1 **Improving behavior monitoring of free moving dairy cows using non invasive**
2 **wireless EEG approach and digital signal processing techniques .**
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13 **ABSTRACT**

14 *Background:* Electroencephalography (EEG) is the most common method to access
 15 brain information. Techniques to monitor and to extract brain signal characteristics in
 16 farm animals are not as developed as in humans and laboratory animals.

17 *New method:* The method comprised two steps. In the first step, the signals were
 18 acquired after the telemetric equipment was developed, the electrodes were positioned
 19 and fixed, the sample frequency was defined, the equipment was positioned, and
 20 artifacts and other acquisition problems were dealt with. Brain signals from six Holstein
 21 heifers that could move freely in free stalls were acquired. The control group consisted
 22 in the same number of bovines, contained in a climatic chamber (restrained group). In
 23 the second step, the signals were characterized by Power Spectral Density, Short-Time
 24 Fourier Transform, and Lempel-Ziv complexity.

25 *Results:* The results indicated that there was an ideal position to attach the electrodes to
 26 the front of the bovine's head, so that longer artifact-free signal sections were acquired.
 27 The signals showed typical EEG frequency bands, like the bands found in humans. The
 28 Lempel-Ziv complexity values indicated that the bovine brain signals contained random
 29 and chaotic components. As expected, the signals acquired from the retained bovine
 30 group displayed sections with a larger number of artifacts.

31 *Comparison with existing methods:* We present the first method that helps to monitor
 32 and to extract brain signal features in unrestrained bovines.

33 *Conclusions:* The method could be applied to investigate changes in brain electrical
 34 activity during animal farming, to monitor brain activity related with animal behavior.

35

36 **Keywords:** cattle, surface electrodes, complexity, Lempel-Ziv, wireless, Bovine.

38

39 **Highlights**

- 40 • A method that allows brain signals to be monitored in freely moving dairy cows
- 41 is described
- 42 • The method uses noninvasive electrodes to minimize stress during EEG
- 43 monitoring and allows bovines to behave normally during the process
- 44 • The method establishes the frequency sampling rate, electrodes positioning and
- 45 fixation, equipment holding, artifact extraction, and signal characterization
- 46 • The brain signals are characterized by PSD, STFT, and Lempel-Ziv normalized
- 47 complexity
- 48 • The method can be applied to relate EEG to animal behavior under normal
- 49 handling conditions

50

51 **Abbreviations**

- 52 AGR Adaptive Gaussian Representation
- 53 EEG Electroencephalogram
- 54 LZC Lempel and Ziv complexity
- 55 C(N) Lempel& Ziv normalized complexity
- 56 DSP Digital Signal Processing
- 57 FFT Fast Fourier Transform
- 58 Fs Sample frequency
- 59 POS1 Electrodes evaluation position 1
- 60 POS2 Electrodes evaluation position 2
- 61 POS3 Electrodes evaluation position 3
- 62 PSD Power spectral density

63 STFT Short-Time Fourier Transform

64 EOG Electroocoulogram

65 ECG Electrocardiogram

66

67 **1. Introduction**

68 Animal behavior plays an important role in precision farming. Precision livestock
69 farming is an approach that enables the farmer with more objective information about
70 the animal to make better choices about the sustainability of their production system.
71 Society in general is demanding closer attention to the needs of individual animal . Due
72 to the increase in consciousness regarding animal welfare, the need for studies on
73 animal behavior has begun to gain importance. In commercial livestock farming, the
74 sustainability and profitability of production is ensured by raising the animals at the
75 tolerable welfare level. The exposure of living organisms to conditions below the
76 tolerable welfare level leads to the occurrence of physiological responses against stress
77 conditions, resulting in increase in health problems and decrease in yield (Cam MA et
78 al., 2018). In this scenario, understanding brain processes in bovines will allow some
79 situations like heat stress (Silanikove, 2000; Sinha, 2007) and pain (McLean et al.,
80 2017; Vuckovic et al., 2018), among others (Small et al., 2019), to be controlled.

