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Heat stress assessment by swine related vocalizations

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A B S T R A C T

In intensive swine farms, stressful conditions in the climate, namely heat stress, affect animal behaviour and welfare. Pigs reared in commercial fattening housing suffer from temperature increases and from their inability to get free to refresh themselves any time they need to. The closed and dense environment further worsens this condition.

This particular study was conducted to determine whether heat stress induces specific vocalisations in a group of piglets bred in standard intensive conditions and whether these vocalizations are acoustically different from other swine normal vocalizations. The temperature increase was aimed to stimulate heat-stress-specific behaviours and vocalisations. For this purpose, the vocal calls were coupled with environmental and physiological parameters (rectal temperature and respiration rate) collected during the temperature-increasing tests.

The study of sound acoustic features such as frequency, duration and amplitude, together with the analysis of the environmental parameters, showed a clear difference between heat-stress-related sounds and other types of vocal calls recorded while the piglets were not stressed by environmental insults. This result shows how animals can communicate emitting specific calls and provides a deeper knowledge of animal behaviour, thereby providing a means toward better animal welfare.

1. Introduction

Heat stress is widely recognised as a stressful condition that affects animal behaviour, animal welfare and production quality.

Intensive modern swine farming deals with animals under extreme climate conditions because of the closed environment, the poor indoor air quality, the use of concrete floors that cannot absorb manure and the deep pits for manure stocking that release gaseous ammonia from underneath (Wathes et al., 2012). The effect of high temperature on pig growth performance and health has been extensively studied and published (Close, 1981; Hillmann et al., 2004; Patience et al., 2005). In most of the published studies on the effect of thermal stress on pig performance, the animal-related measurements were performed with a prior adaptation from 4 to 20 days to the experimental temperature (Renaudeau et al., 2008).

However, during real summer heat waves, especially in temperate climates, pigs are suddenly exposed to high temperatures with negative consequences on their health and their performance (Nienaber and Hahn, 2007). Short-term, high-intensity hot weather patterns are referred to as heat waves.

The physiological responses of pigs have been studied intensively, and the RR and RT have been shown to predictably increase with rising temperature (Brown-Brandl et al., 1998; Liao and Veum, 1994). Because pigs do not sweat, they rely heavily on evaporative losses via the respiratory tract for cooling. Animal mortality has also been related to a certain extent with this phenomenon. Short-term adaptive changes related to behavioural, physiological and immunological functions are the initial

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responses to acute events, whereas longer-term challenges induce performance-oriented responses. Generally, pigs respond to high ambient temperature by nutritional and physiological adaptation to maintain homoeostasis; above the upper limit of the thermo-neutral zone (approximately 29 °C for weaned pigs), an increase in temperature results in a decrease in the average daily feed intake to limit heat production and an increase in the respiratory rate to remove the excess heat (Fuquay, 1981; Kamada and Notsuki, 1987). In addition to the physiological body responses, behavioural reactions to distress include animal vocalisations. Past studies have shown that pigs respond to pain or distress with specific vocalisations, in terms of acoustic parameters e.g. frequency, and that these responses might be used to assess animal welfare in intensive farming conditions (Manteuffel et al., 2004; Marx et al., 2003; Schön et al., 2001; White et al., 1995). Vocal responses are shown to be strictly associated with specific stimuli according to phylogenetic evolution. Animal welfare studies, together with microclimate improvement, must take into account the animals' responses to the severe environment to which they are exposed. Understanding these responses and observing and recognising animals in distress is especially important for implementing appropriate practices to reduce the effects of stress.

A possible means to approach this goal is derived from sound analysis, including the continuous recording and automatic processing of animal sounds, in livestock farming compartments as a tool for the early detection of disease and distress. The strength of this technique is its non-invasiveness and the possibility to work on-line to continuously monitor the animals. The capabilities of such a system, based on the classification algorithms, have been tested in previous studies investigating respiratory diseases (Exadaktylos et al., 2008) or animal welfare (Schön et al., 2004). The aim is to increase our understanding of animal vocalizations and welfare by studying their responses to typical stressful conditions. This particular study was conducted to determine whether heat stress induces specific vocalisations in a group of piglets bred in standard intensive conditions. For this purpose, the vocal calls were coupled with environmental and physiological parameters collected during the temperature-increasing tests.

