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Effects of a veterinary functional music-based enrichment program on the psychophysiological responses of farm pigs

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Intensification of swine production can predispose pigs to chronic stress, with adverse effects on the neuroendocrine and immune systems that can lead to health problems, poor welfare, and reduced production performance. Consequently, there is an interest in developing tools to prevent or eliminate chronic stress. Music is widely used as a therapeutic strategy for stress management in humans and may have similar benefits in non-human animals. This study evaluated the effects of a music-based auditory enrichment program in pigs from a multidimensional perspective by assessing psychophysiological responses. Two experimental groups of 20 pigs each were selected for the study: one enriched, exposed to a program of functional veterinary music designed for pigs, and a control group without auditory stimulation. Qualitative behavior assessment (QBA) and skin lesions indicative of agonistic behavior were used to evaluate the psychological determinants underlying the observed behaviors. Physiological assessment included hemograms, with the determination of the neutrophil:lymphocyte ratio and daily measurements of cortisol and salivary alpha-amylase levels. The results demonstrated a positive effect of a music-based auditory program on psychophysiological responses. Therefore, this strategy developed for environmental enrichment may be beneficial in reducing stress and contributing to the welfare and health of pigs under production conditions.

Keywords Chronic stress, Biomusicology, Environmental enrichment, Swine, Veterinary functional music

Chronic stress is a multifaceted phenomenon that includes cognitive and emotional components that are closely linked to physiological systems. In animals, it has been shown to have a significant impact on physiological, neuroendocrine, and immunological responses, with consequent negative effects on health and welfare[1–4]. In intensive production systems, animals are exposed to multiple stressors of physiological, environmental, and social natures from birth to slaughter, and their effects on behavior, hypothalamic-pituitary-adrenal (HPA) axis reactivity, sympathoadrenal system (SAS), immune response, and mood have been widely described[5–8]. As a result, considerable research effort has been devoted to the development of tools to prevent or manage chronic stress in husbandry.

In humans, the integrated assessment of stress is usually based on psychophysiological measures, understanding the emotional state as the initiation of the individual response, since emotions represent the individual's adaptation to events that have a potential impact on their physical and psychological integrity[9], and physiological measures as the consequence of neuroendocrine activation to cope with the stressor stimulus[10]. In non-human animals, this approach has been approximated by considering numerous physiological variables and behavioral responses. However, emotional assessment can also be included, as we have previously demonstrated a variety of emotional responses in pigs in response to music, a stimulus that can evoke multiple affective experiences in humans and other animals[11].

Music can be defined as a complex and organized stimulus with different spectral and temporal structural elements. The interaction of these components determines the emotional content of music[12–16]. In recent years, music has begun to be considered from a biological perspective[17–20], giving rise to a field of research that explores

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it as a phenomenon and studies its mechanisms and effects, including comparative and interspecific approaches. In humans, music stimulation has been shown to induce a wide range of physiological effects[21–23], with beneficial influences on physiological[24] as well as cognitive and emotional processes[23]. Although the use of music in non-human animals is still an emerging field of research, its effects have been investigated in several species, with some heterogeneous results. These include effects on heart and respiratory rate regulation, blood pressure[25], hormone secretion[26], as well as emotions[11] and behaviour[27–29].

Several studies have shown that some animals can perceive the basic components of music, such as tempo, rhythm, pitch, and harmony[11,16,28,30–32]. However, important interspecies differences in the perception of various acoustic parameters, such as hearing range and sensitivity to sound frequencies, have been reported[33]. Therefore, the importance of evaluating the type of music used in enrichment programs and the need for species-specific settings has been raised as a determinant to optimize the potential effects of music on non-human animals[11,34–36]. To date, most research has been limited to exploring the use of music created for humans and has not evaluated the effectiveness and efficiency of original programs tailored to the needs of animals.

To contribute to this knowledge gap, we previously investigated the effects of musical stimulation and the influences of its structure on the modulation of emotional responses. Our results showed that nursery pigs exposed to music showed a wide variety of emotional responses with different affective valences, depending on the harmonic structure of the stimulus[11]. Next, we performed temporal-spectral analyses of the acoustic characteristics of the music. We demonstrated that musical structure affects emotional responses in pigs and that the valence of modulated emotions depends on integrated and simultaneous interactions of several spectral and temporal structural components of music, which can be modified using music production techniques[16]. Our previous data and analyses were a starting point to design and refine auditory stimulus. From this, we propose a new concept that we call "Veterinary functional music". We define it as a music specially composed or used for a particular purpose, that has undergone validation to ensure a particular effect on non-human species and that incorporates acoustic and musical adjustments appropriate to any species considering their sensory and perceptual characteristics. For our particular interest, the function of the designed music is to decrease stress and consequently positively impact the health and welfare of animals. This music mitigates difficulties previously exposed by other researchers regarding the type of music used in studies of non-human animals and, is presented as a cost-effective and non-invasive intervention strategy for stress management[37] and welfare improvement.

Within this framework, the present study evaluated the effects of a veterinary functional music program for pigs on the regulation of chronic stress from a psychophysiological perspective. For this purpose, two groups of pigs were evaluated, the first one, enriched and exposed to the enrichment program and the second unexposed group, defined as the control. Differences in chronic stress levels between the two groups were compared based on a multifaceted assessment that included the evaluation of emotional responses by QBA and agonistic behavioral indicators such as skin lesions, as well as the evaluation of biomarkers of hormonal axis activation (HPA-SAS) and, indicators of inflammation and immune system activation through complete blood count. Our results should provide a basis for the design and refinement of music-based environmental enrichment programs for non-human animals.

Materials and methods

Ethical considerations

All experimental procedures performed on animals were conducted in accordance with relevant guidelines and regulations and were approved by the Ethics Committee on Animal Experimentation of the University of Antioquia (CEEA) Act N°16, April 10, 2018. The study complies with the ARRIVE guidelines for reporting animal research (https://arriveguidelines.org).

Study location

The study was conducted at the experimental swine farm of the University of Antioquia; located in the municipality of San Pedro de los Milagros (6° 26′59.606 n 75° 32′37.088 W BH-Mb), Antioquia-Colombia). At an altitude of 2350 m, temperatures between 7 and 22 °C and a relative humidity of 70%

Litters

Two groups of 20 pigs each were selected for the study. One group was enriched and received music-based auditory stimulation and the second group, the control, was not stimulated. The animals were commercial crossbred (C29 × PIC 410) and weaned at 27 days of age (range 25 to 28 days). A balanced sex and weight distribution between the groups was verified.

Facilities

The analyses were carried out between 4 and 14 weeks of the production cycle. At the beginning (nursery phase), the animals were housed in of 2.5 × 3 m pens with slatted floors and metal bar walls. In the 12th week (growing phase), the animals were transferred to the rearing and fattening area, with pens of 3.20 × 4.40 m, with a cement floor. All facilities were equipped with nipple drinkers and feeders. Water and feed were available ad libitum. Lighting was maintained between 6 a.m. and 6 p.m., with an average temperature of 20 °C.

Musical pieces

Thirty-nine original instrumental pieces were composed for this study using Ableton live 10 Suite and Sibelius ultimate® (AVID 2022). No equalizers, compressors or spatial effects were used. A series of specific guidelines were established for the composition, each piece considering the auditory perceptual characteristics of pigs (pig auditory frame - 40.5 Hz to 40 kHz), ranges for acoustic parameters[16] (see Table 1), and musical consonant

Acoustic parameter	Definition*	Range
Centroid	Center of mass of the sound spectrum; related to sound brightness and timbre	999.174–1117.021
Amplitude	Distance between the peak of the wave and its base, in decibels (dB); as the wave amplitude increases, dB increase, reflecting an intensification of volume	0.023–0.025
Dissonance	Sensory dissonance measures the perceptual roughness of the sound and is based on the roughness of its spectral peaks. Given the spectral peaks, the algorithm estimates the total dissonance by summing the normalized dissonance values for each pair of peaks. These values are calculated using dissonance curves, which define the dissonance between two spectral peaks as a function of their frequency and amplitude ratios	0.219–0.229
HFC	High frequency content (HFC) of a sound spectrum	37382–48997
ZCR	Zero crossings rate (ZCR): Is a measure of the number of times the value of the signal (audio wave) crosses the zero axis. This value tends to be small for periodic sounds and large for noisy sounds	0.024–0.025
Spectral deviation	A measure of the standard frequency deviation around the spectral centroid, and indicates for how much the frequencies in a spectrum can deviate from the center of gravity	1887.524–1891.628
BPM	Pulse in beats per minute (BPM); the unit of measure for the tempo-rhythm or speed	110–130
Vertical density	Number of instruments simultaneously presented. The number of instruments was always constant over the entire duration of each piece	2–4

Table 1. Summary of the range of acoustic parameters considered in the composition of musical pieces. *Definitions were based on previous literature[38,39].

attributes[11]. These were defined from previous studies conducted by our research group and associated with positive emotional responses in the swine species. The supplementary material includes further elements in this regard.

