VOCAL BEHAVIOUR AS AN
INDICATOR OF
WELFARE IN CATTLE

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfilment of the Requirements
for the Degree of Doctor of Philosophy
in the Department of Large Animal Clinical Sciences
University of Saskatchewan
Saskatoon

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Spring 2001

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ABSTRACT

I investigated the use of vocal behaviour in cattle as an indicator of welfare. The first study (Chapter 3) investigated effects of restraint and branding on 189 beef calves. Branded animals gave more, higher, louder vocalizations than controls. Some unbranded animals vocalized, suggesting that restraint during branding may be aversive. Chapter 4 used 17 full-sibling families of calves (N=130), created using multiple ovulation and embryo transfer, raised by unrelated recipients. Behaviour was measured during visual isolation. Vocalizations varied due to sire and family, suggesting vocal characteristics are partly genetically inherited. Age, weight and sex also influenced responses. Chapter 5 evaluated 307 calves of 4 phenotypic breed groups during routine processing and visual isolation. Phenotype influenced amounts of vocalization, but not acoustic characteristics. More calves vocalized during processing and calls were acoustically different than during isolation. In Chapter 6, steers and bulls (N=119) were evaluated in visual isolation while other unseen cattle were present, or not present, within the handling facility. Proximity and vocal behaviour of unseen companions increases likelihood that an individual will vocalize. Bulls vocalized more than steers. Chapter 7 studied cow-calf pairs while separated and attempting to reunite, in extensive conditions. As time between nursings increases, so does probability that animals will vocalize. Usually both vocalized while searching, or neither. Vocalization probably indicates motivation to reunite, and may be part of their strategy to locate each other.

Measures of the amount of vocalization may be useful in welfare assessment, but should
be used alongside other measurements, and to indicate status of groups not individuals. Measures of acoustic properties are preferred. Vocalization of cattle in testing environments is influenced by genotype, phenotype, age, weight, sex, gender and presence and activities of companions. If not controlled, these factors could confound the effects on vocal behaviour of the conditions being evaluated.
ACKNOWLEDGEMENTS

I wish to thank the following for their direct or indirect contributions to this work:

My advisor, Dr. Joseph Stookey, for his bravery in taking me on as a graduate student,
Dr. Ian Horrell, for introducing me to the fascinating world of farm animal ethology, my
committee members, past and present, Drs. Ken Armstrong, Jan Smith, Harold Gonyou,
Iain Christison, John Campbell and Terry Carruthers. Dr. Sheila Schmutz, whose
creation of the Canadian Beef Reference Herd made possible the study reported in
Chapter 4. James “Barney” Hall and Becky Valentine worked long and hard out at the
community pasture, gathering the observations described in Chapter 7. The staff of the
University of Saskatchewan’s Beef Research Unit feedlot, in Saskatoon, for their care
and handling of the animals used for several of the studies in this thesis. Lisa Loewen
helped to check the reference list. I would like to thank several present and former lab
partners for all their help, whether in handling and observing animals, for their ideas,
encouragement and support, or for tolerating my junk all over the lab. They are: Coreen
Waltz, Dr. Karen Schwartzkopf-Genswein, Dr. Gerrard Flannigan, Derek Haley and
Jean Clavelle.

Funding of my graduate assistantship, and the studies described in this thesis was
provided by the Saskatchewan Agriculture Development Fund.

Most of all I would like to thank my wife Sheila and my son Harvey, for their love,
....and patience.
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<tr>
<td>ANN</td>
<td>Artificial neural network</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>d</td>
<td>Day</td>
</tr>
<tr>
<td>dB</td>
<td>Decibel</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>FGI</td>
<td>Frequency of greatest intensity</td>
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<td>ha</td>
<td>Hectare</td>
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<td>hr</td>
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<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>MMD</td>
<td>Movement measuring device</td>
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<td>mo</td>
<td>Month</td>
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<tr>
<td>OBS</td>
<td>Observing session</td>
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<td>P</td>
<td>Probability</td>
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1.0 INTRODUCTION

Animal welfare has been defined in a number of ways by different authorities. For some, these definitions reflect the experimental approaches of the definers. For example, the late Gary Moberg (1993), a physiologist, defined animal welfare in terms of the risk that an animal would fall into a pre-pathological state in which it would become susceptible to disease or other conditions which would reduce its fitness. Indicators of such a state would include changes in growth, health and reproductive fitness. While it is likely that conditions which would compromise an animal’s ability to fight off disease would also represent a threat to its welfare, I doubt that such vulnerability is truly a defining characteristic of welfare per se. This type of definition invites the production-oriented view that conditions in which animals remain free of disease and suffer no decrements in growth or reproductive performance are necessarily satisfactory from a welfare standpoint. I do not accept this as an adequate definition of welfare.

Donald Broom (1993) views animal welfare as ‘an animal’s state as regards its attempts to cope with its environment’. His view is pragmatic, in that it implies that psychological challenges, as well as threats to physiological functioning, are relevant considerations in discussions of animal welfare. Therefore it offers the prospect of a broader range of measures which could be relevant when considering an individual’s
welfare. Within this definition it is possible to view the incidence of abnormal patterns of behaviour, or significant deviations from a baseline behavioural time budget, as being indicative of changes in the animal’s approach to coping with its environment, and thus as possible indicators of compromised welfare.

The ethicist, Bernard Rollin (1995), has argued that animals have a species-specific nature, which he calls *telos*. For example, the telos of a pig would include the inclination to root in a malleable substrate to obtain much of its food, and to live within an appropriate social structure with other pigs. Production systems which deny animals the opportunity to live according to this telos would tend to pose a threat to their welfare. This view is commendable as an empathetic view of animal experience. The reality is more complicated. In the light of public concerns about animal welfare and the intensification of livestock production, and in part as a commercial gambit to exploit such concerns, there has been increased interest in production methods which do allow animals greater opportunities to engage in “natural” forms of behaviour. But these approaches are not unequivocal solutions to welfare problems. For example, the purchase of free-range eggs may satisfy the humanitarian urges of the fortunate consumers who can afford the privilege. But free-range systems are not chicken utopia. With the freedom to scratch in mud comes increased risk of disease and predation and different forms of social stress.

The most straightforward, and yet the most challenging view of animal welfare is that promoted by Ian Duncan (1993). This is simply that animal welfare is to do with what
animals feel. From this perspective, welfare is a subjective state, and therefore can only apply to organisms that have subjective experiences. Duncan illustrates this by subjecting putative welfare issues to the “pine tree” test. Briefly, this states that if a statement concerning the effect of adverse conditions on an animal could also sensibly be applied to a pine tree, then the problem is not a welfare issue. For example, to say that an animal is diseased is not a statement about its welfare, because a tree also could be diseased. Thus, the presence of disease is not a sufficient criterion to say that a welfare problem exists. But if that disease caused the animal to feel sick, or experience pain, then welfare is at issue because those subjective feelings cannot reasonably be projected onto pine trees. Boredom and frustration, meaningless for pine trees, could also be welfare concerns, even if the animal in question were otherwise functioning optimally as regards its physical health, growth, reproductive function and so forth. To reduce animal welfare to such a simple definition raises a real difficulty over what can be measured to assess it. The traditional physiological and behavioural measures, for example various blood parameters, heart rates or the occurrence of stereotypic behaviours, can, under this definition, only suggest that a welfare problem may exist. They cannot show that the animal is experiencing any unpleasant feelings. Finding a scientifically acceptable way to infer the existence of subjective states based on quantifiable and objective measurement and observation is a serious methodological challenge. Françoise Wemelsfelder (1999) has challenged the notion that judgements about an animal’s condition that invoke conscious processes and subjective experience on the part of the animal, are necessarily unscientific and anthropomorphic. Furthermore, she and co-workers (Wemelsfelder et al., 2000) have begun to investigate how the organisational
processes in the minds of human observers, who readily and reliably form such qualitative judgements, might be formally used in scientific studies.

Each of the schools of thought on animal welfare outlined above, will tend to have its own inherent biases toward certain properties of an animal that should be measured in order to evaluate its welfare. These range from health and performance data, through physiological parameters and more or less objective behaviour measures, to purely subjective assumptions about the animal's condition. Vocal behaviour seems interesting as an indication of animal welfare, because it can be interpreted at a number of levels of explanation. Vocalization may be viewed as a response to physiological events, or as the output of cognitive processes. These levels need not be exclusive. Vocal behaviour could therefore be considered an acceptable phenomenon to measure, by proponents of any of the aforementioned welfare approaches. As a communicative behaviour which has evolved under natural selection, vocalization is certainly adapted for the specific purpose of conveying information between members of a species. The same cannot be said of heart rate or cortisol responses. The important question is whether this information can be made available to eavesdropping human scientists. Vocal behaviour has quite regularly been reported in studies investigating the impact of environmental conditions on farm animals (for an extensive review, see Chapter 2). Usually it is presented alongside other types of measurements, and without any attempt being made to validate its use, other than the intuition that elevated rates of vocalization probably reflect a negative impact on the animal when observed in association with an adverse treatment. Few researchers have been interested in establishing what, if anything, vocal
responses in these situations actually tell us about the animal. One exception has been
the work of Weary and colleagues (Weary and Fraser, 1995; Weary et al., 1997, 1998)
on vocal response in pigs. Until the work described in this thesis began, little had been
done to investigate the proper use of cattle vocalizations as an indicator of their welfare.
Despite this lack of information, simple counts of the proportions of cattle vocalizing
during pre-slaughter handling and stunning had been proposed as a means to classify
slaughter plants as adequate or inadequate in welfare terms (Grandin, 1998), and have
now been accepted and put into practice by Macdonald's.

1.1 Thesis objectives
The major objective of this thesis was to evaluate the usefulness of cattle vocal
behaviour as an indicator of their welfare during the stressful procedures to which they
are subjected under human management. To do this it was necessary to incorporate
several broad areas of inquiry. Firstly, to discover what information already existed in
the public domain, that is relevant to this task. In particular to get some indication as to
what kinds of information are transmitted acoustically between cattle. Secondly, to
determine whether both quantitative and qualitative changes in vocal behaviour could be
measured in response to an acute, painful stimulus. Having noted that vocal response
could vary according to treatment, it was necessary to investigate other sources of
variability in vocalizations which might confound or obscure the effect of the
independent variable in typical experimental conditions, where measurement of vocal
behaviour might be desired. Finally, it was considered appropriate to study the
behaviour of free ranging cattle and their use of vocal behaviour to help accomplish their
ethological needs, in an extensive environment more similar to that in which the vocal behaviour evolved.

In Chapter 2, I present the main literature review section of this thesis. It begins with the premise that the vocalizations of cattle are probably meaningful to other cattle. The review goes on to ask what information cattle could exchange with each other using vocalization. The chapter also contains a discussion of the approaches and techniques which could be used in the study of vocal communication. Chapter 3 introduced the technique of computer-assisted sound spectrogram analysis, which was used, in this and later studies, to help quantify the acoustic variation in cattle vocalizations. This study compared the effect of hot-iron branding versus the degree of restraint required to perform branding on the amount and acoustic properties of vocalizations. Chapter 4 begins to explore sources of variation in vocal response which are due to endogenous characteristics of the individual, rather than the experimental treatment. In this study all animals were given the same treatment; a short period of visual isolation. The technique of multiple ovulation embryo transfer allowed the creation of “families” of full sibling calves, each of which was gestated and raised by different unrelated cow. Using this method it is possible to look for genetically inherited characteristics of vocal response, while controlling to large extent for the effects of learning from the dam. This chapter also looks at the influence of calf sex, age and weight on vocal characteristics. The study presented in Chapter 5 continues to evaluate the extra-experimental influences on vocal behaviour, by comparing the responses of pure and crossbred phenotypic representatives of Angus and Charolais cattle during handling and isolation. Chapter 6
investigates the contribution of the social environment to vocal responses, by looking at the effect of nearby, but unseen, social companions on the vocal behaviour of visually isolated cattle. In chapter 7, I present an account of observations of the vocal behaviour of free ranging cattle in an extensive community pasture. This documents the use of vocal behaviour by cows and their calves while separated and searching for each other.

The studies presented in this thesis will provide new information about the vocal behaviour of cattle in association with distressing events. It will show how vocalization could be used as a tool in welfare assessment, and demonstrate some of the limitations of this approach. It is hoped that this information will be used by other researchers to help them decide whether, how and under what circumstances, measurement of vocal responses should be used as an indicator of welfare in cattle.
2.0 VOCAL BEHAVIOUR IN CATTLE: THE ANIMAL'S COMMENTARY ON ITS BIOLOGICAL PROCESSES AND WELFARE

2.1 ABSTRACT

The vocalizations of cattle provide conspecifics with meaningful information about the caller. If we can learn how to interpret this information correctly, it could be used to improve management and welfare assessment. Vocalization may be viewed as a subjective commentary, by an individual, on its own internal state. The vocal behaviour of cattle is potentially a useful indicator of their physiological and psychological functioning.

The first part of this chapter examines what information is exchanged using auditory cues. Vocalizations provide information on the age, sex, dominance status and reproductive status of the caller. Calves can recognize their mothers using vocal cues but it is not clear whether cows recognize their offspring in this way. Vocal behaviour may play a role in estrus advertisement and competitive display by bulls. Under experimental conditions involving pain or social isolation, vocal response is useful as an indicator of welfare, if properly used. Unlike commonly-used physiological measures, it can be recorded noninvasively and varies on a number of quantitative and qualitative
dimensions.

The second part reviews methodological approaches to the study of vocal behaviour and their application in cattle welfare research. Methods may focus on the actions of the vocalizing animal and the conditions which elicit vocal behaviour, the response of an animal to hearing another's vocalizations, or interactions between sender and receiver.

It is argued that vocal behaviour in cattle may be valuable in welfare studies if the endogenous, exogenous and developmental factors influencing its expression can be more thoroughly investigated and understood.

2.2 INTRODUCTION

The working assumption in writing this chapter is that the calls of cattle reflect the biological status of the animal as it interacts with its environment. Cattle emit vocalizations that are probably meaningful to other cattle. They may signal the physiological and emotional state, motivations and intentions of the calling animal. Increasingly, in applied ethology, there has been interest in the development of objective measurements, and technologies that allow reliable quantification of behaviour, without observer subjectivity, bias or error (e.g. Schwartzkopf-Genswein et al., 1997; 1998). The study of cattle vocalization is an attempt to document an interpretive response to events which is a consequence of the perceptual, cognitive and emotional processes of the animal itself. It can be argued that vocalizations represent a form of commentary by an
animal on its own internal state. The study of vocal behaviour is therefore a potentially useful means by which to investigate the physical and psychological functioning of that animal.

The vocalizations of cattle represent, potentially at least, acts of communication. That is, they may convey information about the calling animal, even if they seem to occur as an involuntary response to environmental stimuli, or in contexts where conspecifics are not present. Communication can be defined as "The imparting of information from one organism to another in a way that evokes a detectable response from the recipient at least some of the time" (Wittenberger, 1981, pp. 613). Where communication has occurred there should be an increase in the probability of some relevant response (whether behavioural or physiological), being shown by the recipient. Therefore, if appropriate measures are employed, acts of communication should often be detectable.

Studies of communication in farm animals are not only practicable, they can make an important contribution to our understanding of the biology of the animals concerned. However, in contrast to the extensive literature for wild vertebrates, particularly birds (See, for example, Kroodsma and Miller, 1996) and primates (Cheney and Seyfarth, 1990), there has been limited research into auditory communication in domestic animals. Data on vocal behaviour of cattle are particularly sparse. It is surprising that they have not received more attention, since vocal responses can be recorded noninvasively, and analysed using simple equipment. Yet until recently there has been little emphasis on vocalization in cattle as a behaviour with potentially useful implications for animal
production or welfare studies. One review of the use of behaviour studies in cattle production (Stricklin and Kautz-Scanavy, 1984) did not mention vocal behaviour as an area of past or future interest in this regard. In their book "The behaviour of cattle", Albright and A rave (1997) did mention some aspects of vocal behaviour. However, their treatment was brief and did not deal with the questions raised in this review.

Recently, some researchers have become more interested in using vocal behaviour in farm animals as a way to evaluate their welfare. For example, the use of vocalizations by swine as indicators of pain (Weary et al., 1998) or need (Weary and Fraser, 1995). In cattle, the use of vocalization rate as a rule-of-thumb indicator of acceptable or unacceptable welfare standards in a slaughter plant has recently been suggested by Grandin (1998).

According to Griffin (1984, 1991), the key to understanding the cognitive processes and subjective experiences, or consciousness, of animals lies in a careful study of the ways in which they communicate. By one widely accepted definition (Duncan and Petherick, 1991), animal welfare is primarily (perhaps solely) concerned with how animals feel. Cattle may have much to tell us about their neurophysiological, affective and cognitive processes if we can learn to interpret their vocal behaviour correctly. Therefore studies of cattle communication are relevant in discussions of welfare evaluation.

This review examines the existing evidence on vocal communication in cattle. With reference to this work and relevant studies on other species, mainly farm animals, I ask
what kinds of information cattle are likely to be able to exchange with each other, based on auditory cues. I discuss ways in which researchers may be able to intercept some of this information and use it to obtain a better understanding of the animal and its responses to human management.

2.3 EVIDENCE FOR THE INFORMATION CONTENT OF CATTLE VOCALIZATIONS

Many of the characteristic vocalizations of cattle have been described verbally, by Schloeth (1961). He also reported the social context in which these were heard and imputed meanings to some of them. A variety of species-typical calls of cattle have also been reported by Kiley (1972). Kiley developed a phonetic classification of these sounds, supplemented, in some instances with sonagrams. She proposed functions for distinct types of vocalization though the evidence for these is not presented. But it seems likely that cattle differentiate between vocal sounds according to different criteria. Some of the call types defined by Kiley’s (1972) system, may be heard in almost any emotional context (i.e. fear, isolation, pain, frustration etc.). It is probable that cattle do produce distinct vocalizations which differ according to context or the emotional state of the individual, and that these calls have characteristics which differ between individuals. The evidence for this general hypothesis is discussed below. It is also possible that differences which make the calls distinctive to other cattle may sometimes be subtle, or even undetectable, to humans with untuned ears and crude measuring equipment. This may be especially true when sounds are considered out of their normal context and
without reference to concurrent visual, olfactory or tactile signals.

The fundamental frequency, or lowest harmonic, of the various calls of spontaneously vocalizing cattle, generally ranges between 50 Hz and 1250 Hz (Kiley 1972). The calls of beef calves during hot-iron branding (Chapter 3) had fundamental frequencies, on average, in the range 120 to 180 Hz, with spectrographically detectable harmonics up to 7 or 8 kHz, and peak sound densities in the range 350 to 420 Hz. Occasionally calls below 50 Hz have been recorded. In recent work (Chapter 4) vocalizations of newly weaned calves with fundamental frequencies as low as 31 Hz have been reported. This is within the 14 to 35 Hz range reported for the infrasonic calls of elephants (Payne et al., 1986: Poole et al., 1988). Low frequency sounds propagate extremely well through the atmosphere. Computer models predict that the infrasound calls of elephants can exceed 10 km in range (Larom et al., 1997).

The normal hearing range of cattle for a standard 60 dB tone is between 23 Hz and 37 kHz. The greatest sensitivity occurs at around 8 kHz (Heffner, 1998). Their sensitivity to sound at higher frequencies is considerably greater than that of humans. Cattle are thus sensible to environmental or animal noises that are inaudible to people. The extended frequency range may well allow them to detect and monitor the activity of predators at a considerable distance. Delpietro (1989) reports cases in which cattle showed defensive responses to the screams of vampire bats (Desmodus rotundus). Free-ranging cattle are apparently more successful at avoiding vampire bat predation than confined cattle, thus there may have been a selective advantage among the
ancestors of domestic cattle in being able to detect sounds higher in frequency than they were able themselves to produce. The full range is not used in intraspecific communication, since cattle do not appear to be capable of producing very high-pitched vocalizations. It has recently been shown by Heffner and Heffner (1992) that mammals use high-frequency hearing as an aid to localizing the source of sounds. The authors report that despite the sensitivity of cattle to high frequencies the orientation of the head towards a sudden noise is relatively inaccurate. However, directional movement of the ears may have been a more revealing indicator of their ability to localize sounds.

People who work with cattle know that they are responsive to human vocalizations. Waynert et al. (1999) observed behavioural and heart rate changes in response to recordings of the kinds of vocalizations often given by humans when attempting to move cattle. Animals were more responsive to human voice than to mechanical chute noises played at the same overall volume. It may be that sounds of a biogenic, or vocal origin have special significance (possibly as signals of danger) that transcends species boundaries. Cattle learn to come in from pasture for milking when called (Albright et al., 1966) and calves may learn to respond to their individual names (Murphey and Duarte, 1983). Thus they are capable of learning to associate specific referential information with distinct vocalizations even of another species. It would not be surprising if, as part of their normal development, they could learn to obtain a variety of information about herdmates based on their vocalizations.
2.3.1 Age, sex and dominance status

The vocalizations of any individual may provide conspecifics with important information about the calling animal. For example, male, female and young feral asses (*Equus africanus*) each produce distinctive sounds. These also vary with social context and territory-holding status (Moehlman, 1998). Similarly in cattle, just as they may be able to discern visually that a certain animal is say, an adult bull or a calf, the sound of the call may reveal the gender or age of a nearby animal, even when it is not visible. For the extinct aurochs (*Bos primigenius*), ancestors of domestic humpless cattle (Clutton-Brock, 1987), this may have been an important factor in maintaining spatial and social cohesion in the postglacial forests of pleistocene Europe. Even the rate of calling may be somewhat characteristic of a particular class of animals. For example, Hinch et al. (1982) reported that bulls vocalized more often than steers. However there was significant individual variability. Hall et al. (1988) analysed vocalizations of the Chillingham cattle, minimally managed descendants of a medieval population. In a study of sound spectra, they found that bulls, cows and calves each make characteristic calls. The voice of any individual must therefore undergo change during its lifespan. The calls of American bison (*Bison bison*) bulls also differ in frequency and duration from those of calves or cows (Gunderson and Mahan, 1980), being longer and of lower frequency. Hall et al. (1988) noted that the Chillingham bulls tended to call in response to the calls of other bulls, suggesting that they are capable of distinguishing the vocalizations of bulls from those of cows. They also found that the type of vocalization given was influenced by dominance rank. Koene (1997) reported that dominant bulls
gave longer calls.

2.3.2 Individual identity

The significance of any act of animal communication is dependent on its social context. Cattle are highly social animals. Individuals form distinctive relationships with others. The relationship between mother and offspring is an obvious example, but, where herd management permits, there are relationships between cows and successive offspring, as well as social partnerships between unrelated adults (Reinhardt and Reinhardt, 1981; Swanson and Stricklin, 1985). Such relationships are not possible without mutual recognition. If individual differences in vocalization are as discernable to cattle as they are to a listening human, then they may, potentially be used, at least in conjunction with other evidence, as clues to an individual's identity. The relevance of a call to any particular listener may well depend on the social relationship of that animal to the caller. For example, the voice of a cow ought to be more significant to her own calf than it is to other calves. If the calf is capable of discriminating the voice of its own dam from all other cattle voices, then we should see a preferential, and probably adaptive response to hearing that voice.

In sheep, vocal recognition has been studied by Shillito Walser et al. (1981, 1982, 1983). In playback experiments (1981) they found that ewes of three breeds bleated more in response to the recordings of their own lambs than to alien lambs. A later study (1982) showed that lambs are likewise able to recognise their own ewe by voice. Arnold et al.
(1975) report that recognition of the mother's voice by lambs occurs within 7 days and that lambs use auditory cues to locate their dam thereafter. Dwyer et al. (1998) note that ewes' lamb-directed vocal behaviour may be influenced by breed and maternal experience. Domestic sows give acoustically distinctive nursing grunts which may allow them to be distinguished by piglets (Blackshaw et al., 1996).

Individually distinctive calls in cattle have been noted by several researchers (e.g. Kiley, 1972; Hall et al., 1988; Koene, 1997). Visual recognition by Bos indicus calves of their mothers has been investigated by Murphey et al. (1990). When these calves made errors of identification, the cows that they approached often resembled their dams in coat colour. The authors proposed that these calves use elements of their mothers' phenotypes including their colouration and possibly their odour to identify them. The errors that they make suggest the use of something like a phenotype-matching algorithm, with which they select the best available "mother" candidate from available cows in their vicinity. It is likely that matching criteria would include elements of the mother's behaviour, including her vocal behaviour.

