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Analysis of pain-related vocalization in young pigs

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Abstract

The assessment of pain constitutes a major issue for animal welfare research. The objective of this study was to classify vocalizations during castration pain and to assess alterations in vocalizations under local anaesthesia. The alterations in vocalization were measured by multiparametric call analysis. A total of 4537 calls of 70 young pigs were evaluated. With the data of this study three call types are distinguishable (grunt, squeal, scream). A high percentage (94.64%) of calls that could be classified in one of the three call types during the castration process within the confidence level of 95% was found. The comparison of the occurrence of the call types during treatments gives evidence for pain-related use of screams. The piglets castrated without local anaesthesia produced almost double the number of screams as piglets castrated with anaesthesia. The comparison of the recorded sound parameters reveals the particular position of screams in the call repertoire of young pigs. Screams are significantly different in their sound parameters than grunts or squeals. Castration in comparison to mere restraint produced a comprehensive change in sound parameters, with castration calls becoming more extended and more powerful. The findings in this study also show differences in the effectiveness of the parameters which indicate pain. Parameters that describe a single event in a call, such as peak level or peak frequency give better results than parameters that describe an average, such as weighted frequency and main frequency. The research indicated that pain-related changes of calls in piglets can be identified. On the basis of the results, automatic classification of call types during management operations may be developed. This could contribute to objective animal welfare assessment.

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1. Introduction

The assessment of pain constitutes a major issue for animal welfare research. The vocalization of pigs during the castration procedure is a well-documented behaviour [1]. Methods, originally developed for speech analysis, have been successfully applied to call analysis [2–5]. In this study, the analysis of pig calls was based on these methods; the pain that results from castration of young male pigs was used as a model for the analysis of call characteristics during pain. Regulations in many European countries do not require anaesthesia for the castration of piglets during the first four weeks of life. Castration of male pigs is a standard practice in many countries to prevent the negative effect of male hormone metabolites (boar taint) on pork. Wemelsfelder and van Putten [1] described differences in frequency and intensity of spectrograms from pig calls during different treatments in the castration process. More recently, several studies on the effect of castration have been conducted that were mainly based on the analysis of call frequency or, more specifically on the frequency of maximal energy, as a means for stress assessment [6–8]. Weary et al. [7] suggested a critical threshold for the frequency of maximal energy of more than 1000 Hz in order to assess pain intensity for the different aspects of castration [6]. The procedure of pulling and severing of the spermatic cord caused most high-frequency calls. These aspects seem to be most painful as in another study local anaesthesia was most effective in reducing behavioural resistance when the spermatic cord was cut [9]. Therefore, local anaesthesia seems to be an appropriate treatment for minimising pain during castration.

Although several authors suggest that the vocalization during castration is variable, this type of vocalization was not compared to vocalization elicited by pigs during suckling as described by Jensen and Algers [10], who described 5 call types in the context of suckling. Similar call structures were also observed in relation to acoustical and visual separation of pigs [11].

The objective of this study was to classify vocalizations during castration pain according to the acoustic structure and to assess alterations in vocalizations under local anaesthesia. The methodology of vocalization analysis based on the acoustic structure is different from the methods used by other researchers such as White et al. [8]. The advantage is that the alterations in vocalization and call types were measured by multiparametric procedures focusing on the energetic and temporal characteristics of calls.

2. Methods

Four male pigs per litter, for a total of 70 pigs from 19 litters, were assigned to each of 4 treatments. Treatments were conducted on day 7 (28 pigs), day 13 (16 pigs) and on day 19 (26 pigs). In some litters there were less than four male piglets that could be assigned to the treatments. Treatments were: (a) castration without anaesthesia [C], (b) castration with local anaesthesia [CA], (c) restraint without anaesthesia [R], and (d) restraint with local anaesthesia [RA]. Restraint consisted of tying the hind legs of the piglets with 2 loops to a rack. Piglets in all four treatments were tied the same way. Piglets in groups R and RA were not handled during treatment other than by disinfecting the scrotum and administering local anaesthesia. Piglets in group RA were always uncastrated animals.