81 Several DSP techniques have already been applied to humans and laboratory
82 animals; e.g., FFT (Fast Fourier Transform) (Jalilifar et al., 2016), wavelets (Subasi et
83 al., 2005), AGR (Adaptive Gaussian Representation) (Hashida et al., 2005), ANN
84 (Artificial Neural Network) (Robert et al., 2002; Vuckovic et al., 2002), Lempel-Ziv
85 complexity (Zhang and Roy, 1999), and entropy (Mazza et al., 2002), among others
86 (Khosla et al., 2020; Motamedi-Fakhr et al., 2014). This kind of technology has also
87 been employed to investigate some diseases in domestic pets (Bassett et al., 2014;
88 Bergamasco et al., 2003; Brauer et al., 2012). Concerning farmed animals, studies
89 involve pre-slaughter sensitization (Gibson et al., 2019; Small et al., 2019), sleep stages
90 (Hänninen et al., 2008; Ternman et al., 2012) and pain (BERGAMASCO et al., 2011)
91 most of them with animal kept in cages. This situation is changing and a recent study on

92 sheep (Perentos et al., 2017), showed a method that did not use restraint and which
93 employed implanted electrodes to analyze animal brain signals instead.

94 Acquiring brain signals in animals that can move freely presents various
95 challenges, and the number of research studies dealing with bovine brain electrical
96 activity is limited. Most of these studies have been conducted in laboratory conditions
97 (Suzuki et al., 1990) and used subdermal electrodes (Jones et al., 1988; Suzuki et al.,
98 1990) in animals that were subjected to some type of containment (Jones et al., 1988;
99 Small et al., 2019). These conditions are unreal when we consider farmed animals,
100 mainly large ones (Perentos et al., 2017).

101 Telemetric recording systems can monitor physiological parameters in freely
102 moving animals without any restrictions in their exploratory behavior (Lapray et al.,
103 2008). In 1966, West and Merrick (West and Merrick, 1966) presented a telemetric
104 equipment for EEG acquisition in large animals and presented the test results obtained
105 for cattle with their equipment. The authors showed the temporal characteristics of the
106 signal, but they did not mention the characteristics in terms of frequency. After that, the
107 need for long-term, continuous monitoring of EEG signals encouraged increasing use of
108 telemetric systems, to provide continuous and reliable data for studies on epilepsy
109 (Chang et al., 2011), sleep disorders (Weiergräber et al., 2005), and other pathologies
110 (de Araujo Furtado et al., 2009) in freely moving animals. In almost five decades,
111 different systems have been developed, from large systems (West and Merrick, 1966) to
112 transmitters that can be implanted beneath the skin of small animals (e.g., rats) (Chang
113 et al., 2011).

114 Another challenge regarding electroencephalography is the presence of artifacts
115 in the signal. Due to the nature of the acquisition process, the EEG signal can mix with
116 other biological potentials. The greatest influence is on the ocular signal (EOG), which

117 is in the same amplitude range as the EEG (i.e., millivolts) (Urigüen and Garcia-
118 Zapirain, 2015; Vidal et al., 2011), but it can still be contaminated by muscle (EMG)
119 and cardiac (ECG) signals, both in the millivolt range (Reaz et al., 2006; Xie et al.,
120 2021). Broadly speaking, artifacts can originate from internal (physiological activities
121 of the subject) and external sources (environmental interference, equipment, electrode
122 pop-up, and cable movement) and contaminate recordings in both temporal and spectral
123 domains (Islam et al., 2016). When signals from animals are concerned, the presence of
124 artifact is hardly avoided.

125 Given the number of research studies on bovine brain signal analysis and the
126 kind of signal processing techniques they apply (Bager et al., 1990; Gibson et al., 2019;
127 Jones et al., 1988; Merrick and Scharp, 1971; Small et al., 2019; Suzuki et al., 1990;
128 Takeuchi et al., 1998; West and Merrick, 1966), a good strategy to characterize these
129 signals is to assume that they are nonstationary, as in humans, and to employ time–
130 frequency techniques to estimate the spectra.