Veterinary functional music-based enrichment program

To design the enrichment program, targets, objectives, and general acoustic and musical characteristics were established to be included in the stimuli to produce the desired effects in the pigs through a variety of Music Creation and Production Resources (MCPRs). The goals of the program were divided into the following categories: Psychological and cognitive, social, sensory, homeostasis, and health; details of the main features are presented in (Table 2).

The Table 2 summarizes the structural characteristics of the music-based enrichment program designed for this study. Each of the major MCPRs is referenced based on relevant research. Most MCPRs are quantifiable: e.g., melodic, and harmonic intervals, metric impulses, rhythms, articulatory groups, and acoustic parameters. The pig vocalizations emitted in a positive context were kindly provided by Dr. Celine Tallet[53,54]. These were used as a reference for the synthesis of effects and artificial vocalizations. Supplementary material includes audios and descriptions that illustrate some of the characteristics of the music created.

The general considerations for the structure of the enrichment program are as follows:

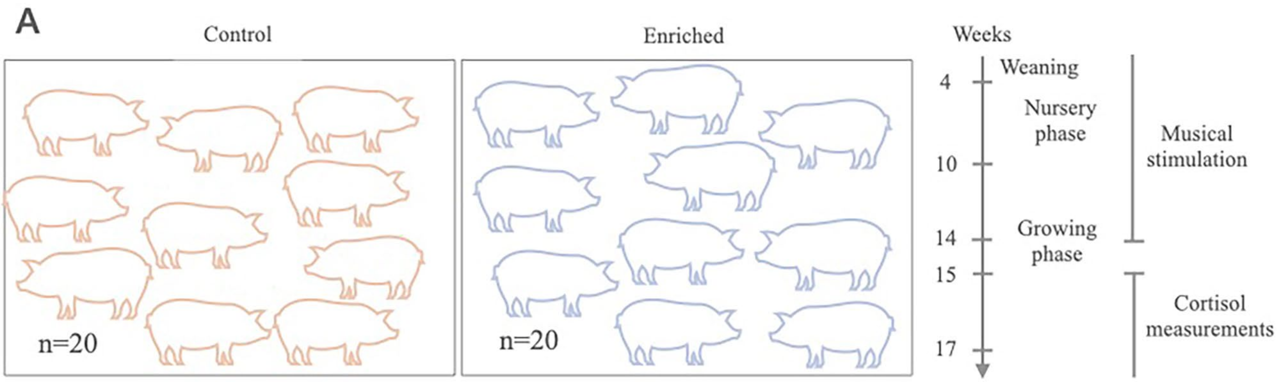
1. Definition of swine hearing requirements: these were defined on the basis of the species audiogram and comparative differences with humans, including a range of audible frequencies between 250 Hz and 16 kHz, avoiding thresholds that cause discomfort in pigs[55,56].
2. Definition of the acoustic characteristics of the musical pieces: Previous studies were carried out to evaluate the emotional response of pigs to musical compositions with harmony and with variable temporal spectrum parameters capable of inducing positive or negative emotional states[11,16]. In the production of the stimuli, the characteristics and parameters that induce positive emotional states were prioritized, and fragments designed to elicit attention were incorporated as a strategy to induce novelty.
3. Definition of the duration of the implementation of the program: The study was conducted in growing pigs. The animals were exposed to music for 10 consecutive weeks (between 4 and 14 weeks of pig life).
4. Definition of the periodicity and application times of the musical stimuli: The stimuli were changed weekly and each had a non-consecutive repetition. The maximum exposure duration was 45 min and was performed twice a day, between 9:00 and 10:00 am and between 3:00 and 4:00 pm. Each week, music exposure occurred on six consecutive days. The last day of the week was a rest day before exposure to new stimuli.
5. Dosage formulation to avoid habituation to stimuli: The program was designed for growing pigs and included musical stimuli that were varied and novel. Five musical arrangements were designed with progressive variations in rhythm, harmony, and instrumentation to avoid habituation. Other elements such as duration, dynamics, acoustic parameters, variability, and complexity were also considered in the design and dosage of the stimulus.
6. Plan the distribution of the musical works: For the application of the music in the enriched group, the previously defined music arrangements were assigned weekly. Each arrangement consisted of between 7 and 8 pieces that had a duration of 5 min, separated from each other by rest periods (silence) of 3 min. The distribution of the arrangements was intentional, taking into account the dosage and other modifications described previously.

Treatment target	Objectives	General characteristics for musical pieces	Music creation and production resources (MCPRs)
Psychological and Cognitive	Creation of a complex cognitive environment	Unknown acoustic information, including sounds that are not characteristic of the environment	The creation of sounds is achieved through the use of software synthesizers[40,41]. In sound design, various elements, whether periodic functions or mathematical functions, are combined in order to create the required sounds. This is achieved using various additive, subtractive, granular, and frequency-modulated synthesis procedures. For example (Supplementary Material- Audio 1)
	Challenging stimuli with increasing complexity[42]	Inclusion of effects, sounds, textures, and dissonance without exceeding the predefined limits	
	Generate gradual assimilation and adaptation to stimuli	Use musical patterns or shapes with weekly addition of layers of acoustic and musical information	Functional harmony is used as a structural template for the composition[43], then ancient Greek modes[44], exotic scales, and atonality are incorporated to increase sensory and musical dissonance[45]. The "vertical construction" of the chords is also changed using extended chords[44]. Each modification in the construction generates changes in the acoustic parameters and the dissonance value[46]. Occasionally the counterpoint level of a work is changed by using two or more melodies simultaneously[47]. When several melodies are heard superimposed, an effect of textural complexity is generated. In a similar way, syncopations, polymetries and polyrhythms are used to raise the level of information and rhythmic complexity[48]. For example (Supplementary Material- Audio 2)
Social	Encouragement of play and exploration behaviors	Unexpected and novel sound events	New sounds are created using synthesizers and virtual instruments[49]. For example (Supplementary Material- Audios 1, 2)
	Reduce aggressive behavior	Avoid ranges of acoustic parameters that create negative emotional states	The values of the acoustic parameters to be avoided are given in previous publications[16]. Audio 3 is an example of music with acoustic patterns that elicit negative responses in pigs
Sensory	Provide rewarding and pleasurable stimulation	Use ranges of acoustic parameters that create a positive emotional state	The values of the acoustic parameters to be used were listed in Table 1. (Supplementary material- Audio 4)
Homeostasis	Reduce stress	Intersperse relaxing musical information with another that generates motor activation to promote the regulation of the nervous, cardiovascular, endocrine, and immune systems	The amount of repeated and new parts is dosed to generate predictability or uncertainty[50,51]. Music is designed by integrating and dosing multiple harmonic, melodic and rhythmic elements in a mix, from simpler to more complex forms[42,52]. (Supplementary material- Audio Audio 5)
Health	Reduction in skin lesions associated with aggression	Use affiliative musical information by performing the digital synthesis of positive context vocalizations	Synthesizer-generated emulations of pig vocalizations were used[40,41]. These contain the acoustic parameters of pig vocalizations emitted in an affiliative context. Supplementary material- Audio 6)

Table 2. Treatment target, objectives, and general musical characteristics that are included in the auditory enrichment program.