Sows were housed in commercial farrowing crates on a commercial farm in Saxony-Anhalt, Germany. Experiments were conducted in a room that had been equipped with sound absorbent walls. After disinfecting the scrotum with rubbing alcohol, the skin was cut with a scalpel blade. An emasculator was used to squeeze the spermatic cord and for subsequent cutting of the spermatic cord. All castrations were performed by experienced assistants.

During the CA and RA treatments, disinfection was followed immediately by an intratesticular injection with lidocain (Ursocain 2%; 0.5 ml per testis) and 2 min waiting time. Due to the waiting time after injection of the anaesthetic, the duration of the treatment lasted, on the average, for C: 148 $\pm$ 10 s; CA: 282 $\pm$ 16 s; R: 161 $\pm$ 7 s, and RA: 200 $\pm$ 4 s.

A DAT-recorder (TCD-D8, SONY, Berlin, FRG) recorded the calls via a calibrated two-channel microphone system (B&K 4191/2669/5935, Brüel & Kjaer, Naerum, DK). The microphone was placed at a distance of approx. 50 cm away from the mouth of the restrained pig. A second microphone was placed outside the experimental area in the room and was used to record background noise in the building.

At the beginning of each tape, a signal (1 kHz, 94 dB) was recorded with an acoustic calibrator (B&K 4231, Brüel & Kjaer, Naerum, DK) that was mounted to the microphone.

The vocalizations were analyzed using a workstation (INDY, Silicon Graphics, Mountain View, CA) with Entropics signal processing software (ESPS 5.3, Entropic Inc., Washington, DC, USA). All vocalizations of each animal and treatment were digitally transferred from the DAT cassette to the hard disk of the workstation. Sampling frequency was 48 kHz. The resulting waveform file was then filtered with a high pass filter (-5 dB at 500 Hz; 10.6 Hz/dB). All acoustic events were then selected with the ESPS function find_ep (ESPS 5.3, Entropic Inc., Washington DC, USA) using an algorithm published by Rabiner and Sambur [12] based on the power of time function. Background noises were eliminated by comparing the waveform amplitudes between the two channels. Events were excluded when waveform amplitude of background noise was similar or greater than the amplitude of call signal. A total of 14 from the 4551 calls were detected to be superimposed by background noise. Some acoustic events contained more than one call. These were manually separated into single calls.

A spectrogram was calculated with a 1024 point fast Fourier transformation of the time function of a window sequence of 2 ms in order to measure the call characteristics. This resulted in a frequency resolution of 46.875 Hz. Fig. 1(a) shows an example of a spectrogram on which the amplitude values are encoded by a grey scale. A basis statistic with minimum, maximum, mean, and standard deviation across all spectra of each call was calculated for each frequency line of the spectrogram (Fig. 1(b)). Six call characteristics were extracted from this averaged spectrum.

The level of the total power (dB) for each spectrum was calculated from the total power of the spectrum and the mean total power of the calibration signal as reference. Values for 5 additional call characteristics were extracted from time function (Fig. 1(c)). The definitions of all call characteristics are shown in Table 1.

A sample of 145 calls from 19 animals that could be obviously assigned to one of the three call types grunt, squeal, or scream was used as a basis for discrimination analysis. This assignment was done according to distinct differences in the spectrograms as seen in Fig. 2. The categorization revealed 54 grunts, 33 squeals, and 58 screams that were used for the reference sample.