2. Materials and methods

Heat stress trials were performed at the experimental farm "Centro Zootecnico Didattico Sperimentale" of the Milan Veterinary facility in Lodi (Mi). The experiment utilised litter mates of crossbred (Large White × Landrace) females and males with an average initial live weight of 4.92 ± 0.35 kg. The piglets were observed for 120 days from their age of about 25 days. The piglets were housed in a mechanically ventilated building in two adjacent pens of $2 \times 2.5\ m^2$ dimensions and the floor was constructed from fully slatted PVC. The piglets were fed a pelleted diet, formulated to meet or exceed NRC (1998) recommendations for all nutrients, twice a day, and water was available ad libitum. Each piglet's weight was measured, using an industrial livestock scale, at their arrival in the experimental facility, during the period of the heat stress trials and at the end of the experimental trials. A total of 20 piglets were housed in groups of ten per box for the first month of the trial. The groups were then split into three smaller groups of 7, 7 and 6 animals. This allowed a space allowance from $0.2\ m^2$ to $0.3\ m^2$ as per the CEE/88/2001 requirements.

The room temperature was adjusted for the thermal comfort zone of the weaned piglets, ranging from 32.2 °C when the animals were 4.92 ± 0.35 kg to 25.2 °C in the last week of the trials. The relative humidity ranged from 20% to 30%, and the ventilation rate was $3\ m^3$/h. These climate parameters were controlled in real time from a central control panel placed outside the room. The climate parameters were also measured at the animal level using a portable datalogger (Delta OHM). Animals were feeded, cleaned and managed for the trial from one single operator.

2.1. Heat stress trial

Groups of two or three piglets, out of the 20 piglets available, were assigned to high-temperature treatments (29 °C up to 41 °C) to simulate specific behaviours and vocalisations. This increase was designed to induce heat stress because normally the temperature in the pen was maintained within the comfort zone throughout the time that the animals were kept in the experiment room. During the trial, a small, randomly selected, group of 2 or 3 piglets was placed into a mobile solid-wall crate with dimensions of $0.5 \times 0.5\ m^2$ up to $0.7 \times 0.7\ m^2$ to isolate them from the rest of the group and facilitate our measurements. These dimensions also allowed a space allowance of $0.2\ m^2$ to $0.3\ m^2$ as per the CEE/88/2001 requirements such that a certain separation among animals was kept possible and avoiding further stress and competitive vocalizations.

This crate was placed inside the pen where the animals were normally living. On top of this crate, two infrared lamps (150 W) were hung 50 cm above the animals (Figs. 1 and 2). These types of lamps are routinely used for nest heating in farrowing rooms.

Each piglet was tested for heat stress reaction two, up to five times during the whole experimental trial period. Animals turnover was random such that groups were not of fixed mates.

The piglets were placed inside the crate and allowed to adapt to the smaller environment of the crate for approximately 30 min. Their reaction to newer environment was visually observed, from above the crate, by the operator (crate exploration, interaction with mates) as well as their vocalizations were recorded. As they started lying down, the infrared lights were turned on, and the measurements began. The temperature inside the crate was measured at time 0 (t_0) and after 20 (t_1), 40 (t_2) and 60 (t_3) min using a standard mercury-glass thermometer placed on the wall of the crate at floor level. Either water or feed was available in the test crate. In total 25 trials were repeated along the 120 days and each piglets was on average tested three times during the whole experimental period.