131 EEG is typically described in terms of rhythms and transients. The rhythmic
132 activity of EEG is divided into frequency bands (Islam et al., 2016; Ramadan and
133 Vasilakos, 2017). This characteristic makes traditional analysis rely mainly on the
134 detection of spectral power changes (Derya Übeyli, 2009). The Power Spectral Density
135 (PSD) is calculated by Fourier Transform, the estimated autocorrelation sequence that is
136 found by nonparametric methods. One of these methods is Welch's method (Al-
137 Fahoum and Al-Fraihat, 2014). Because the EEG signal is nonstationary, the most
138 suitable way to extract feature from raw data is to use time–frequency domain methods,
139 such as STFT (Short-Time Fourier Transform) (Kıymık et al., 2005; Marchant, 2003;
140 Ramos-Aguilar et al., 2020). STFT is a time-dependent Fourier Transform that may be
141 calculated by sliding a window over the signal to compute the FFT (Marchant, 2003). It

142 is represented by a two-dimensional time–frequency plot, called spectrogram. The
143 major drawback of STFT is a compromise between time and frequency resolution. If a
144 short window is applied, information derived from each FFT will be well localized in
145 time, but frequency resolution will be poor.

146 In addition, the electrical activity of the brain (EEG) exhibits significant
147 complex behavior with strong nonlinear and dynamic properties (Jeong, 2004; Zhang et
148 al., 2001). Non-linearity in the brain is introduced even at the cellular level because the
149 dynamic behavior of individual neurons is governed by threshold and saturation
150 phenomena (Abásolo et al., 2006). This implies that nonlinear methods can be applied
151 to investigate EEG dynamics (Zhang et al., 2001). The Lempel-Ziv complexity (LZC)
152 (Lempel and Ziv, 1976) uses symbolic techniques to map a time series into a sequence
153 that retains its dynamics. LZC has been widely used in biomedical applications to
154 estimate the complexity of discrete-time signals (Gómez et al., 2006). Normalized
155 complexity can be used to quantify nonlinear and nondeterministic data (Costa et al.,
156 2017) .

157 This study aimed to develop a method to monitor bovine brain electrical activity
158 by noninvasive techniques in animals in an experimental free stall. Another objective
159 was to apply techniques like PSD (Power Spectral Density), STFT, and LZC to
160 characterize the obtained signals, a necessary step to expand this field of study.

161

162 **2. Material and Methods**

163 This section is divided into three subsections: (1) developing a portable and low-cost
164 wireless electroencephalograph; (2) developing a method to monitor bovine brain
165 electrical activity; and (3) extracting the features of these signals.

166

167 2.1. *Wireless electroencephalograph*

168 The EEG acquisition system consisted of two modules: the first module, which was
169 embedded in the animal, concerned the recording, conditioning, and wireless
170 transmission of the EEG signals. The second module, which was called base module,
171 received data from the embedded module and connected the system to a computer
172 (Silva et al., 2005).

173

174 2.1.1. Animal module

175 This module could be attached to the animal's neck, and it performed three tasks: (1)
176 signal amplification and conditioning, (2) analog-to-digital conversion, and (3) wireless
177 transmission of the digital data. A microcontroller sampled the EEG analog signal,
178 converted the analog signal to a digital signal, and transmitted the recorded data (Silva
179 et al., 2005). Each of these parts will be detailed below.

180 The electronic amplification and conditioning circuit consisted of (i) an
181 amplifier that pre-amplified the EEG signal and (ii) three analog active filters: a 0.05-Hz
182 high-pass filter, a 1.5-kHz low-pass filter for antialiasing, and a band-pass filter to
183 remove 60-Hz electromagnetic interference. The total gain of the circuit was 1×10^4 .
184 The A/D converter stage of the circuit used a 10-bit analog-to-digital converter of a
185 Microchip PIC16F877A microprocessor and allowed frequency up to 120 Hz to be
186 sampled.