The variables for the discrimination analysis was selected using a stepwise selection of quantitative variables (procedure stepdisc: SAS 6.12; SAS Institute Inc., Cary, NC). The

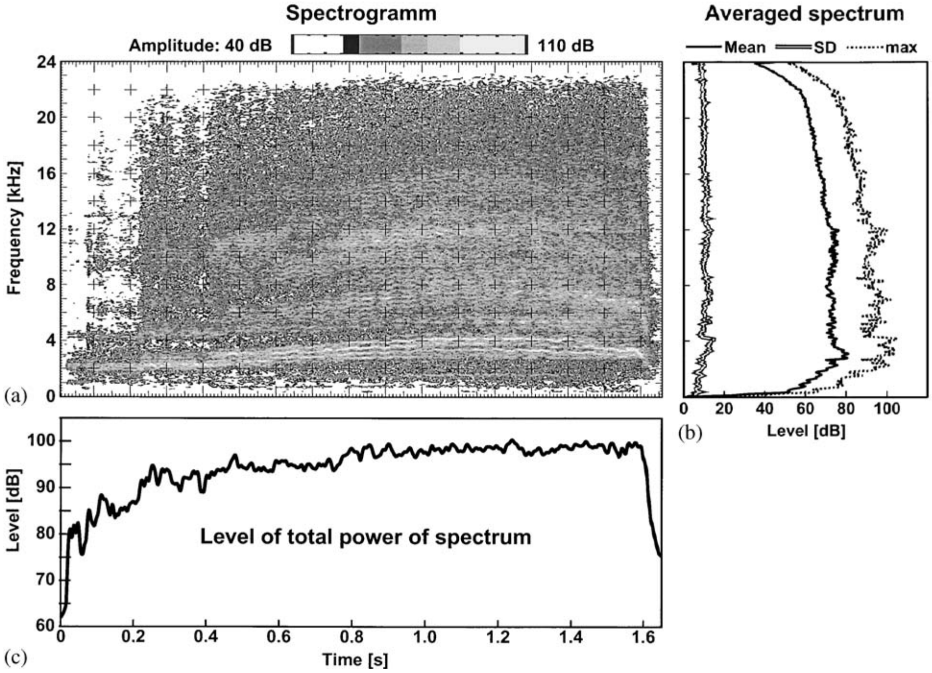

Fig. 1. Spectrogram, average spectrum, and level curve of an example call.

calculation of the classificatory discriminate function was performed by using a SAS 6.12 discrimination procedure. The resulting discriminate functions were used to calculate the probability for the assignment to each of the three call types.

A mixed linear model with the fixed effects of treatment, age, call type and the three pair interactions between the effects and the random effect of the litter was used for statistical analysis. The random effects of the litter considers the biological variability of the behavioural traits. Least-square means were compared by a t-test with the Tukey–Kramer adjustment. Related degrees of freedom were estimated by Satterthwaite approximation. Calculations were done by the SAS 6.12 procedure mixed.

The number of calls within 10 s intervals were recorded in order to compare the distribution of vocalizations within the treatment period. The comparison of the mean number of calls per animal between the four treatments was separately assessed for each interval by an analysis of variance with multiple comparisons of means.

3. Results

Four of the 70 piglets tested did not vocalise during treatment. Two of these four piglets were treated with restraint plus local anaesthesia (RA), one was treated with castration plus local anaesthesia (CA), and one was treated with restraint alone (R). Therefore a total of 4537 calls of the remaining 66 piglets were evaluated.

A model with 11 variables resulted from the stepwise selection of variables. All but the occurrence of peak amplitude and occurrence of peak level were integrated into the model. The

Table 1
Definition of parameters for call quality

Parameter	Description	Unit	Abbreviation
Call duration	Duration of utterance	ms	CL
Peak amplitude	Highest amplitude of a single time–frequency cell in the call	dB[a]	PA
Peak frequency	Frequency of peak amplitude	Hz	PF
Occurrence of peak amplitude	Time point of peak amplitude as percentage of call duration	%	OPA
Main amplitude	Highest amplitude in the mean spectra of the call	dB[a]	MA
Main frequency	Frequency of highest amplitude in the mean spectra	Hz	MF
Coefficient of energy concentration	Power of peak amplitude as percentage of the sum of power of mean spectrum[b]	%	CEC
Weighted frequency	Mean frequency weighted by the level of each frequency of the mean spectrum[b]	kHz	WF
Peak level	Level of the time window with the highest total power in the call	dB[c]	PL
Occurrence of peak level	Time point of peak level as percentage of call duration	%	OPL
Standard deviation of power	Standard deviation of total power of all time windows of the call		SDP
Call level	Level of mean total power of all time windows in the call	dB[c]	CL
Call energy	Emitted call energy standardised for a duration of 1 s	dB(SEL)[d]	CE

[a] These values are the differences between the recorded data and the mean level of peak frequency of the calibration system. To improve clarity, 94 dB of the calibration signal are added to the data in these tables and figures.

