vocalization has been shown through use of an electric stimulus in restricted regions of the brain. This revealed that specific neural sites were responsible for different emotional vocalization. It has also been shown that injections of hormones and other drugs can elicit vocalizations in farm animals. This directly suggests that vocalizations are indicative of an internal state of stress or pain and therefore can be used to evaluate welfare. In order to categorize vocalizations, they must be subject to phonetic analysis to determine its meaning. This can be done through modern bioacoustics methods allowing for interpretation of vocalizations. Cattle vocalizations have undergone analysis and revealed that high-pitched tonal sounds signal fear, whereas harsh low-pitched vocalizations are correlated with aggression. From this, the authors hypothesized that specific vocalizations can be categorized and correlated with different welfare concerns, ultimately improving individual welfare. The authors proposed future experiments using neurobiological research, and pharmacological injections alongside bioacoustics analysis, to allow for categorization of vocalizations. This controlled experimental approach would allow for different vocalizations to be generalized and used to indicate welfare-relevant stressors.

Contribution: The article reviews the current understanding of cattle vocalizations and their meaning. It identifies that average scoring of total vocalizations in herds is used to identify poor welfare, however it suggests that categorizing vocalizations and correlating them with welfare concerns would be a more accurate indicator of welfare. This article was chosen as it explains the importance of conducting future research in a controlled setting, where different vocalizations can be associated with specific welfare concerns. If vocalizations can be well defined and classified, they can be applied to multiple environments such as, practical farming, transport, and slaughter.

Watts, J. M., & Stookey, J. M. (2000). Vocal behaviour in cattle: The animal's commentary on its biological processes and welfare. *Applied Animal Behaviour Science*, 67(1–2), 15–33. https://doi.org/10.1016/S0168-1591(99)00108-2

Summary: Watts and Stookey (2000) review the methodological approaches that have been used to study cattle vocalizations. The authors reviewed research where vocalizations are correlated with specific behavior to determine meaning, the response of a conspecific or herd t specific vocalizations, and interactions between the sender and receiver of several different vocalizations. The authors highlight that the majority of studies reveal the same conclusion that aversive or stressful events result in a higher rate and percentage of cattle vocalizing. They review other parameters that have been used to determine welfare, such as, heart rate and cortisol levels, but note that these results can occur due to positive or negative stimuli. Vocalizations on the other hand, have the possibility to indicate specific animal conditions. The provide results from a previous experiment indicating that isolated calves' vocalizations were shown to have lower frequencies compared to vocalizations from branded calves. However, the isolated calf vocalizations had a greater amplitude possibly indicating that an isolation call is used in an attempt to locate a herd, whereas a vocalization from branding occurs involuntarily due to pain. Since vocalizations have the possibility of encoding so much specific information, tea authors suggest that future research needs to focus on how vocalization is used as a tool in herds, social competition, reproduction, etc., in order to gain an understanding of how these calls differ.

Contribution: This article reviews the methodological approaches previously used to determine cattle welfare and explain through results of previous studies that cattle vocalization is able to provide the most specific and reliable information. Due to this, the authors have a unique perspective that individual calls can be analyzed to make specific predictions about a specific animals rather than a herd, I included this article because it shows that there is a controversy between whether vocalizations are more effective when conclusion are applied to the herd compared the an individual animal.