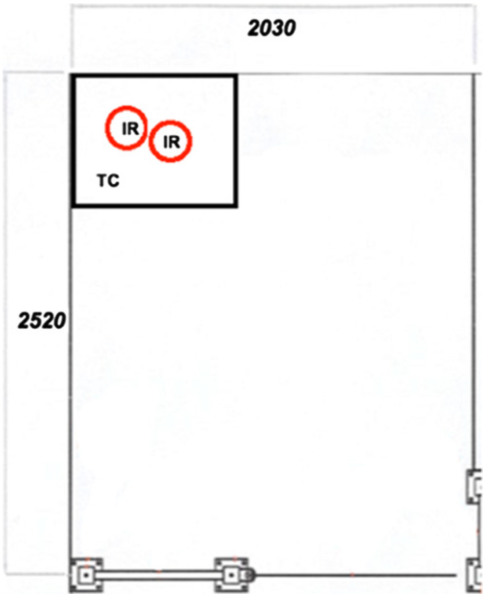

Fig. 1. Planimetry of the box hosting the 10 piglets of the trial (dimensions 2520×2030 mm^2), TC is the 0.5×0.8 m^2 test crate where the temperature was increased by means of the two infrared lamps (IR).

Fig. 2. Heat stress test crate with two infrared lamps and microphone hanged 50 cm above the animals.

2.2. Physiologic measures

The piglets' rectal temperature (RT) and respiration rate (RR, breaths per minute)were recorded as physiologic indicators of heat distress. The rectal temperature was measured at t_0 when the animals were about to be placed in the crate and at t_3 as they were moved out using a commercial digital pocket thermometer. The respiration rates were determined by counting the flank movements when the animals were in lateral decubitus and recorded as the frequency per minute at t_1, t_2 and t_3 assuming 35–40 breaths per minute as a normal physiological respiratory rate (Brown-Brandl et al., 2001; Renaudeau et al., 2007). Two simultaneous observers did the counting.

2.3. Behaviour observations

The groups of piglets were observed to annotate specific behaviours happening during the audio recordings both in normal conditions and in heat stress experiments.

Observations, in normal conditions, were made, both from direct view or from recorded video, in days when no stress trials were performed, to label vocalizations happening while animals were fed, when they were playing or when they were interacting towards each other. The time and the causes of vocalizations were noted such that during audio playback listening and labelling it was possible to associate a specific behaviour to a specific vocalization.

Observations, in heat stress conditions, were done to analyse the behaviour of piglets in the test crate. At first, their reaction to a newer environment was observed: it normally consisted in exploration of the crate walls and larger interaction towards mates. Generally they get used to this environment in the first 25($\pm$4) min after which they started to lay resting in sternal position. The heating started at the moment piglets laid down for resting as well as sounds recordings and during the trial hour the piglets showed different lying behaviours as temperature increased, respectively they moved from sternal to lateral position and progressively avoiding the physical contact towards the other 2 mates. In the same time pigs started to emit low frequency vocalizations.

2.4. Sound recordings

All vocal calls were recorded with a long-gun directional microphone (Sennheiser ME 67) that was placed on the top of the crate 50 cm above the animals or hung over the box at the same height. The sensor was connected to the external microphone input of a hand-held digital recorder (Marantz PMD 620), and the sounds were recorded on an SD flash media. The sounds were recorded as uncompressed (.WAV) 24-bit files at a 44.1 kHz sample rate. Piglets vocalisations, for control without heat stress, were recorded for about one hour of time repeated as many times as many heat stress trials. A total of about 20 h of recordings were available from these recordings.

Vocalisations, from the piglets kept in the trial crate, for heat stress experiments, were recorded from the moment the heat stress was given, approximately 30 min after the piglets entered the crate, to avoid recordings of non-heat stress related vocalizations caused from the newer environment. The recordings were run for one hour in every test performed so that nearly 20 h of audio files were available for analysis. The sounds of normal activity (e.g., play, socialisation) were recorded in several moments during piglets normal life and included screams and grunts of the piglets playing with each other with an enrichment tool (chopped wood), hierarchic fight vocalisations and the sounds emitted when the piglets were accessing at the trough (15 h in total). These sounds were collected as a pool of sounds named as non-heat stress sounds since they belong to a large acoustic repertoire of vocalizations that pigs emit in standard breeding conditions.