[b] These data were calculated using the mean levels (ML_i) of frequency (i) according to the following equation:

$$CEC = \frac{10^{MA/10}}{\sum_{i=1}^{n} 10^{ML_i/10}}, \qquad WF = \frac{\sum_{i=1}^{n}(f_i \cdot ML_i)}{\sum_{i=1}^{n} ML_i}.$$

[c] These values are calculated to the basis of the mean total power of the calibration system in accordance to the definition of the unit dB. To improve clarity, 94 dB of the calibration signal are added to the data in these tables and figures.

[d] These values are calculated by dividing the total power emission by one second, according to the definition of SEL.

discriminate function was determined by the generalized squared distance method based on these variables. A 100% relation between the 145 calls of the labelled sample and the three call types was the result of the discriminate functions. The calibration information from the labelled samples were used to associate each call in the test to one of the three call types.

Of the calls, 3714 (81.86%) could be positively associated with one single call type. In addition, 4294 calls (94.64%) could be associated with a 95% confidence level of one single call type. In 25 calls (0.55%) the probability for the association to all three call types was more than zero.

Fig. 3 shows the distribution of the three call types in the four treatment groups. Castration without local anaesthesia has the highest number of calls. The number of calls decreases with local anaesthesia, even more with restraint and the most with both restraint and local anaesthesia. The differences between the treatment groups were most distinct in the scream call type. Here the

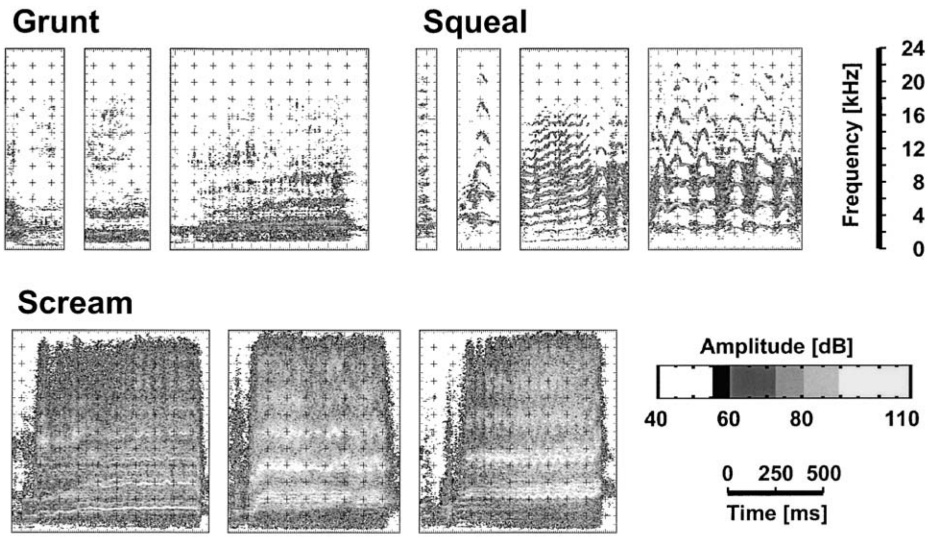

Fig. 2. Spectrograms of three call types of pigs under stress.