The first experimental evidence that cattle are capable of individual recognition by voice has been provided by Barfield et al. (1994). They found that 3 to 5 week old calves were able to discriminate a played-back recording of their own mothers' voices from the voices of another cows. Thus for calves of this age it appears that auditory cues alone are sufficient for maternal recognition. There is as yet no comparable evidence that cows can recognise their calves by sound. A report that the recorded calls of a single
calf stimulated an increase in milk yield from a group of 16 dairy cows (Pollock and Hurnik, 1978) suggests a lack of individual recognition. Anecdotal sources seem to agree that undisturbed newborn calves hiding in a natural environment seldom vocalize. Thus auditory cues for recognition may not be available to cows in the immediate post-partum period. Barfield et al. (1994) were unable to secure enough recordings of calf vocalizations from 2 week old animals for a playback experiment because they occurred too infrequently. Thus they were unable to demonstrate whether cows could recognize the voices of their offspring. It is not always the case that mother and offspring are equally capable of recognizing the other by sound. For example in red deer (Vanková et al., 1997) calves recognize their mothers' voices better than hinds can recognize their calves' calls. It may turn out to be a general rule that offspring more readily learn to recognise their mothers' voice than vice versa, even in more vocal species. This may be due in part to the time taken for individual voice characteristics to develop in the young. For example, the vocalizations of goat kids show little acoustic individuality during the first few days of life, and their mothers do not learn to identify them by sound until after this time (Lenhardt, 1977). For hideer species, like cattle, it may be advantageous for the young animal to emerge from hiding in response to its mother's call, as is the case in sable antelope (Thompson, 1996), rather than exposing itself to predation by calling to solicit her attention. This might help to explain why there might be a specific selective advantage to calves in being able to recognize the voice of their dam.
2.3.3 Reproductive status

In adult cattle, calls may give information about reproductive fitness or estrous condition. Fraser and Broom (1990) report that female cattle and goats "... employ vocalizations in oestrus, presumably to summon and maintain the attendance of the male". However they cite no evidence to support either the contention that vocal behaviour does change during estrus in these species, or their hypothesis concerning its function. Blaschke et al. (1984) found that artificially impairing the hearing of bulls did reduce their ability to detect estrus in heifers. The authors felt that hearing was less important than sight and olfaction in the detection of estrus. However, their trials were conducted in small pens where proximity would ensure that visual and olfactory stimuli were particularly strong. In an extensive pasture environment, where other cues are attenuated, hearing could be much more important. Here sound might be the earliest indication of a female's status to an approaching bull. Unfortunately, there is no evidence to show whether the vocalizations of cows in estrus are distinctive in form or in their frequency of occurrence, or whether other cattle are particularly responsive to them. Berger and Cunningham (1991) report that male bison bellow more on days when females are in estrus. These calls seem to function as displays aimed at other males as they only bellowed before and after copulation when rival males were present. Vocal displays, such as these, might also serve as a positive feedback mechanism, a form of autocommunication, which enables an individual to raise its own emotional and motivational state to an appropriate state of arousal for a highly competitive activity.
The word "autocommunication" is normally used to describe an animal's use of its own signals to extract information about the external environment. Examples include echolocation in bats and cetaceans and electrolocation in some fish (Bradbury and Vehrenkamp, 1998, pp.345-348 and pp.851-882). If animals are capable of a physiological response following their own signals or the act of vocalizing, then this also could appropriately be termed autocommunication. There is no direct evidence that animals do use vocalizations for the sole purpose of modulating their internal state. However, given the complexity of interaction between cognition, physiology and behaviour, there might be instances when vocalizations precede and facilitate changes in sympathetic activity. This phenomenon certainly exists among human athletes (Hardy et al., 1996) who employ a range of behaviours, including vocalization, and cognitive strategies intended to manipulate their own arousal state to a level where optimal performance is possible.

Vocalizations of males may influence the reproductive status of females. In red deer, the playing of recordings of stag vocalizations to hinds had the effect of advancing their date of ovulation (McComb, 1987). Veeckman and Odberg (1978) found that recordings of the vocalizations of courting stallions influenced the behavioural expressions of estrus in mares. However in a more recent study, McCall (1991) did not show this effect. Usually females will be subject to several kinds of possibly-stimulating cues. It is not always clear whether auditory, tactile, visual or auditory signals are effective alone in eliciting changes in reproductive state, or only in combination and within the right context. Signoret (1970) and Pearce and Hughes (1987a) have suggested that auditory cues from
males may have some influence on the expression of estrus in female swine. However
Tilbrook and Hemsworth (1990) reported that exposure of gilts to male stimuli did not
affect their sexual behaviour. Pearce and Hughes (1987b) found that exposure to a boar
accelerated the onset of puberty in gilts, whereas exposure to a castrated male with
recorded auditory cues and artificially-presented olfactory stimuli did not have this
effect. If auditory signals do influence pubertal onset in swine it is likely only in
combination with a variety of other subtle signals. It would be interesting to investigate
these areas systematically in cattle. In particular the idea that characteristic vocalizations
might serve as a further behavioural indicator of estrus, or that estrous responses might
be stimulated by the sound of a vocalizing male could be of practical value to producers.

2.3.4 Fitness or need for resources

The vocalizations of infants of some species, apparently including humans (Furlow,
1997), may primarily indicate the phenotypical fitness of the caller. The frequency and
intensity of piglet vocalizations (Weary and Fraser, 1995) has been invoked as a signal
of need for the sow's resources (i.e. of relatively poor condition). The theoretical concept
of "honest signalling" (Maynard Smith, 1991, Johnstone, 1998) suggests that the calls of
such distressed animals will usually evolve to be reliable reflections of the animals'
condition. Weary and Fraser (1995) indicate that variations in the vocal responses of
piglets seem related to their degree of need, and are therefore consistent with this model.
No comparable studies are available for cattle. Calves do vocalize frequently following
forced separation and weaning. These vocalizations may convey specific information
about the experiences of the animal concerned (hunger, isolation, separation, fear etc.), or else may express a general degree of negative feeling about the situation it is in. The extent to which these calls may be regarded as honestly indicating need, as they appear to do in pigs, is not known. As cattle are usually born singly, they only have to compete for the dam's resources with the dam herself (and arguably with her unborn offspring, if she becomes pregnant again). Since they will have evolved neither to compete nor to cooperate with many siblings, one might expect a tendency toward a more "selfish" strategy of attempting to maximise their acquisition of maternal resources.

2.3.5 Expression of pain

Vocalization may well be a good behavioural indicator of pain. It is not neccessary here to discuss the differences or similarities in subjective experiences, including pain, in humans and other animals. This question has been debated extensively elsewhere (e.g. Nagel, 1974; Bateson, 1991). For the purposes of this article it is assumed that domestic animals do feel pain as an "unpleasant sensory and emotional experience" (Bateson, 1991) and that if this is severe or prolonged it is to the detriment of their welfare.

The proportion of animals vocalizing in response to painful procedures have previously been reported for cattle. For example, responses to hot-iron and freeze branding, including vocalization, have been documented by Schwartzkopf-Genswein et al. (1997, 1998) and Lay et al. (1992 a,b). In general these studies suggest the straightforward conclusion that treatments assessed as more painful, on a variety of behavioural and
physiological measures, tend to elicit vocal responses from a higher proportion of animals experiencing them. Vocal response is potentially a more revealing source of information about an animal's experience than other measures commonly employed as indicators of pain or distress. Heart rate or cortisol levels, for example, can only vary along a single dimension from low to high. This may give information about the relative aversiveness of procedures. But either measure may increase in response to both pleasant and unpleasant stimuli. Vocal response can vary along a number of dimensions, both quantitatively and qualitatively. In Chapter 3 it is reported that a painful stimulus (hot-iron branding) evokes vocalizations that are different in terms of several measurable acoustic parameters, as well as being more numerous, than those occurring during a painless control treatment. Characteristic vocalizations, among other behavioural changes, may be used as indications of pain in various experimental animals (Merton and Griffiths, 1985). Weary et al. (1998) have suggested that a greater rate of high frequency calls by piglets is a reliable response to the pain of castration. Their work also shows that even within a procedure such as castration, vocal response may help to indicate which parts of the operation are most painful.

2.3.6 Isolation

An animal's vocal behaviour may also provide an index of its response to psychosocial challenges such as social isolation. All domesticated livestock are social animals. Although individuals of some species may sometimes seek isolation for specific purposes (e.g. parturition), artificially imposed separation from companions is expected
to be a distressing experience. An individual's response to such treatment may consist of
behavioural and physiological changes associated with fear, attempts to regain the
company of conspecifics, or both. Consequently, vocal responses of domestic animals
during enforced isolation may be either a general or situationally-specific distress
reaction or an adaptive attempt to communicate with fellows. Arguably, the same
vocalization may fulfil both functions. The tendency to vocalize during social isolation
may well be influenced by previous social experience and rearing conditions. Warnick et
al. (1977) found that group-reared dairy calves vocalized more frequently during an
open field test than those raised in isolation or in individual hutches. Dairy goats
(Carbonaro et al., 1992) give more frequent and intense vocalizations during isolation
and showed elevated plasma norepinephrine levels. Three-week-old lambs bleat more
frequently following maternal separation when they are held in isolation. When provided
with an agemate companion, they vocalized less if the companion was their twin than if
it was an unfamiliar lamb (Porter et al., 1995). Cattle also vocalize more frequently
when visually isolated from companions (Hopster et al., 1995; Boissy and Le Neindre,
1997; Watts et al., 1998). These vocalizations may be acoustically distinctive, even
possibly diagnostic of a psychophysiological state of fear, but this has not yet been
demonstrated.
2.4 APPROACHES TO THE STUDY OF VOCAL COMMUNICATION

2.4.1 Ethological field studies.

Observation and detailed descriptions of the spontaneous activities of animals under extensive conditions have contributed a good deal to our knowledge of their social behaviour. Scott (1967) wrote that "Such a descriptive study inevitably leads to the conclusion that the members of an animal society communicate with each other". Of special interest to applied ethologists is research on free-ranging domestic animals, feral livestock and closely-related wild species. Unfortunately, few such studies have looked in any detail at vocal behaviour.

Moehlmann (1998) studied two populations of feral asses (*Equus africanus*) in the United States. She used field recordings, later analysed in the laboratory, and field notes to develop a detailed description of vocal behaviour in this species. The clear advantage of a study of this kind is that the social context of the vocalizations can be observed and is not influenced or constrained by management conditions.

Schloeth (1961) studied the social behaviour of the semi-wild Camargue cattle under extensive conditions. His description of the vocal behaviour of these animals was purely qualitative. Based on prolonged observations of these cattle, Schloeth clearly felt that he had attained sufficient insight to be able to assign meaning to some bovine sounds. Reexamination of descriptive, anecdotal monographs such as this may be valuable to
contemporary researchers in that they can be a resource to inspire new thinking and from which to generate hypotheses. Certainly Schloeth's work has been drawn upon and cited by others such as Kiley (1972) and Hall et al (1988) who have conducted more systematic analyses of cattle vocal behaviour.

Hall et al. (1988) studied the Chillingham cattle in England. Although the park in which these cattle are kept is only 134-ha in area (Hall, 1989), they are an interesting population for this type of study, because they have been subject to very little management for a long time. Therefore the herd has what probably approaches a "natural" sex ratio and age distribution. This provides an opportunity to study vocal behaviour in a similar social context to that in which it evolved. The context-dependent variability in vocal behaviour under these conditions, while presumably not being indicative of welfare per se, may give clues as to how vocal responses might be interpreted as indicating good or poor welfare under experimental conditions.

Studying how vocalization is involved in social cohesion of the herd, defense against predators, social competition, mother-young relationships and reproduction in groups which conduct these activities with little human interference remain relevant objectives for future observational studies. Such knowledge contributes to a more complete evaluation of the impact of different management systems on the expression of normal behaviour.
2.4.2 Studies of vocal production (behaviour of the animal as sender)

Communication requires, by definition, both a sender of information and a receiver by whom the information may be used. Most studies in which vocal behaviour of cattle has been reported have given information only about the sender. These studies make it possible to identify the external forces acting upon an animal that will cause it to vocalize. They allow us to compare levels of vocal response with other behavioural and physiological changes associated with external events which act on the animal. At a higher level of sophistication the acoustic structure of vocalizations can be described and compared in different situations. Qualitative variations in vocal response may be observed in association with changing levels of stress hormones (e.g. Schrader and Todt, 1998). However, without reference to their effects on the behaviour of an audience they can tell us little (logically perhaps even nothing) about their meaning. Nonetheless, studies that focus on the vocal activities of the sender may still be important in welfare evaluation.

The simplest use of vocal response seen in the literature occurs in the form of anecdotal reporting. For example, Hudson and Mullord (1977) note that in one experimental situation cows "vocalized continually" and in another they "seldom vocalized". In this case the vocalization was implicitly reported as an indication of distress, but the response was not quantified.

A slightly more sophisticated analysis is possible when one knows how many animals
out of a treatment group vocalize when the treatment is applied. Grandin (1998) has used this approach to compare the welfare of cattle in different slaughter plants. The general premise is that the greater the proportion of animals that vocalize during pre-slaughter handling and stunning, the more pain or fear they are probably experiencing. Grandin advocates this method as a simple way to detect welfare problems. All else being equal, this would seem a reasonable approach. There are a number of intrinsic factors including breed (Chapter 5), age and sex (Chapter 4) which may influence the probability that an animal will vocalize during a certain treatment. Extrinsic factors also, including the proximity and numbers of conspecifics and humans, previous experiences and so forth, probably influence the propensity to vocalize in more or less subtle ways. Also it is usually the case that, given an experience of a certain severity, some animals will vocalize and others will not, even when identically treated. Obviously one can not say, in relation to administration of an electric shock, for example, that it was painful to those animals that vocalized but not to those that remained silent. Because of this latter difficulty, it is suggested (Chapter 3) that measures of the probability of vocalizing or the proportion of animals vocalizing should be used to make inferences about the treatment group as a whole rather than implying very large differences in the experience of individuals within that group. This type of reporting is of greater interest as supporting evidence when presented alongside other ostensible measures of distress, with which the vocal response tends to covary in a consistent way (e.g. Schwartzkopf-Genswein et al., 1997).

The number of calls given during the treatment period, the rate of calling, assuming a
standardised treatment duration (as, for example, in Chapter 3), and temporal relationships (e.g. Berger and Cunningham, 1991) can give additional information that is not obtainable from simple reports of the occurrence or non-occurrence of a vocalization. It may be more appropriate to draw conclusions about individuals based on this level of analysis. Comparisons of the average number of vocalizations given by animals between treatments, without any data about individual responses (e.g. by Price and Thos, 1980) have similarly restricted applicability, since the standard deviation or standard error of the mean offers only a crude indicator of the within-group variability. Often, there will be a wide range of individual responses, with many animals remaining silent or vocalizing only once, an intermediate group of responders who call several times, and a few who vocalize almost continually. Where only numerical counts of vocalization are practicable, a more useful approach might be to develop a simple classification of subjects as low, intermediate, high or non-responders and compare the proportions of each between treatment groups.

Individual vocalizations may be tape-recorded which allows for subsequent measurement in the laboratory. By playing tapes through an oscillograph, Shillito Walser et al. (1982, 1983) were able to investigate the timing of sheep bleats. This technique also allowed them to study the temporal relationships between the calls of ewes and their lambs (see below). The acoustic components of calls can be displayed graphically in various ways which allow for objective measurement. Older studies (e.g. Kiley, 1972; Gunderson and Mahan, 1980; Hall et al., 1987) have used dedicated electronic devices such as a sound spectrograph or frequency analyser to generate
audiospectrograms or power spectra from recorded calls. Modern personal computers enable such work to be done more cheaply and flexibly than before (e.g. Blackshaw et al., 1996; Weary et al., 1998; see also Chapter 3 in this thesis). It is a technically straightforward (though time-consuming) task to describe animal vocalizations in terms of a handful of measurable acoustic features. Most modern PCs come equipped with a standard sound card with an input capability which is quite adequate for digital sampling of audio recordings. Software is readily available which can generate audiospectrograms and power spectra from the digital files. Analyses can be carried out successfully on recordings of indifferent sound quality. Equipment need not therefore be expensive or elaborate and useable recordings can be obtained in diverse and acoustically cluttered environments.

2.4.3 Neuroethological approaches

The neuroethology of sensory perception and cognition in domestic livestock is an area with considerable potential which has been explored rather little to date. A notable exception is work on visual cognition in the sheep (Kendrick and Baldwin, 1987). Single cell recording from areas of the sheep visual cortex was used to explore the neurological response to visual images. The sheep visual system (if not consciously the sheep itself) responds differently to familiar and unfamiliar sheep's faces and the presence or absence of horns. Parallel studies are possible on auditory image processing. However this approach has not yet been used with farm animals. Uetake et al. (1996) have recorded gross event-related electrical activity in the auditory pathways of calves. They used the
evoked auditory brainstem response to an electronically generated tone simply as an
indication of which sound frequencies the auditory system was capable of processing.
Recently Jarvinen et al. (1998) found that pigs raised in an outdoor environment
developed differences in neuronal morphology compared with pigs raised indoors. They
examined cells in the somatosensory, visual and auditory cortices and found differences
in all areas. Neurons from the auditory cortices of outdoor-reared animals had more
dendrites with greater numbers of dendritic spines. This may have been an adaptation to
the greater acoustic complexity of the outdoor environment, though the sound properties
of the two environments were not formally compared.

Computer simulation approaches may also be used in studies of animal communication.
It has recently been demonstrated that artificial neural networks (ANNs) can learn to
recognise the voices of individual deer (Reby et al., 1997). Studies of this kind may
become an important means to test hypotheses concerning the neurological mechanisms
subserving individual auditory recognition. Xin et al. (1989) has shown that it is possible
for pig vocalizations to be classified by acoustic features which seem to occur
consistently within specific contexts such as nursing, farrowing or anticipation of food.
It may also be possible to use ANNs as a means to classify digitized sound recordings
into discrete categories based on consistent context-dependent characteristics. This could
enormously increase the efficiency of a valuable, but extremely tedious and
time-consuming method of investigation. While there seems little doubt that ANNs
could be used to group together calls which sound the same, a greater challenge would
be to incorporate contextual information alongside the auditory data at the input stage in
an effort to achieve a classification of calls that effectively mean the same. The use of computers to compare power spectra of digitized cattle vocalizations has been attempted by Jahns et al. (1997) in an effort to assign vocalizations to common categories according to the context in which they were produced. The intent of their work is to try to develop a technique for monitoring the internal state of an animal, for example its need to be milked, or hunger, based on variations in its vocalizations.

2.4.4 Responses to heard vocalization (behaviour of the animal as receiver)

The natural neural networks of cattle are continually at work analysing and organizing representations of the external world based on sensory inputs which include auditory information. In experimental situations there are numerous options for manipulating these inputs. What is required is an effective means by which cattle can be coerced into reporting the results of this organizational process. In other words, are we able to observe specific responses to a heard vocalization that indicates something about the significance of that particular sound? It is probable that the best evidence for the meaning of a call is to be found by observation of an animal's response to hearing it.

One possible reaction to the vocal behaviour of another animal, is of course, to vocalize, thereby initiating an exchange of calls. In this situation, one might consider the role of an individual as alternating in time between acting as "sender" at one instant and "receiver" at another. However, for the purpose of this discussion, participation in such interactive situations is looked at mainly from a receiver's perspective. In other words, as
a (possibly adaptive) response to hearing the vocalizations of the initiating animal.

One possibility for evaluating an animal's response to heard auditory stimuli is through the use of operant conditioning techniques. The use of operant studies on farm animals has been reviewed by Kilgour et al. (1991). The premise is simple. The animal is trained (and farm animals, in general, have proven quite tractable subjects for this purpose) to respond to a chosen stimulus by acting on a device in the testing environment. Within the auditory realm this technique has chiefly been used to investigate the psychophysics of hearing itself. For example operant techniques have been used to estimate auditory thresholds to different sound frequencies by Uetake and Kudo (1991) and also by Heffner and Heffner (1992).

A more common method is to observe the behavioural or physiological changes which may occur in association with the presentation of a vocal stimulus. The rebroadcasting, or playback, of recorded signals to animals is a technique which has been used in studies of animal communication for many years and is now increasingly being attempted with domestic animals. Playback offers the means to present a sound in isolation, in a repeatable fashion and in a controlled environment. This can be seen as a simplifying advantage in experimental design. However, much animal communication is probably multimodal, combining, for example, sound with posture or visible display. There may well be reason to question the validity of findings based on observations of an animal's response to a single-channel signal, presented without its normal context. Use of insufficient examples of the sound in question in the playback schedule may
result in pseudoreplication, casting further doubt upon findings (Kroodsma, 1989).

Despite these problems, playbacks have been used in several of the studies cited earlier in this review (e.g. McComb, 1987; McCall, 1991; Weary and Fraser, 1995; Vanková et al., 1997). The first use of playback of cattle vocalizations appears to be the study of Barfield et al. (1994) on auditory recognition. Weary (1992) has discussed the use of playback stimuli in conjunction with operant responding as a means to investigate song perception in birds. This interesting approach ought to permit a researcher to demonstrate something about an animal's cognitive categorization of sounds and its ability to discriminate between categories. This technique has not yet been applied to farm animals.

2.5 CONCLUSIONS, IMPLICATIONS AND FUTURE RESEARCH NEEDS

Vocal behaviour in cattle is poorly understood. Yet intuitively, it is presumed to reflect the affective state of the animal and perhaps to convey important information between animals. For these reasons, and perhaps also due to the ease with which it can be observed, vocal response has been reported a number of times alongside other behavioural and physiological measures considered to be indicative of their wellbeing. The assumption that increased rates of vocalization indicate an unpleasant experience is supported only by the frequent (but not invariable) covariation with these other measures. We have no independent evidence as to what a vocalizing bovine is actually saying.
Cattle are commonly thought of as being among the least vocal domestic animals. In terms of the amount of spontaneous vocal behaviour they show under unconfined conditions, or the threshold level of human interference which would induce them to vocalize, this is probably true. Rather than dismiss vocalization in cattle as an infrequent and therefore unimportant behaviour, its very rarity suggests something about the biological significance of conditions under which they do vocalize. Conditions which have an unpleasant emotional component such as isolation, pain or fear do seem to be associated with elevated vocal responses.

It is conceivable, as Grandin (1998) proposes in relation to commercial slaughter operations, that simple comparison of the proportion of animals that vocalize during a procedure may provide a meaningful indicator of relative welfare. Statements concerning the acceptability or unacceptability of welfare levels between slaughter plants or any other comparison of this kind should be subject to a number of qualifications however. There may be considerable breed, gender and age differences in the likelihood of vocalizing during a particular handling operation (see Chapters 4 and 5). Failure to evaluate differences of this kind might leave us in the illogical position of concluding that identical operations carried out by the same personnel, in the same place, could be acceptable one day and unacceptable the next, depending on the type of animals that are being dealt with. Obviously endogenous factors influencing the propensity to vocalize should be investigated thoroughly if we are to appreciate the significance of any particular animal's vocal response.
There may be considerable intrinsic variations in the acoustic quality of cattle vocalizations. The calls of some breeds, for example, may prove to be subtly, yet measurably distinct from others. Vocal expression may be mediated (quantitatively and qualitatively) by learning, through exposure to the vocal behaviour of other cattle. The considerable literature on the development of bird song indicates that there is a complex interaction between innate tendencies and learning (See Catchpole and Slater, 1995, Chapter 3). It is likely that the development of vocal behaviour in cattle is influenced by the social environment and perhaps by the physical environment also (for example whether an animal is raised in extensive or intensive conditions). But no research has yet been carried out in this area. The social context in which vocal behaviour occurs is of considerable importance if we are to properly evaluate the significance of vocalizations as a tool in welfare assessment. Inevitably, different types of managed environments (for example a feedlot pen compared with a tie stall system) afford different opportunities for social interaction. Animals maintained in these systems for any length of time are probably going to use vocal behaviour differently. Use of vocal response as a welfare indicator should take into account not only the conditions applied to the animal at the time of testing, but also the management system from which they originate.

It is probable that vocal behaviour in most farm animals is influenced by the presence and activities of people. For example; both goats and sheep have been observed to show a greater vocal response to isolation from conspecifics when human observers were not present (Price and Thos, 1980). Similarly, Rushen et al. (1998) found that isolated cows milked in an unfamiliar room vocalized less while being brushed by a person, than when
the person was absent. Cows also defecated less and exhibited lower heart rates when
the human was present. Such vocal inhibition might indicate a reduction of fear, with the
animal accepting a human as a social substitute, or perhaps a tactic to avoid drawing the
attention of a potential predator. In either case human presence could be a confounding
factor in experimental designs involving the assessment of vocal responses.

Quantitative evaluation of vocal response is probably of scientific value, certainly in
welfare assessment. However more detailed analysis of the acoustic components of calls
is possible, and certainly preferable if one is to make inferences about the experience of
individual animals rather than populations. The acoustic structure of calls may give
important information about their "meaning" or function. For example, the calls of
isolated calves (Chapter 4) seem to have lower fundamental frequencies than those of
calves during branding (Chapter 3) and may be audible at a greater distance (Larom et
al., 1997). The same animal is capable of producing sounds with different acoustic
properties in visual isolation than while restrained in a headgate and squeeze for
processing (Chapter 5). This suggests that the use of the isolation call may be more
purposeful, specifically to contact other individuals, whereas the call of a branded animal
may be a relatively involuntary response to an extreme state of rage, fear or pain.

Efforts to use vocal response appropriately in welfare studies would benefit from greater
knowledge of vocal communication among cattle under extensive conditions. In
particular we need to know how vocalization is used as a tool in herd cohesion, predator
defense, social competition, mother-offspring interactions and reproduction. It would be

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most interesting to know which aspects of the bovine vocal repertoire pertain to the
conscious imparting and acquisition of information and which are more closely
associated with affect and sympathetic activity. Both kinds of processes probably occur
and modulate the other. The former allows us the potential to learn more about
cognition, consciousness and subjective experience in cattle, the latter may be a valuable
indicator of the psychophysiological impact of harsh treatment upon these animals.
3.0  EFFECTS OF RESTRAINT AND BRANDING ON RATES AND ACOUSTIC PARAMETERS OF VOCALIZATION IN BEEF CATTLE

3.1  ABSTRACT

An animal's vocalizations may be a useful reflection of its subjective state of welfare. This study was an attempt to evaluate vocal response as an indication of animal distress. Calves (n=189) were randomly allotted to 4 treatment groups in a 2X2 factorial design, the factors being Branded versus Not Branded and Restrained versus Not Restrained. On four consecutive days calves were brought through a headgate and squeeze apparatus. Restrained calves were caught in the headgate for 3 to 5 s. The remainder were stopped at the headgate but not restrained. On d 5 all calves were captured and restrained. Half the animals were hot-iron branded and half were sham branded using an unheated iron. During branding, vocalizations were recorded. Digitized files were used to generate an audiospectrogram and a power spectrum for each call. One hundred and sixty seven calls were analysed. During treatment 65 calves vocalized. More branded than non-branded animals vocalized (58/95 compared with 7/94, P<0.0001). Branded animals showed a greater frequency range in the fundamental, or lowest harmonic, of the audiospectrogram, (68.04 Hz ± 5.33 compared with 28 Hz ± 8.74, P<0.05), a higher maximum frequency (186.66 Hz ± 5.19 compared with 141.6 Hz ± 6.6, P<0.01) and a
higher peak sound level ($P<0.05$). The previous 4 d of restraint did not alter the probability of vocalizing, or any characteristics of the calls. This suggests that measuring vocal response may be particularly useful when the effects of relatively severe stressors are being investigated. The insensitivity of vocal response to moderate levels of stress may offer an advantage over cortisol or heart rate measurements which can reach high values in response to situational factors, leaving little capacity to respond to treatment. Analysis of vocalization may be among the more reliable and least invasive methods of assessing acute distress in cattle. Based on findings of this study, it is suggested that vocalization data should be interpreted as statistical properties of a treatment group, rather than indicating the condition of any individual animal.