Experimental design
Two experimental groups were included in the study, an enriched group exposed to the previously described Veterinary functional music-based enrichment program for a period of 10 weeks (between 4 and 14 wks of age), and a control group with no exposure. Multiple psychophysiological evaluations were conducted during this period, including quantitative behavioral assessment (QBA), examination of skin lesions and measurement of hematological parameters. Subsequent biomarkers testing was conducted at 15–17 weeks. The details of these evaluations are presented below. For playback, QSC speakers (AD-S10T-WH) were used, fixed on the wall, and controlled by a YAMAKI 240 W amplifier-mixer (LIN-Z240TR MKII). The decibel level was monitored to avoid exceeding 80 db. A summary of the experimental design is presented in (Fig. 1).

Psychological evaluation
Evaluation of emotional responses
Emotional responses were assessed in the entire litter. Both experimental groups of animals, enriched and the control, were videotaped in their pen during the 10 weeks of the experiment. Each video was recorded between 9:00 to 10:00 am, and 3:00 to 4:00 pm. Subsequently, from the recordings of the enriched group, the segments with music were separated from the breaks. Similar time segments were selected from the recordings of the control group. These video segments were used to perform the quality behavior assessment (QBA) as described in previous studies[11]. From each recording and per group, scores were obtained for 15 QBA terms (active, relaxed, agitated, calm, content, indifferent, friendly, playful, positively occupied, lively, inquisitive, irritable, uneasy, sociable, happy). The items fearful, bored, apathetic, frustrated, and distressed were not scored during the observations; hence they were excluded from further analysis. For the evaluation, the videos were processed,



B

Musical stimulation																
Arrangements = 5	Master 1	P # 1	R	P # 2	R	P # 3	R	P # 4	R	P # 5	R	P # 6	R	P # 7	R	P # 8
	Master 2	P # 9	R	P # 10	R	P # 11	R	P # 12	R	P # 13	R	P # 14	R	P # 15		
	Master 3	P # 16	R	P # 17	R	P # 18	R	P # 19	R	P # 20	R	P # 21	R	P # 22	R	P # 23
	Master 4	P # 25	R	P # 26	R	P # 27	R	P # 28	R	P # 29	R	P # 30	R	P # 31		
	Master 5	P # 32	R	P # 33	R	P # 34	R	P # 35	R	P # 36	R	P # 37	R	P # 38	R	P # 39

P #: Music piece code
R: Rest

Figure 1. Schematic representations of the experimental design and program of veterinary functional music for environmental enrichment. (**A**) Two groups: control and enriched were included in the study. The enriched group was exposed to musical stimulation for ten weeks. Some measures were taken until week 17. (**B**) Music arrangement of the functional veterinary music program. A total of 39 musical pieces were composed and distributed in five arrangements with seven or eight pieces each. Between the pieces, interspersed periods of rest (silence) were included.

coded, and the observations were performed by a single trained experimenter. The analysis was performed blind to the experimental groups, randomized and without sound. Each session had an interval of 4 days. Before the analyses, an intraobserver reliability assessment was performed with Pearson's correlation coefficient, using a subsample of 20 video clips obtaining values between high ($r \geq 0.90$) and moderate ($0.50 \geq r < 0.80$). The data obtained were collected in a matrix for statistical evaluation.

Evaluation of the skin lesions
Direct observation by two trained and blinded observers was used to evaluate the skin. The observed lesions were recorded on lateral, dorsal, caudal, and frontal profile images. The anatomical areas considered were the ears, forehead (head to back of shoulder), midline (back of shoulder to hindquarters), hindquarters, legs (from the accessory digit upward), tail, snout, and genitalia. Two types of temporal comparisons were made between experimental groups. The first evaluation was performed weekly (during all 10 weeks) for each body part of each pig, and a score of 0 or 1 was assigned based on the absence or presence of a lesion. From this score, the proportion of pigs with skin lesions was calculated for each body part and for each group. In addition, another score (including the sum of the lesions of the 8 body parts of each animal) was used to compare the specific effect of group and week on the number of skin lesions. The second evaluation included a comparison only of the proportion of skin lesions between the first and last week of the enrichment program (4 and 14 weeks of age of the animals specifically). Cohen's kappa coefficient was used to evaluate the agreement between the observations, obtaining high values (≥ 0.90).

Physiological evaluation

Stress markers
Two biomarkers were evaluated, cortisol as a biochemical biomarker of HPA axis activation[57] and salivary alpha-amylase (sAA) as a biomarker of autonomic nervous system (ANS) activity, i.e., the sympathoadrenal system (SAS)[58,59]. Cortisol displays fluctuations associated with the circadian rhythm, and its diurnal variation was considered in this study. Cortisol in saliva is in a free biologically active form and therefore, this fluid is a good indicator of cortisol levels in blood plasma[60]. In contrast, the sAA hormone does not present diurnal variations associated with the circadian rhythm, its isolated use is mainly related to acute stress and SAS activation, but its comparison with cortisol provides information on the balance in neuroendocrine functions.

The assessment of both biomarkers was performed on saliva samples collected at 15, 16, and 17 weeks of age in both experimental groups (once the enrichment with music was completed). Measurements were performed from week 15 onwards because, in general, for mammals at early ages, the HPA axis is less sensitive to stress-induced modulations[61–63].

Sampling was performed on the last day of the selected weeks, with one sample taken every hour between 8:00 and 17:00 (for 10 samples per day, each with duplicates). Saliva was collected without restraining the pigs, by letting them chew on a large cotton swab, until it was completely wet (approximately 30 s per sample). The samples were immediately stored in a nitrogen-charged thermos flask at a temperature of −196 °C until analysis. The concentration of cortisol was determined using an ELISA method (Cortisol Enzyme Immunoassay Kit—Salimetrics, USA). sAA was measured using a kinetic enzyme assay kit (Salimetrics, USA).

For the comparative analysis of the data obtained from both biomarkers, we initially calculated the following indices: area under the curve (AUC), for cortisol (AUCc) and sAA (AUCa), these indices have been previously evaluated in numerous studies[64,65], as sensitive indicators with respect to basal levels and reactivity for each hormone. The ratio of sAA over cortisol (AOC), an index obtained by dividing AUCa by AUCc was also calculated. The aforementioned indices have been widely implemented in research and medical practice as reliable indicators to assess the balance of neuroendocrine function, involving the HPA-SAS axes[66–68]. The literature provides abundant evidence of dysfunctions in the relationship between these two axes in individuals with chronic stress; however, and despite the relevance of the indices, their use in animals has not yet been explored, and there are no previous studies in swine species.

Hemograms

In both experimental groups, blood samples were collected at 28 days of age (before starting the stimulation with music) and again at 170 days of age just before transport to the slaughter. Samples were obtained between 08:00 and 10:00 am using a puncture in the jugular vein, collected in EDTA tubes, refrigerated, and sent immediately to the laboratory for basic hematological analysis. From the data obtained, the proportions of the different blood cell populations and the neutrophil:lymphocyte ratio (N:L–calculated from the mean absolute values of these populations), were evaluated. This index is considered a proxy for inflammation and immune system activation and as a potential indirect indicator of stress response[69,70].

Statistical analysis

The data obtained were longitudinal, corresponding to measurements repeated over time, the experimental unit was the pen ($n = 2$), with two experimental groups, each with 20 individuals. The data were evaluated by descriptive statistical metrics and normality analysis using graphs and hypothesis testing (Shapiro–Wilk test and Bartlett or Levene test), which allowed us to define subsequent inferential analyses using parametric and nonparametric methods. The data from the QBA were analyzed using Principal Component Analysis (PCA, with correlation matrix and varimax rotation), allowing the conformation of two principal components (PC) or index. To analyze the variation of each index obtained along the evaluated groups, a general linear mixed model (GLMM) with repeated measures was fitted, including condition group ("control" and "enriched") as fixed effects. For hematological analysis, the Kruskal–Wallis test was used as a nonparametric alternative to analysis of variance, and paired comparisons were performed using the Mann–Whitney U test with correction for multiple comparisons. Z-test for proportions with correction and generalized linear model (GLM) with Poisson distribution and ANOVA followed by Tukey's post hoc comparison tests were used for the analysis of skin lesions. Descriptive analysis of diurnal variation for cortisol was used to evaluate biomarkers, and concentrations were compared between experimental groups using a three-way ANOVA with treatment, hour, and week as fixed effects. In addition, the area under the curve (AUC) index was calculated for cortisol (AUCc) and salivary alpha-amylase (AUCa) levels. Both indices were then related to the extract of AOC (amylase over cortisol) and compared by Student's t-test. $p \leq 0.05$ was chosen as the limit of statistical significance. All analyses were performed with the R statistical software (version 4.0.2; https://www.R-project.org)[71] implementing, among others, the FactoMineR, tidyverse, tidymodels, DALEX, splines, mgcv, and vip libraries.