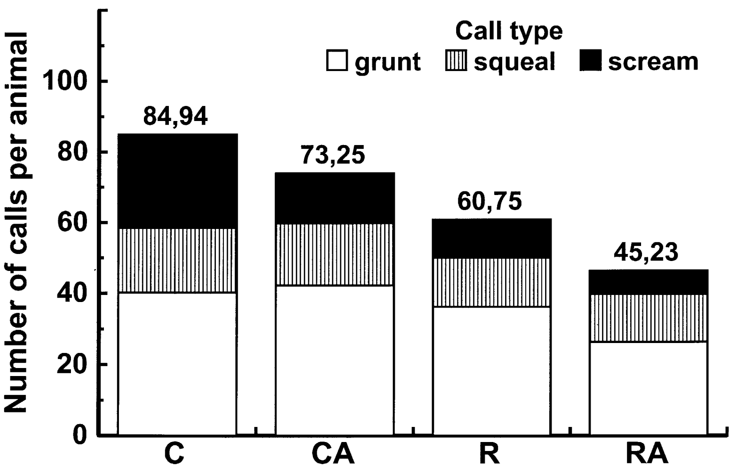

Fig. 3. Number of recorded calls of three different call types in pigs under stress. (Abbreviation of treatments: C—castration without anaesthesia, CA—castration with local anaesthesia, R—restraint without anaesthesia, RA—restraint with local anaesthesia castration.)

treatment of castration had significantly higher screams; no significant difference between other call types and other treatments could be observed.

Fig. 4 shows the distribution of screams within a treatment group. It indicates the mean number of screams, recorded in 10 s intervals after the start of the treatment. Differences between the treatment groups CA, R, and RA are not significant; even the procedure of intratesticular injection in the treatment group CA and RA does not significantly increase the number of screams during the first 20 s of the procedure. Castration without local anaesthesia (C), however, significantly increases the number of screams 30–110 s after the start.

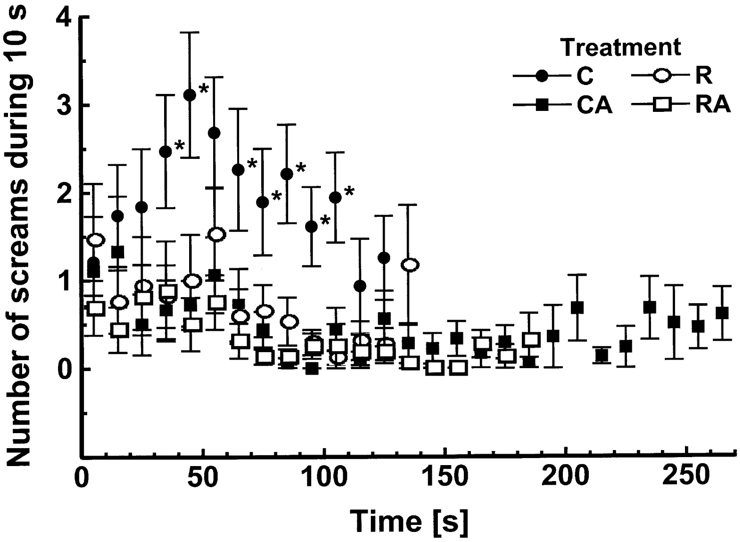

Fig. 4. Average number of screams in 10 s intervals during the four treatments. Asterisks (*) indicate significant differences. (Abbreviation of treatments: C—castration without anaesthesia, CA—castration with local anaesthesia, R—restraint without anaesthesia, RA—restraint with local anaesthesia castration.)

Table 2
The influence of age on parameters of energy

	Age in days			Unit
	7	13	19	
Call energy	89.7 ± 0.54^{a}	86.7 ± 0.71^{b}	91.5 ± 0.56^{c}	dB(SEL)
Call level	92.1 ± 0.62^{a}	89.3 ± 0.83^{b}	93.8 ± 0.63^{a}	dB
Peak amplitude	93.4 ± 0.60^{a}	90.7 ± 0.79^{b}	95.4 ± 0.62^{a}	dB
Peak level	97.2 ± 0.61^{ab}	94.8 ± 0.80^{a}	98.9 ± 0.61^{b}	dB
Main amplitude	74.2 ± 0.66^{a}	71.1 ± 0.87^{b}	75.4 ± 0.65^{a}	dB
n	2076	1342	1119	

Means within a row with no common superscript differ significantly ($P < 0.05$).