3.2 INTRODUCTION

The purpose of this study was to assess the value of the vocal response as an indication of pain in beef cattle during branding, and to determine how the information might be interpreted most appropriately. The rate of calling and acoustic parameters of the calls were measured. Also the extent to which prior experience of the type of restraint required to perform branding influenced the vocal response.

Several types of bovine vocalization have been described by Kiley (1972), who also attempted to link these to the contexts in which they are usually heard. Hall et al. (1988) described interindividual differences in call characteristics of the Chillingham cattle as well as some contextual information. However, the role of vocalization in intraspecific
communication by cattle is still poorly understood.

An animal's vocalizations may provide an important source of information about its condition (Weary et al., 1997). Studies of the effects of painful or otherwise stressful protocols upon cattle frequently report the vocalization associated with these procedures, as an indicator that an animal is suffering. Some authors report rates of vocalization among a variety of physiological or behavioural measures of an animal's response to a treatment (e.g. Boissy and Le Neindre, 1997; Schwartzkopf-Genswein et al., 1997a). Others mention vocal activity of the subjects anecdotally, as collateral evidence of the (presumably) negative quality of their experience, without giving numerical values (e.g. Hudson and Mullard, 1977). An uncritical and circular argument would be that when observed in association with what the researcher assumes to be a noxious stimulus, vocalization probably indicates an unpleasant experience (e.g. Moloney and Kent, 1997). However, cattle also vocalize during mother-offspring interactions, intermale rivalry and the anticipation of feeding (Kiley, 1972). Therefore cattle vocalizations are probably not always indicative of an unpleasant experience. This contradiction afflicts several common measures often invoked as indices of stress. These include changes in an animal's normal time budget with regard to behaviours such as eating, lying or locomotion. Physiological responses such as heart rate and serum cortisol concentrations also fluctuate in response to the level of excitement or arousal experienced by the animal, even during mating which would not normally be considered distressing. Measures such as these can be very responsive to changes in an animal's internal condition. This is not always to the researcher's advantage when studying the effects of
severe stressors because cattle are fearful of novel environments, human handling and physical restraint. In some experimental situations these factors and the invasiveness of a sampling technique contribute significantly to variation in the parameters being measured, obscuring the effect of the independent variable (e.g. Lay et al., 1992a). Thus the very sensitivity of some measurements to changes in an animal's physiological or psychological state makes them unsuitable as evidence of acute and severe pain or emotional responses. One way to address these problems is to use an animal model which, through prior experience of human handling, is less reactive to the experimental situation (e.g. Lay et al., 1992b). Alternatively one could seek an indicator of stress which is relatively insensitive to low and moderate degrees of distress and is only observed in any significant degree when the animal perceives itself to be in serious difficulty. In the case of cattle, the propensity to vocalize in response to an unpleasant treatment may be just such an indicator.

In cattle, the presence of mild to moderately distressing conditions may not be associated with an increase in vocalization rates. A reasonable evolutionary explanation might be that, as a prey species, they gain a selective advantage by not needlessly broadcasting their location, or weakness due to illness or injury, to a potential predator (Livingston et al., 1992). Presumably pain and situational factors are additive, in terms of how unpleasant the animals perceive a situation to be. Recent studies have suggested that vocalization by cattle (Grandin, 1998) and also swine (Weary et al., 1998) could be a useful indicator of the distress experienced during traumatic procedures. The work of Weary and Fraser (1995a,b) has shown that the distress calls of piglets appear to be
"honest" signals, with respect to the degree of adversity encountered by an individual. The vocalization of a distressed animal might therefore be regarded as a valid statement about its own instantaneously perceived state of wellbeing. An important objective in contemporary animal welfare research should be the correct interpretation of such statements.

3.3 MATERIALS AND METHODS

Data presented in this article are derived from audio recordings made during an earlier study (Stookey et al., 1996), which investigated the effects of branding and prior experience of restraint on calves' subsequent willingness to reenter the treatment area. The handling and restraint procedures and the branding treatments described in this article were approved by the University of Saskatchewan's Committee on Animal Care and Supply (UCACS Protocol 940108).

3.3.1 Animals and treatment groups

One hundred and eighty nine crossbred beef calves of both sexes (mean weight 279.78 ± 1.54 kg on arrival) were purchased from several livestock auction markets over the course of one week, and brought to the Beef Research Unit feedlot at the University of Saskatchewan. Animals were ear-tagged and vaccinated on arrival at the feedlot. Following a one week (±3 d) adjustment period, calves were randomly allotted to 4 treatment groups in a 2 X 2 factorial design, the factors being "Branded" versus "Not
Branded" and "Restrained" versus "Not Restrained". Animals were housed in feedlot pens of 10 animals each which were mixed randomly with respect to treatment. On each day of handling (described below) the calves were brought to the handling point one pen at a time. As the treatment to be given each animal was preassigned, the actual order of treatments given on any day was dependent on the order in which animals entered the treatment facility.

### 3.3.2 Pre-branding treatments

Once on each of 4 consecutive days prior to branding, calves were brought from their pens along a chute into a treatment shed and into a squeeze chute and headgate handling apparatus (W & W Manufacturing, Dodge City, KA). Animals assigned to the Restrained treatments were caught in the manual headgate and confined for 3 to 5 s before being released and returned to their home pens. Not Restrained animals had the headgate closed in front of them as they approached it, impeding their forward progress for 3 to 5 s, before being allowed to proceed through the gate and back to their home pens.

### 3.3.3 Branding

On day 5, calves were brought into the treatment shed as before. Inside the shed, the chute leading up to the headgate area held 3 to 4 animals. Therefore each animal was able to see, to some extent, and perhaps smell (in the case of actual branding which
burns the hair and skin of the animal) the treatments given to several animals ahead in the treatment line. It would also have been able to hear the vocal responses of these animals. On this occasion all animals were captured in the headgate and enough side pressure was applied by the squeeze to minimise movement during branding. Animals in the Branded treatment groups were branded with the University of Saskatchewan's registered "US" brand. Branding was performed using a single element electric iron in the shape of the brand applied to the right rib area of the animal. Branding took approximately 5 to 8 s for each animal. The same procedure was performed on calves in the Not Branded groups except that an unheated iron was applied.

3.3.4 Sound recording and measurement

During the branding procedures all vocalizations were recorded onto audio tape (Sony TC135 SD stereo cassette recorder) using a single microphone (Shure 588SD dynamic cardioid) placed approximately 1.2 m from the headgate. All audible vocalizations recorded from the instant the iron was applied to the animal until it was released from the headgate (about 20 s). Sounds were sampled by computer (CompuPartner 90MHz, Pentium IBM compatible PC) at a rate of 22 kHz using a 16 bit sound card (Sound Blaster 16, Creative Labs Ltd). The sound files thus generated were analysed using "Spectrogram v2.3" software (Horne, R.S. 1995) and "Cool Edit v1.53" (Syntrillium Software Corp, 1996). "Spectrogram" uses a Fast Fourier Transform routine to display sound files as an audiospectrogram plot of frequency over time with sound density represented as a variable-density gray scale (See Figure 3.1a). This program was used to
Figure 3.1. (a) Typical audiospectrogram representation showing a calf vocalizing twice while being branded. (b) Power spectrum plot of one of the calls.
measure the duration of the call together with the minimum and maximum frequencies of the fundamental, or lowest visible harmonic band of the audiospectrogram (for a good explanation of Fourier analysis see Ch 3 in Bradbury and Vehrencamp, 1998). The "Cool Edit" program was used to compute an average power density profile on a selected portion of the audiospectrogram. Sound intensity (on an arbitrary dB scale) is plotted graphically at approximately 7Hz frequency increments between 0Hz and 11kHz (see Figure 3.1b). This program was used to derive the frequency at which the sound intensity was highest, and the relative loudness at that frequency. When animals vocalized more than once the values used for statistical purposes were mean values for that animal. No attempt was made to calculate absolute sound pressure levels, the sound intensity values given by the software are regarded as a relative rather than an absolute measure and were therefore converted to a rank for each animal prior to statistical analysis.

3.3.5 Statistical analysis

All statistical analyses were performed using Statistix Version 1 for Windows (Analytical Software, 1996). Analysis of variance to compare the effects of prior restraint and branding and interactions between the two on the rate of vocalizing used the General AOV/AOCV procedure. Number of animals which vocalized in each condition were compared using the Two by Two contingency table procedure. P-values given are the Fisher Exact (two-tailed) probabilities. Treatment differences in all other vocal parameters shown were calculated by Mann-Whitney U test. P-values are
two-tailed with correction for continuity.

3.4 RESULTS

Of 189 calves in the study, 65 vocalized during treatment. In total, 167 calls were recorded. The analysis of these is presented in Figs. 3.2 and 3.3. Sex of animal was not found to influence any of the measures reported and was therefore excluded from the analysis. Significantly more branded than non-branded animals vocalized (58/95 compared with 7/94, \( P<0.0001 \)). Experience of restraint on days 1-4 did not alter significantly the probability that an animal would vocalize. Analysis of variance on the number of vocalizations given by each animal showed a highly significant effect of branding (\( P<0.0001 \)) but no effect of the prior restraint treatment (\( P=0.3434 \)) or evidence of an interaction between brand and restraint status (\( P=0.6366 \)).

Despite the relatively low incidence of vocalization among the non-branded animals, enough calls (\( n=15 \)) were recorded for a useful analysis. A point of caution in interpreting these results is that only a single sound file was obtained from the Not Branded/Not Restrained group. Thus this group alone could not be statistically compared with any other on any measure (except number of calves vocalizing). Figure 3.2 summarises the analysis performed on the calls, by treatment group. Figure 3.3 shows the data grouped by branding status versus restraint status. Here it was clear that, on most measures, whether animals were branded or not had a greater effect on both the quantity and quality of their vocalizations than whether they were restrained on the four
Figure 3.2. Vocal data presented by treatment group.
Figure 3.3. Vocal data presented by branding and restraint status.
previous days. Branded animals' vocalizations showed a higher maximum frequency (186.66 Hz ± 5.19 compared with 141.6 Hz ± 6.6 P<0.01) and greater frequency range (68.04 Hz ± 5.33 compared with 28 Hz ± 8.74 P<0.05) within the fundamental band of the audiospectrogram, than unbranded animals. The vocalizations of branded animals also showed higher relative intensity (P<0.05), which was in accord with the subjective judgement that they were, in fact, louder. There were no significant differences on any of the parameters measured between animals which were restrained on days 1-4 and those which were not.

3.5 DISCUSSION

Welfare assessment in farm animals has been subject to methodological difficulties (Rushen, 1991; Barnett and Hemsworth, 1990). When studying the effects of a traumatic procedure or stressor upon an animal it is common to measure a number of physiological and/or behavioural parameters.

In the present study it was found that the application of a hot branding iron to the animal yielded a much higher rate of vocal response than a sham branding treatment. Branded animals also produced vocalizations that were different in some measurable acoustic parameters.

Prior experience of restraint on the four days before branding could have had a number of effects. Animals may have habituated, to some extent, to the restraint component of
the branding or sham branding treatment, resulting in less reactivity under restraint alone. Alternatively, repeated restraint might have contributed to an active process of anticipation of an unpleasant event, perhaps priming the animal to react more vociferously to the branding treatment. Neither scenario is supported by this study.

Though some animals did vocalize without being branded, the clear increase in the rate of calling by branded animals shows that the effect of handling, restraint and human presence did not account for an unreasonably large proportion of the potential to respond vocally. In order to account for the effects of situational factors, animals usually need to be habituated to the testing environment. Furthermore, repeated sampling is often required before, during and after a treatment, when physiological measures are employed (e.g. Schwartzkopf-Genswein et al., 1997b; Lay et al., 1992b). Vocalization might be considered a more useful measure than either cortisol or heart rate, when the effects of fairly severe stressors such as acute pain are being studied. In this study at least, rates of vocalization did not approach the maximum the animals were capable of producing.

No single measure, behavioural or physiological can serve reliably as a universal index of pain in all circumstances. So how is vocal response best used as an indicator of pain? Clearly an individual's vocalization is not an unambiguous indicator that it is in pain. It would be absurd to conclude that because 37 animals did not vocalize during hot-iron branding they felt no pain. Equally the fact that 7 animals did vocalize during the sham branding treatment cannot be taken to indicate that those calves did experience pain.
while being touched with an unheated iron. As a means of assessing the welfare of an individual animal, the amount it vocalizes may not always be a reliable indication of the degree of suffering it is experiencing. Nevertheless, in this study, many more calves did vocalize when subjected to what common sense tells us must be the more unpleasant experience. In an experimental situation it would be possible to confuse vocalization due to pain with vocalization due to some other cause of arousal or agitation, in some individuals. As a precaution against making this kind of error, measurements of vocalization should probably refer to a population or sample of animals experiencing that condition, rather than to an individual animal. The important piece of information which changes when animals are given an unpleasant treatment is not whether a certain animal vocalizes or not, but rather, as Grandin (1998) has given, the proportion of animals that vocalize.

The type of vocalization given may be a better indication of the quality of an animals's experience than the mere fact that it utters a sound. In several species, particular kinds of vocalization are considered to be characteristic of animals experiencing pain (see Morton and Griffiths, 1985). Here I attempted to discover whether the calls made by branded animals were acoustically distinctive in any measurable way. Another potential advantage of studying vocalization over some physiological measures is that the latter are unidimensional traits (for example cortisol can only be higher or lower than under some control condition), whereas the acoustic variation in calls may convey useful qualitative information. Some acoustic factors of the vocalizations did differ between branding and non-branding treatments. The lower band, or fundamental, of the
audio spectrogram tended to cover a wider frequency range, or extend to higher frequencies for branded than unbranded animals. Branded animals also made louder calls. It is not clear from these data whether the form of call given by a branded animal is a distinctive and specific signal indicative of pain. Visual inspection of the sound spectra indicates that, though there is some variability between individuals and between treatments in the factors measured, the calls of branded and unbranded animals are of similar general type. These appear to correspond to the "men(h)" or "(m)enh" calls described and depicted sonographically by Kiley (1972). Kiley associated such calls with a variety of emotionally significant situations, not just painful stimuli. This would be consistent with the view that the vocalization of a branded animal does not convey the specific information that it is in pain, but rather signals a high degree of general negative affect. Pain is not just an unpleasant sensation, the associated concepts of suffering and distress refer to unpleasant emotional conditions also (Morton and Griffiths, 1985; Moloney and Kent, 1997). It may be that vocalization, induced by unpleasant treatments in cattle is indicative more of the negative emotional state than the sensory experience itself. A noteworthy point is that cattle which often vocalize when subjected to acute social or psychological stress (when visually isolated, for example), may be indicating a similar affective state to that experienced during branding. We may have, in vocalization, a direct means of comparing the aversiveness of physical challenges such as pain, cold, thirst etc, and psychological states such as acute fear as perceived by the animals.

Does the vocalization of a branded calf constitute an act of communication?
Communication is usually said to occur when the activities of an individual influence the behaviour of others (Johnstone, 1997). It is not clear from this study whether the vocalizations of a branded animal influenced the behaviour of audience animals, or modified their responses to the treatment.

Animals frequently signal to solicit help from conspecifics. Weary and Fraser (1995a,b) have investigated the honesty of care soliciting calls of isolated piglets. These authors argue that such responses appear to be "honest" with respect to the degree of need of the individual and may therefore be useful as an indicator of welfare. Whether, by vocalizing during a painful procedure, cattle are emitting a signal which is "designed" to elicit a particular response from conspecifics is unclear. Models of honest signalling suggest that calls given in these circumstances will, in general, evolve to be true reflections of an animal's state (Johnstone, 1998). However, there is insufficient evidence as yet to make strong claims for the honesty of distress vocalizations in cattle.

Analysis of vocalization, properly used, may be among the more reliable and least invasive methods of assessing distress in cattle. Propensity to vocalize, or rate of vocalization and acoustic measurements of the calls are probably most valid as indications of distress when considered as statistical properties of a population of animals subject to a particular treatment, rather than indicating the condition of any individual animal within that group. More study is needed to explore the relationship between the vocalizations of domestic animals and the qualities of subjective experience which they may reflect.
4.0 VARIABILITY IN VOCAL AND BEHAVIOURAL RESPONSES TO
VISUAL ISOLATION BETWEEN FULL-SIBLING FAMILIES OF BEEF
CALVES

4.1 ABSTRACT

In order to assess the value of vocal response as an indicator of welfare in cattle, it is
necessary to investigate factors which influence vocal behaviour of individuals that are
independent of the specific environmental conditions to which they are exposed. The
effect of parental differences, sex, age and weight on vocal responses of calves to visual
isolation were examined. The relationship between vocal response and the amount of
body movement during isolation was also evaluated.

Seventeen full-sibling families of beef calves (n=130) were created by breeding 5 sires
with 13 superovulated dams. Embryos were transferred into unrelated cows, which
reared the calves to weaning age. Vocal response of calves and body movement were
measured on four occasions, while they were visually isolated for 1 min on a single
animal scale platform. The first two observations were made on the day of weaning
(mean age 166 d) and the following day. At the third and fourth observations their
average ages were 278 d and 350 d. On the four occasions 33.3%, 34.8%, 27.8% and
38.6% of calves respectively vocalized. Both sire and family had a significant influence on the number of vocalizations and acoustic properties of the sounds. Electronically-measured movement while on the scale was also influenced by sire and family, but there was no relationship between vocal responses and body movement. There was a tendency ($P=0.08$) for older and therefore heavier calves to produce longer vocalizations. In the final observing session, age and weight were positively correlated with fundamental frequency and the sound pressure level at the frequency of greatest intensity, and negatively correlated with the loudest harmonic. At the last two observations, significantly more heifer calves than bull calves vocalized. There was little difference between the acoustic properties of heifers' and bulls' vocalizations.

The considerable individual variability in vocal response within a group of identically-treated calves is partly due to parental genetic influences that are independent of rearing and early experience. Other endogenous characteristics such as sex, age and weight also influenced vocal response. But there was no clear relationship between individual temperament (as assessed by the amount of body movement during isolation) and characteristics of vocal response.

### 4.2 INTRODUCTION

Vocalization is a common feature of bovine responses to both physically and psychologically stressful situations. Measuring vocal responses during unpleasant
handling procedures as an indicator of animal welfare has the advantage, compared with physiological measurements, of being entirely noninvasive. Cattle show increased rates of vocalization in a variety of painful and distressing situations including branding (Schwartzkopf-Genswein et al., 1997, 1998), weaning (Stookey et al., 1997) and isolation (Boissy and Le Neindre, 1997). Vocalizations can also differ qualitatively in a number of acoustic parameters that can be measured objectively (Chapter 3). Thus vocal response can be quantified in several ways, possibly reflecting shades of affect or specific emotional states. To date there has been insufficient research to establish whether, or how best, vocal response should be used as an indicator of welfare in cattle. The vocalizations of cattle have previously been described by Schloeth (1961), Kiley (1972) and Hall et al. (1988). Vocal responses of cattle during distressing procedures have occasionally been reported in the literature among other measures considered to be relevant to their welfare. In Chapter 2 it was argued that measures of vocal behaviour in cattle will be more useful in welfare studies if the many factors influencing its expression are better understood. These factors might include breed and parental influences, sex differences, developmental changes within the individual, social factors such as dominance, and also learning.

Historically, psychological distress in cattle has been of less interest to animal welfare researchers than painful procedures and physiological stressors. In social animals such as cattle, sudden enforced separation from conspecifics evokes characteristic behavioural and physiological changes which are suggestive of a state of distress or fear (Kondo and
Hurnik, 1988; Veissier and Le Neindre, 1992; Hopster and Blokhuis, 1994a). These changes include increased struggling, heart rate, plasma cortisol and vocalizations (Boissy and Le Neindre, 1997). Beef cattle are seldom raised in isolation or held isolated for extended periods of time. However, they are confined in handling facilities for routine procedures such as vaccination, weighing and ear tagging. As a result they are likely to be exposed to brief periods without visual contact with other cattle. In general, the behavioural responses of cattle to social isolation are similar to those of other domestic species. Goats and sheep vocalize more and make escape attempts when isolated (Price and Thos, 1980). Dogs show increased vocalization and movement when isolated (Hefts et al., 1992) and increased heart rate and movement have been reported in alpacas (Pollard and Littlejohn, 1995). Given the similarities between the behavioural and physiological responses to isolation and to painful procedures, it is reasonable to assume that isolation causes a reduction in welfare in social animals.

In Chapter 2 it was suggested that the vocal behaviour of cattle can be regarded as a form of subjective commentary, by the animals, on their own biological functioning. The present study is part of an attempt to determine the extent to which this commentary is accessible, and of possible value as a tool in welfare assessment. This effort includes investigation of the endogenous factors influencing an animal's vocal behavior.

During 1997 and 1998 a multiple trait gene mapping study on beef cattle was conducted (see Schmutz, 2000). For this project, quantitative measurements of animal movement
during visual isolation were made, as an objective indicator of temperament. The herd studied was produced by multiple ovulation embryo transfer. It comprised a number of "families" of several full genetic siblings of similar age. This situation obviously does not occur naturally in cattle as it does, for example, in swine. This was a very rare opportunity to assess the influence of genetics, as an endogenous factor influencing vocal behaviour in cattle. An additional benefit was that parental behavioural influences were diluted to a large extent, because the calves within each family were mothered by unrelated cows from shortly after conception. However, in taking this opportunity, it was necessary to accommodate a preexisting experimental design which was less than ideal for the purpose. It was not possible to influence the breed composition of the families within the herd, and it was necessary to house the calves in single sex pens after weaning. It was also expected that on any one observing occasion, only a proportion of calves would exhibit the behaviour of central interest (i.e. the vocalization). These restrictions placed some constraints on the possibilities for statistical analysis. These limitations were accepted in order to gain at least some information about genetic influences on cattle vocal behaviour which could not practicably have been obtained otherwise.

The study reported in Chapter 3 examined the effects of hot-iron branding on vocal behaviour. In the present study, unlike branding, the period of visual isolation imposed on the animals, while assumed to be unpleasant, does not cause pain or tissue damage. It may therefore be argued that reactions to isolation reflect more a psychological than a
physiological state of distress. The primary goal of this study was to determine whether an animal's genotype contributes to the characteristics of its vocal responses. A secondary objective was to look for developmental changes in vocal behaviour associated with increasing age and weight of the animal, and sex differences. An additional question was whether the type or magnitude of vocal response is related to their general degree of exciteability, as indicated by the amount of body movement shown during the isolation period.

4.3 ANIMALS, MATERIALS AND METHODS

The animal handling procedures described in this section were approved by the University of Saskatchewan's Committee on Animal Care and Supply (UCACS Protocol 19970043).

4.3.1 Animals

The study population comprised 130 purebred and crossbred beef calves, 62 females and 68 uncastrated males. Information on the design of this herd is available electronically online (Schmutz, 2000). Breeds represented in the herd included Angus, Charolais, Hereford, Simmental, Limousin and Belgian Blue. The calves were conceived by natural breeding of 13 superovulated dams to 5 sires. The embryos were implanted into recipient cows to which they were unrelated. These recipients carried the
pregnancies to term and reared the calves to weaning. Throughout this paper, the term "dam" will refer to the embryo donor rather than the recipient. This process resulted in 17 full-sibling biological families of between 2 and 17 calves as shown in Table 4.1. Calves were weaned at approximately 6 mo of age and transported 2 hr by road to the University of Saskatchewan's Beef Research Unit feedlot. Newly-weaned calves arrived in six groups of similarly-aged animals over a period of 4 months. Each group was segregated by sex into two feedlot pens.

4.3.2 Animal handling

The behavioural responses of each calf to a short period of visual isolation was assessed on four occasions. These observations are referred to by the abbreviations "OBS 1", "OBS 2", "OBS 3" and "OBS 4". Each of the 6 groups was first observed upon arrival at the feedlot (OBS 1) and again on the following day (OBS 2). Two further observation sessions, separated by 3 months, were conducted in which all calves were evaluated on the same day. Thus at OBS 3 their ages ranged from 8 to 12 months and at OBS 4 between 11 and 15 months. On each occasion the animals were handled similarly. Animals were brought from their home pens to the handling area and moved into a crowd pen. They walked single file into a curved chute which turned through 180 degrees before entering the handling facility. Inside they passed through a 5m straight section of chute, through an open-sided headgate and squeeze section and onto a single-animal scale platform with solid steel sides and doors.
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<td>C</td>
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<td>C7</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>C8</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>C4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>D9</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>D10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>D11</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
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<td>E12</td>
<td>5</td>
<td>3</td>
</tr>
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<td></td>
<td>1</td>
<td>E1</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>E13</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>E9</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

1 "Dam" refers to the animal from which embryos were taken, not the individual into which they were transferred.

2 Note that the family identification is obtained by concatenating the sire and dam identifications.

Three dams (1, 4 and 9) were superovulated on more than one occasion and bred by different sires. Thus, for example, families A4, B4 and C4 had the same genetic dam but different sires.
For testing, one animal was moved onto the scale platform while the next three stood single file in the straight chute. The subject animal was confined within the scale compartment, out of visual contact with other cattle or humans for a period of 1 min while behavioural observations were made as detailed below. At the end of the 1 min test, the animal exited the building through the front door of the scale and was moved to a nearby holding pen, to await its group's return to their home pen. The next animal was then moved onto the scale and one more brought into the straight chute inside the building.

Throughout the study period the calves were brought into this same facility to be weighed every 14 days. Therefore they had been exposed to the testing environment, though not the 1 min period of isolation, on several additional occasions prior to OBS 3 and OBS 4.