Results

Psychological evaluation

Evaluation of emotional responses

Descriptive analysis of the QBA terms shows significant differences between groups for the means of items (see Table 3). Means scores for emotional states such as content, friendly, playful, positively occupied, sociable, and happy were higher in the enriched group. Indifferent and uneasy had a high mean score in the group without music, and a tendency as well was observed to calm, lively, and irritable items. The standard deviations and coefficients of variation recorded for several of the terms were higher in the enriched group, indicating a greater range of emotional responses during enrichment.

The PCA suggests two factors or principal components that explain 59.36% of the total variance of the dataset (Table 4). Mean loadings were used as a criterion to select the QBA terms with major contributions to each PC (0.49 and 0.38 mean loadings, respectively). The first component (PC1) included content, friendly, playful, positively occupied, lively, sociable, and happy terms with positive loadings and an upper mean load. This was referred to as the emotional valence index, with items with higher positive loading representing more positive emotional valence. The second component (PC2) included the terms active, agitated, and irritable with positive loadings and relaxed, agitated and calm, with negative loadings. This resulted in an arousal or psychomotor activity axis (from low to very active).

Figure 2A,B summarizes the results of the PCA and shows the relationship between the emotional terms contributing to each component and the spatial location of the responses of the litters in both experimental groups. In the enriched group, responses were expressed and widely distributed in the four PCA quadrants and

Emotional state	Enriched				Control				p-value
	Mean ± SD	Min	Max	CV (%)	Mean ± SD	Min	Max	CV (%)	
Active	3.63 ± 2.35	0.0	11.0	64.7	2.81 ± 1.79	0.4	7	63.7	0.072
Relaxed	3.68 ± 1.65	0.3	8.4	44.8	4 ± 1.98	0	7.2	49.5	0.388
Agitated	0.78 ± 1.87	0.0	9.5	239.7	0	0	0	0	0.130
Calm	3.39 ± 1.57	0.0	7.0	46.3	4.1 ± 1.21	1.2	6	29.51	**0.021**
Content	1.84 ± 1.13	0.3	6	61.41	0.94 ± 0.62	0	3.4	65.96	**0.000**
Indifferent	0.52 ± 0.6	0.0	1.8	115.4	1.81 ± 1.16	0	5	64.09	**0.000**
Friendly	1.82 ± 0.95	1	5	52.2	0.98 ± 1.01	0	3.4	103.06	**0.000**
Playful	1.79 ± 1.12	0.8	6.6	62.57	0.71 ± 0.65	0	2	91.55	**0.000**
Positively occupied	3.02 ± 1.69	0.5	7.0	55.96	1.84 ± 1.11	0.6	4.6	60.33	**0.000**
Lively	3.17 ± 2.35	0.3	8.2	50.79	2.33 ± 1.71	0	5	73.39	**0.017**
Inquisitive	0.3 ± 0.97	0.0	6.3	323.3	0.22 ± 0.6	0	2.5	272.73	0.671
Irritable	0.73 ± 1.44	0.0	6.0	197.3	0.12 ± 0.37	0	1.4	308.33	**0.012**
Uneasy	0.02 ± 0.13	0.0	1.0	650.0	0.15 ± 0.36	0	2	240	**0.006**
Sociable	1.82 ± 1.16	0.2	6.2	63.74	1.15 ± 0.76	0	3	66.09	**0.002**
Happy	1.71 ± 0.98	0	5.3	57.31	0.64 ± 0.49	0	2	76.56	**0.000**

Table 3. Descriptive analysis for adjectives based on emotional states used for QBA [mean, standard deviations (SD), maximum (max), minimum (min), and percent coefficient of variation (CV -%)]. P-value calculated using Student's *t*-test, n=2, two groups. 20 animals per group. Values with significant statistical differences are highlighted in bold.

Emotional state	PC1-index for emotional valence	PC2-index for psychomotor activity
Active	0.53	**0.70**
Relaxed	0.02	**−0.82**
Agitated	0.37	**0.62**
Calm	−0.09	**−0.83**
Content	**0.82**	0.24
Indifferent	−0.43	−0.29
Friendly	**0.88**	−0.03
Playful	**0.82**	0.33
Positively occupied	**0.61**	0.09
Lively	**0.58**	0.54
Inquisitive	−0.09	−0.29
Irritable	0.07	**0.66**
Uneasy	−0.23	0.11
Sociable	**0.84**	−0.05
Happy	**0.89**	0.08
Mean loadings	0.49	0.38
SS loadings	5.11	3.35
Cumulative percentage of variance	34%	59%

Table 4. Principal component analysis of the QBA. Mean loadings were used as a criterion to select the QBA terms with major contributions to each PC. Terms with loadings greater than the mean loading of the respective components are shown in bold. Mean loadings: Average for the loads (in absolute value) of each component. SS loadings: Sum of squared loadings.

were related to both emotional valence and the psychomotor activity index. In contrast, the responses of the control group were densely clustered, mainly occupying quadrants III and IV (see Fig. 2B). Furthermore, the location of these observations for the control group indicated low values for the emotional valence index, and the observations were predominantly calm, relaxed, and indifferent (see Fig. 2A). The inferential analysis indicated significant differences between the evaluated groups for the emotional valence index ($p = 0.000$), with higher values for the enriched group. There were no differences ($p = 0.300$) in the psychomotor activity index. (Table 5).

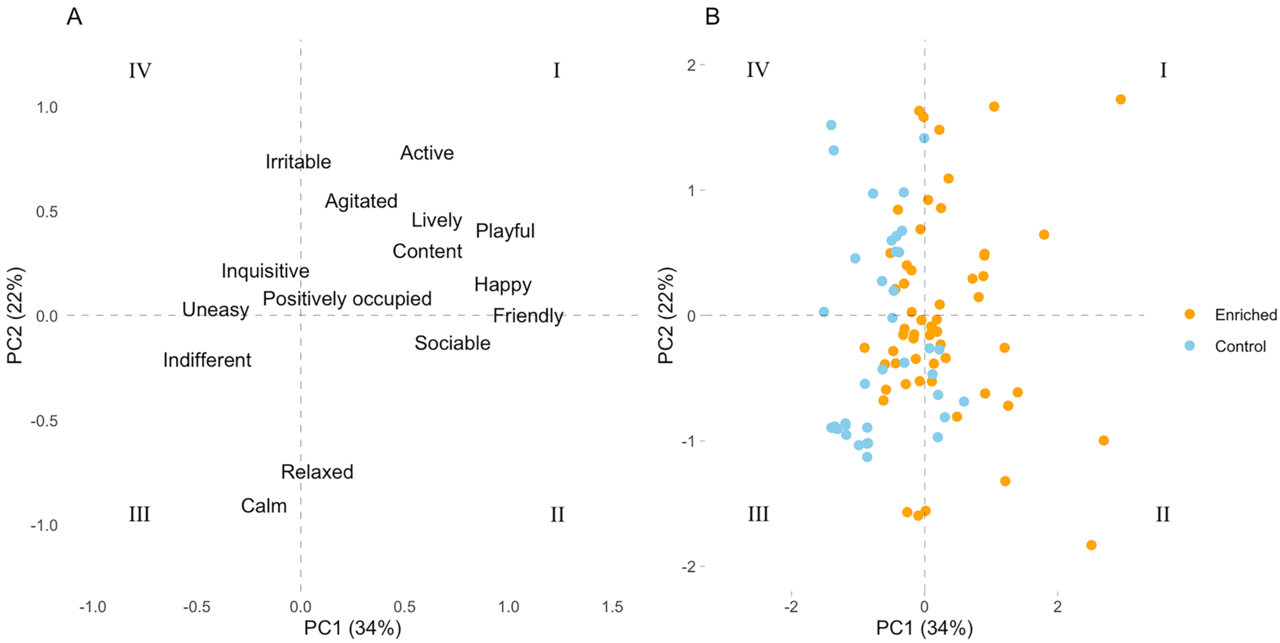


Figure 2. (**A**) Plots of loadings for the qualitative behavior assessment (QBA). Emotional responses on dimensions PC1 (emotional valence index) and PC2 (psychomotor activity index). (**B**) Individual loadings associated with the evaluated groups. The points indicate the coordinates for the responses of each observation for the entire litter, and the colors refer to the groups: Enriched (orange), Control (blue).