Call energy was age dependent. Only parameters for energetic call qualities showed significant differences (Table 2). Differences in power between calls were observed in call energy, the energy sent off with each call. Four other parameters of call quality were significantly different between day 13 and day 19. However, these parameters were not significantly different in the comparison of day 7 and day 19.

Table 3 lists the means of the recorded data (±SEM) and the comparison of means for each treatment. The different treatments significantly influenced all measured parameters. This difference was particularly observed between C and the other treatment groups. Calls of the piglets that were castrated without local anaesthesia (C) had a longer duration, a higher call energy, reached peak levels and peak amplitude later in time and had a higher standard deviation of power than the calls of piglets in treatment groups R and RA. Calls of piglets that were

Table 3
The influence of treatment on call quality

	Treatment				Unit
	C	CA	R	RA	
Call duration	698.5 ± 26.3^{a}	665.2 ± 26.9^{ab}	647.8 ± 27.7^{bc}	601.4 ± 31.0^{c}	ms
Call energy	90.2 ± 0.37^{a}	89.6 ± 0.40^{ab}	88.8 ± 0.42^{b}	88.5 ± 0.52^{b}	dB
Peak level	97.8 ± 0.41^{a}	97.1 ± 0.42^{ab}	96.3 ± 0.44^{b}	96.6 ± 0.52^{ab}	dB
Peak frequency	3895 ± 121^{a}	4349 ± 125^{bc}	4227 ± 129^{b}	4528 ± 147^{c}	Hz
Main frequency	3943 ± 120^{a}	4430 ± 124^{b}	4104 ± 128^{ac}	4361 ± 148^{bc}	Hz
Occurrence of peak level	67.17 ± 1.21^{a}	60.49 ± 1.30^{b}	61.98 ± 1.38^{b}	58.55 ± 1.74^{b}	%
Occurrence of peak amplitude	61.80 ± 1.17^{a}	57.52 ± 1.26^{b}	58.07 ± 1.35^{b}	54.92 ± 1.77^{b}	%
Weighted frequency	11.31 ± 0.034^{ab}	11.34 ± 0.035^{ab}	11.29 ± 0.035^{a}	11.37 ± 0.038^{b}	kHz
Standard deviation of power	317244 ± 41978^{a}	228675 ± 42793^{b}	164090 ± 43666^{cd}	238303 ± 47783^{bd}	
n	1510	1191	1043	793	

Means within a row with no common superscript differ significantly ($P < 0.05$).
C—castration without anaesthesia, CA—castration with local anaesthesia, R—restraint without anaesthesia, RA—restraint with local anaesthesia castration.

castrated after local anaesthesia (CA) had similar call duration, energy, peak frequency to piglets that were not castrated. Differences between CA and RA were small; significant differences between these groups existed only in call duration.

All frequency parameters show the influence of local anaesthesia on call quality. Peak frequency increases with local anaesthesia both in the comparison of C–CA and R–RA. Main frequency increases with local anaesthesia in the comparison of C–CA. Weighted frequency increased with local anaesthesia in the comparison of R–RA.

Analysis of variance revealed a significant influence of call type on call parameters. The comparison of means result in two groups of parameters (Table 4). The first group consisted of the parameters of call duration, occurrence of peak level, occurrence of peak amplitude, and standard deviation of power. This group of parameters yield no difference between the call types of grunt and squeal and show significantly higher results in screams. The other group of parameters, referring to frequencies and energy emission during calls, resulted in significant differences between all three call types. Call energy and weighted mean frequency increase from grunt to squeal to scream, whereas squeals show the highest main frequency of all three call types.

Fig. 5 demonstrates the comparison of peak frequency of the three call types in all four treatments. Significant differences between call types were obtained in a comparison of all four treatment groups. Only two significant differences, however, were obtained in a comparison between treatment groups. One difference was found in the call type of grunt, the other in squeal. RA produced an increase in peak frequency of grunts, castration without local anaesthesia lowered peak frequency in squeals.