4.3.3 Animal Movement Measurements

An electronic movement measuring device (MMD) was employed, to obtain an objective behavioural measure of general exciteability or reactivity. This machine (Stookey et al., 1994) analyses the electrical analogue signal generated by the weigh scale upon which the animal stands, to calculate the amount of movement during a 1 min sampling period. Any slight movement of the animal as it stands on the scale causes this signal to fluctuate. The device measures the signal voltage 122 times each second and
records a "peak" every time a trend of 10 or more voltage changes in the same direction is reversed. It has previously been determined that the number of peaks recorded in 1 min is positively correlated with heart rate (Waynert et al., 1999; Piller et al., 1999). The number of MMD peaks was recorded for each animal at each of the four observing sessions.

4.3.4 Vocal responses: recording and measurement.

During OBS 1 and OBS2 it was simply noted whether or not each animal vocalized during its 1 min isolation period. During OBS 3 and OBS 4 all vocalizations were recorded for subsequent analysis. Sounds were recorded using the audio channel of a video cassette recorder (Panasonic model AG6730) running in 6 hr mode and a single microphone (Shure 588SD) suspended from the ceiling within approximately 1m of the animal's head. A camera was also used to provide visual confirmation of events which facilitated later work in the laboratory. From the recordings, vocalizations were sampled by computer at a rate of 11kHz using an IBM compatible PC with 16-bit sound card. Each vocalization was stored as a separate computer file in ".WAV" format. Acoustic measurements were made from 235 such files. Sound files were analysed using "Spectrogram v4.2.3c" (R.S. Horne, 1998). This software was used to generate an audiospectrogram plot of frequency over time with sound level represented visually by a variable density grey scale (Figure 4.1). This was used to calculate the duration of each call (in milliseconds). A 1 s subsample of each call centered on the midpoint (or the
Figure 4.1 Audiospectrogram plot of calf vocalization
entire call if it was less than 1 s in length) was used to generate a power spectrum plot of relative sound intensity (on an arbitrary dB scale) over frequency. This graphically represents the intensity of sound emitted at each frequency in the call. From this plot were derived the frequency in hertz at which maximum sound energy was emitted, the intensity of this energy peak (in arbitrary dB units), the fundamental frequency (in hertz) and the loudest harmonic of the call (see Figure 4.2).

This recording system, while not of the sensitivity which would be required in a playback study, proved quite adequate to allow imaging of the harmonic structure of calls on the audiospectrogram and power spectrum plots. Using this system, harmonics down to 80 Hz or below could be observed. The overall frequency response of the recording and sampling system was not adequate to allow imaging of lower frequencies. With sounds which exhibit a periodic harmonic pattern, as most vocalizations of cattle do, it is possible to calculate the value of fundamental frequencies which are below the detection limits of the recording system. This is achieved by calculating the mean frequency interval between a number of successive harmonic bands within a higher frequency range. This procedure has been used by Berg (1983) to reveal fundamental frequencies of elephant calls in the range 18-28 Hz, which her equipment would have been unable to record. This method was used to calculate the fundamental frequencies reported in this article because it yielded a more precise value than that which could be measured by reading the cursor frequency value directly from the power spectrum plot and also because it allowed the very rare calls below 50 Hz which cattle occasionally
Figure 4.2. Power spectrum plot of calf vocalization.
produce to be included in the data set.

4.3.5 Statistical analysis

Due in part to design constraints and in some instances to unbalanced and abnormally distributed data, a simple, and predominantly nonparametric analysis was considered most appropriate. It was not possible to examine interaction effects between variables.

Statistical tests were computed using Statistix V4.1 (Analytical Software, 1996). Rank sum (Mann-Whitney) tests were used to test for differences in the mean values of vocal measurements between OBS 3 and OBS 4. Pearson correlation coefficients were determined between individuals' acoustic measurements on data which met the criteria for this test (i.e. latency to vocalize, call duration, frequency of greatest intensity and fundamental frequency). Otherwise Spearman's rank correlation was used. One-way nonparametric analysis of variance (Kruskall-Wallis) was used to estimate the variability due to sire and family in vocal measurements and mean movement scores. Pearson correlation coefficients were calculated on individual calves's movement scores between observing sessions. The Mann-Whitney test was used to test for differences in the mean ages and weights of vocal and nonvocal calves during the four observing sessions. The relationships of age and weight to the vocal response measurements used either Pearson or Spearman correlation coefficients as appropriate for each measure. Comparison of the distribution of vocal and nonvocal calves between sexes during each
session used a 2X2 chi square procedure. The Mann-Whitney was used to test for sex differences in the acoustic measurements.

4.4 RESULTS

4.4.1 Vocal responses: general description.

On the four days of observation 33.3%, 34.8%, 27.8% and 38.6% of the calves vocalized respectively during the 1 min sampling period. During OBS 1 and OBS 2 the number of cattle which vocalized were recorded. During OBS 3 and OBS 4, when the cattle were between 8-12 months and between 11-15 months of age respectively, several additional parameters were measured. These data are summarised in Table 4.2. Although the number of vocalizations per bout and the duration of calls did not alter significantly between OBS 3 and OBS 4, the frequency at which the maximum sound intensity was seen, and therefore also the harmonic classified as loudest, did increase between these two sets of observations. Because of difficulty in ensuring consistency in the absolute sound sensitivity of the recording and sampling process between OBS 3 and OBS 4 no attempt was made to compare the loudness of calls between these two sets of observations. For animals which vocalized on both occasions, correlation coefficients for the other acoustic measurements are given in Table 4.3. Between OBS 3 and OBS 4 the number of vocalizations, the mean frequency of greatest intensity, and the mean fundamental frequency were significantly positively correlated.
### Table 4.2 Summary of acoustic measurements of vocalizations recorded during OBS 3 and OBS 4.

<table>
<thead>
<tr>
<th></th>
<th>OBS 3 Age 8-12 Months</th>
<th>OBS 4 Age 11-15 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n¹</td>
<td>Mean ± S.E</td>
</tr>
<tr>
<td>Latency to vocalize (s)</td>
<td>34</td>
<td>26.971±3.09</td>
</tr>
<tr>
<td>Calls per bout²</td>
<td>35</td>
<td>2.09 ±0.28</td>
</tr>
<tr>
<td>Duration of call (msec)</td>
<td>83</td>
<td>1273.8 ±49.11</td>
</tr>
<tr>
<td>Frequency of greatest sound intensity (Hz)</td>
<td>70</td>
<td>272.86±14.43ᵃ</td>
</tr>
<tr>
<td>Fundamental frequency (Hz)</td>
<td>72</td>
<td>96.46 ± 3.14</td>
</tr>
<tr>
<td>Loudest Harmonic</td>
<td>64</td>
<td>1.92 ± 0.17ᵇ</td>
</tr>
<tr>
<td>Latency to vocalize (s)</td>
<td>49</td>
<td>21.82 ± 2.19</td>
</tr>
<tr>
<td>Calls per bout²</td>
<td>49</td>
<td>2.39 ± 0.26</td>
</tr>
<tr>
<td>Duration of call (msec)</td>
<td>129</td>
<td>1289.3 ± 43.48</td>
</tr>
<tr>
<td>Frequency of greatest sound intensity (Hz)</td>
<td>124</td>
<td>365.1 ± 13.56ᵃ</td>
</tr>
<tr>
<td>Fundamental frequency (Hz)</td>
<td>120</td>
<td>98.70 ± 3.11</td>
</tr>
<tr>
<td>Loudest Harmonic</td>
<td>115</td>
<td>2.65 ± 0.14ᵇ</td>
</tr>
</tbody>
</table>

¹Pairs of numbers with a common superscript differ (P≤0.001)

²n indicates the number of observations from which each mean was derived. For latency to vocalize and calls per bout there should ideally be one observation per vocalizing animal. For the other measurements there should be one observation per vocalization. The statistical unit used to compute P-values was the number of means used in each comparison.

²These are the mean numbers of vocalizations for calves which did vocalize at least once during the observation period.
Table 4.3
Correlation between vocal response parameters measured during OBS 3 and OBS 4 for calves that vocalized on both occasions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation (^a)</th>
<th>(n)^b</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vocalizations</td>
<td>0.430 (S)</td>
<td>26</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Latency to vocalize</td>
<td>0.317 (P)</td>
<td>26</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mean call duration</td>
<td>0.208 (P)</td>
<td>23</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mean frequency of greatest intensity (FGI)</td>
<td>0.624 (P)</td>
<td>21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean sound pressure level at FGI</td>
<td>-0.012 (S)</td>
<td>21</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mean fundamental frequency</td>
<td>0.7394 (P)</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean loudest harmonic</td>
<td>0.232 (S)</td>
<td>20</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

\(^a\) The letter in parentheses indicates the type of correlation coefficient calculated:
(P) = Pearson product moment correlation coefficient, (S) = Spearman rank order correlation coefficient.

\(^b\) \(n\) represents the number of comparisons used in calculating the correlation coefficient. Not all measurements could be obtained for every animal and every vocalization. A comparison could only be made when the parameter in question could be calculated for an individual calf for both OBS 3 and OBS 4.
4.4.2 Influence of sire on vocal response.

The results of the nonparametric analysis of variance used to estimate the influence of sire on several measures of vocal response are shown in Table 4.4. Sire accounted for a large proportion of the variability in the calves' vocal responses. The effect of sire was significant for all the measures shown, with the exception of the loudest frequency.

4.4.3 Influence of family on vocal response.

Figure 4.3 illustrates the variation due to family in the vocal measurements. The variation between families was significant for all measures tested.

4.4.4 Relationship between vocal response and age and weight.

Table 4.5 shows the mean ages and weights of calves at each observing session separated according to whether they vocalized or not on that occasion. It was hypothesized that vocal animals would be of a different mean age or weight than nonvocal animals. Vocal animals tended to be older and thus also heavier at each observing session than nonvocal animals. The difference was significant for both age and weight during OBS 2 and OBS 3. Table 4.6 shows the relationships of both age and weight to vocalization measurements made during OBS 3 and OBS 4. There was a tendency for increasing age and weight to be associated with increasingly long and
Figure 4.3. Mean values of vocal measurements and MMD peak scores ± Standard Error for each family of calves. P-values given are based on a one-way nonparametric analysis of variance (KruskalWallis) test. Absence of a standard error bar on any column indicates that it represents a single observation.
Table 4.4
Mean values ± standard errors of vocal response measurements of the calves compared by sire (number of measurements contributing to each mean given in parentheses).

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>P-value&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occasions on which they</td>
<td>1.71±</td>
<td>1.67±</td>
<td>0.92±</td>
<td>0.63±</td>
<td>1.59±</td>
<td>0.0014</td>
</tr>
<tr>
<td>vocalized</td>
<td>0.24&lt;sup&gt;a&lt;/sup&gt;(31)</td>
<td>0.20&lt;sup&gt;a&lt;/sup&gt;(33)</td>
<td>0.26&lt;sup&gt;ab&lt;/sup&gt;(13)</td>
<td>0.16&lt;sup&gt;a&lt;/sup&gt;(30)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;a&lt;/sup&gt;(17)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Duration of calls (s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1474±</td>
<td>1296±</td>
<td>1164±</td>
<td>1047±</td>
<td>1049±</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>68&lt;sup&gt;a&lt;/sup&gt;(62)</td>
<td>47&lt;sup&gt;a&lt;/sup&gt;(93)</td>
<td>389&lt;sup&gt;ab&lt;/sup&gt;(3)</td>
<td>111&lt;sup&gt;a&lt;/sup&gt;(14)</td>
<td>41&lt;sup&gt;a&lt;/sup&gt;(40)</td>
<td></td>
</tr>
<tr>
<td>Fundamental frequency (Hz)</td>
<td>103.8±</td>
<td>103.5±</td>
<td>90.1±</td>
<td>85.41±</td>
<td>78.98±</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3.13&lt;sup&gt;a&lt;/sup&gt;(59)</td>
<td>4.19&lt;sup&gt;a&lt;/sup&gt;(84)</td>
<td>4.76&lt;sup&gt;ab&lt;/sup&gt;(3)</td>
<td>7.08&lt;sup&gt;a&lt;/sup&gt;(10)</td>
<td>2.70&lt;sup&gt;a&lt;/sup&gt;(36)</td>
<td></td>
</tr>
<tr>
<td>Loudest frequency (Hz)</td>
<td>342.9±</td>
<td>301.6±</td>
<td>370±</td>
<td>320.3±</td>
<td>382.9±</td>
<td>0.0687</td>
</tr>
<tr>
<td></td>
<td>24.5&lt;sup&gt;ab&lt;/sup&gt;(60)</td>
<td>12.7&lt;sup&gt;a&lt;/sup&gt;(85)</td>
<td>122.6&lt;sup&gt;ab&lt;/sup&gt;(3)</td>
<td>38.3&lt;sup&gt;ab&lt;/sup&gt;(9)</td>
<td>20.0&lt;sup&gt;a&lt;/sup&gt;(37)</td>
<td></td>
</tr>
<tr>
<td>Loudest harmonic</td>
<td>2.18±</td>
<td>1.91±</td>
<td>3.00±</td>
<td>2.67±</td>
<td>3.71±</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>0.15&lt;sup&gt;b&lt;/sup&gt;(55)</td>
<td>0.14&lt;sup&gt;b&lt;/sup&gt;(78)</td>
<td>1.15&lt;sup&gt;ab&lt;/sup&gt;(3)</td>
<td>0.60&lt;sup&gt;b&lt;/sup&gt;(9)</td>
<td>0.31&lt;sup&gt;a&lt;/sup&gt;(34)</td>
<td></td>
</tr>
<tr>
<td>Intensity at loudest</td>
<td>29.56±</td>
<td>33.68±</td>
<td>72.5±</td>
<td>9.5±</td>
<td>58.15±</td>
<td>0.0017</td>
</tr>
<tr>
<td>frequency (OBS 3)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.01&lt;sup&gt;a&lt;/sup&gt;(24)</td>
<td>4.00&lt;sup&gt;a&lt;/sup&gt;(33)</td>
<td>(1)</td>
<td>(1)</td>
<td>3.41&lt;sup&gt;b&lt;/sup&gt;(10)</td>
<td></td>
</tr>
<tr>
<td>Intensity at loudest</td>
<td>90.40±</td>
<td>62.03±</td>
<td>37.5±</td>
<td>73.12±</td>
<td>40.13±</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>frequency (OBS 4)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5.66&lt;sup&gt;a&lt;/sup&gt;(35)</td>
<td>5.15&lt;sup&gt;b&lt;/sup&gt;(52)</td>
<td>22&lt;sup&gt;ab&lt;/sup&gt;(2)</td>
<td>17.96&lt;sup&gt;ab&lt;/sup&gt;(8)</td>
<td>4.15&lt;sup&gt;a&lt;/sup&gt;(27)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>P-value for the overall sire effect calculated by Kruskal-Wallis nonparametric anova.

<sup>2</sup>Means given for sound intensity are mean ranks. Because the absolute recording level during OBS 3 and OBS 4 may not have been equal, intensity measures from the two sessions are compared separately. The values of the other parameters shown in this table are means of all available measurements pooled.

<sup>ab</sup>Within a row pairs of means with no superscript letter in common are statistically different (P<0.05)
Table 4.5
Comparison of mean age and weight of calves which vocalized or did not vocalize during four observation sessions.

<table>
<thead>
<tr>
<th></th>
<th>Vocalized</th>
<th>Did not vocalize</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean age (days) ±</td>
<td>Mean age (days) ±</td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td>standard error</td>
<td>standard error</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>OBS 1</td>
<td>43 168.14 ± 1.34</td>
<td>86 165.95 ± 0.87</td>
<td>0.1702</td>
</tr>
<tr>
<td>OBS 2</td>
<td>44 169.14 ± 1.22</td>
<td>84 165.52 ± 0.90</td>
<td>0.0146</td>
</tr>
<tr>
<td>OBS 3</td>
<td>34 296.62 ± 7.27</td>
<td>94 272.22 ± 3.75</td>
<td>0.0047</td>
</tr>
<tr>
<td>OBS 4</td>
<td>49 353.31 ± 6.05</td>
<td>78 348.41 ± 4.32</td>
<td>0.5033</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Vocalized</th>
<th>Did not vocalize</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean weight (kg) ±</td>
<td>Mean weight (kg) ±</td>
<td></td>
</tr>
<tr>
<td>WEIGHT</td>
<td>standard error</td>
<td>standard error</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>OBS 1</td>
<td>43 243.94 ± 4.66</td>
<td>86 233.82 ± 3.20</td>
<td>0.0992</td>
</tr>
<tr>
<td>OBS 2</td>
<td>44 247.10 ± 5.00</td>
<td>84 232.42 ± 3.00</td>
<td>0.0146</td>
</tr>
<tr>
<td>OBS 3</td>
<td>34 362.49 ± 9.48</td>
<td>91 336.91 ± 5.77</td>
<td>0.0204</td>
</tr>
<tr>
<td>OBS 4</td>
<td>49 421.03 ± 7.57</td>
<td>76 415.11 ± 7.72</td>
<td>0.4496</td>
</tr>
</tbody>
</table>

\(^1P\)-values based on Mann-Whitney rank sum test (two-tailed)
Table 4.6. Correlations of age and weight with vocal response parameters measured during OBS 3 and OBS 4.

<table>
<thead>
<tr>
<th>OBS 3</th>
<th>AGE</th>
<th>WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Correlation</td>
</tr>
<tr>
<td>Number of vocalizations</td>
<td>34</td>
<td>0.1814 (S)</td>
</tr>
<tr>
<td>Latency to vocalize</td>
<td>34</td>
<td>-0.1321 (S)</td>
</tr>
<tr>
<td>Mean call duration</td>
<td>34</td>
<td>0.3921 (P)</td>
</tr>
<tr>
<td>Mean frequency of</td>
<td>30</td>
<td>0.0090 (P)</td>
</tr>
<tr>
<td>greatest intensity (FGI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean sound pressure</td>
<td>30</td>
<td>-0.0930 (S)</td>
</tr>
<tr>
<td>level at FGI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean fundamental</td>
<td>29</td>
<td>0.3567 (P)</td>
</tr>
<tr>
<td>frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean loudest harmonic</td>
<td>27</td>
<td>-0.2308 (S)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OBS 4</th>
<th></th>
<th>AGE</th>
<th>WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of vocalizations</td>
<td>49</td>
<td>0.2241 (S)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Latency to vocalize</td>
<td>49</td>
<td>-0.1363 (P)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mean call duration</td>
<td>47</td>
<td>0.2826 (P)</td>
<td>0.0543</td>
</tr>
<tr>
<td>Mean frequency of</td>
<td>45</td>
<td>0.104 (P)</td>
<td>N.S.</td>
</tr>
<tr>
<td>greatest intensity (FGI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean sound pressure</td>
<td>45</td>
<td>0.4094 (S)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>level at FGI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean fundamental</td>
<td>43</td>
<td>0.5456 (P)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean loudest harmonic</td>
<td>43</td>
<td>-0.5138 (S)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 The letter in parentheses in these columns indicates the type of correlation coefficient calculated: (P) = Pearson product moment correlation coefficient, (S) = Spearman rank order correlation coefficient.

2 \( P \) - values less than 0.1 are shown for illustrative purposes in addition to significant (<0.05) values. Values of \( P \geq 0.1 \) are labelled "N.S." (i.e. "not significant").
higher frequency calls. The relationship between age/weight and fundamental frequency was highly significant during OBS 4. The ranked values for maximum sound intensity were highly \( (P<0.001) \) correlated with both age and weight during OBS 4. Since the calls were ranked from loudest (Rank 1) to quietest (Rank 133), this correlation was negative. In other words, larger, older animals produced calls with a lower maximum sound pressure at any single frequency.

4.4.5 Animal movement data.

The numbers of MMD peaks recorded for individual calves were highly correlated between observing sessions, suggesting that physical agitation during isolation is a stable characteristic of individuals over time. The mean number of MMD peaks for each calf over all four sessions was therefore taken as the measure best representing this characteristic. Nonparametric analysis of variance showed that mean peaks scores for calves varied significantly between sires and between families \( (P<0.001) \).

4.4.6 Relationship between vocal response and animal movement scores.

There were no differences in movement scores between animals which vocalized and those which did not, during any of the four observing sessions. Neither were MMD peaks during OBS 3 and OBS 4 correlated with any of the acoustic parameters measured.
4.4.7 Relationship between vocal response and sex

Table 4.7 shows the proportions of calves of each sex which vocalized during the four observing sessions. During the first two tests there were no differences. In the two later tests, a larger proportion of female calves vocalized. There was little difference between sexes in the acoustic properties of the calls. During OBS 3 there was a tendency for heifers to give longer calls ($P=0.083$) and a trend toward a higher sound pressure level at the frequency of maximum intensity ($P=0.061$) compared with bulls.
Table 4.7
Comparison of the numbers of calves of each sex which vocalized or did not vocalize at each observing session.

<table>
<thead>
<tr>
<th></th>
<th>Vocalized</th>
<th>Did not vocalize</th>
<th>Vocalized</th>
<th>Did not vocalize</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>OBS 1</td>
<td>21</td>
<td>47</td>
<td>22</td>
<td>39</td>
<td>0.39</td>
<td>0.530</td>
</tr>
<tr>
<td>OBS 2</td>
<td>24</td>
<td>44</td>
<td>20</td>
<td>40</td>
<td>0.05</td>
<td>0.815</td>
</tr>
<tr>
<td>OBS 3</td>
<td>12</td>
<td>56</td>
<td>22</td>
<td>38</td>
<td>5.91</td>
<td>0.015</td>
</tr>
<tr>
<td>OBS 4</td>
<td>19</td>
<td>48</td>
<td>30</td>
<td>30</td>
<td>6.26</td>
<td>0.012</td>
</tr>
</tbody>
</table>
4.5 DISCUSSION

This study represents an attempt to account for some factors intrinsic to individual cattle which help to explain variability in their vocal behavior. With most behavioural or physiological measures one would expect to find individual differences and therefore a range of responses. For example, dairy cows (Hopster and Blokhuis, 1994b) showed wide variations in heart rate, vocal, locomotor and plasma cortisol responses during social isolation. Their responses were highly repeatable, indicating that they were characteristic for the individual. This is consistent with the notion of individual psychological profiles, or "personality" in domestic animals (Boissy and Bouissou, 1995). It is important to investigate the sources of this variability in order to gauge the applicability of any particular measure as an indicator of welfare.

In the present study, multiple ovulation embryo transfer allowed the creation of relatively large groups of siblings, similar in numbers to litters of piglets. They were raised by surrogate mothers in a process analogous to the fostering of a neonate, with the important difference that the offspring were "adopted" soon after conception rather than after birth. These calves had no opportunity to learn to imitate their genetic dams' individual behaviour patterns. Within a "family", although the calves were siblings in a genetic sense, each was raised by a different surrogate parent. This made it possible to look for innate tendencies in behaviour with the confounding effects of early experience somewhat diluted.
The vocalizations of these isolated calves did sound different from those of calves during branding (Chapter 3). The calls of the isolated calves sounded lower in pitch and quieter, with less frequency modulation. Spectrographically, they show a lower mean fundamental frequency than those of cattle measured in earlier studies, of comparable age, during branding. In the present study calls as low as 31 Hz were recorded. These lie outside the 50 to 1250 Hz range previously reported for cattle (Kiley, 1972) and fall within the 14 to 35 Hz range of the infrasound calls of elephants (Payne et al., 1986; Poole et al., 1988). Low frequency sounds propagate efficiently through the atmosphere (Larom et al., 1997) which means that they are appropriate for communication at a distance. It is possible that the differences in sound between branded and isolated cattle reveal differences in the motivational or affective states evoked by these conditions. It may be that while the calls of a calf during branding are relatively involuntary expressions of pain, rage or fear, the calls of an isolated animal amount to a voluntary attempt to communicate.

These results show that there can be considerable individual variation in vocal response among animals that have all been subject to an identical treatment. As Table 4.2 shows, all the measurements of vocal response vary over a considerable range. The voices of individual animals can be very distinctive. There were also indications that these individual characteristics are persistent over time. On any one observing occasion, only about a third of the calves vocalized. But as Table 4.3 illustrates, among those that did vocalize, the rate of vocalizing is correlated between occasions, as are at least some of
the acoustic properties of the sounds. The vocal characteristics of individual cattle, on this evidence, could be regarded as another factor in an animal's overall personality profile.

Both sire and family influenced the amount of vocal behaviour shown and also the acoustical quality of the sounds they produced. Table 4.4 shows that in almost every acoustic measurement taken, there was significant variation between the offspring of different sires. The category of family, of course, is not completely independent of the category of sire. Each family reflected a unique combination of sire and dam influences. Figure 4.3 shows that there was a significant family effect in all the measurements made. Since most dams were used to generate only one family, it was not possible to test for significance of a dam effect. Therefore it is not possible to tell whether one parent exerts a greater genetic influence over the offspring's vocal characteristics. Nevertheless, it is certain that some proportion of a calf's vocal behavior is inherited from the parents, independent of rearing conditions and learning. It is unclear whether the variations observed are characteristic of specific breeds or just the inherited idiosyncrasies of the particular few sires and dams used in this study. In fact, in this study, the breed of individual parents have deliberately not been disclosed, since, with few exemplars of each, no conclusions as to the effect of breed would be possible.

Vocal behaviour also appears to change in measurable ways over time. The relationship of vocal response to both age and weight of animal was examined. These two factors are
naturally so highly correlated that it is difficult to know whether it is the size of the animal or the length of its development which has most influence on its vocal behaviour. This is a question worth considering, as changes in vocalization over time in a young animal may be influenced by distinct physical and psychological developmental processes. Up to the time of OBS 3, vocal animals were the older and heavier members of the herd, as Table 4.5 indicates. By the last observation there was no difference. In juvenile and adolescent cattle there may be a tendency to become more vocal over time, up to a certain stage of development where no further increase takes place. Conceivably, the propensity of individual cattle to vocalize during isolation reaches a relatively stable state somewhere around age one year (i.e. between OBS 3 and OBS 4). During OBS 4, the animals may have been fairly homogeneous as regards the state of development of this characteristic which they had reached. The significant correlations of age/weight with sound pressure level, fundamental frequency and loudest harmonic during OBS 4 (see Table 4.6), would then tend to be interpreted as being more functionally associated with the size of the animal than with its present state of psychological development.