Index	Enriched		Control		Difference between periods p-value
	Mean ± SEM	ICC (lower limit-upper limit)	Mean ± SEM	ICC (lower limit-upper limit)	
Emotional valence	$0.38^{a} \pm 0.20$	[−0.09, 0.85]	$-0.63^{b} \pm 0.13$	[−0.93, −0.34]	**0.000**
Psychomotor activity	$0.12^{b} \pm 0.16$	[−0.24, 0.48]	$-0.18^{a} \pm 0.19$	[−0.62, 0.27]	0.300

Table 5. Adjusted means (± SEM) and confidence intervals (ICC) of the PCA indexes of emotional valence and psychomotor activity for the enriched and control groups. The value with significant statistical differences is highlighted in bold. [ab]Within a row means without a common superscript differed ($P < 0.001$).

Evaluation of the skin lesions

The first analysis of skin lesions examined a weekly comparison (see Fig. 3), including all 10 weeks of the enrichment program (between 4 and 14 wks of age). A lower proportion of lesion presentation was observed in the enriched group, which was more remarkable from week 2 onwards (fifth week of pig age). A GLM was used to compare the groups and significant differences were observed in the anterior and middle anatomical areas ($p = 0.021$ and $p = 0.002$, respectively). The score (including summatory of lesions of the 8 body areas of each animal) was used to compare the specific effect of group and week on the number of skin lesions. An effect of group was observed ($p = 0.00182$), with a minor score for enriched. The comparison between weeks shows differences with the second week ($p = 0.00271$).

The second analysis of skin lesions (Table 6) includes the specific differences between the first and last week of the enrichment program (4 and 14 wk of age specific). At the beginning of the program, there were no significant statistical differences between the groups in any body area ($p > 0.05$). In contrast, in the last week of enrichment, a comparative reduction in skin lesions was observed in the enriched group on the ears, front, and tail areas that was significant ($p = 0.001$, 0.020, 0.020, respectively).

Physiological evaluation

Evaluation of stress markers

Diurnal salivary cortisol concentrations were assessed between 8 am and 5 pm and the results are shown in (Fig. 4). In the enriched group the diurnal fluctuations of the metabolite had a similar and consistent dynamic in the weeks evaluated between 8 and 10 am. Cortisol levels had a downward trend, with values between 0.5 and 0.2 ug/dL. At 11 am, a first peak is observed, reaching values up to 0.64 ug/dL. The levels decreased again between 12 and 1 pm, between 0.2 -0.3 ug/dL. A new increase is observed at 2 pm, reaching 0.5 ug/dL, and finally at 4 pm the highest peak of the diurnal dynamics is observed, a maximum value of 0.85 ug/dL. This reflects fluctuations in the diurnal secretion of the metabolite. In contrast, the group control, during the three weeks, presented a much

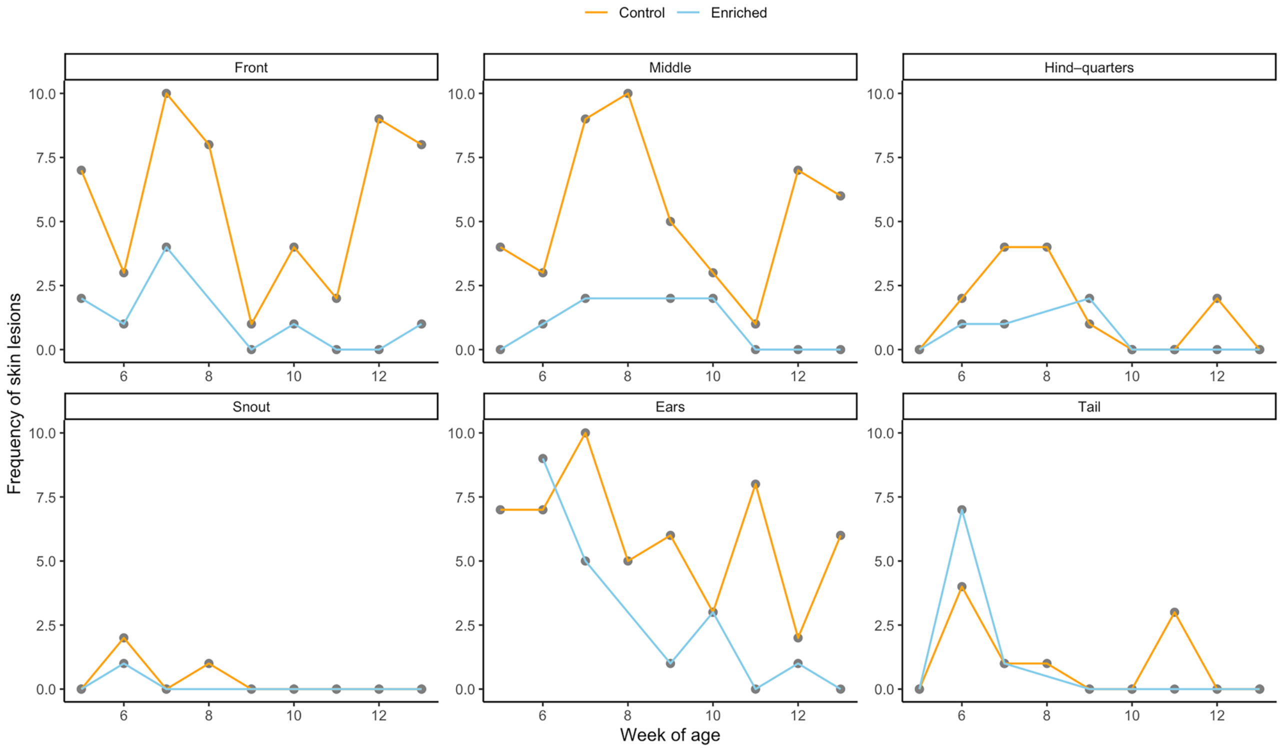


Figure 3. Comparison of the frequency of skin lesions presentation between the enriched and control groups in the weekly evaluation. From week 2 of the experiment (fifth week of pigs age), a tendency to decrease in the number of skin lesions was observed in the enriched group. Differences between groups were significant in front and middle ($p=0.021$ and $p=0.002$, respectively) anatomical areas, $n=2$, two groups. 20 animals per group.

Body area	First week			Final week		
	Proportion of pigs injured			Proportion of pigs injured		
	Control	Enriched	p-value	Control	Enriched	p-value
Ears	0.6	0.86	0.205	0.55	0.05	**0.001**
Front (head to back of shoulder)	0.45	0.48	0.735	0.35	0.05	**0.020**
Middle (back of shoulder to hind–quarters)	0.3	0.38	0.195	0.05	0.29	0.131
Hind–quarters	0.1	0.0	0.226	0.15	0.95	1.000
Legs (from the accessory digit upwards)	0.1	0.0	0.026	0.1	0	0.226
Tail	0.05	0.0	0.474	0.25	0	**0.020**
Snout	0.5	0.19	0.668	0.1	0	0.226
Genitals	0.5	0.0	1.000	0	0	1.000

Table 6. Proportions of pigs with skin lesions in the experimental groups at the first week (4 wk of age) and at the end of the enrichment program (14 wk of age). The p-value was calculated using the Z-test to proportions. Values with significant statistical differences are highlighted in bold.

flatter dynamic, without notable peaks and with oscillations between 0.2 and 0.4 ug/dL and sometimes reaching values of 0.5 ug/dL. A three-way ANOVA for cortisol statistical analysis was applied, using as main factors: (1) conditions (control vs enriched), (2) time of the day, (3) week. The conditions were the factor statistically significant ($p=0.01$), with a global media of 0.41 and 0.33 ug/dL to enriched and control conditions, respectively.

Figure 5 shows the comparative evaluation of the cortisol curves in both experimental groups in each week evaluated. The flattening of the curve in the pigs of the control group is highlighted, with very slight modulations in the cortisol level that do not reach the maximum levels observed in the enriched group. In contrast, the curve of the enriched group showed notable fluctuations in the diurnal secretion of the metabolite. The differences were maintained for the three weeks evaluated.