187 Through its USART interface, the microprocessor also controlled telemetric
188 transmission. The circuit used the BIM2-433-160 transceiver (Radiometrix Ltd, 2004),
189 which transmitted and received the data at 433 MHz with low energy consumption in a
190 range of up to 200 m.

191

192 2.1.2. Base module

193 The base module received and stored data arriving from the embedded modules. This
194 module consisted of one 433-MHz channel that used a BIM2 transceiver (Radiometrix
195 Ltd, 2004). Dedicated software in the computer stored the data in a database and
196 showed the EEG in real-time on the display monitor.

197

198 2.1.3. Equipment validation

199 To validate the test equipment (TE), the TE ability to amplify signals of low amplitude
200 and frequency was evaluated and compared to the control equipment (CE), which
201 consisted of a 32-channel digital electroencephalograph from the company EMSA
202 Medical Equipment S/A, model BRAINNET BNT-EEG. The following signals were
203 evaluated: (1) 3.90-Hz and 30.20-Hz sine waves generated by a sine generator and (2)
204 brain signals from a 26-year-old volunteer free of neurological problems. Stretches of 5
205 and 10 s were collected at a sampling frequency of 100 Hz for sine waves and brain
206 signals, respectively.

207 In the frequency domain, the PSD was estimated by the Welch method for each
208 signal. In the time domain, the comparison was made by considering the Signal-to-Error
209 Ratio (SER) (Equation 02). The method considered the developed equipment as the
210 output of a nonlinear predictor of the EEG signal acquired by the control equipment
211 (Kavitha and Narayana Dutt, 1999).

212

$$213 \quad SER = 10 \log_{10} \left[\frac{\sum_{i=1}^N (x(i))^2}{\sum_{i=1}^N (x(i) - p(i))^2} \right] dB \quad (2)$$

214

215 2.2. Signal monitoring

216 To develop a method to monitor bovine brain electrical activity, it was necessary to
217 define sample frequency (F_s), where to place the electrodes (positioning and fixation)
218 and equipment, and to deal with artifacts and other acquisition issues. These conditions
219 were established by conducting measurements with two animals. The second stage
220 involved 12 animals, which were divided into two groups of six, and brain signals were
221 acquired in two different conditions: from animals moving freely in a free stall and from
222 animals kept in a climatic chamber.

223 Considering that the frequency interval of human brain signals ranges from 0.4
224 Hz to almost 40 Hz (Islam et al., 2016; Ramadan and Vasilakos, 2017), F_s was initially
225 set to 100 Hz, to ensure that frequencies were mapped between 0 and 50 Hz without
226 aliasing. This allowed the presence of frequencies higher than the frequencies detected
227 in humans to be investigated. The signals were digitized in the embedded module.

228 The experiment was carried out in the city of Pirassununga, state of São Paulo,
229 Brazil (latitude 21°59'46" south and longitude 47°25'33" west, altitude of 627 meters).
230 The signals were acquired from Holstein heifers.

231 This study was conducted by using noninvasive electrodes consisting of 1.0-cm
232 gold discs. Electrode fixation was attempted with either strong adhesive tapes or
233 cyanoacrylate, also known as super glue (Super bonder - Loctite®). First, the area at the
234 forehead of the cows where the electrodes would be fixed was shaved. Then, the
235 electrodes were filled with conductive gel for EEG. Next, the animal module was placed
236 in a small bag around the animal's neck. Fixation was tested for a whole month.