Table 4
Comparison of call types

	Call type			Unit
	Grunt	Squeal	Scream	
Call duration	557.6 ± 26.0^{a}	555.7 ± 27.0^{a}	828.0 ± 27.7^{b}	ms
Call energy	83.4 ± 0.37^{a}	87.1 ± 0.40^{b}	97.3 ± 0.42^{c}	dB(SEL)
Peak level	91.8 ± 0.40^{a}	95.5 ± 0.42^{b}	103.6 ± 0.44^{c}	dB
Peak frequency	3087 ± 120^{a}	5269 ± 125^{b}	4393 ± 129^{c}	Hz
Main frequency	2934 ± 118^{a}	5326 ± 124^{b}	4368 ± 128^{c}	Hz
Occurrence of peak level	60.28 ± 1.18^{a}	60.88 ± 1.31^{a}	65.02 ± 1.39^{b}	%
Occurrence of peak amplitude	55.62 ± 1.13^{a}	56.52 ± 1.27^{a}	62.09 ± 1.37^{b}	%
Coefficient of energy concentration	6.80 ± 0.21^{a}	6.10 ± 0.23^{b}	4.21 ± 0.24^{c}	%
Weighted frequency	11.31 ± 0.034^{a}	11.34 ± 0.035^{b}	11.48 ± 0.035^{c}	kHz
Standard deviation of power	45651 ± 41706^{a}	65570 ± 42888^{a}	600013 ± 43745^{b}	
n	2450	1066	1021	

Means within a row with no common superscript differ significantly ($P < 0.05$).

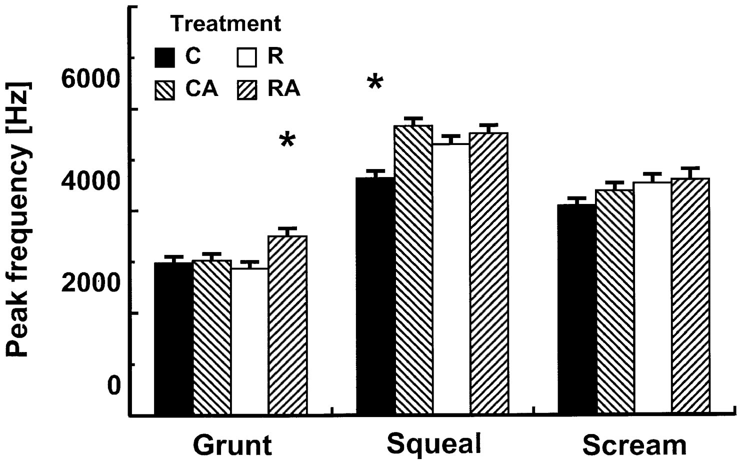

Fig. 5. Comparison of peak frequency of three call types in four treatments of pigs under stress. Asterisks (*) indicate significant differences. (Abbreviation of treatments: C—castration without anaesthesia, CA—castration with local anaesthesia, R—restraint without anaesthesia, RA—restraint with local anaesthesia castration.)

4. Discussion

The vocalization of piglets under stress is less homogenous than that of chicks in stressful situations [13]. With the data of this study three call types are distinguishable in pigs. They were named in accordance with earlier descriptions of call structure [2,10] grunt, squeal, and scream. With a numeric description of sound quality a discriminate function for each of the three call types was successfully produced. All calls of the sample were assigned to one of the three call types according to their visual features; all these calls could be classified by discriminate analysis. This fact provides evidence for the consistency of the visual classification.

A high percentage (94.64%) of calls that could be classified in one of the three call types during the castration process within the confidence level of 95% was found. This fact supports the contention that piglet calls, even under stress, are predominantly discrete and can be classified accordingly. This observation is in concurrence with that of Jensen and Algers [10] for the vocalization during suckling and the observations by Klingholtz and Meynhardt [14] for the vocalizations of wild boars.