The values of diurnal secretion of sAA, were variable between groups and weeks, and were used to calculate the index called area under the curve (AUCa). A similar procedure was carried out with the data obtained for cortisol (AUCc). Subsequently, both indices were related to the extract of AOC (amylase over cortisol) and

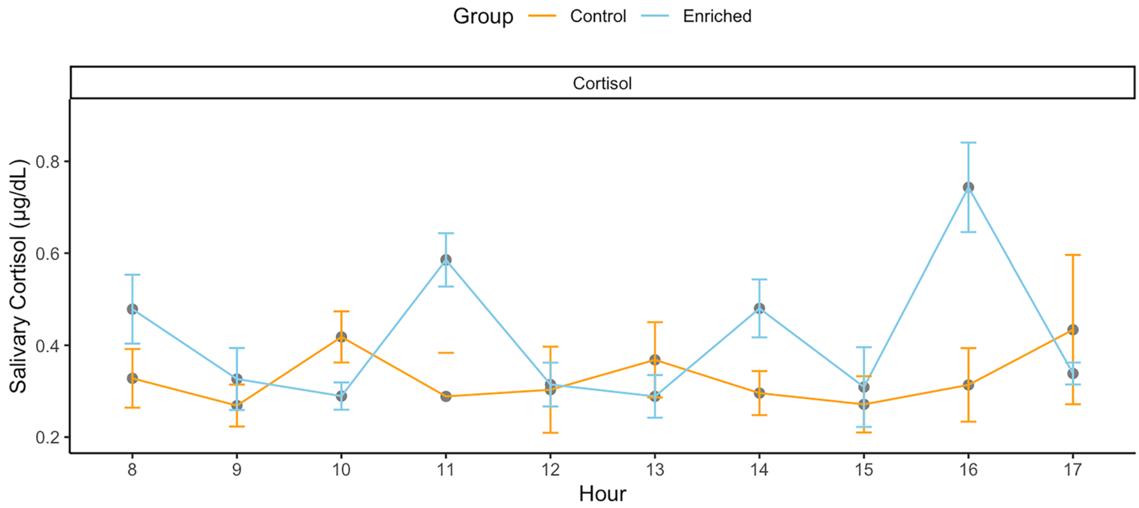


Figure 4. Mean cortisol curves. Enriched (blue), control (orange). Salivary cortisol concentrations (mean ± sem) measured during 10 diurnal hours, at 15, 16, and 17 weeks. n = 2, two groups. 20 animals per group.

compared using Student's t-test. The results obtained from the evaluation of these indices are presented in (Table 7). The AUCc was higher in the enriched versus control pigs (3.74 and 2.91 ug/dL, respectively; p = 0.01), and there was an effect of treatment (p = 0.01) on cortisol concentrations. In contrast, AUCa were not statistically significant between groups with amylase concentrations 28.67 and 28.75 ug/dL. The AOC index was lower in the enriched group with a significant difference (p = 0.01), suggesting a better balance between the two stress systems (HPA-SAM) for this group.

Evaluation of hemograms
Comparison of hematological values between the groups at the beginning of the experiment showed no significant differences. In the evaluation performed at the end of the productive cycle, a higher cell count was observed in all leukocyte cell lines in the enriched group. Figure 6 shows the most relevant populations, and the difference in the absolute value of lymphocytes, neutrophils, and eosinophils, using the Kruskal–Wallis test, which verified that these differences were significant (Table 8). In erythrocytes, hematocrit, platelets, and the indices related to these blood populations, there were no significant differences between the groups. In addition, the enriched group presented higher plasma protein levels with significant differences with respect to the control group (p < 0.01).

In addition, the evaluation of the neutrophil:lymphocyte ratio (N:L) was considered. From this evaluation, it was observed that the enriched group presented a lower value (0.29) than the control group (0.47).

Discussion
To the best of our knowledge, this is the first study to present an environmental enrichment program that incorporates acoustic and musical adjustments to porcine species considering their perceptual characteristics. The results obtained indicate that pigs housed in music-enriched conditions exhibit psychophysiological responses that differ from those of non-enriched animals. In this context, enrichment affected the presentation of positive emotional responses and the reduction of skin lesions associated with agonistic behaviors. In addition, from the physiological perspective, differences in diurnal cortisol dynamics and in the AOC index were evidenced, suggesting a better balance in neuroendocrine functions for the enriched group. The findings obtained from the integrated evaluation of all these variables highlight the beneficial impact of veterinary functional music in the reduction of chronic stress in farm pigs as well as plaid in favor of its use for environmental enrichment in husbandry.

The concept of functional music is not new in the literature, with an application in the human context and is defined as that which is composed or used for a particular purpose, which in humans is applied to generate atmospheres and ambiance[72]. However, the extension of the term to music used in animals can be interesting from the perspective that music can be useful and usable in different species to promote particular states and behaviors. Previous studies have demonstrated the effects of music on emotional[11], behavioral[28,35,73] and physiological parameters[26,74] in various animal species, providing a basis for its use with particular objectives or functions, such as stress regulation.

Additionally, and considering that the experience of stress is a multifaceted phenomenon comprising cognitive and emotional components closely related to physiological systems[75], in this study, the potential usefulness of veterinary functional music in stress regulation was demonstrated from a psychophysiological perspective. The multidimensional approach to the study of the relationship between music and stress is well supported, and several studies that support this approach are presented together in a model of interactions (Fig. 7). Here, music is proposed as a physical stimulus, containing information that is processed and translated in the central nervous system through neurocognitive processing. It then determines emotional responses that activate different areas