During OBS 3 and OBS 4 heifers were more vocal than bulls. For obvious management reasons the cattle, which were sexually intact, were kept in single sex pens. By one interpretation, to avoid pseudoreplication, the pen should be used as the individual unit in the statistical comparison between sexes. However, this would leave too few observations for meaningful comparison. Besides which, there is a great deal of variability within each pen, due to the unique characteristics of individuals. Since the
pens were all essentially the same, whereas the individuals within each were certainly not, the individual animal was used as the statistical unit for this analysis. There were few indications that males and females gave acoustically different calls. A higher proportion of females vocalized during the latter two observations. Under conditions of social isolation, females, being possibly more gregarious than males, may be more strongly motivated to vocalize to make social contact with other herd members.

The animal movement data also showed individual variations attributable to sire and family. These scores were used as an indicator of general physical reactivity to the testing situation. The MMD scores of individuals correlate well over time, perhaps illustrating another stable individual characteristic. A factor which, like vocalization, is partly inherited from the parents. The lack of any relationship between physical movement on the scale and either the amount or acoustic properties of the vocalizations was surprising. It was initially assumed that vocal responses and high MMD scores could both be interpreted as indicating fear. This may be too simplistic a view. In sheep, vocalization may indicate an intermediate level of response to social isolation (Syme and Elphick, 1982), the most fearful individuals may actually be less likely to vocalize. Some evidence suggests that certain domestic species vocalize less frequently in the presence of human observers. This has been shown in sheep (Syme and Elphick, 1982), sheep and goats (Price and Thos, 1980) and dairy cows (Rushen et al., 1998). In this study the high-sided scale platform minimised visual contact between calf and observer during testing, which may have reduced any inhibition against vocalizing in the presence of a
human. The extent of visual and perhaps auditory and olfactory exposure to humans should also be considered as a possible source of influence on their vocal behaviour. It may be that vocalization and physical reactivity, while both being stable endogenous characteristics, do not reflect similar emotional or mind states. If not, it would be illogical to invoke both (or perhaps either) as evidence of fear induced by isolation. This study seems to indicate that the tendencies to be vocal or to be reactive are both somewhat heritable. But perhaps these characteristics are inherited independently of one another. The expression of both types of response will also be influenced by previous experience and learning. The psychological processes subserving vocal and reactive responses to isolation may also be different. The behaviour of an isolated individual will be influenced by inherited tendencies. However, the "decision" as to whether to flee, communicate, or try both simultaneously will also be a product of the animal's cognitive representation of the threat status of the situation and the appropriate goal-oriented response.

It is tempting to see vocal response as a useful way to circumvent some of the problems associated with other measures used to indicate welfare. Vocal response can be measured noninvasively and can be variable in both the amount of vocalization and in a variety of qualitative parameters. In this sense it offers advantages over heart rate measurements or any measure which requires blood sampling. However there remains uncertainty about the validity and reliability of vocal response when used as a measure of distress or suffering. One implication of this study is that, given the existence of such
individual variability, any group of animals being studied must be regarded as more or less unique in its vocal tendencies, at any particular time, even before the effect of an experimental treatment is considered. This would appear to be an argument against the use of comparisons in vocal response rate between different groups of animals as a valid indicator of their relative states of welfare. Similarly, it implies difficulties in the use of vocal response in longitudinal studies, where repeated measures separated by intervals of weeks or months are desired.

One problem with using vocal response as an indicator of welfare of cattle during a stressful procedure is that normally only a proportion of the animals will exhibit any vocal response at all. This remains true whether the procedure is pre-slaughter handling (Grandin, 1998), branding (Chapter 3) or short periods of isolation, as in the present study. Unlike measuring blood parameters, for example, where a proportion of spoiled samples would mean missing data in the statistical analysis, a null vocal response is a relevant data bit. But what can be said, at the individual level, of animals (often a majority) that do not vocalize? It would be unreasonable to argue that an animal feels no pain or fear associated with the procedure, simply because it does not vocalize; the majority of cattle do not vocalize during hot-iron branding and castration. It is probably sensible to conclude that measures of vocal response should be used to supplement other forms of information rather than as a sole criterion to assess welfare. At least where the impact of a treatment on individuals is at issue. The proportion of animals within a group which vocalize is more indicative of the welfare of the group than of any single
animal within it.

In conclusion, these findings suggest that there are endogenous sources of variability in the vocal responses of cattle, which are independent of previous experience and learning. These attributes were influenced by both sire and family, which suggests that they are transmissible genetically, from parents to offspring. There are individual vocal characteristics which are stable over time, and developmental processes which tend to change vocal behaviour over time. In respect of their propensity to vocalize, males and females responded similarly at the start of the study, but during the two later observations females were more vocal, implying that the development of individual vocal characteristics proceeds differently for females than for males. A calf’s predisposition to vocalize and the acoustic quality of its calls will depend on a combination of its parents’ attributes, its age, physical size and sex, as well as the stimuli acting upon it at any moment.
5.0 THE PROPENSITY OF CATTLE TO VOCALIZE DURING HANDLING AND ISOLATION IS AFFECTED BY PHENOTYPE, BUT CALL CHARACTERISTICS DEPEND ON THE TESTING SITUATION.

5.1 ABSTRACT

This study investigated whether phenotype affects vocal and behavioural responses of newly-weaned beef calves during handling situations and whether vocal response differs between two types of handling situation. Steers phenotypically representative of "pure" Angus (121 animals), "pure" Charolais (114), crossbred Angus (35) or crossbred Charolais (37) were used. Cattle designated crossbred had body colours consistent with being of Angus or Charolais parentage, with contrasting head or face colouration resulting from crossbreeding with a Hereford.

In Trial 1, vocal responses of 307 steers were recorded for 1 minute, while each was restrained for ear tagging, implantation, rectal temperature measurement, vaccination and placement of electrodes for subsequent heart rate measurement. The proportion of calves vocalizing was 36.2%. This proportion varied among phenotype groups ($P=0.042$). More "pure" Angus (45.4%) than "pure" Charolais (29.8%) vocalized
(P=0.014). Calls did not differ between phenotype groupings in any acoustic properties.

In Trial 2, conducted on d 2 and d 3 after Trial 1, 111 steers were confined individually, in visual isolation on a scale platform for 1 min. Heart rate was measured at the beginning and end of this period. Vocal behaviour was recorded and movement was measured using an electronic device connected to the load sensing circuit of the scale. During isolation 16.2% of steers vocalized; a smaller proportion than in Trial 1 (P=0.0001). Angus-type cattle (i.e. both “pure” and crossbred Angus) tended to be more vocal than Charolais-type (22.4% and 9.4%, respectively, vocalized, P=0.0639) There were no phenotype-related differences in movement. Angus-type cattle tended to have higher initial heart rates (151.4±3.7 versus 140.7±2.8 beats/min, P=0.063) and greater reduction in heart rate (27.4±1.9 versus 22.9±1.7 beats/min, P=0.076) over the 1 min than Charolais-type. The amount of movement positively correlated with heart rate. Calves that vocalized showed greater reduction in heart rates than silent calves (32.4 ±2.1 versus 24.5 ±1.4 beats/min, P=0.0373).

Cattle that vocalized during Trial 1 were more likely to vocalize during Trial 2 (P=0.0373). Calls recorded during Trial 2 were shorter (1.29 ±0.16 s versus 1.80 ±0.1 s, P=0.0075) with lower fundamental frequencies (88.1 ±2.8 Hz versus 136.8 ±10.9 Hz, P=0.001) than during Trial 1. These results indicate that there are differences in the propensity to vocalize between phenotypic groups. This should be considered when
making welfare judgements based on a proportion of animals that vocalize. The vocal propensity of individuals is a stable characteristic over different occasions and situations. Acoustic properties of calls differ between conditions and may provide information about how those conditions affect the internal states of the animals.

5.2 INTRODUCTION

Cattle frequently vocalize during unpleasant management procedures. Their vocal responses to painful or psychologically disturbing events may be a valid and useable indicator of their welfare. Measurement of vocal behaviour is an attractive approach to welfare assessment for several reasons. It is noninvasive, requiring only the presence of a microphone or an unobtrusive observer. At its simplest, it is cheap and technically undemanding. In Chapter 2 it was argued that vocal behaviour may be interpreted as a form of subjective commentary by an individual animal on its own internal condition. Therefore vocal behaviour may reflect an interpretive cognitive process, more directly associated with the emotional and psychological states evoked by an unpleasant treatment than other common measures. It is likely that such an interpretive process, and the consequent vocal response will be influenced by individual experience and learning. Similarly there may be genetic influences, either on the detailed functioning of that process, or on how the results of that process are expressed vocally. In Chapter 3 it was reported that the vocal responses of beef cattle to visual isolation are influenced by age and genetic factors. This variability imposes some limitations on how vocal response
should be used in welfare measurement.

Vocal response is appealing as a welfare measure, in part because it might reflect the processes underlying subjective phenomena such as "feelings". Animals' subjective experiences are undoubtedly an important aspect of their welfare. Perhaps, as Duncan and Petherick (1991) have argued, the only important aspect. But they are difficult to study. Studies of animal communication have been invoked by Griffin (1984) as a possible means to investigate animal consciousness and subjective phenomena. The use of vocal response is problematic as a practical indicator of welfare, because individual variation may result in a wide range of responses even among a group of animals that are all identically treated. For example, it seems fairly certain that all cattle subjected to hot-iron branding will experience acute pain. However, Chapter 3 showed that there was a wide range of individual vocal responses to branding, both quantitatively and qualitatively, with most animals not vocalizing at all. In Chapter 2, I concluded that vocal response should preferably be considered alongside other measures when evaluating welfare, rather than as the sole criterion for comparison. I also felt that more research into the sources of individual variability in vocal response was necessary.

It is possible for specific phenotypic characteristics to be associated with behavioural traits. For example, the position of hair whorl patterns on the foreheads of cattle appears to be related to excitability (Grandin et al., 1996; Randle, 1998). It is also well established that there are breed-related differences in the temperament characteristics of
cattle and their behavioural responses to handling and restraint (discussed by Grandin and Deesing, 1998). Hereford cattle were rated by Stricklin et al. (1980) as more docile than other British breeds and Galloway more excitable. Tulloh (1961) rated Herefords as quieter during restraint than either Angus or Shorthorns. Wagnon et al. (1966) noted breed differences in dominance ranking within a mixed-breed cow herd, with Angus cows being most dominant, Hereford least and Shorthorn intermediate in rank. Voisinet et al. (1997) found differences in temperament scores between different breeds of Bos taurus cattle and between different B. taurus - B. indicus crosses. There is some evidence suggesting that different cattle breeds use vocal behaviour differently. For example, Narasimha Rao et al. (1996) compared the behaviour of breeding bulls associated with semen collection (using an artificial vagina and male decoy) and found that Ongole and Murrah bulls vocalized more frequently than Jersey or Jersey-Ongole crossbred bulls. In one test, Le Neindre (1989) found that Salers cattle vocalized more often than Friesians when tested singly in a novel enclosure. One objective of the study presented in this chapter was to examine the effect of phenotypic characteristics, on vocal responses to stressful handling procedures.

Behavioural responses, including vocal responses may differ markedly between different handling situations. Such differences may reflect varying degrees of distress or diminished welfare as experienced by the subjects. They may also represent the output of a perceptual-cognitive process in which animals evaluate the challenges posed by the present situation and attempt an appropriate response. For example, an animal which is
being restrained and directly handled by people might “appropriately” attempt to struggle free, whereas an isolated, but not directly threatened individual might attempt to initiate communication with other herd members.

Vocal behaviour does vary with changes in the physiological condition of an animal. Jahns et al. (1998) developed a computer program which was able to classify power spectra of dairy cows’ vocalizations into categories such as “hungry”, “thirsty” or “delayed milking” which agreed well with their apparent condition. Schrader and Todt (1998) found changes in the rates of specific types of pig vocalizations associated with changes in levels of plasma adrenaline and cortisol during isolation and restraint.

In the present study the same group of cattle were tested under two quite distinct sets of conditions. Firstly, firm mechanical restraint while several routine management procedures were performed, and secondly, a period of visual isolation. This was done in order to discover whether they would use vocal behaviour differently between situations which appear to pose different problems, either in terms of the level of distress involved, or in the selection of an appropriate response.

5.3 ANIMALS, MATERIALS AND METHODS

5.3.1 Animals
The cattle used in this study were 307 newly-weaned steers (274.15 ± 1.15 kg.) aged approximately six months. They were purchased as commercial beef feeder calves from local livestock auctions and transported to the University of Saskatchewan's Beef Research Unit feedlot.

Although they were bred from unknown stock, they were selected by a cattle buyer as being phenotypically representative of two beef breeds common in Western Canada; Angus and Charolais. Within each breed group some calves appeared to be fairly pure representatives of their breed, in terms of their colouration. These were designated "pure" Angus and "pure" Charolais for the purposes of this study. A proportion of the group (72 calves) displayed the appearance which typically results from crossbreeding with a white-face (i.e. Hereford) animal. With the Angus, this crossing frequently produces a calf which is predominantly black in the body, but with a partially or totally white head. Black-bodied animals displaying any degree of white face were designated as "Angus cross" cattle. When Charolais and Hereford cattle are crossbred the offspring typically show evidence of the Hereford pattern of colouration, much diluted by the white of the Charolais. The body may range from light brown, through tan to off-white, with a lighter coloured head or face. Animals displaying these characteristics were designated "Charolais cross". The numbers of each type used in the study were as follows: 121 "pure" Angus, 35 Angus cross, 114 "pure" Charolais and 37 Charolais cross. This classification was made purely on the visible expression of certain common phenotypic features and represents something of a simplification. It is likely that breeds
other than Angus, Charolais and Hereford contributed influences to both the appearance and the behaviour of some of these animals, whether designated as "pure" or crossbred.

5.3.2 Handling Procedures and observation methods

5.3.2.1 Trial 1; Initial processing trial

5.3.2.1.1 Handling and treatment during trial 1

The calves arrived at the feedlot in several truckloads over a period of 7 days. The handling procedure used in trial 1 was a series of operations normally performed at this facility for the initial processing of newly arrived calves. These animals were processed in 3 batches, comprising 112, 92 and 103 animals respectively. Each batch was processed on a separate day. The proportions of animals of each phenotypic category did not differ significantly between days.

Each batch of animals was moved from the single pen in which they had been held since arrival (12-48 hr), through a curved, solid-sided chute into an indoor handling facility. After entering the building each calf in turn was moved forward until captured in a headgate and side pressure was applied with a mechanical squeeze to assist in restraining the animal. At the same time another calf was moved into the building. During each
treatment there would normally be three steers in the building: one receiving the
treatment and two standing behind it in the chute. Once the lead animal was restrained, a
stopwatch was started and the processing treatment commenced. This consisted of a
number of common management operations carried out in quick succession by several
operators. This was sufficient to elicit vigorous escape movements and vocalization
from many of the animals. Once restrained, each animal had any existing ear tags
removed with wirecutters and a new tag placed through the left ear. A growth-promoting
implant (Synovex S®, 200 mg) was introduced subcutaneously into the right ear. All
animals were injected intramuscularly with IBR-PI3 (Smithkline Beecham, Missisauga,
Ontario) and eight-way Clostridium (Tasvax 8®, Mallinkrodt Veterinary Inc., Ajax,
Ontario) vaccines. An antiparasitic medication (Ivomec Pour-on) was applied to the
back. Body temperature was measured by an electronic thermometer and rectal probe.
Hair was removed from a 5cm by 5cm area approximately 10 cm behind the right
shoulder and a similar area on the left flank using an electric clipper and a stainless steel
surgical skin closure staple was inserted into the skin in each of these locations. These
staples served as electrodes for subsequent heart rate measurements (described below).
In most instances these procedures took less than 1 min from the time the animal was
catched in the headgate (typically about 50 s). In such cases the calf was held in the
headgate until a full minute had elapsed. The animal was then released from the
headgate and moved into a closed-sided single animal scale where it was confined for 5-
10 s while it was weighed. After weighing, they exited the building and were allotted to
pens. The animals were divided among 21 pens. Thirteen pens held 14 calves each,
seven held 15 and another had 20 animals. Animals were allocated such that each pen had approximately equal proportions of the four phenotypes. Ten calves were found to be bulls, rather than steers. These were castrated immediately after the 1 min observing period and before being released to the weigh scale.

5.3.2.1.2 Vocal responses during trial 1: recording and measurement

While they were confined in the headgate, cattle were videotaped from a head-on perspective using a black and white camera (Panasonic wv-BL204) placed approximately 1.5 m in front of the headgate and a time-lapse recorder (Panasonic AG6730P) running in 6 hr mode. Vocalizations were recorded on the audio channel of the video recorder via a single unidirectional, high impedance dynamic microphone (Sony F-V9). The microphone was placed in front of and above the normal head position of an animal restrained in a standing position in the headgate. Depending on an animal's head movements and the posture it assumed in the chute, the microphone would be between approximately 1 m and 1.5 m distant from the mouth. A stopwatch was started when the animal was caught in the headgate. Each time the animal vocalized, the elapsed time, to the nearest second, was noted.

From the recordings, 480 vocalizations were sampled by computer at a rate of 11025 Hz using a computer with a 16-bit sound card. Each vocalization was stored as a separate
computer file in "WAV" format. Acoustic measurements were made from 470 of these files. Sound files were analyzed using Spectrogram Version 5.0.5.(® R.S. Horne, 1999). This software was used to generate an audiospectrogram plot of frequency over time with sound level represented visually by a variable density gray scale. A short computer program, named "Moocrunch" was written (using Turbo Pascal® 7.0, DOS version, Borland International Inc, 1992) to facilitate calculation of acoustic parameters and record sound data in a file for later analysis. The source code of this program is given in Appendix A. Using the cursor function on the audiospectrogram plot, the start and end points of the call in microseconds were entered into the Moocrunch program which calculated the duration and midpoint in time of each vocalization. "Spectrogram" was used to generate an instantaneous power spectrum plot of sound intensity over frequency occurring at the midpoint of the call. The sound intensity was expressed in decibel (dB) units. Since there was no absolute calibration of this scale against real sound pressure levels present at any position relative to the animal, the dB values displayed are arbitrary. All sound files were produced using an identical process (equipment, recording levels, sampling rate, brand of videotape, etc.), therefore this scale represents a relative indicator of sound intensity only between calls so recorded. Fundamental frequency was calculated as the mean frequency interval (in Herz) between several amplitude peaks (representing harmonics) in the power spectrum. This value was calculated by Moocrunch using information entered by the user from the power spectrum plot. Spectrogram’s data logging function generated a text file of dB measurements at approximately 10.8 Hz frequency intervals between 5Hz and 2750Hz.
Moocrunch read this file and used the information to derive the frequency of greatest intensity (FGI), the maximum sound pressure level at FGI, the loudest harmonic and the total sound intensity. To calculate total sound intensity the program added 100 to each value to ensure that each was a positive number, and summed all the values. Since the dB scale was not calibrated against any absolute measure of loudness, and is a nonlinear scale, data derived using this scale (total sound intensity and sound pressure level at FGI) were analyzed as ranks.

5.3.2.2 Trial 2; response to 1 min visual isolation.

5.3.2.2.1 Handling and observations during isolation testing

The first batch of animals that had been tested in Trial 1 was brought back to the same facility for a test of their vocal, heart rate and movement responses during visual isolation. These 111 calves (one was removed from the study prior to trial 2), from 9 pens were tested over a 2 d period. For 56 animals there was a 2 d interval between Trial 1 and Trial 2 and for 55 animals there was a 3d interval.

Steers were brought one pen at a time from their home pen to the crowd pen outside the handling facility and moved single file along the curved chute to the door. One animal at a time was brought into the facility and, as quietly as possible, walked along the straight chute until stopped by the closed headgate. A wireless electrocardiogram transmitter
fitted with a spring-loaded plastic clip with rubber coated jaws was attached to the hair on the animal’s back. The clip held the transmitter in place without appearing to cause discomfort to the animal. Leads from the transmitter were attached, using small alligator clips, to the skin staples that had been placed on the right shoulder and left flank during Trial 1. The intent was to minimise the invasiveness of the transmitter attachment process. The animal was then moved forwards onto the scale platform and held there, without visual contact with humans or other cattle, for 1 min while observations of its movement, heart rate and vocal response (as described below) were recorded. After this period, the calf was released from the scale and moved out of the building where the transmitter and leads were removed.

5.3.2.2.2 Movement measurements during isolation

Objective measurements of the amount of movement of each calf were obtained using an electronic movement measuring device (MMD). The MMD is connected to the load-sensitive circuit of an electronic scale. Movement of the animal while on the scale causes resistance in the load cells to vary and consequently the voltage in the circuit fluctuates. The MMD measures this voltage 122 times each second for 1 min, recording a "peak" each time a trend of 10 or more voltage changes in the same direction is reversed. The number of peaks is positively correlated with visual estimates of the amount of animal movement (Stookey et al., 1994).
5.3.2.2.3 Audiovisual recording during isolation

A black and white video camera and microphone (as used during Trial 1) were fixed above the scale. The microphone was suspended above the normal head position of an animal standing on the scale platform. The microphone was approximately 1 m distant from the animal’s mouth. A second camera recorded the screen output of a 4-channel heart monitor (Paragon 420, Quinton Instrument Co., Seattle WA, USA) that displayed a real-time electrocardiogram (ECG) trace of the animal on the scale. The camera was placed so that this display almost filled its field of view. The microphone was fed directly to a video recorder (as used in Trial 1) running in 6 hr mode. The two video signals were combined using a video quad unit (Panasonic WJ-MS424) which passed a single video signal to the recorder. When the single video recording was played back, the images from the two cameras (one of the animal and one of the ECG) appeared side-by-side as the two upper quadrants of a 4-way split screen, with time and date information displayed in a lower quadrant.

5.3.2.2.4 Heart rate measurement

Two heart rate measurements were recorded for each animal during the 1 min period of isolation. The first measurement was taken as early as possible in the 1 min period and the second measurement as late as possible. Due to electrical artifacts associated with the movements of the animal which sometimes obscured parts of the ECG trace, it was
not always possible to obtain a measurement precisely at the preferred time. In such instances we accepted a measurement obtained within either the first 15 s or last 15 s of the isolation period, or else declared that measurement as missing. The method of measurement took advantage of the fact that at its fixed sweep speed the heart monitor displayed 5.7 s of ECG trace at any instant (this was determined empirically and differs slightly from the manufacturer's value of 5.3 s). To obtain a segment of ECG data for measurement of the initial heart rate, the video frame was first frozen 6 s after the start of the observation period (i.e. when the trace on the screen represented cardiac activity between 0.3 s and 6 s into the 1 min period). If this trace was unsatisfactory, the tape was slowly advanced to the earliest time (up to 15 s into the isolation period, which would represent activity between 9.3 s and 15 s into the 1 min period) that a satisfactory trace could be seen. The same procedure was applied in reverse, working backwards from the end of the isolation period, to select a portion of ECG for the second heart rate measurement. From each of these segments the heart rate was derived by first counting the number of complete cycles of cardiac activity visible on the screen (i.e. QRS-QRS intervals) and estimating any remaining fraction to one tenth of an interval. The number of intervals was multiplied by 60/5.7 to obtain the mean heart rate in beats/min for the 5.7 s period. At typical heart rates measured during this study an estimation error of ±0.2 intervals would correspond to an approximate heart rate measurement error of ±2 beats/min.
5.3.2.2.5 Vocal responses during isolation: recording and measurement

Techniques and equipment for recording and measuring vocal responses were identical to those used in Trial 1.

5.3.3 Statistical analysis

Data were analysed using Statistix V1.0 (Analytical Software, 1996) for Windows. Many of the quantities available for statistical comparison in this study were either in the form of categorical or ordinal data. Among the continuous variables recorded, most of the acoustic measurements, movement measurements and time intervals either deviated from a normal distribution (as evaluated visually by frequency histogram and rankit plot, and Wilks-Shapiro statistic) or else important comparison groups possessed unequal variances. Therefore only nonparametric procedures have been used in this analysis. Comparisons of the numbers of animals which vocalized between phenotype groups and between trials used the chi-square procedure. Matched pairs comparisons used the Wilcoxon test. Other two sample comparisons used the Mann-Whitney rank sum test. Tests of more than two samples used the Kruskal-Wallis nonparametric analysis of variance. Clustering of vocal animals within the entry order was assessed using the runs test. All correlations were calculated using the Spearman rank order correlation procedure.
5.4 RESULTS

5.4.1 Trial 1. Vocal responses during 1 min restraint for processing

During the 1 min observation interval, 111 of 307 calves (36.2%) uttered at least one audible vocalization. On the 3 days when the 3 batches of calves were processed, the proportions which vocalized were 34.8%, 41.3% and 33% respectively (not statistically different). Of 480 calls recorded, 470 were of sufficient quality to allow some quantitative analysis. Calves that did vocalize gave between 1 and 13 calls (mean 4.32 ± 0.31). In some cases they continued a series of vocalizations beyond the 1 min period, if they were still under restraint at that time. However calls after 1 min were neither counted nor analysed acoustically. Table 5.1 reports an analysis of the numbers of animals which vocalized or did not vocalize during Trial 1. There were significant differences among the four phenotype groupings in the proportions which vocalized ($P=0.042$). Comparing all Angus-type (i.e. "pure" Angus and crossbred Angus groups combined) with all Charolais-type calves revealed a tendency for more of the Angus-type to be vocal ($P=0.071$). The "pure" Angus phenotype class had a significantly higher proportion of vocalizers than the "pure" Charolais type ($P=0.014$).