2.5. Sound labelling and analysis

The audio playbacks were processed using an audio recording and editing home made tool to label all of the piglets' recorded vocalisations from their time and frequency spectrum domain. The labelling procedure consisted of audio playback listening and single sounds selection and extraction. The procedure was manually performed, as the tool was able to show the sounds images and play its noise simultaneously. The labelling was eased from the observations annotations and videos confirming time and type of happening as well as the acoustic source of vocalizations. The sounds were thus manually selected, copied and pasted into two main groups in separate folders: heat stress (HS) derived from the test crate session when the temperature was increased and non-stress (NS) from the session performed in standard condition including more categories of sounds (games, interactions, etc.). Both categories were further divided into subcategories screams and grunts on the basis of sound type, identified by the operator, and sound duration (Table 1). In total 4 groups of sounds were available for the following sound analysis.

Each vocalization, in every category, was analysed for the average peak frequency (Hz), fundamental frequency (Hz) and sound duration (s). The peak frequency and fundamental frequency were calculated by 20th order Auto Regressive estimation and FFT Power Spectrum Density (Figs. 3 and 4). The values were extracted by sliding a moving window (Hamming window with a length of 128 samples and with a 50% overlap) over the total vocal call. In every window, the fundamental frequency was derived simultaneously with its energy envelope using an AR spectral estimation method (Pardey et al., 1996; Silva et al., 2010). The order of the AR model will influence the number of peaks that are to be modelled. A higher order is associated with more estimated peaks. In our case, the model order was set to 20 based on Akaike's information criterion (AIC). Using peak detection, the fundamental frequency was extracted for every window. Signals were analysed using Matlab R14 (Mathworks).The sound duration was measured by manually selecting the beginning and the end of the sound waveform showed by the spectrogram.

Table 1
Type of sounds collected during the trials.

Heat stress	Non-heat stress
Grunts	Grunts for interaction (grooming)
Screams	Screams for game Screams at feeding

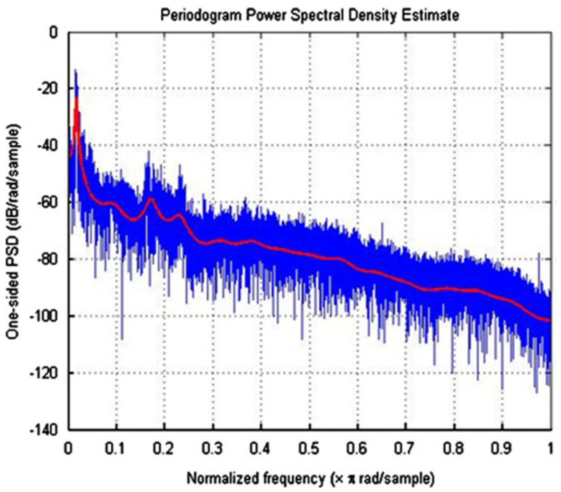

Fig. 3. Power spectrum density of a heat stress grunt and the automatic extraction of the peak frequency (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

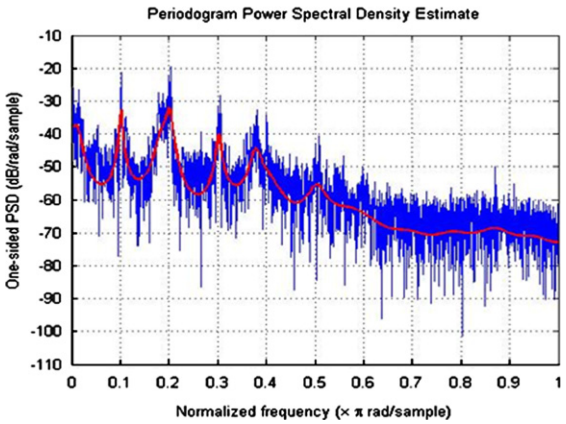

Fig. 4. Power spectrum density of a non-heat stress sound and the automatic extraction of the peak frequency (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.6. Statistical analysis