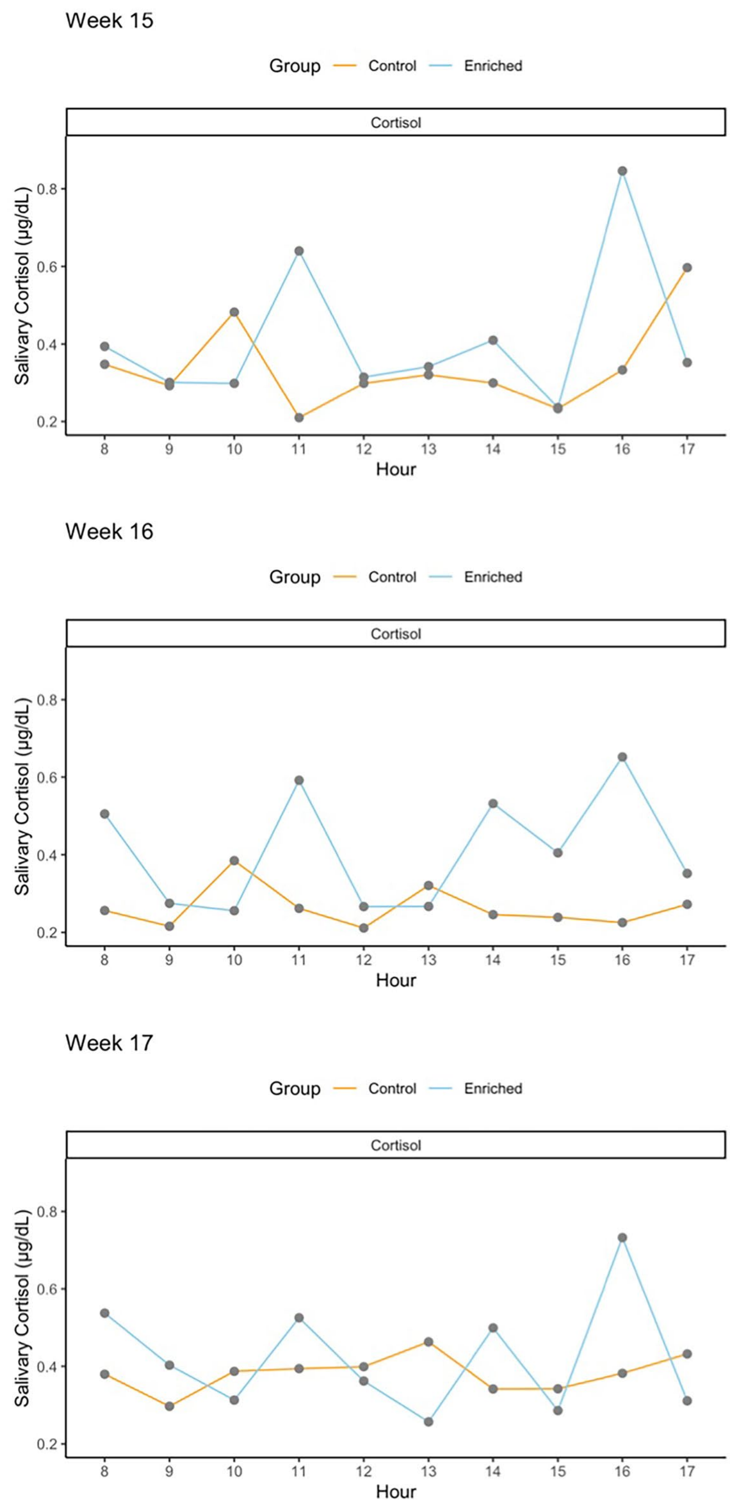


Figure 5. Comparisons of basal salivary cortisol concentrations (mean ± sem) measured during 10 h of daytime at 15 (upper panel), 16 (middle panel), and 17 weeks of age (lower panel) for pigs with auditory enrichment (enriched) and control, n = 2, two groups. 20 animals per group.

related to cognitive, neuroendocrine, and immune functions, and impact different subsystems and organs that modulate the stress response. Therefore, music, by sharing dynamics with stress responses, can be efficient in counteracting it and thus substantiating its usefulness.

Group					
Week	Enriched group mean value	SD	Control group mean value	SD	p- value
AUCc	3.74	0.07	2.91	0.52	**0.010**
AUCa	28.67	3.51	28.75	12.92	0.062
AOC	7.65	5.23	10.65	12.86	**0.010**

Table 7. Indices of cortisol and salivary amylase curves for enriched and control (T student) group pigs, n=2, two groups. 20 animals per group. *AUCc* area under the cortisol curve, *AUCa* area under the amylase curve, *AOC* Amylase over cortisol ratio. Values with significant statistical differences are highlighted in bold.

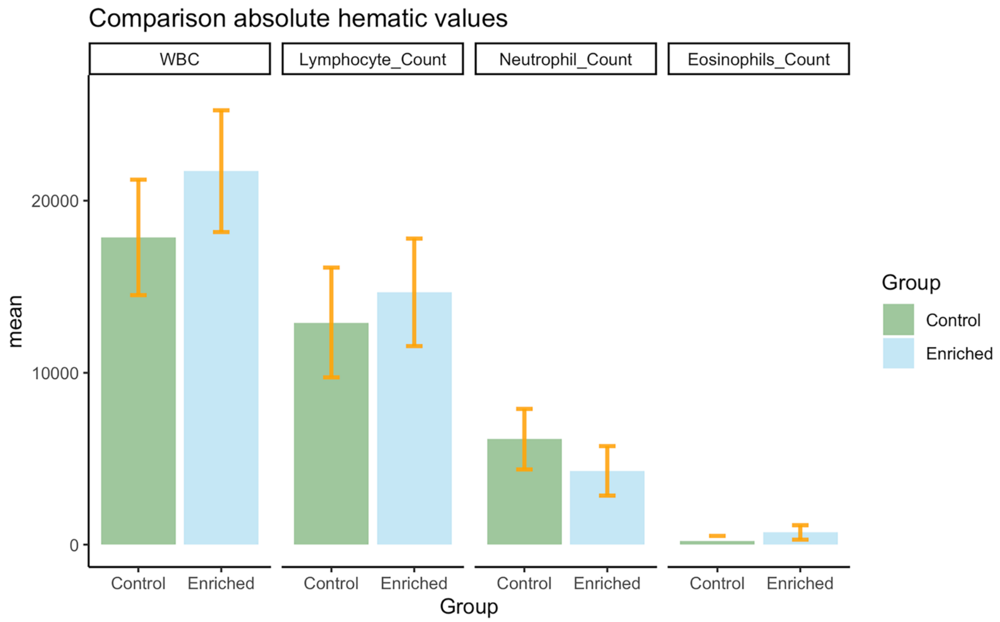


Figure 6. Comparative averages of leukocyte lines in both experimental groups at the end of the production cycle, n = 2, two groups. 20 animals per group.

Psychological effects

The results obtained showed that the designed music influenced the presentation of positive emotional states in the enriched group, finding significant differences with respect to the control group in terms of QBA such as content, happy and friendly, which were associated with the index for emotional valence. A previous study conducted by this research group had demonstrated that pigs are capable of showing a wide variety of emotional responses to music with different affective valence[11]; and the findings obtained indicate that the acoustic and musical adjustments made to the musical pieces in the framework of functional music were adequate, limiting the presentation of negative emotional states and favoring the expression of states with an intensely positive emotional valence.

Considering the assessment of emotional responses in the evaluation of the effect of music on stress has a relevant justification because, as previously mentioned in the model, emotions in music are a critical element and trigger of other neurophysiological responses and therefore constitute a point of great relevance in the evaluation of stress. Therefore, this may be the basis for explaining the differences in the evaluation of other behavioral and physiological parameters in the control group.

The results obtained from the evaluation of the skin lesions provide information in the same sense. In the group enriched, a lower frequency in the presentation of lesions associated mainly with anatomical areas such as ears, tail, middle and front was observed, and therefore, a decrease in agonistic behaviors in the pigs was observed. Pigs are gregarious animals that establish hierarchical relationships to determine the order of access to resources[93,94]. Fighting and avoidance behaviors are the way the hierarchy is exercised. In this way, skin lesions are considered as a sensitive and relevant indicator of the presentation of aggressive behaviors on the farm, generated by stressful conditions[95–97]. Previously, a study demonstrated the efficacy of music as strategy to reduce aggressive behavior during the regrouping of pigs[98] and multiple studies have evaluated the decrease in skin lesions as a positive indicator of the effect of environmental enrichment[96,99].

Previous studies, although using other types of environmental enrichment, mainly substrate, have also reported a decrease in lesions in the middle and front[100] and in the ears and tail[96,99,100]. This suggests that environmental enrichment is a protective factor, limiting the presentation of aggressive behavior and subsequent

Hematic parameter	Pairwise comparison	p-value
White blood cell count	C1 vs C2	**0.006**
	C1 vs T1	1.000
	C1 vs T2	1.000
	C2 vs T1	1.000
	C2 vs T2	**0.004**
	T1 vs T2	0.115
Lymphocyte count	C1 vs C2	0.057
	C1 vs T1	0.061
	C1 vs T2	0.314
	C2 vs T1	0.027
	C2 vs T2	**0.000**
	T1 vs T2	**0.001**
Neutrophil count	C1 vs C2	0.028
	C1 vs T1	0.161
	C1 vs T2	0.700
	C2 vs T1	**0.000**
	C2 vs T2	**0.005**
	T1 vs T2	**0.002**
Plasma Proteins	C1 vs C2	0.054
	C1 vs T1	0.081
	C1 vs T2	**0.006**
	C2 vs T1	0.064
	C2 vs T2	**0.006**
	T1 vs T2	**0.000**

Table 8. Hematic parameters enriched vs control group. Kruskal–Wallis test and Mann–Whitney U test corrected for multiple comparisons. n = 2, two groups. 20 animals per group. C1: Sampling at the beginning of the cycle-control group, C2: Sampling at the end of the cycle-control group, T1: Sampling at the beginning of the cycle-enriched group, T2: Sampling at the end of the cycle-enriched group. Values with significant statistical differences are highlighted in bold.