The measured acoustic properties of the calls are reported by phenotype group in Table 5.2. Statistical tests revealed no significant differences between groups in any of the measurements. Animals which vocalized did not differ in their mean weight on entry.
Table 5.1
Chi-square analysis of the numbers of animals which vocalized or did not vocalize during trial 1

<table>
<thead>
<tr>
<th></th>
<th>Vocalized</th>
<th>Did not vocalize</th>
<th>Total</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>55 (43.7)</td>
<td>66 (77.2)</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angus cross$^2$</td>
<td>9 (12.6)</td>
<td>26 (22.3)</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charolais</td>
<td>34 (41.2)</td>
<td>80 (72.8)</td>
<td>114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charolais cross$^2$</td>
<td>13 (13.4)</td>
<td>24 (23.6)</td>
<td>37</td>
<td>8.182</td>
<td>0.042</td>
</tr>
<tr>
<td>All Angus</td>
<td>64 (56.4)</td>
<td>92 (99.6)</td>
<td>156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Charolais</td>
<td>47 (54.6)</td>
<td>104 (96.4)</td>
<td>151</td>
<td>3.258</td>
<td>0.071</td>
</tr>
<tr>
<td>&quot;Pure&quot; Angus$^3$</td>
<td>55 (45.8)</td>
<td>66 (75.2)</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Pure&quot; Charolais$^3$</td>
<td>34 (43.2)</td>
<td>80 (70.8)</td>
<td>114</td>
<td>6.094</td>
<td>0.014</td>
</tr>
<tr>
<td>Angus cross</td>
<td>9 (10.7)</td>
<td>26 (24.3)</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charolais cross</td>
<td>13 (11.3)</td>
<td>24 (26.7)</td>
<td>37</td>
<td>0.752</td>
<td>0.386</td>
</tr>
<tr>
<td>All &quot;pure&quot; breeds</td>
<td>89 (85.0)</td>
<td>146 (150.0)</td>
<td>235</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All crossbred</td>
<td>22 (26.0)</td>
<td>50 (46.0)</td>
<td>72</td>
<td>1.278</td>
<td>0.258</td>
</tr>
</tbody>
</table>

$^1$ In these columns the first number is the observed frequency and the number in parentheses is the expected frequency.

$^2$ "Cross" refers to cattle that phenotypically expressed some light head or face coloration that contrasted with the colour of the rest of the body, as would be expected if one parent had been a Hereford animal.

$^3$ "Pure" refers to cattle that phenotypically expressed uniform coat and face colouration that was consistent with the registered breed characteristics.
### Table 5.2
Mean values ±standard errors of calf vocalization measurements during trial 1, compared by phenotype group.

<table>
<thead>
<tr>
<th></th>
<th>Angus</th>
<th>Charolais</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;Pure&quot;</td>
<td>Crossbred</td>
<td>&quot;Pure&quot;</td>
</tr>
<tr>
<td>Number of vocalizations</td>
<td>4.32±0.45</td>
<td>5.11±1.40</td>
<td>4.47±0.53</td>
</tr>
<tr>
<td>Time before first call (s)</td>
<td>21.36±1.69</td>
<td>28.78±6.18</td>
<td>18.36±2.02</td>
</tr>
<tr>
<td>Interval between calls (s)</td>
<td>9.70±1.49</td>
<td>6.04±1.36</td>
<td>8.71±1.18</td>
</tr>
<tr>
<td>Length of call (s)</td>
<td>1.70±0.09</td>
<td>2.22±0.36</td>
<td>1.60±0.13</td>
</tr>
<tr>
<td>Fundamental frequency (Hz)</td>
<td>126.8±6.7</td>
<td>140.4±20.4</td>
<td>132.9±17.7</td>
</tr>
<tr>
<td>Frequency of greatest intensity (Hz) [FGI]</td>
<td>565.1±68.6</td>
<td>446.0±67.9</td>
<td>435.4±34.5</td>
</tr>
<tr>
<td>Sound pressure level at FGI (mean rank)(^1)</td>
<td>48.56±4.18</td>
<td>37.22±10.1</td>
<td>57.21±5.27</td>
</tr>
<tr>
<td>Total sound intensity (mean rank)(^1)</td>
<td>48.86±0.62</td>
<td>32.78±10.3</td>
<td>56.10±4.96</td>
</tr>
<tr>
<td>Loudest harmonic</td>
<td>4.87±0.62</td>
<td>3.39±0.39</td>
<td>4.03±0.36</td>
</tr>
</tbody>
</table>

\(^1\)Mean ranking within the 100 animals for which these two measures could be calculated. Lower mean values indicate higher intensity levels (louder calls).
into the feedlot, or in their rectal temperature at processing time, compared with
nonvocal animals. Neither weight nor rectal temperature were significantly correlated
with any of the acoustic measurements of the vocalizations. Within each of the 3 groups
that were processed, the mean rank order of vocal calves as they entered the handling
chute was not significantly different from that of silent animals, though within the
second batch vocal animals tended to be earlier in the entry order (\(P=0.07\)). Within each
batch, animals which vocalized were not significantly clustered in the entry order.

5.4.2 Trial 2; Responses during 1 min isolation period.

During the 1 min isolation trials, 18 of the 111 animals vocalized (16.2%). A breakdown
of these by phenotype group is given in Table 5.3. There was a tendency for more Angus
type calves to be vocal (\(P=0.0639\)). Table 5.3 also illustrates the average number of
vocalizations and latency to vocalize for those that did call.

Movement scores (number of MMD peaks) are compared by phenotype group in Table
5.4. There were no phenotype-related differences in movement. Table 5.4 also compares
heart rate measurements between groups. Angus cattle tended to show higher initial
heart rates and a greater reduction between initial and final measurements than
Charolais. The number of MMD peaks was correlated with both initial (\(r=0.23, P<0.05\))
and final (\(r=0.30, P<0.005\)) heart rate values.
Table 5.3
Vocal responses of cattle by phenotype group during 1 min of visual isolation.

<table>
<thead>
<tr>
<th></th>
<th>Number that vocalized</th>
<th>Number of vocalizations(^1)</th>
<th>Time to first vocalization (s)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Pure&quot; Angus</td>
<td>10 of 41</td>
<td>3.9 ± 1.1</td>
<td>18.4 ± 5.9</td>
</tr>
<tr>
<td>Crossbred Angus</td>
<td>3 of 17</td>
<td>2.0 ± 1.0</td>
<td>35.3 ± 14.4</td>
</tr>
<tr>
<td>&quot;Pure&quot; Charolais</td>
<td>5 of 44</td>
<td>3.8 ± 1.9</td>
<td>30 ± 6.9</td>
</tr>
<tr>
<td>Crossbred Charolais</td>
<td>0 of 9</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>All Angus</td>
<td>13 of 58(^a)</td>
<td>3.5 ± 1.9</td>
<td>22.3 ± 5.7</td>
</tr>
<tr>
<td>All Charolais</td>
<td>5 of 53(^b)</td>
<td>3.8 ± 1.9</td>
<td>30 ± 6.9</td>
</tr>
<tr>
<td>All Calves</td>
<td>18 of 111</td>
<td>3.56 ± 0.79</td>
<td>24.44 ± 4.51</td>
</tr>
</tbody>
</table>

\(^1\)Mean ± standard error.
\(^a^p=0.0639\)
Table 5.4.
Means ± standard errors of movement score and heart rates by phenotype group, during trial 2

<table>
<thead>
<tr>
<th></th>
<th>Movement score(^1)</th>
<th>Initial heart rate (HR1)</th>
<th>Final heart rate (HR2)</th>
<th>HR change (HR2-HR1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(peaks)</td>
<td>(beats/min)</td>
<td>(beats/min)</td>
<td>(beats/min)</td>
</tr>
<tr>
<td>&quot;Pure&quot; Angus</td>
<td>17.63 ± 2.52</td>
<td>151.4 ± 4.8</td>
<td>124.1 ± 4.2</td>
<td>-26.8 ± 2.2</td>
</tr>
<tr>
<td>Crossbred Angus</td>
<td>20.35 ± 4.90</td>
<td>151.2 ± 5.3</td>
<td>122.3 ± 5.1</td>
<td>-29.3 ± 4.1</td>
</tr>
<tr>
<td>&quot;Pure&quot; Charolais</td>
<td>21.98 ± 3.64</td>
<td>140.7 ± 3.0</td>
<td>121.3 ± 4.2</td>
<td>-22.3 ± 1.8</td>
</tr>
<tr>
<td>Crossbred Charolais</td>
<td>21.22 ± 9.85</td>
<td>140.3 ± 8.8</td>
<td>117.4 ± 7.3</td>
<td>-25.7 ± 4.6</td>
</tr>
<tr>
<td>All Angus</td>
<td>18.43 ± 2.27</td>
<td>151.4 ± 3.7(^a)</td>
<td>123.6 ± 3.4</td>
<td>-27.4 ± 1.9(^c)</td>
</tr>
<tr>
<td>All Charolais</td>
<td>21.85 ± 3.41</td>
<td>140.7 ± 2.8(^b)</td>
<td>120.5 ± 3.6</td>
<td>-22.9 ± 1.7(^d)</td>
</tr>
<tr>
<td>All Calves</td>
<td>20.06 ± 2.01</td>
<td>146.4 ± 2.4</td>
<td>122.2 ± 2.5</td>
<td>-25.4 ± 1.3</td>
</tr>
</tbody>
</table>

\(^{ab}P = 0.063; \quad ^{cd}P = 0.076\)

\(^1\)Movement scores were calculated by an electronic device connected to the load-sensitive circuit of the scale. The device sampled the fluctuating voltage in this circuit 122 times per second for 1 min and recorded a peak each time a trend of 10 consecutive voltage changes in the same direction was reversed.
Table 5.5 compares the movement and heart rate data between vocal and nonvocal animals. Calves that vocalized during the 1 min isolation period showed a greater reduction in heart rate during this time. Neither the pen in which the calves had been held between Trials 1 and 2 nor the day of testing influenced the measurements recorded during Trial 2.

5.4.3 Relationship between responses shown in Trials 1 and 2.

A larger proportion of the cattle vocalized during Trial 1 than in Trial 2 (36.2% versus 16.2%; \( \chi^2 = 15.19, P = 0.0001 \)). Animals that vocalized during Trial 1 were more likely to vocalize again during Trial 2 (\( \chi^2 = 4.34, P = 0.0373 \)). Between the 10 individuals that vocalized during both trials there was no correlation in the number of calls they gave.

Acoustic measurements were made of 64 calls. The calls did differ very significantly in some measurable parameters between trials. Table 5.6 compares mean call properties for the comparison group of 111 animals which were used in both trials. In Trial 2, calves gave shorter calls (\( P = 0.0075 \)) with lower fundamental frequencies (\( P = 0.001 \)) than in Trial 1.

A matched pairs comparison between sound measurements for the 10 animals that vocalized in both trials showed that the same calves did produce different calls in different situations. Individuals gave shorter (\( P = 0.0249 \)) vocalizations with lower fundamental frequencies (\( P = 0.0191 \)) during Trial 2 than they did during Trial 1.
Table 5.5
Comparison of movement score and heart rates (means ± standard errors) between calves that vocalized and calves that did not vocalize during trial 2.

<table>
<thead>
<tr>
<th></th>
<th>Movement score(^1) (peaks)</th>
<th>Initial heart rate (HR1) (beats/min)</th>
<th>Final heart rate (HR2) (beats/min)</th>
<th>HR change (HR2-HR1) (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vocalized</td>
<td>22.56 ± 4.41</td>
<td>149.4 ± 7.1</td>
<td>119.1 ± 7.1</td>
<td>-32.4 ± 2.1</td>
</tr>
<tr>
<td>Did not vocalize</td>
<td>19.58 ± 2.25</td>
<td>146.0 ± 2.6</td>
<td>122.6 ± 2.6</td>
<td>-24.5 ± 1.4</td>
</tr>
<tr>
<td>(P)</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>0.0373</td>
</tr>
</tbody>
</table>

\(^1\)Movement scores were calculated by an electronic device connected to the load-sensitive circuit of the scale. The device sampled the fluctuating voltage in this circuit 122 times per second for 1 min and recorded a peak each time a trend of 10 consecutive voltage changes in the same direction was reversed.
Table 5.6
Mean values ± standard errors of calf vocalization measurements\(^1\) during Trial 1 (processing) and Trial 2 (isolation) for the 111 calves that were tested in both trials.

<table>
<thead>
<tr>
<th></th>
<th>Trial 1 (38 vocal calves)</th>
<th>Trial 2 (18 vocal calves)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (s)</td>
<td>1.805 ± 0.100</td>
<td>1.2886 ± 0.163</td>
<td>0.0075</td>
</tr>
<tr>
<td>Fundamental frequency (Hz)</td>
<td>136.77 ± 10.88</td>
<td>88.10 ± 2.76</td>
<td>0.001</td>
</tr>
<tr>
<td>Loudest Harmonic</td>
<td>3.76 ± 0.26</td>
<td>5.68 ± 1.21</td>
<td>N.S.</td>
</tr>
<tr>
<td>Frequency of greatest</td>
<td>451.92 ± 31.08</td>
<td>482.79 ± 99.83</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

\(^1\)Measurements of sound intensity have not been compared between trials because microphone position relative to the subject's mouth was not the same in both testing locations.
Calves that vocalized during Trial 1 did not show different movement scores or heart rates in the subsequent trial than those that did not vocalize. Among those that did vocalize during Trial 1, the number of calls they made was not correlated with later movement or heart rate scores. However, the number of calls in Trial 1 was highly correlated with the amount of change between initial and final heart rates during Trial 2 ($r = -0.503, P < 0.01$), in the sense that animals that vocalized more showed a greater reduction in heart rate. Eliminating the calves that also vocalized during Trial 2, the relationship remained similar ($r = -0.463, P < 0.05$). In other words, animals that were more vocal in the first trial reduced their heart rate more during 1 min of isolation, whether they vocalized during that time or not.

There were no significant relationships between any of the acoustic measurements of the call obtained during Trial 1 and the movement or heart rate values during Trial 2.
5.5 DISCUSSION

During Trial 1 the calves used in this study were subjected to a barrage of stimuli, that was certainly distressing and, in parts painful. They were physically restrained in close proximity to several human operators, who worked all around them including right beside their heads. The tasks these people performed included implanting, ear-tagging and vaccination. This procedure elicited struggling and clear indications of distress in many instances, and a vocal response from more than a third of the animals. This trial was not set up with the intent to create an artificially severe procedure for experimental purposes. With the exception of the staple placement for the heart rate transmitters, what was done to the animals was a routine set of processing tasks performed on virtually all cattle arriving at a feedlot. It should be remembered that processing occurred shortly after a series of other stressful events during the preceding 2 to 3 days, that included weaning and separation from familiar adult animals, transport, mixing with unfamiliar cattle and placement into a novel environment (Tarrant and Grandin, 1993). These prior events may also have contributed to their reactions during the processing trial. These few days probably represent one of the most stressful periods that feedlot cattle will experience.

It is probable that the high proportion of animals that vocalized during Trial 1 indicates a reduction in welfare during this trial. Any change in the procedure which significantly reduced this proportion would likely be an improvement in welfare terms. Vocal
response has been used in this way by Grandin (1998) to detect welfare problems in
slaughter plants and to monitor improvements resulting from changes in practice.

However levels of vocal response by cattle are certainly influenced by factors other than
the treatment being considered (discussed in Chapter 2).

Recently, it has been shown that, to some extent, the vocal characteristics of individual
cattle are heritable from the parents (Chapter 4). Cattle may also adapt some of their
vocal characteristics through exposure to the mother’s vocal behaviour and that of the
natal group. By creating families of siblings through embryo transfer from superovulated
cows into unrelated surrogate mothers, it was shown that some genetically inherited
tendencies in vocal response can still be observed despite the effects of the rearing
environment. Although the offspring of particular individuals shared aspects of their
vocal behaviour, because of the small number of parent animals involved in the Chapter
4 study (5 sires and 13 dams) it was not possible to say whether what was inherited were
characteristics of particular breeds or peculiarities of particular parents. In the present
study the exact pedigrees of the calves were unknown. It would therefore be imprecise to
say breeds have been compared. However, animals were compared that phenotypically
expressed an outward appearance similar to purebred cattle, or else that were clearly
crossbred. On the basis of this phenotypic classification it was found that the vocal
behaviour of those classed as “pure” Angus and “pure” Charolais cattle was different.
But the only difference detected was in the proportion of animals that called. The effect
of crossbreeding, involving what we assumed to be Hereford cattle, was apparently to
dilute this difference. If breeds can be said to have a hierarchy from greater to lesser propensity to vocalize, then possibly Hereford cattle rank between Angus and Charolais on this scale. Alternatively, it may be that purebred animals tend to be at one extreme or the other in their propensity to vocalize, with crossbred animals tending to be intermediate. It would be necessary to study pure and crossbred phenotypic representatives of other breeds to achieve a full description of variability in the tendency to be vocal. Even without such information one can say that it is possible for two groups of cattle with different phenotypic breed compositions to be differently-biased in their vocal tendencies, even when given the same treatment. This is potentially a confounding factor in studies that use the proportion of vocal animals as a measure, unless the phenotypic composition of treatment groups is balanced. Acoustically, although there was considerable variation between individuals, the call properties were not dissimilar at the level of the phenotype group. This finding is consistent with an interpretation that the familial differences in sound properties found in the embryo transfer study (Chapter 4) were, in fact, the inherited characteristics of individuals, not breeds.

Isolated cattle typically exhibit behavioural and physiological changes suggestive of fear or distress that include struggling, increased heart rate and vocalizations (Hopster and Blokhuis, 1994; Boissy and Le Neindre, 1997). During the one minute isolation trials, a smaller proportion of the animals tested vocalized compared with the proportion observed during the processing trial. Nevertheless, the Angus cattle still tended to be more vocal. Though nonsignificant, this further implies that there exists a difference
between these phenotypic groups in their propensity to vocalize. Another nonsignificant
trend, in the heart rate data, suggested that Angus cattle may be more fearful than
Charolais cattle in handling situations such as these.

The movement scores showed no phenotype-related differences. The relationship
between vocal response and physical reactivity during handling procedures may not be a
simple one. It was interesting to find that the number of vocalizations made by calves
during isolation was correlated with the amount of reduction in heart rate between the
first and second measurements. One can only speculate as to the reasons for this.
Possibly vocalization by a distressed animal may serve a self-calming function. I
previously discussed the possibility that an animal might use its own signals to modify
its internal state; a form of autocommunication (Chapter 2). This may have been an
example of the phenomenon. Alternatively, if the vocalizations of isolated cattle
represent an adaptive attempt to communicate, rather than simply a fear response, an
appropriate adjunct to this behaviour would be a phase of active listening for a reply,
necessitating stillness and calm attentiveness, during which the heart rate might be
expected to decline more than during a period of continued fearful, agitated behaviour.
That animals with a propensity to vocalize have some different temperamental
characteristics than silent animals is further suggested by the intriguing discovery that
calves that called more during Trial 1 also showed a higher reduction in heartrate during
Trial 2, even if they did not vocalize during the isolation period. For a more
communicative animal, attempting to initiate a vocal exchange by calling may not be the
only good strategy when isolated. In this situation, some individuals may choose instead to stand quietly and listen for the calls of others. This could have the same lowering effect on heart rate. The tendency to be more or less vocal may well be an individual characteristic that persists over time and between different environments and conditions. This is suggested by the finding that calves that vocalized during processing were more likely to vocalize during the isolation trial.

Calls differed both qualitatively and quantitatively between trials. It was quite apparent to observers present during these trials that the calls of the calves during isolation sound different from calls made during the processing. On visual inspection the sound spectra of calls made during isolation typically showed much flatter harmonic bands (i.e. less variation in frequency within a call) than those made during processing. It was also found that the calls differed significantly in their fundamental frequency and duration between the two conditions. Those few animals that vocalized under both circumstances were apparently able to produce a different type of call in each condition. This supports the view that the calls of cattle are situation-specific and may thus give information about the caller’s emotional and motivational state which should be meaningful to other cattle. The use of computer algorithms to differentiate between internal states in cattle on the basis of properties of their vocalizations has been pioneered by Jahns et al. (1998). Their system was able to discriminate between vocalizations of dairy cows that were hungry, thirsty or that were overdue to be milked. Eventually, this work could have important implications for animal welfare assessment in cattle and other animals.
Results of the present study are consistent with their premise that cattle produce sounds with specific properties when specific conditions are experienced.

Based on these results, several conclusions may be made about the vocal responses of cattle during stressful management practices. Vocal behaviour does differ between groups possessing phenotypic features typical of different breeds. However, in this study, phenotype grouping only influenced the likelihood of vocalizing, not the acoustic properties of the sounds. The propensity to vocalize is a persistent individual characteristic, in the sense that animals that vocalize in one situation are more likely to vocalize in others. Different situations can elicit different proportions of vocalizing animals, even when the same group of animals is being tested in both conditions. Cattle produce acoustically different calls in different situations. These are potentially a source of information about an animal's perception of those situations. This information could be exploited by other cattle, or by human researchers.
6.0 EFFECTS OF CLOSE PROXIMITY OF SOCIAL COMPANIONS ON VOCAL BEHAVIOUR AND MOVEMENT OF VISUALLY-ISOLATED CATTLE.

6.1 ABSTRACT

When cattle are visually isolated, their behaviour may be influenced by the proximity of other animals that they cannot see, but that they may know are present. This study tested the effect of presence or absence of familiar animals in a testing environment on vocal responses of visually isolated cattle. One hundred and forty nine male cattle were used, of which 30 were employed as decoys to provide the social conditions required for the test. One hundred steers and 19 bulls were actually tested. Animals were kept in 5 feedlot pens. One pen was tested each day for 5 days. Each day of testing was divided into two treatment series. In one series, known as the “Alone” treatment (A), each animal entered the handling facility and stood alone in a straight chute for 5 min. It was then moved into a solid sided scale platform where it remained for 1 min before being released from the building. In the other series, known as the “Not Alone” treatment (NA), each animal also stood for 5 min in the straight chute, but in a queue with 2 other animals. Every 100 s, the lead animal moved forward into the scale, where it remained for 1 min, the other two moved forward one place in the queue and another was brought
into the chute behind them. Twelve animals were tested in each treatment series each
day. For each series of NA treatments the chute was "primed" with 3 decoy animals
before the first that was actually tested, and 3 decoys followed the last test animal. The
order of the test series was determined randomly on the first day and alternated each
subsequent day. The number and timing of vocalizations was recorded for all animals in
the chute and on the scale. Their movements on the scale were monitored using an
electronic movement measuring device.

Only 8 animals (6.7%) vocalized in the handling facility. Four only vocalized on the
scale, 3 only in the chute and one in both locations. Proportionally more bulls than steers
vocalized (4 out of 19 bulls vs 4 out of 100 steers, $P=0.022$). All animals that vocalized
on the scale were in the NA condition ($P=0.0573$). Three out of 4 that called in the chute
were in the NA condition ($P=0.06186$). Of the 8 animals that vocalized in either
position, 7 were in the NA condition ($P=0.0612$). In the NA condition, cattle that heard
another animal inside the building vocalize tended to be more likely to vocalize (4 out of
15 which heard another animal vocalizing called, compared with 3 out of 45 which did
not hear another animal vocalize, $P=0.0585$). There were no treatment-related
differences in the amount of movement measured during isolation.

Despite the low level of vocal responses, these results are interpreted as supporting the
idea that the calls of visually-isolated cattle are a form of social behaviour, facilitated by
both the proximity, and the vocal activity of other individuals.
6.2 INTRODUCTION

During the sire and family genetic study reported in Chapter 4 of this thesis over 30 percent of the calves vocalized during one minute of visual isolation. This high level of response was similar to that reported during the processing task described in Chapter 5, and not much less than that evoked by the branding treatment in the experiment described in Chapter 3. When cattle were again placed in a similar situation of visual isolation (Chapter 5) it was found that only 16 percent of the animals tested vocalized. It may have been that the calves of the Canadian Beef Reference Herd used in the study (Chapter 4) were, for some reason, an unusually vocal group. One difference between this and other groups we have studied is that, at the time of testing, the cattle were not newly mixed, but rather had been together since birth. A later study (Chapter 5) revealed that there probably are real herd-related differences in the propensity to vocalize. Another important difference which may have contributed to this finding was the manner in which testing was carried out. In the Chapter 4 study, responses to isolation were evaluated while three other animals were also within the building; standing in the straight chute that led up to the scale compartment. In the Chapter 5 study and on other occasions when we have performed isolation testing, the subject animal was alone in the building.

In Chapters 4 and 5 it was speculated that the vocal responses heard during visual isolation were more of an intentional attempt to communicate with other animals than
the vocal responses recorded during acute treatments such as branding and processing. Accordingly, it was hypothesized that vocal responses during isolation would be facilitated by the presence of a potential receiver of those calls, even if the receiving animal were out of sight of the caller. It was assumed that the calves tested in the genetic study were aware of the presence of other animals in the building, possibly by hearing their movements, but also by the fact that they were in close proximity and visual contact with them right up to the moment when they were moved into the scale and visually isolated. It seemed reasonable that the vocal behaviour of these isolated calves would, in fact, be directed at the animals behind them from which they had just recently been separated. If this is the case then vocal responses ought to be more frequent if other animals are present within the building than if they are absent.

The study reported in this chapter represents an attempt to systematically test the difference in the level of vocal response to visual isolation in the presence or absence of other animals within the building at the time of testing.

6.3 ANIMALS, MATERIALS AND METHODS

6.3.1 Animals

One hundred and forty nine steers and bulls (420 ± 2.5 kg) of 4 “breed” types were used in this study. Data are reported on the behaviour of 119 (100 steers and 19 bulls).
Phenotypically these were classified, based on colour pattern, as Charolais (n=53), Simmental (n=16), Hereford (n=14) and “British” (resembling either black Angus or a white-faced, black-bodied Angus-Hereford cross, n=36). As with the animals in Chapter 5, this classification was made on the basis of visible external features. Their actual pedigrees were unknown. The remaining 30 animals were used only as social companions, required in order to create consistent social conditions for the others in the experiment (see section 6.3.2). Cattle were held in 5 feedlot pens (4 pens of 30 animals and 1 pen of 29) at the University of Saskatchewan’s Beef Research Unit. Five weeks before this study was carried out these animals had been used in a study of their responses to late age castration, carried out in the same handling facility where the present observations took place. The design of the castration study was such that each animal had experienced, with or without epidural anaesthesia, one of the following treatments: surgical castration, rubber ring castration, a sham castration procedure performed either on a bull or on a steer that had been castrated several months earlier. The proportions of animals having received each treatment were similar for each pen.