A first variance analysis was made to distinguish sounds from the heat stress trial, normal sounds and test crate control group, then the climatic, physiologic and acoustic data were analysed using the variance analysis procedure of SAS 9.2 to investigate the most significant associations. The pig weight; room temperature (T); relative humidity (RH); crate temperature measured during the trial at t_0, t_1, t_2 and t_3; respiratory rate (RR) at t_1, t_2 and t_3; rectal temperature (RT) at t_0 and t$_3$; number of heat-stress vocalisations; number of non-heat-stress sounds and acoustic features, such as frequency (peak frequency, PF and fundamental frequency, $F0$) and duration were considered in the model. The repetition and nesting structure of the experiment was also included in the model to assess the dependence of number and type of vocalizations with the grouping of animals in the test crate. The sounds were also run into a proc Chart to assess the distribution of their acoustic features. All the parameters were also studied for correlations by a statistic Pearson test.

3. Results

3.1. Physiologic responses

The rectal temperature measured at t_0 and t_3 of the HS trial showed an average increase of 0.41 °C from 39.3 °C to

Table 2
Physiologic parameters collected at different periods (t_0, t_1, t_2, t_3) during the heat stress trials.

Parameter	t_0	t_1	t_2	t_3
RT (°C)	39.3	nr	nr	39.7
RR (bpm)	33	37	69	116
Trial Δt (°C)	29	35	37	41

39.71 °C over the entire pool of animals tested. From Pearson's Correlation analysis, this rise in body temperature was increased ($r=+61\%$, $P<0.001$) by the increase in air temperature from t_0 to t_3 ($\mu\ \Delta t=5\pm3$ °C) during the time of the trial, increased by the heat exposure time ($r=+53\%$, $P<0.001$) and increased by to the RR ($r=+47$, $P<0.05$). The RR increased from t_1 to t_3, ranging from an average of 33 to an average of 116 bpm. The RR measured at t_2 was observed as a marker to test whether the breathing was increasing constantly, according to the increase of temperature, or was stable or even decreased with thermal physiologic adaptation. The RR and Δt were increased ($r=+72\%$, $P<0.001$) during HS (Table 2). The RR was also correlated ($P<0.05$) with the animal weight, showing an increase in bpm as the animals were growing ($r=+52\%$; $P<0.05$). The animal weight varied from 4.92 kg $\pm$ 0.35 at the beginning of the housing period for the trials to an average of 17 kg $\pm$ 0.59 at the end of the experiment.

3.2. Sound analysis

Twenty heat stress trials were performed, during which 20 h of recordings were collected. Audio-playback labelling allowed the identification of 1990 heat-stress sounds, of which 1708 were "heat stress grunts" (HSg) and 282 were "heat stress screams" (HSs). In total, 870 various vocal calls were also identified from recordings taken in the control group when the animals were together in their pen and when the animals were placed in the test crate without the heating conditions.

The results from the proc Chart showed that all of the acoustic parameters (peak frequency, fundamental frequency and sound duration) had a normal distribution. The first variance analysis made to distinguish sounds from the heat stress trial, normal sounds and test crate control group confirmed that heat stress sounds had a significant difference from normal sounds and control sounds for $P<0.001$, for all the parameters investigated. Differences were confirmed for all of the parameters (PF, $F0$ and duration) among the four different sound classes (HSs, HSg, NSs and NSg). On the other side, no statistical difference was observed when comparing normal sounds and test crate control group sounds both for length and frequency (respectively $P<0.05$ and $P<0.001$); for this reason although the two groups of sounds recorded in non-heat stress conditions had different sources, for statistical analysis, they were grouped into a large pool of "non-heat-stress sounds" (NS). From the proc Chart it is demonstrated that the length of the NS sounds ranged from 0.3 to 1.2 s, and 45% of them measured between 0.3 and 0.7 s. The HSg ranged from 0.2 to 1 s, and 43% of the values measured between 0.2 and 0.5 s. The HSs ranged from 0.2 to 2.4 s, and 56% of the values measured between 0.1 and 0.2 s.