One hundred and twenty seven (52.3%) of the remaining 243 calls that were less clearly classified were transitions from grunt to squeals. This transition stage was observed by Igney [11] after piglets were separated visually and acoustically from their group. He described a call quality with the structure of a grunt at the beginning and a structure of a squeal in a second part of the call. Thus, the existence of a combination of grunt and subsequent squeal in a single call may explain the number of calls with this particular quality.

The comparison of the occurrence of the call types during treatments gives evidence for pain related use of different call types. Piglets being castrated without local anaesthesia produced almost double the number of screams as castration after anaesthesia or mere restraint. Differences of other call types in all treatment groups were not significant. Likewise the comparison of call frequencies in 10 s intervals produced increased scream rates in the castration group (C). The increase in screams during unanaesthetized castration during the first 40 s gives evidence that pulling the testes produces the highest stress. This observation is in line with the results found by Taylor and Weary [6]. The distribution of calls over the treatment period reveals that the increase in screams of piglets in CA compared with R is the result of the increase in treatment length due to the 2 min waiting period for the anaesthetic effect.

The comparison of the recorded sound parameters reveals the particular position of screams in the call repertoire of pigs. Screams are significantly different in their sound parameters than grunts or squeals. Screams have a lower peak frequency and main frequency than squeals. Hence, these data do not support the contention of White et al. [8] that an increase in frequency indicates an increase in stress. Contrarily, according to the results of this study, the decrease in frequency at the time in the castration process when the spermatic cord is severed may be caused by an increase in screams, which may reveal an increase in stress. Grunts and squeals show similarities in length and variability of power. This may indicate a close relation in the basic control mechanism of these two call types.

Castration in comparison to mere restraint produced a comprehensive change in sound parameters, with castration calls becoming more extended and more powerful. The maximal energy emission of the calls shifted towards the end of the call, a phenomenon which is a sign of a choked voice in babies [15]. Also peak frequency decreases during pain, a phenomenon which may indicate an increase in energy, as was seen in babies in the frequency range between 1500 and 2000 Hz [15]. The increase in the standard deviation of power (SDP) during pain may indicate higher tension.

The findings in this study also show differences in the effectiveness of the parameters which indicate pain. Parameters that describe a single event in a call, such as peak level or peak frequency give better results than parameters that describe an average, such as weighted frequency and main frequency. No pain related difference could be observed in main amplitude and CEC. With the use of local anaesthesia prior to castration (CA) all parameters shift towards the control

(R). This gives further evidence for the assertion that pain in piglets influences call quality. We observed an increase in peak frequency after local anaesthesia. However, White et al. [8] observed a reduction of high energy frequency after intratesticular local anaesthesia, a comparable parameter to the peak frequency measured in the present study. These apparent contradictions may be due to differences in restraint applied to the piglets during treatments. An increase in peak frequency was observed in both treatments with local anaesthesia (CA and RA) in correlation with the characteristic delay in the effect of the anaesthetic. Restraint in the present study consisted of hanging the piglets from a rack tied with loops to the hind legs. In this position, vocal organs of the animals may be displaced due to systemic effects of the anaesthetic. Thus, a relaxation of the vocal organs may be due to the anaesthetic effect and may have subsequently altered peak frequency.

5. Conclusions

The research has indicated that pain-related call types in piglets can be identified. Screaming, a call type that differs significantly from two other characteristic call types of piglets increases when the piglet is suffering pain. Parameters of energy emission, frequency and call duration are particularly appropriate to characterise call types. The technique reported in this study can be transferred to other applications to measure discomfort of animals. This technique based on separated calls is applicable to all situations with low density of vocalization. Hence, calibrated analysis of call types are necessary when energy emission in different studies are compared. On the basis of the results, the automatic classification of call types during management operations becomes feasible. The development of an automatic classification of call types would be useful as a tool to compare stress situations for animals by an objective measurement. This could contribute to objective animal welfare assessment.

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