6.3.2 Experimental treatments and animal handling

A single pen of cattle was observed on each of 5 consecutive days. On each day, the observing session was divided into two series of observations. In one series, animals were observed consecutively in visual isolation while the handling facility building was otherwise empty of cattle. This is referred to as the “Alone” treatment. In the other series
cattle were observed consecutively in visual isolation while three other penmates stood within the building, but out of sight (referred to as the "Not Alone" treatment).

On each day cattle were moved from their home pen to the handling area where they were lined up in single file, in their self-determined entry order, in a curved chute which led to the entry door of the facility. Animals were admitted one at a time to the handling facility. During the Alone testing series, animals were admitted to the building at approximately 6 min intervals. Each animal was stood, unrestrained within a 5m straight section of chute, inside the building for 5 min. During this time it could not see any other cattle, but could see one, or sometimes both of the two observers (depending where in the chute it chose to stand). During this time an observer noted the number and timing of any vocalizations it made. It was then moved forward onto a solid-sided single animal scale platform, with front and rear sliding doors, which were closed to isolate it. It remained there for 1 min, out of visual contact with cattle and humans. It was then released from the scale, while at the same time another single animal was admitted to the empty chute.

Each Not Alone testing series required the use of 6 decoy animals per block of observations, whose behaviours are not included in this study because their handling experience did not correspond exactly to either experimental condition. The Not Alone treatment phase was started by moving 3 animals into the straight chute, where they stood in single file for 1 min. A fourth animal was then admitted to the chute and the
first animal was moved onto the scale platform where it stood out of sight of the others. This fourth animal was the first one for which actual data were recorded. After 1 minute, the animal on the scale exited the building while another was brought into the straight chute. A waiting period of approximately 40 s occurred, after the previous animal had left the scale, during which an electronic movement measuring device (see below) attached to the scale made computations. Following this delay, the lead animal in the straight chute was moved onto the scale. This cycle continued until no more cattle were present in the outside chute. Following the release of the fourth from last animal from the scale platform (the last for which actual data were recorded), the remaining three decoys in the straight chute were also run out of the building. This handling procedure was required to ensure that cattle spent the same amount of time standing in the straight chute (5 min, equivalent to three 1 min scale observing periods and three 40 s computation delays) and in the scale (1 min) whether they were in the Alone or Not Alone treatment. Also that every Not Alone animal stood with two others in the straight chute prior to isolation, and had three others present in the building while it was visually isolated on the scale. Dividing each day's treatments into two contiguous blocks, rather than randomizing individual treatments within days, meant that it was possible to test 12 cattle in the Alone condition and 12 in the Not Alone condition from each pen of 30 animals at a cost of 6 decoys, whose behaviour could not be used. Setting up appropriate conditions to test just one animal in the Not Alone treatment would still have required 6 decoys. Blocking like treatments together in this way, was considered preferable to the wastage of the available subject pool which would have occurred if individual
treatments had been performed in random order. The order of the two blocks was
decided by a coin toss on the first day, and alternated each subsequent day to mitigate
any effect of entry order.

6.3.3 Recording of vocal responses and movement

Vocalizations were noted by two observers, one of whom recorded vocal behaviour of
the animal or animals in the straight chute while the other observer recorded vocal
responses of the animal on the scale platform. The observers reset stopwatches each time
a new isolation period started. They recorded the elapsed time in seconds for each
vocalization and, in the case of the chute observer during Not Alone treatments, which
of the three animals in the chute had vocalized.

Objective measurements of the amount of movement of each calf were obtained using an
electronic movement measuring device (MMD). The MMD was connected to the load-
sensitive circuit of the electronic scale. Movement of the animal while on the scale
causes resistance in the load cells to vary and consequently the voltage in the circuit
fluctuates. The MMD measures this voltage 122 times each second for 1 min, recording
a "peak" each time a trend of 10 or more voltage changes in the same direction is
reversed. The number of peaks is positively correlated with visual estimates of the
amount of animal movement (Stookey et al., 1994). Following the 1 min sampling
period the MMD required approximately 40s to process the sampled data and be reset

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for another animal. In the Alone condition this could be carried out while the next animal was held in the chute for its 5 min waiting period. During the Not Alone series a delay of 40s was required before the next animal could be placed on the scale. To equalize the time in the straight chute for all cattle, the 5 min duration of the standing period in the chute for Alone animals was chosen to match the requirement for each Not Alone animal to be held in the chute throughout three 1 min scale observing periods and three 40s data processing periods.

A black and white video camera (Panasonic wv-BL204) and microphone (Sony F-V9) were fixed above the scale. The microphone was suspended above the head position of an animal standing on the scale platform in which position it would normally be approximately 1m distant from the animal’s mouth. Sound and picture were recorded on a time-lapse video recorder (Panasonic AG6730P) running in 6hr mode.

6.3.4 Statistical analysis

Data were analysed using Statistix V1.0 (Analytical Software, 1996) for Windows. All P-values given are 2-tailed. Relative frequency of vocalizing and non-vocalizing animals between conditions was assessed by Fishers exact test. Comparison of movement scores between conditions used the Mann-Whitney rank sum test.
6.4 RESULTS

6.4.1. Vocal responses

Irrespective of treatment, the level of vocal response was very low. Only 8 animals out of 119 (6.7%) vocalized during the 6 min spent inside the handling facility. Four only vocalized on the scale, three only in the chute and one called in both locations. A higher proportion of bulls than steers vocalized (4 out of 19 bulls vs 4 out of 100 steers, \(P=0.022\)). The numbers of animals that vocalized in either position are summarised by treatment in table 6.1. All of the 5 animals that vocalized during 1 min of visual isolation on the scale were in the Not Alone treatment \( (P=0.0573) \). Out of 4 animals that vocalized in the chute, 3 were in the Not Alone treatment \( (P=0.06186) \). Of the 8 animals that vocalized in either the scale or chute positions, 7 were in the Not Alone condition \( (P=0.0612) \). In the Not Alone treatment, cattle that heard another animal vocalize inside the building tended to be more likely to vocalize themselves, irrespective of where either animal was standing (4 out of 15 which heard another animal vocalizing called, compared with 3 out of 45 which did not hear another animal vocalize, \(P=0.0585\)).

There was no difference in the proportions of each breed that vocalized.
Table 6.1 Numbers of cattle which vocalized in scale, chute and either position compared between Alone and Not Alone treatments.

<table>
<thead>
<tr>
<th></th>
<th>Alone</th>
<th></th>
<th>Not Alone</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vocal</td>
<td>Silent</td>
<td>Vocal</td>
<td>Silent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>On scale</td>
<td>0</td>
<td>59</td>
<td>5</td>
<td>55</td>
<td>0.0573</td>
<td></td>
</tr>
<tr>
<td>In chute</td>
<td>1</td>
<td>58</td>
<td>3</td>
<td>57</td>
<td>0.06186</td>
<td></td>
</tr>
<tr>
<td>Either position</td>
<td>1</td>
<td>58</td>
<td>7</td>
<td>53</td>
<td>0.0612</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) \(P\)-values given are the two-tailed Fisher exact probabilities.
6.4.2 Movement scores

Movement scores did not differ between treatments, breeds, days (pens), vocal and nonvocal animals, or between those which heard another animal vocalize and those which did not. Nor were they different between first and second treatment series within days.

6.5 DISCUSSION

The vocal response level reported here was, once again, rather low. Despite this, the idea that more isolated calves would vocalize when other animals were in close proximity appears to have been borne out. There was no evidence to contradict this central hypothesis, that vocal responses during isolation would be facilitated by the presence of a potential receiver. These data offer some support for the view that vocal responses to isolation are socially-facilitated, or directed at other animals as acts of communication. In several key tests, the difference between calves in the Alone and Not Alone conditions fell slightly short of the alpha criterion only because of the very low overall level of response. One of the major problems with using vocal response in a situation such as this, throughout these studies, has been that isolated calves often do not vocalize within such a short (1 min) period as that which we have usually measured before. In this study, even 6 min of isolation from other cattle (for animals in the Alone treatment) was insufficient to elicit vocalization from more than a small proportion. It has been
noted that, in human epidemiological studies for example, to detect an uncommon event, such as a rare complication of a drug, many people must be observed (Fletcher et al. 1996, p200). To measure the rate of vocal response in isolated cattle it is necessary, not only to observe many cattle, but also to watch each for a long time. The sparse responses observed in this study are a further indication that counting vocal responses during moderately stressful experiences of a psychological, rather than a physical nature, as was done in this study, is of little value as a tool with which to evaluate welfare in such conditions.

In several earlier chapters I suggested that vocal responses to isolation might be differently motivated than responses to severely traumatic experiences such as branding and processing. Specifically, I believed that vocal behaviour in this situation would be performed in order to communicate with others, rather than as an involuntary expression of pain or negative emotion. In this study I presented evidence (albeit falling just short of significance) that vocal behaviour was stimulated by both the presence and the vocal activity of conspecifics. This had not been found in earlier studies of either type of experience. These results appear to fulfil the criteria for an act of communication (Wittenberger, 1981 and Chapter 2 of this thesis).

The most important implication arising from these results, for the thesis as a whole, concerns the use of vocal behaviour for assessing welfare. In earlier chapters it has been shown that there are a great many factors influencing vocal response in testing situations
which are independent of the treatment being considered. Given these confounding factors, would seem that the simple production of a vocalization by an individual bovine, ought not to be considered an indicator of its welfare in any particular testing situation. These results lend further support to this argument, by indicating that an animal’s vocal response to treatment can also be influenced by social conditions in the testing environment, including the proximity and vocal behaviour of other animals.
7.0 VOCAL COMMUNICATION BETWEEN COW AND CALF DURING VISUAL SEPARATION AND REUNITING, IN AN EXTENSIVE PASTURE ENVIRONMENT

7.1 ABSTRACT

Cattle vocalizations may serve as an indication of their welfare. To understand more fully the possible use of vocal behaviour measurements in evaluating welfare, it is appropriate to study the use of vocalization by free-ranging cattle. Vocal interaction may assist cow-calf pairs to locate each other in an extensive mixed-terrain environment. This study investigated the role of vocal behaviour in maintaining and regaining social contact between a cow and calf and the relationship between vocal behaviour and nursing.

Cow-calf pairs (208) from 7 different local farms were pastured together at a community pasture. Calves were aged between 2 and 10 wk at the beginning of the study, which lasted 2 mo. Two observers recorded the behaviour of 44 cow-calf pairs from the end of one nursing to the start of the next (mean interval 4 hr 59 min ± 16 min, mean duration of nursing 12.02 ±3.07 min.). During 19 observing sessions, cow and calf became
visually separated (mean separation time 41.35 ± 8.42 min). The most common scenario leading to separation was that the cow would gradually move away as she grazed, while the calf lay down and did not rise to follow her (11 of 19 pairs). Pairs that separated averaged 17.1% of their time separated and 10.1% of time searching for each other prior to reuniting. The proportion of time out of visual contact was negatively correlated with calf age ($P<0.02$). Numbers of vocalizations given by individual cows and their own calves were highly correlated ($P<0.001$). Among pairs that did not separate, the numbers of cow and calf vocalizations were correlated with increasing nursing intervals ($P<0.05$ for cows and $P<0.002$ for calves). Whether separation occurred or not, the number of vocalizations of the calf was highly correlated with the nursing interval ($P<0.01$).

Searches during which the cow vocalized averaged 69.0 ± 15.13 min compared with 14.4 ± 6.05 min when the cow was silent ($P=0.0235$). Calves that vocalized at shorter intervals while searching, achieved shorter search times ($P<0.05$). The average time from the end of visual separation to nursing was over 1 hr.

Vocal behaviour of free-ranging cow calf pairs, while separated or searching for each other may serve more than one function. Vocalization may indicate a state of need, of either cow or calf, to nurse. The vocalizations of one pair member may also enhance the motivation of the other, inducing it to vocalize. The vocalizations themselves probably also serve as a reference to the location and distance of the partner which is used during the searching process.
7.2 INTRODUCTION

The mother-offspring relationship in cattle is a state of emotional, social, nutritional and physiological interdependence. The vocal behaviour of cow and calf is an important factor in the maintenance of this bond. Between a cow and her offspring, vocalization is possibly involved in recognition and location of the partner as well as communication of physical and emotional states such as hunger and fear. If cow and calf are permitted to remain together, natural weaning will occur by around 260 days after the birth of the calf, ending the nutritional relationship, but strong social bonds may persist for many years (Reinhardt and Reinhardt, 1981). It is common, in beef production, to end the cow-calf relationship prematurely, by abruptly separating cow and calf when the latter is 6 to 8 months old. However, even within this restricted time it is possible to see that the relationship changes as the calf becomes more nutritionally and socially independent of its dam. Lidfors (1994) estimated that the natural weaning process may actually begin at around 123 days after the birth of the calf. In part these changes may be driven by an increasing parent-offspring conflict (Trivers, 1985), in which the calf demands more maternal resources than the cow, with her need to invest in future offspring, and who may already be pregnant, is selected to provide. As calves get older, suckling bouts become shorter in duration and tend to be initiated more often by the calf (Lidfors et al, 1994).

Despite the dependence of the calf on its mother for protection and milk, and the
mother's need to preserve her investment, cow and calf are not inseparable. In fact, they will be spatially separated and performing activities independently at certain times, from the earliest days of the calf's life. Frequently a young calf will remain lying while its mother moves off to graze. Schloeth (1961) reports “creching” in which calves rest together for long periods while mothers return to nurse their young at fixed times and often together. Although cows often graze in company with their own offspring, the calves do form grazing associations with other calves (Reinhardt and Reinhardt, 1981). Being part of such an association would result in a calf becoming separated from its mother during some grazing periods. Cows and calves may differ in the way they exploit grazing areas. Kiley-Worthington and de la Plain (1983) report that cows usually moved first to new areas, followed later by the calves. These authors indicated that this mainly occurred because calves spent more time lying down in a group. When they woke they searched for their mothers to obtain milk. Individual cattle do show persistent home range use (Howery et al, 1996).

There are probably several factors that contribute to the reuniting of a separated cow and calf. If herd members tend to congregate in certain areas at a certain time of day, for example to drink, or to rest in preferred shade areas, then members of separated pairs will probably encounter each other as they approach these areas. Another major factor leading to reuniting is the need to nurse. Both members of the pair will be motivated to do this, the calf by hunger and the cow, by increasing pressure in her udder. But they may not always be equally motivated at the same time. Therefore on any occasion,
attempts to locate and reunite could be initiated by either animal. Parent-offspring conflict theory (Trivers, 1985) would suggest that, as the calf gets older, reuniting for the purpose of nursing should increasingly be initiated by the calf, rather than the cow.

In order to reunite, the cow and calf will need to search for, locate and ultimately recognise each other. At close range, cattle may identify each other primarily by sight (Murphey et al., 1990). However visual recognition is only possible when there is an unobstructed line of sight between two animals, and perhaps only within a limited distance. In extensive pastures, these conditions often will not be met. Vocal interaction is one mode of communication that cow-calf pairs could use when attempting to maintain or regain contact and locate each other. This study attempted to investigate the role of vocal behaviour in maintaining and regaining social contact between a cow and calf, and the relationship between vocal behaviour and nursing, in an extensive, mixed terrain environment.

7.3 ANIMALS, MATERIALS AND METHODS

7.3.1 Animals and study area

The study was carried out at a community pasture approximately 50 km south of Saskatoon, Saskatchewan at 51°40' N, 107°15' W. Two hundred and eight crossbred
cow-calf pairs, one pregnant cow, which gave birth during the study period, and 6 Charolais breeding bulls were pastured together. The cows and calves originated from 7 different local farms. The bulls belonged to the Prairie Farm Rehabilitation Administration (PFRA), the managing authority of this government-owned communal grazing area. At the start of the study these animals were pastured on 777 hectares of native pasture, and were moved 3 wk later to another field of 510 hectares of native and crested wheat grass pasture. Both areas consisted of undulating, mixed terrain, with open grazing areas, small sand dunes and stands of poplar and alder. Thus there were ample opportunities for pair members to become visually separated. Water was available in each field from troughs filled by wind driven pumps from adjacent wells. Mineral blocks were scattered on the ground close to the water sources. Calves were aged between 2 and 10 wk at the beginning of the study. All cattle had been branded with the owner’s registered mark and had numbered ear tags. These together with distinctive phenotypic features such as colour markings and size were used to identify them in the field.

7.3.2 Observing methods

Observations were conducted between 27 May and 25 July 1998. In each observing session a focal cow and calf pair was selected and watched from one suckling period to the next. The pair selected was the first pair seen suckling after the observers entered the field. Observations were made on horseback, or while walking with the horse, by two observers, one of whom watched the cow while the other watched the calf. Cattle in this
environment seemed to accept the presence of people more readily when they were mounted on, or accompanied by, horses than when horses were not present. Activities of the focal animals were continuously recorded as well as the time that they occurred. Behaviour recorded included standing, walking, running, lying, grazing, nursing, grooming, drinking and vocalizing. Observers maintained a minimum distance of 50 m from the focal animals and used binoculars when necessary, allowing observation from as far as 100 m. The observers attempted to maintain a distance that would allow them to record behaviour without their presence influencing the animals. This distance varied, depending on the animal, and was increased if the focal animals showed signs of being attentive to the observers. When cow and calf became separated, each observer followed his or her focal animal and maintained contact with the other by portable two-way radio. Field notes were handwritten using a pocket notebook and pencil, or in some instances dictated onto microcassette and transcribed later.

7.3.3 Post-observation data manipulation

After the period of fieldwork was completed, information from the field notebooks was transferred to a computer spreadsheet file, structured to allow comparison of all instances of separation, searching, reuniting and vocal behaviour occurring during each of the 44 nursing to nursing periods. Table 7.1 gives definitions of the terms used in data processing, statistical analysis and the results sections of this chapter.
<table>
<thead>
<tr>
<th>Term</th>
<th>Operational definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursing interval</td>
<td>The time between the end of one nursing period and the start of the next.</td>
</tr>
<tr>
<td>Nursing time</td>
<td>The time between the first calf-udder contact in a nursing period and the last.</td>
</tr>
<tr>
<td>Separated</td>
<td>Pairs were said to be separated if, in the observers' judgement, visual contact between them would not be possible due to intervening terrain features.</td>
</tr>
<tr>
<td>Separation time</td>
<td>The time from when a pair first became separated until they ceased to be separated.</td>
</tr>
<tr>
<td>Searching</td>
<td>A separated pair were judged to be searching (for each other) from the time when either pair member ceased its former activity (e.g. lying or grazing) and began moving about with the apparent intent of locating its partner.</td>
</tr>
<tr>
<td>Searching time</td>
<td>The time from when a pair began searching until they reunited.</td>
</tr>
<tr>
<td>Reunited</td>
<td>A pair which had separated were said to be reunitied when they came close enough to physically interact with each other (e.g. sniff each other).</td>
</tr>
</tbody>
</table>
7.3.4 Statistical analysis

All statistical tests were computed using Statistix V1.0 for Windows (Analytical Software, 1996). Possibilities for analysis were limited by the non-normal distribution of much of the data, and the fact that cows' and calves' vocal behaviour, within a pair, cannot be considered independent measures. Despite these limitations some statistics were computed, using mainly nonparametric procedures. Only nursing interval, nursing time and calf ages met the criteria for parametric procedures, therefore 1-way ANOVA was used to compare nursing intervals by which members of the pair vocalized and two-sample t-tests were used to compare nursing interval and nursing time between pairs that separated and pairs that remained together. Otherwise two sample comparisons used the Mann-Whitney rank sum test, and comparisons of more than two samples used the Kruskall-Wallis one-way nonparametric analysis of variance. The limitations of the data meant that it was not possible to examine interactions between variables and the effect of covariates. All correlations were calculated using Spearman's rank correlation procedure. Although the assumptions of the procedure were not met, partial correlation coefficients were used to compare search times with the intervals between vocalizations with cow vocal behaviour corrected for calf vocal behaviour and vice versa. These results are offered as a matter of interest, rather than as evidence. All comparisons of frequency counts of numbers of animals or observations used chi-square or Fisher's exact test as appropriate.
7.4 RESULTS

7.4.1 General description

A total of 456 hr of observation were logged following 44 cow-calf pairs between one nursing and the next. During 19 of these observing sessions, cow and calf became separated, to the extent of appearing to be out of visual contact with each other, at least once. Separations lasted on average \(41.35 \pm 8.42\) min, with a minimum of zero minutes (momentary separation) and a maximum of 176 min. Separation and reuniting between nursings occurred more than once on a few occasions. Therefore 26 search and reuniting sequences are reported. On one occasion, search and reuniting occurred without cow and calf ever appearing to be out of sight of each other, from the observers' point of view. The mean time interval between nursings was 4 hr 59 min \(\pm 16\) min and the mean duration of nursing was \(12.02 \pm 3.07\) min. Neither value was influenced by age or sex of calf.

The most common scenario leading to visual separation between cow and calf was that the cow would gradually move away as she grazed, while the calf lay down and did not rise to follow her (11 of 19 pairs). Also common was a situation in which both animals were standing and active, and became separated while grazing or otherwise moving independently (7 of 19 pairs). Only once was a calf seen to move out of sight while its dam was lying down. Pairs which did separate averaged 17.1% of their time between
nursings beyond visual range, and 10.1% of time apparently searching for each other prior to reuniting. Among pairs that separated, the proportion of time out of visual contact was negatively correlated with calf age ($r_{\text{Spearman}} = -0.5693$, $P < 0.02$).

As the time between nursings increased, so did the probability that one or both members of a pair would vocalize (see Table 7.2). Nursing intervals, duration of nursings and calf age between pairs that separated and those which did not, are compared in Table 7.3. Nursing intervals and nursing time tended both to be longer for pairs that separated, and pairs that separated had significantly older calves. There was also a greater tendency for male calves to separate from the cow. The sex of one calf was not recorded. Of the remainder, 13 of 23 males separated, compared with 6 of 20 females ($P = 0.0807$).

### 7.4.2 Vocal behaviour

During the 44 cows-calf pair observations reported, 20 cows and 26 calves were heard to vocalize at least once (not significantly different). A total of 474 vocalizations were noted. The numbers of vocalizations given by individual cows and their own calves were highly correlated ($r_{\text{Spearman}} = 0.605$, $P < 0.001$). The number of vocalizations given by a calf or its dam was not related to calf age or sex. Among pairs that did not separate, the numbers of both cow and calf vocalizations were correlated with increasing nursing intervals ($r_{\text{Spearman}} = 0.447$, $P < 0.05$ for cows and $r_{\text{Spearman}} = 0.6146$, $P < 0.002$ for calves). The same relationship was not seen in pairs that did separate. Figure 7.1 shows the proportion
<table>
<thead>
<tr>
<th>Which pair members vocalized between nursings</th>
<th>Time between nursings (min) ±S.E.M.¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither</td>
<td>251.32 ± 21.21</td>
</tr>
<tr>
<td>Cow only</td>
<td>295.33 ± 88.054</td>
</tr>
<tr>
<td>Calf only</td>
<td>330.00 ± 36.416</td>
</tr>
<tr>
<td>Cow and calf</td>
<td>344.38 ± 26.9</td>
</tr>
</tbody>
</table>

¹ Column $P=0.0625$, 1-way ANOVA
Table 7.3
Comparison of mean nursing intervals, length of nursings and calf age between cow-calf pairs that separated and pairs that remained within visual contact.

<table>
<thead>
<tr>
<th>Pair cohesion between nursings</th>
<th>Number of cow-calf pairs</th>
<th>Interval between nursings (min)</th>
<th>Nursing time (min)</th>
<th>Age of calf (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never separated</td>
<td>25</td>
<td>274 ± 20</td>
<td>11.4 ± 0.47</td>
<td>94.8 ± 5.16</td>
</tr>
<tr>
<td>Separated at least once</td>
<td>19</td>
<td>332 ± 25</td>
<td>12.8 ± 0.57</td>
<td>113.8 ± 5.11</td>
</tr>
</tbody>
</table>

$P^1$  
0.076  0.054  0.012

$P^1$-values obtained by two sample t-test.
Figure 7.1.
The proportion of cows and calves which vocalized while searching, compared by separation time.
of cows and calves which vocalized during searching compared by length of separation time. The proportion of vocal animals does appear to increase as separation time increases. No difference in nursing time or nursing interval between separated pairs were found which could be attributed simply to whether either the cow or calf vocalized. However, across all observations, whether or not separation occurred, the number of vocalizations of the calf was highly correlated with the nursing interval ($r_{\text{Spearman}} = 0.4148$, $P < 0.01$). A similar trend was seen with the number of cow vocalizations ($r_{\text{Spearman}} = 0.2761$, $0.1 > P > 0.05$). Table 7.4 shows that the animals, calves in particular, ($P = 0.0138$) vocalized more while searching, prior to uniting, than before any separation occurred. Table 7.4 also shows that the overall frequency of vocalization, in each situation, differed little between cows and calves. In the period between separation and reuniting, most commonly either both members of a pair, or neither member vocalized, as Table 7.5 illustrates. The vocal behaviour of the cow appeared to be related to the duration of searching. Searches during which the cow vocalized averaged $69 \pm 15.13$ min compared with $14.4 \pm 6.05$ when the cow was silent ($P = 0.0235$).

When a cow or calf vocalized two or more times during the pre-separation or search phases it was possible to calculate the mean interval between vocalizations for an individual. During the pre-separation period, too few animals vocalized twice or more for any meaningful comparisons to be made of mean interval between vocalizations. During searching, calves vocalized at $5.76 \pm 1.62$ min intervals and cows at $9.47 \pm 4.57$ min intervals (not significantly different). Calves that vocalized at shorter intervals while
Table 7.4
Vocalizations of pairs that separated between nursing, comparing mean numbers ± S.E.M of vocalizations before separation with numbers during searching.