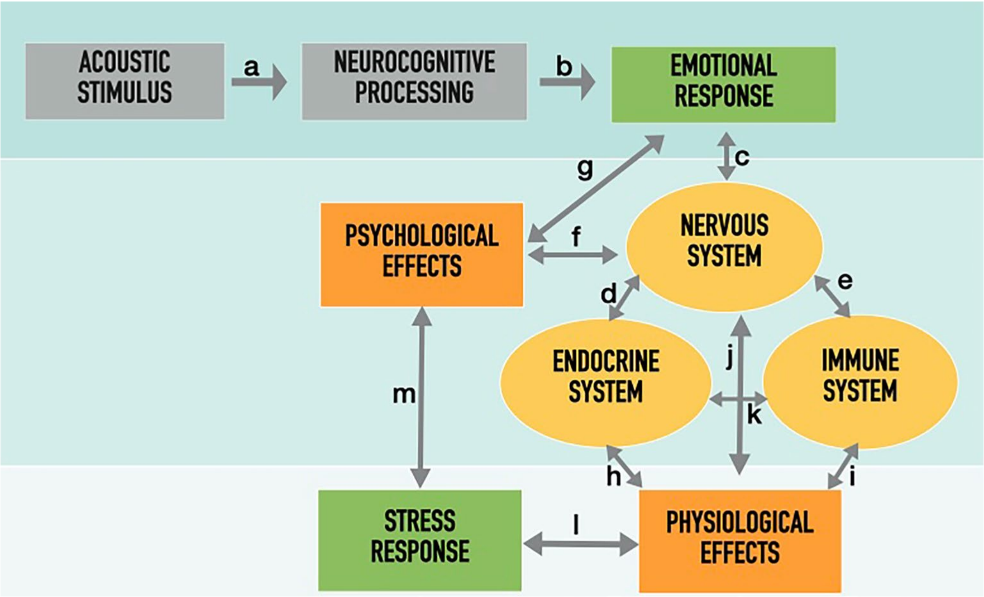


Figure 7. A model of the system interactions involved in modulation of music on stress. Note: **(a)**[76,77], **(b)**[78,79], **(c)**[80], **(d)**[81], **(e)**[82,83], **(f)**[84], **(g)**[22,85], **(j)**[86], **(h)**[81], **(i)**[87],**(k)**[24,83], **(l)**[88,89], **(m)**[90–92].

lesions. In this study, the findings indicate that similar to what was observed with physical enrichment, adjusted music influences behavior.

Physiological effects

Our results showed that auditory-enriched pigs differed from unstimulated pigs in the diurnal dynamics of stress biomarkers and thus in neuroendocrine regulation. The enriched group had the highest cortisol levels, and with detailed analysis of the metabolite dynamics during the day, notable fluctuations were observed that resulted in several peaks. This finding was very consistent in this group and was maintained for the three weeks that were compared. Previous studies on the species applying other types of enrichment have reported similar findings[62,101]. In contrast, the unstimulated pigs had decreased circadian peaks, with a flattened curve. Other studies have shown that the analysis of variations in salivary cortisol presents blunted (blunt-flattened) curves in situations of chronic stress in pigs, rodents rodents[102–104] and in humans during pathological states such as certain types of depression[105]. The observed attenuation of cortisol secretion during chronic stress may reflect adaptive habituation or a pathological disruption of the hypothalamic-pituitary axis (HPA)[106].

Cortisol has often been used as a critical parameter for assessing the HPA axis and chronic stress. However, stress assessment should not be based solely on cortisol levels but should consider integrated neuroendocrine function. Therefore, the measurement of salivary alpha-amylase, which is considered a significant and reliable marker of stress, and the assessment of the SAM axis[107] were included in this study. This allows comparison with the HPA axis, by using indices such as the AOC, providing valuable information on the balance and regulation of neuroendocrine function.

The calculation of the hormone requires the quantification of the area under the curve of both hormones. The enriched group presented higher AUCc and lower AUCa than the control group. The relationship between the axes was asymmetric, and under conditions of acoustic enrichment, the HPA axis was favored, therefore, the calculation of AOC was lower in this group. Thus, and considering that a strong correlation has been reported between AOC and chronic stress[65], the results observed in this study indicate a favorable effect of music-based auditory enrichment on the reduction of this type of stress. In animals and specifically in the porcine species, no previous studies were found that could serve as a comparison for these findings, since most of them evaluated biomarkers in isolation without having this type of interaction. However, in humans, it is indicated that the ratio of sAA to cortisol can capture the specific nature of the dissociation of the SAM-HPA axes and is a more sensitive marker of dysregulation of the stress system[65,107]. It has been reported that the AOC index has a stronger and more positive correlation with levels of stress, anxiety, and depressive symptoms than other indices such as COA (cortisol over amylase) or any other stress marker evaluated in isolation[65].

For the interpretation of the simultaneous regulation of the SAM-HPA axes, several models have been proposed, for example that of Munck et al[108], which suggests that the two endocrine axes function in a complementary manner, so that greater activity of the HPA axis is associated with a greater response to SAS stress, or the additive model of Bauer[109], which suggests that the optimal adaptation to stress occurs when both systems show moderate activity, or when the systems show a high-low pattern, that is, one system has high activity and the other shows low activity. In this study, higher AOC levels were observed in the control group, indicating attenuated the HPA axis activity, with a complementary increase in SAM stress responses. These results would provide evidence for the complementary model. However, due to the lack of previous work in swine that has explored the potentially important interactions between the physiological systems, more studies are needed to provide information on concurrent activity in the two endocrine axes and the information presented here can serve as a reference.

However, in this study, significant quantitative alterations were observed in the relative and absolute leukocyte populations, indicating differences between the experimental groups in the distribution of blood cells, reflected in a lower neutrophil—to lymphocyte ratio (N:L) in the enriched group. The effects of stress and the hormones that regulate it on the components of the immune system have been extensively described[110–112]. It has been suggested that leukocyte profiles are particularly useful in the field of physiology, showing quantitative changes during stress[113]. Specifically, in chronic conditions, an increase in N:L radio, with an increment in the number of neutrophils (neutrophilia) and decreases in the number of lymphocytes (lymphopenia or lymphocytopenia) has been observed[114,115]. In addition, researchers have considered the N:L ratio as a composite measure of the stress response.

For the swine species in particular, no reference values were found for the N:L ratio, however, in other species, a lower value such as that observed in the enriched group is associated with lower levels of stress. For example, in birds, values of 0.2, 0.5, and 0.8 are indicators of low, optimal and high stress levels, respectively[116]. This ratio is considered a sensitive indicator of the proportion between both leukocyte compartments and, therefore, of the functional balance of the immune system. These changes may be related to the attenuation of the cortisol curve reported in the enriched group. In the literature, the reduction of lymphocytes and the contrasting increase in the number of neutrophils in stress, therefore an increase in the N:F ratio, has been associated with the effect of an increase in glucocorticoid concentrations and its consequent effect on the "trafficking" or redistribution of lymphocytes from the blood to other body compartments[117].

Stress is a complex psychophysiological response, and music in turn is a stimulus capable of regulating in an integrated way the emotional and physiological states of the organism, therefore, its implementation for stress management seems a consistent and appropriate approach. As a therapeutic technique in the veterinary community, music can be designed to fulfill defined functions from clearly delimited objectives, bringing with it multiple benefits, including availability, minimal cost, convenience, and low invasiveness.

Finally, it should be noted that the concept of veterinary functional music presented in this study is currently being worked on in other pig farms, with the intention of improving some methodological conditions, such as a larger number of animals, some complementary measurements at the individual level, and the inclusion of additional experimental control conditions. It is also necessary to highlight that, in previous work of the team, we have already evaluated other types of control conditions, such as white and pink noise (considering its wide variation in spectral and temporal content, which allows its use as a non-musical acoustic stimulation) (unpublished results). However, the responses of the animals exposed to this stimulation were similar to those observed in the absence of music. This finding may be explained by the high noise levels that are constantly present in pig production systems, and habituation may occur. In non-human species, continuous noise may be more easily habituated[118,119] so noise does not appear to be a relevant problem condition for this species, at least under production conditions.

Conclusion

The experimental findings obtained allow us to conclude that a music-based program, induces psychophysiological changes in pigs, which may determine its therapeutic potential and support the usefulness of music in environmental enrichment for the swine species, with an impact on health and welfare by reducing stress that also has a translational value for other species.

Data availability

The datasets generated during the current study and code implemented for its analysis are available at (one).

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Author contributions

J.Z. and B.R. carried out the conceptualization; the investigation and methodology were developed by J.Z., B.R., and S.D.; J.Z. wrote the main text of the manuscript. E.D. and J.Z. performed the statistical analyses. B.R. composed and designed the music enrichment program used in the study. J.Z., B.R., S.D., Y.I., and A.S. contributed to drafting and refining the manuscript. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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