Regarding the peak frequency, 90% of the NS sounds had values less than 750 Hz, as did the HSg sounds. The HSs sounds showed a higher peak frequency distribution, with 55% of the samples higher than 750 Hz. The fundamental frequency values showed that the NS sounds ranged from 230 to 770 Hz, with 50% in the range inferior of 400 Hz. The HSg showed a 95% distribution from 180 to 600 Hz, of which 72% was in the 400-Hz range (average 400 ± 125). The HSs showed a higher fundamental frequency distribution, with 54% of the values higher than 1000 Hz.

After the descriptive statistical analysis, a further analysis of variance was performed. The model was used to study the effect of the class of sounds (type of sound: NS, HSg, HSs) on the different acoustic parameters and the results confirmed a strong association between the increase in temperature of the test crate and the number of vocalizations and the values of fundamental and peak frequency (respectively $P<0.001$ and $P<0.01$) confirming both that heat waves induce a typical vocalization and that this vocalization has a specific footprint. This allows using this parameter as a discriminant for an automatic sound classification.

From the Pearson Correlation the HSg sounds were positively related to the RT and the rising temperature in the test crate, (all for $P<0.001$) showing a linear increase of approximately 80% from t_0 to t_3. The HS screams occurred the most during the central 20-min period (from minute 20 to minute 40) and were negatively correlated ($P<0.001$, -78%) with the room temperature, which, in normal situations when no tests were performed, was kept at the optimal level for thermal comfort given the age and weight of the piglets. The HS screams and all NS sounds did not show any relevant correlation with the climate parameters involved in heat stress. The NS sounds values, of all the acoustic parameters investigated, showed a significant correlation with weight increase ($P<0.001$).

4. Discussion

Short-term HS trials were performed to simulate real summer heat waves for a group of pigs. Vocalizations and physiologic parameters were recorded. During the trials, the increase in the RT was directly associated with the higher temperature in the trial crate and with the RR, which shows the efficacy of this method to induce heat stress in the piglets. The RT linearly increased during the trial, which indicates that the mechanisms used for heat reduction are not sufficient to prevent an increase in body temperature in short term heat waves. In previous studies, a decrease in body temperature was observed on long-term exposure to heat-stressful conditions showing a body adaptation to this stress, for this reason the RR, which provides an easily observable measure of animal's thermal state, was measured at three different periods. The correlation between the RR and the Δt of the test crate ($P<0.001$) excluded thermal adaptation in this type of trial. Because the physiologic parameters show stressful conditions in the piglets, we can conclude that there is indeed a causal effect of heat stress on the vocalization behaviour, definitely confirmed by the large increase of vocalization (+80% from t_0 to t_3). The positive correlation between the number of vocalisations and the rectal temperature showed that animals in distress emit a great number of sounds (1990 HS vs. 870 NS). The variance analysis, made to compare the two main groups of sounds achieved in the study, showed a clear, statistically relevant, distinction between the HS and NS sounds for parameters such as frequency ($F0$ and PF) and length of sounds.

5. Conclusions

Research and livestock-addressed legislation aim to enhance animal welfare and production. Heat stress may be evaluated using a continuous acoustic analysis to improve management by warning farmers about a higher level of vocalisations during the hot summer season. An added value of this sound recording and analysis technique is that it is non-invasive, inexpensive, and can be used for the development of an intelligent monitoring algorithm for heat-distress sounds. Intensive sustainable livestock farming requires the advanced planning of production management systems that may adapt dynamically to the animals' conditions. On-line stress levels, as well as disease condition, must be recorded to study the timing for appropriate action. Shade, irrigation, air movement and active cooling are among the corrections that may be helpful in cases of heat stress and might be automated by calls from animals in need. Such use of sound based technology can improve our understanding of animals to reduce negative impacts on modern breeding systems.

Conflict of interest statement

There is no conflict of interest.

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