<table>
<thead>
<tr>
<th></th>
<th>Calf vocalizations</th>
<th>Cow vocalizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to visual separation</td>
<td>0.23 ± 0.13</td>
<td>0.27 ± 0.13</td>
</tr>
<tr>
<td>During search</td>
<td>6.15 ± 3.13</td>
<td>5.15 ± 3.93</td>
</tr>
<tr>
<td>$P$ (Mann-Whitney, 2-tailed)</td>
<td>0.0138</td>
<td>0.1358 (NS)</td>
</tr>
</tbody>
</table>
Table 7.5
Contingency table comparing the numbers of cows and calves which vocalized or did not vocalize between initial separation and reuniting.

<table>
<thead>
<tr>
<th></th>
<th>Calf vocalized</th>
<th>Calf did not vocalize</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow vocalized</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Cow did not vocalize</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

$P=0.0414$, Fisher exact test, two-tailed
searching, achieved shorter search times ($r_{\text{Spearman}}^{\text{Spearman}}=0.5576, P<0.05$). Cows’ vocalization intervals and search times were correlated in the same sense, but not significantly ($r_{\text{Spearman}}^{\text{Spearman}}=0.5021, 0.2>P>0.1$). The vocalization intervals of individual cows and their calves were not highly correlated with each other, but since both seem related to search time, partial correlations were used as a means to suggest which was more likely to exert the greater influence. The partial correlation coefficient for search time with calf vocalization intervals, controlled for cow vocalization intervals, was rather high (0.8661). When search time was compared to cow vocalization intervals, controlled for calf vocalization intervals, the partial correlation coefficient was very low (0.0793).

The average interval between coming back within visual range following separation, and actually starting to nurse was 63.58 ± 16.6 min. The interval between the end of the search phase (i.e. when the calf regained its mother’s side) and starting to nurse was 17.1 ± 9.38 min. Therefore there was, on average, an interval of about 46 min between coming within visual range and physically reuniting. These intervals are compared for cows and calves which did or did not vocalize during searching, in Table 7.6. The main feature of note in the table is that the period from visual contact to nursing, or from reuniting to nursing, tends to be shorter when the animals vocalized during the search. Table 7.7 suggests that it may be the vocal behaviour of the calf which is more closely related to the time taken to approach each other following visual separation.
Table 7.6
Mean intervals ± S.E. between coming within visual range and the start of nursing following visual separation, compared by vocal behaviour of cow and calf during the search.

<table>
<thead>
<tr>
<th></th>
<th>Interval between coming within visual range and nursing (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow vocalized</td>
<td>48.44±18.31</td>
</tr>
<tr>
<td>Cow quiet</td>
<td>77.2 ± 27.18</td>
</tr>
<tr>
<td>(P)</td>
<td>Not significantly different</td>
</tr>
<tr>
<td>Calf vocalized</td>
<td>35.09 ± 13.88</td>
</tr>
<tr>
<td>Calf quiet</td>
<td>102.75 ± 30.42</td>
</tr>
</tbody>
</table>

\[P\]\(^1\) 0.0258

\(^1\)\(P\)-value is the 2-tailed probability, based on Mann-Whitney rank sum test.
Table 7.7
Mean intervals ± S.E. between coming into visual range and start of nursing compared by which pair members vocalized during search.

<table>
<thead>
<tr>
<th>Members of pair which vocalized during search</th>
<th>Interval between coming into visual range and start of nursing (min)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow and calf vocalized</td>
<td>44.29 ± 20.3</td>
</tr>
<tr>
<td>Only calf vocalized</td>
<td>19.00 ± 13.693</td>
</tr>
<tr>
<td>Only cow vocalized</td>
<td>63.00 ± 56</td>
</tr>
<tr>
<td>Neither cow nor calf vocalized</td>
<td>116.0 ± 37.12</td>
</tr>
</tbody>
</table>

¹Column P=0.1056 (Kruskall-Wallis 1-way nonparametric ANOVA)
7.5 DISCUSSION

The extensive community pasture environment offers an excellent opportunity to study cattle social behaviour, including vocal behaviour. The main theme of this thesis has been the vocal behaviour of cattle during aversive procedures, isolation, proximity to people, and always within indoor handling facilities. The purpose of these studies has been to explore the suggestion that measurement of vocal responses could be a useful indicator of welfare. Implicit in this suggestion is the notion that cattle, in common with other species, have evolved a system of vocal communication which has the capacity to transmit information between individuals concerning their physical and emotional states. In Chapter 2 this use of vocalization was described as an animal’s “commentary” on aspects of its own functioning, which could perhaps be exploited by applied ethologists to gain information about that functioning. In trying to understand more fully the content of this commentary, and its possible uses in evaluating welfare, it is appropriate to study the spontaneous use of vocal behaviour by free-ranging cattle, in an environment closer to that in which, and for which, this behaviour system evolved.

Cattle use vocalizations in a variety of social contexts and they probably serve a number of biologically significant purposes. These are discussed in Chapter 2, but probably include recognition between individuals and maintaining social cohesion. This study examined a single context; the visual separation and reuniting of a cow and its calf. Even within this narrow subset of all possible contexts in which vocal activity occurs, it is
likely that vocalization serves more than a single function for the animal. It is tempting to try to interpret vocalization, solely on the basis of its context, in terms of simple statements which are comprehensible to the anthropocentric human mind. For example, when a calf strides across the pasture, calling as it goes, approaches its dam and immediately begins to suck, one might be forgiven for assigning simple equivalence between those vocalizations and a statement such as "I am hungry" or "I want milk". In my opinion, such transcription of animal signals into human language should be avoided. This is anthropomorphism of an unhelpful kind, which fails to address a greater gestalt in the relationship between cow and calf, which is inadequately expressed in the mechanistic description "calf demands milk, cow gives milk". Therefore no attempt is made in this discussion to assign a specific function to vocalization heard in the context of cow-calf separation and reunion, as though the two animals were exchanging verbal signals.

Calves of the age range observed here are no longer solely dependent on their dam nutritionally. They are capable of independent grazing and travel, although they continue to move with other herd members and may often maintain close spatial proximity to the mother much of the time. These calves nursed for about 10 to 15 min every 5 hours on average. This left plenty of time between nursings during which a pair, behaving semi-independently of each other, could get far enough apart that it would not be possible for them to see each other. This happened once or more in nearly half of the nursing to nursing intervals observed. The fact that these periods of separation had a substantial and
variable duration (nearly 3 hr in the longest case) indicates that cows and calves do not try particularly to avoid becoming visually separated, nor is inadvertent separation necessarily a motivation for immediate attempts to rejoin each other. However they must be together to nurse, and it may be that the need to nurse, by either or both partners, is one stimulus to reunite. It is likely not the only motive for reunion though, since the average time from the end of visual separation to nursing was over 1 hr. It was expected that as calves became older they would display greater independence from their dams. In this study it was found that, although calves that separated were older than calves that did not, among those that did separate the amount of time separated from their mothers was negatively correlated with age.

About half the animals observed were heard to vocalize at least once during the interval between nursings. Compared with the studies described in Chapters 3, 4, 5 and 6 of this thesis this would appear to be a useful response rate for the purpose of studying vocal phenomena. On the other hand, 474 vocalizations were recorded in 228 hours of observing two animals; an average of a vocalization per animal every 58 minutes. If this can be considered a background rate for the spontaneous vocal activity of typical cattle reasonably undisturbed by people or artificial conditions, then the proportions of animals vocalizing during the short observing intervals used in the other studies were relatively high.

There was a significant degree of agreement within pairs in the level of their vocal
activity. This is suggested by the finding that during searching, within most pairs usually either both were vocal or both were silent. Also the numbers of vocalizations were highly correlated between cow and calf. This information is insufficient to discriminate between competing explanations. One is that the more one pair member vocalizes the more the other also vocalizes because they are actively communicating with each other. Another is that mother and offspring have a similar propensity to vocalize in any particular situation, which is partly inherited genetically (see Chapter 4). A third possibility is that vocalization initiated by one party serves to stimulate a common motivational state in the other. Thus by expressing its own need, a calf could elevate its mother’s need also, and thus her motivation to reunite.

With longer nursing intervals the number of vocalizations of calves increased, regardless of whether separation occurred or not. Curiously, this relationship was more pronounced for both cows and calves during observations where separation did not occur. To an extent this would be expected even if the overall vocalization rate remained constant for all animals. But this was not the case, since vocalizations were more frequent during searching than before pairs became separated. The mean numbers of vocalizations were quite similar between cow and calf; lower before separation and higher while searching. Vocal behaviour appeared to be related to the length of search time also. Searches during which cows vocalized were significantly longer than those in which they did not. Taken together these results support the conclusion that vocalization and nursing intervals are at least causally related. An obvious inference would be that
they reflect greater degrees of need to reunite. Possibly increasing indications of hunger on the part of the calf and an increasing need to nurse by the cow are important components, but probably these are not the only functions of the sounds. As Figure 1 shows, at the longest separation times seen in this study two thirds of the animals vocalized. If the trend continues, a separation time would be reached when all animals would vocalize. It may be that increasing rates of vocalization serve to limit the duration of overly long nursing intervals. This might explain why almost all cows and calves vocalize following separation for weaning (personal observation).

During searching, calves that vocalized at a higher rate (i.e. with shorter intervals between calls) achieved shorter search times. The same relationship was seen for cows, but it was not significant. These observations suggest that vocal behaviour serves a function in reuniting, beyond simply expressing the need to do so. It is probable that, for some pairs at least, vocalization is part of an adaptive strategy to facilitate the search and reunion process. That the relationship is so much stronger for calves than for cows may indicate that it is primarily the interests of calves that control when and how urgently pairs reunite. This may be due to the age range of the calves observed here. As calf age increases it might be expected, in view of Trivers' (1985) theory of parent-offspring conflict, that attempts to gain nursing access will increasingly be driven by the calf. It would be interesting to observe younger calves to discover if there is an age range at which the relationship is reversed or equally strong for both cows and calves.
In conclusion it is thought that the vocal behaviour exhibited by free-ranging cow calf pairs, while separated or searching for each other may simultaneously serve more than one function. In part, vocalization may indicate a state of need, either to obtain milk or to have milk removed from the full udder. The vocalizations of one pair member, in this study primarily the calf, may also enhance the motivation of the other, inducing it too to vocalize. The vocalizations themselves probably also serve as a reference to the location and distance of the partner which is used during the searching process.
8.0 SUMMARY AND CONCLUSIONS

The studies presented in this thesis have mainly explored the uses and limitations of cattle vocal behaviour as an indicator of welfare during handling and intensive management procedures. The thesis has also attempted to address some aspects of the use of vocal behaviour as a medium of animal communication.

Beef cattle in feedlots are, at various times in the production cycle, subjected to management procedures which may cause varying degrees of pain and fear. These include branding, restraint during routine processing and brief periods of visual isolation. Vocal responses during these specific practices have been described in this thesis.

The literature review presented in Chapter 2 revealed that although studies on vocal behaviour in cattle are fairly sparse in the literature, vocal response has been reported in a number of studies; implicitly as an indication of distress, fear or pain. In other words, the idea that elevated vocal responses during intensive procedures indicate poorer welfare seems self-evident in the minds of some researchers. The scientific basis for this assumption has yet to be presented. There is widespread interest in noninvasive
techniques to measure welfare. Work done in recent years on the vocal behaviour of pigs has shown that measurements of vocal response can give important information about the experiences of animals during distressing procedures.

Chapter 3 presented an account of the vocal responses of calves during hot-iron branding; a common management procedure in the beef industry in Canada and elsewhere which, on a variety of other evidence, is believed to cause acute pain and distress at the time of application. The experiment compared vocal responses recorded during branding and during a sham procedure in which the animals were restrained in an identical way and instead touched with an unheated branding iron. Unsurprisingly, more calves vocalized during the hot iron treatment. However several vocalized during the sham procedure. This may indicate that the type of restraint and handling required to perform branding is, in itself, somewhat distressing. Any person present in the handling facility during these treatments might have concluded that the calls of the branded animals were more intense and sounded different. Analysis of the sound spectra showed that the calls of branded and unbranded animals were acoustically different also. Branded animals produced louder calls with more frequency modulation and with peak sound energy emitted at higher frequencies. Analysis of sound spectrograms proved to be a useful technique for measuring these differences objectively by quantifying these qualitative differences.

In evaluating the usefulness of any physiological or behavioural measurement as a tool
for measuring the difference between specific environmental situations or experimental
treatments, it is necessary to investigate other sources of variation in the measurement in
question. In respect of vocal response, these studies have revealed that there are a
number of factors contributing to vocal response which are quite independent of the
conditions in which welfare is purportedly measured.

One possible source of variation which might be expected to modify an animal’s vocal
response during treatment is genetic influence. We have observed a wide range of
individuals’ vocal responses. In part this variability could be due to innate predisposing
factors which are inherited from the parents, rather than learned from the mother and
other members of the social group. The development, through multiple ovulation and
embryo transfer, of the Canadian Beef Reference Herd, comprising 17 full-sibling
families of calves, provided a rare opportunity to study genetic effects on vocal
behaviour. While half-sibling analysis could be used to give information about sire
effects on vocalization, full-sibling comparisons would normally be impracticable for a
species like cattle, with a long reproductive cycle and usually single offspring. Also this
allowed an opportunity to control for the effect of maternal behaviour on development of
vocal behaviour in the calf since each sibling was gestated and raised by a different,
unrelated, cow.

The study reported in Chapter 4 made use of this opportunity, although some
compromises were necessary in the vocalization study in order to accommodate a pre-
existing experimental design. The testing paradigm used in this study was a period of visual isolation, during which calves stood on a single animal scale platform which was equipped with a device to measure their movement electronically. Both sire and family had a significant influence on the numbers of vocalizations and the acoustic properties of the sounds produced by the calf during isolation. These findings highlight a difficulty with the use of vocal response as a welfare measurement. There are variations between individuals in the propensity to vocalize and the acoustic qualities of sound produced which have nothing to do with the effect of conditions being evaluated. The most important finding of this study is that part of this variation is inherited, not learned, and is independent of the rearing environment. It also suggests the probability that some breeding lines will be characteristically more, or less, vocal than others. Other information gathered in the course of the study indicated that age, weight and sex also contribute to variation in vocal response between individuals.

If some groups of related individuals share aspects of their vocal behaviour then it may be that phenotypically similar animals, for example representatives of a particular breed, show characteristic vocal responses in common, which differ from those of phenotypically dissimilar animals. Chapter 5 compared the responses of four distinct phenotypic groups of cattle. These were typical representatives of commercial Angus and Charolais type cattle and typical crosses between either of these phenotypes and a Hereford animal. The study also compared the performance of the same group of animals during two types of stressful situation. Firstly, their vocal behaviour was
recorded during the standard processing given to all cattle on arrival at our feedlot. This treatment includes restraint, ear-tagging vaccination etc. Later they were tested during a period of visual isolation, as in Chapter 4, while their movement and heart rate were simultaneously measured. Differences in vocal behaviour were found between these phenotype groupings. More cattle vocalized during the processing than during the isolation test. The calls were also significantly different. Calls recorded during isolation were shorter, with lower fundamental frequencies than during processing. A similar pattern was seen between trials just in respect of those cattle that vocalized on both occasions. Thus individuals are capable of producing acoustically distinct vocalizations in different circumstances. The Angus type cattle were more vocal in both testing situations. However, no phenotype-related differences in the acoustic properties of calls were found. Cattle that vocalized in one testing situation were more likely to vocalize in the other, showing that the overall propensity of an individual to vocalize may persist over time and between different circumstances. There were no differences between phenotypes in the amount of movement recorded during the isolation trial. Animals that vocalized in either trial showed a greater reduction in heart rate during the 1 min isolation period than those which did not. This study showed that phenotype is another factor that can influence vocal responses, at least as regards the proportion of animals that vocalize.

It might be expected that one factor influencing whether an individual vocalizes or not during a distressing experience (or indeed at any other time), would be the presence or
absence of potential audience animals in the vicinity. In two of the earlier studies (Chapters 4 and 5) we had observed animals in isolation for 1 min using the same facilities. In the Chapter 4 study, more than 30% of the calves vocalized on each of the 4 occasions of testing. In Chapter 5 it was reported that only 16% vocalized. It is probable that there is substantial variation between groups of cattle, with some, for the reasons given earlier, being inherently more vocal than others. However, one known difference between the two studies was that in one (Chapter 5) the visually isolated animal was the only animal present in the building. In the other study, while the subject stood on the scale, three other calves stood in line in the chute behind it. Though they were out of sight, they may not have been out of mind of the subject, since it had occupied the chute itself only moments before. Chapter 6 was an attempt to test whether the absence or presence of unseen companions influenced the proportion which vocalized. The numbers or animals which vocalized, both on the scale and in the chute, were very low. The results therefore fell very slightly short of the 5% criterion. However they did seem to indicate that cattle were more prone to vocalize when other animals were present in the building. They also appeared more likely to vocalize if another animal occupying the building at the same time vocalized, than if the other animals were silent. The social environment probably exerts a significant influence on the probability that an animal will vocalize in any given situation. Another result, which actually did reach significance, was that among the animals tested, bulls were more likely to vocalize than steers. This is another example of an endogenous (i.e. within the animal, not a consequence of the experimental treatment) source of variability in vocal response.
Chapter 7 examined how cows and their calves use vocal behaviour in extensive range conditions. As an adjunct to the observations of vocal response under confinement and during unpleasant treatments, it was considered useful to include some information about the spontaneous vocal behaviour of relatively unconfined animals. This study looked at how vocalizations are used to maintain contact and find one another in a large, complex environment, and the relationship between vocal behaviour and nursing. Pairs most commonly became separated when the cow moved away while the calf was lying down. The occurrence of visual separation between cow and calf did not stimulate immediate attempts to reunite. Periods of separation appear normal and tolerable to the animals. The numbers of vocalizations were highly correlated between cow and calf, suggesting that an active process of communication was occurring. As length of separation increased, so did the proportion of animals that vocalized. Vocalization is probably part of an adaptive strategy to facilitate searching for and reuniting with the partner. It may, in part, indicate a need on the part of one or both animals to reunite, due to a negative condition; possibly hunger, or udder fullness are often involved. The vocalizations of one animal may also influence the motivation level of its partner to reunite.

Throughout these studies, a persistent problem for analysis and interpretation was that, in most of the experimental situations used, a significant number (usually the majority) of animals did not vocalize. Even before considering higher level measurements of vocal behaviour, whether an animal vocalized or not is a useable piece of information about its
response to the experimental situation. The response rates seen here, though low, were probably reasonably high relative to the background rate of cattle vocalizing under extensive conditions, reported in Chapter 7. While vocal responses, when they occur, can be described in terms of several parameters, as these studies have done, the absence of a vocalization offers only a limited basis for comparison. A substantial proportion of the hot-iron branded cattle observed in the Chapter 3 study did not vocalize, during what is undoubtedly a painful experience. Therefore, I argue that simple counts of animals vocalizing should never be used as the sole criterion to assess welfare, or detect welfare problems. Especially in view of the many other factors in any testing situation which are known, based on the information in this thesis, to influence the propensity to vocalize. There is probably some value in comparing how many of a group vocalize in a given situation, under appropriately controlled experimental conditions, especially in combination with other behavioural and physiological measures. However, nothing can be inferred about the status of an individual, based solely on whether or not it vocalizes.

The studies reported in this thesis have shown that cattle vocalizations do vary in both quantity and acoustic quality between different types or degrees of stressful events. Individual cattle produce qualitatively different sounds in different testing situations. These findings suggest that measurement of vocal response could be a useful approach to assessing their welfare. However, the tendency to be more or less vocal is an individual characteristic that persists across different testing situations. These observations reveal that the propensity to vocalize is influenced by genetics, age, weight,
sex, gender and phenotypic features. In an experimental situation, or an attempt to
evaluate animal welfare in commercial environments, these factors may well serve to
confound the effects of the treatment being investigated. Therefore it is suggested that
measurements of vocal response should never be used as the sole criterion to assess
welfare. It may be best used as an adjunct to other types of measurement, especially
when it is possible to control for the confounding factors listed above. Where possible,
recordings and subsequent acoustic measurements should be considered. This method
contributes a number of different dimensions on which vocal responses can vary. Some
of these dimensions, for example fundamental frequency, call length and loudness, may
relate more directly to the experience of an individual, than whether or how often it
vocalized.
9.0 REFERENCES


APPENDIX A  ANNOTATED SOURCE CODE OF “MOOCRUNCH”

PROGRAM USED TO FACILITATE THE ACOUSTIC
ANALYSIS DESCRIBED IN CHAPTER 5

A1  INTRODUCTION

The Moocrunch program was designed to improve speed and accuracy when
analysing cattle vocalizations and to eliminate the time-consuming and error-
prone task of transcribing acoustic values into a file readable by statistical
software. The program is used in conjunction with the Spectrogram V5.05 (R.S.
Horne, 1999) sound analysis software. Moocrunch runs within a small MSDOS
window placed adjacent on the screen to the window in which Spectrogram is
running. The program prompts the user to carry out each step in the analysis
procedure for each .WAV file, starting with identifying the animal and the call
and finishing with writing the values of several computed acoustic parameters to a text file for subsequent statistical analysis. The straightforward top-down structure of the program reflects the sequence of tasks involved in this analysis. The program was written using Turbo Pascal® version 7.0 for DOS (Borland International Inc, 1992). Normal Pascal programming convention is to contain explanatory comments within the program between left and right braces. In the listing below they have also been italicised for additional clarity, thus: \{this is a comment\}. The source code has been annotated with comments in this fashion, to explain what task the program is doing at each step.

A2 PROGRAM LISTING

program moocrunch (input, output);

var

Id, Vocno, Lopeak, Hipeak, Nints, Fftpt : Integer;
Start, Finish, Duration : Longint;
Freq, Db, Hidb, Midpoint, Hifreq, Hint, Totaldb: Real;
F, G: Text;

Ch : Char;

Done : Boolean;

begin

Done:=False;

while not Done do

begin

Clrscr;

{Prompts user to enter call identification information}

Writeln("** 1 Enter Identification **");

Write('Animal ID: ');

Readln(Id);

193
Write ('Vocalization # ');

Readln (Vocno);

Writeln;

{Reminds user to check the settings in Spectrogram and get it to display the required audiospectrogram}

Writeln (** 2 Check analysis parameters and analyse file **);

Writeln ('30dB : TS 10msec : Linear : 1024 pt');

Writeln ('10.8 Hz res : 0 - 5512 Hz : Spec Ave 1');

Writeln;

{Asks user to enter the start and finish cursor coordinates of the selected vocalization and calculates the middle point}

Writeln (** 3 Compute Midpoint of call **);

Write ('Moo starts : ');

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Readln (Start);

Write ('Moo ends : ');

Readln (Finish);

Duration := Finish-Start;

Midpoint := Duration/2+Start;

Writeln;

{Instructs user to set the cursor to the calculated midpoint and click the mouse to display the power spectrum plot}

Writeln (** 4 Generate power spectrum **);

Writeln ('Set cursor near ', midpoint:5:0,' msec and L-click');

Writeln;

{Instructs user to select a series of amplitude peaks in the power spectrum, enter the frequency of the highest and lowest and the number of harmonic intervals between them.}
It then calculates the mean frequency interval between peaks, i.e. the fundamental frequency)

Writeln (** 5 Compute harmonic interval **);

Writeln ('Find a series of peaks');

Write ('Enter freq of lowest peak : ');

Readln (Lpeak);

Write ('Enter number of interpeak intervals : ');

Readln (Nints);

Write ('Enter freq of highest peak : ');

Readln (Hpeak);

Hint:=(Hpeak-Lpeak)/Nints;

Delay (500);
Writeln;

{Instructs user to invoke Spectrogram's data logging function to create a text file of dB values at 10.8 Hz intervals between 5Hz and 2750 Hz. It then reads these values from the file and uses them to identify the highest dB value and the frequency at which it occurs. It also totals all the dB values in the text file.}

Writeln (** 6 Auto-analyze power spectrum **);

Writeln ('Log power spectrum as "spectrum.txt". Press Return when done');

Readln;

Assign (F,'spectrum.txt');

Reset (F);

Hidb:=0; Hifreq:=0; Totaldb:=0;

Readln(F); Readln(F); Readln(F); Readln(F);

Readln(F); Readln(F); Readln(F); Readln(F);
while not Eof (F) do

begin

Read (F, Fftpt);

Read (F, Freq);

Readln (F, Db);

Db:=Db+100;

if Db>=Hidb then

begin

if freq>24 then

begin

Hidb:=Db;

Hifreq:=Freq;

end
end;

end;

Totaldb:=Totaldb+Db;

end;

Close (F);

Writeln ('OK');

Clrscr;

{ Writes a summary of calculated acoustic values to the screen }

Writeln; Writeln ('Data summary for animal # ',Id,' call # ',Vocno);

Writeln ('-----------------------------------------------');

Writeln ('Duration: ',Duration,' msec. Harmonic Interval: ',Hint:6:2,' Hz');

Writeln ('Max dBs: ',Hidb-100:6:1,' at ',Hifreq:6:2,' Hz');
WriteLn ('Total sound level score: ',Totaldb:6:1,',',Hifreq/Hint:3:1);

WriteLn ('---------------------------------');

{Opens the output text file and adds the calculated values at the bottom line}

WriteLn; WriteLn ('** 7 Write to data file **');

Assign (G, 'procdata.txt');

Append (G);

WriteLn (G,Id:3,',',Vocno:5,',',Duration:8,',',Hint:6:2,',',Hidb-100:6:1,

',Hifreq:6:2,',',Totaldb:6:1,',',Hifreq/Hint:3:1);

Close (G);

{Asks user whether another call is to be processed at this time. If yes, then the program returns to the beginning and continues running, otherwise it stops}

Write ('Process another moo? (y/n)');
Ch:=Readkey;

if Ch='n' then

Done:=True

end;

end.