237 The equipment comprised a single-channel electroencephalograph (bipolar
238 measurement), so the sensor consisted of two electrodes, to acquire the biopotential
239 signal acquisition, and a grounding electrode. The grounding electrode was positioned
240 at the back of the animal's neck, whereas the other two electrodes were tested at three

different positions (POS1, POS2, and POS3) of the animal's forehead. These positions were selected on the basis of the bovine head anatomy. Anatomical head sections were analyzed to investigate positions for electrode fixation. The images of heads that were used for this analysis were obtained at a local slaughterhouse, so they are not the images of heads of the animals that were used in the experiments.

To minimize artefacts from eyes and muscle movements, the test was performed on animals sedated with 1 mL/kg Rompun from Bayer®. A total of 6 mL was enough to achieve deep sedation for 40 min. The positioning test data were acquired from two cows during a whole day.

After the best electrode position was determined, the signals were acquired in two different situations: 1) from animals contained in a climatic chamber (cage containment) and 2) from animals in pasture (animals with freedom of movement). In the first situation, the animals were contained in 1.0 x 2.0 m individual cages arranged as a 2x3 matrix inside a climatic chamber. In the second situation, the animals were contained in free stalls measuring 4.0 x 3.0 m and arranged side by side as a 1x6 matrix. The cows had free access to food and water during the experiment. For each situation, the signals were acquired from six Holstein heifers.

During the experiment, the temperature and relative air humidity were collected and used to determine the enthalpy of the place, as proposed by Albright (1990). The enthalpy is a variable that is considered an index of thermal comfort—it indicates the environmental conditions related to the thermal stress suffered by the animals (Moura, D. J.; Naas, I. A.; Silva, I. J. O.; Sevegnani, 1997; Rodrigues et al., 2011). This stage of data acquisition lasted three full days.

2.3. Signal characterization

For both groups of animals, all the stages of brain signal characterization were performed with the Signal Processing ToolboxTM from MATLAB[®] software releases R2015a and 2019a, developed by The Mathworks, Inc.

In pre-processing stages, different methods can be employed to handle artifacts. One of them rejects the epoch or segment of EEG data that is labelled as artifactual (Motamedi-Fakhr et al., 2014). Sauter et al. (Sauter et al., 1990) proposed a sequential method where spectral analysis is performed after corrupted segments are extracted. For this purpose, artifacts are detected by visual inspection, and then rejected. Welch's method was applied to artifact-free signal segments to estimate the signal PSD and to verify whether the spectral content is due to brain signal.

PSD makes the frequency spectrum smoother than the raw FFT output ,and the Welch algorithm (Monson H. Hayes, 1996) is a nonparametric method to estimate PSD. Let us consider that the FFT is calculated over the entire duration of the signal. In the Welch algorithm, instead of processing the FFT over the entire time domain, the signal is separated in windows with the same size. Window size affects the clarity of the result by cutting frequencies with periods larger than the window. Windowing is taking a sample of a larger dataset and tapering the signal at the edges of each interval. This makes the signal smoother without sharp transitions that can disturb the frequency spectrum representation (Same et al., 2020). Welch's method was applied to artifact-free signal segments to estimate the signal PSD.

Considering that STFT is a sequence of Fourier Transforms of a windowed signal, it can be represented as in Equation (3) (Kehtarnavaz, 2008).

$$X_{STFT}[m,n] = \sum_{k=0}^{L-1} x[k]g[k-m]e^{-j2\pi n k/L} (3)$$

291 where $x[k]$ denotes the signal, and $g[k]$ denotes an L-point window function.

292 STFT was also employed to artifact-free signal segments, so that data could be
293 mapped in the time–frequency domain.

294 For LZC calculation, each signal segment is converted into a binary sequence
295 $s(n)$, as follows (Bachmann et al., 2018; Gómez et al., 2006):

296

$$297 \quad s(n) = \begin{cases} 1, & \text{if } x(n) > m \\ 0, & \text{if } x(n) < m \end{cases} \quad (4)$$

298

299 where $x(n)$ is the signal segment, and n is the segment sample index from 1 to N
300 (segment size). The segment length N was chosen for at least 400 samples – a value for
301 which normalized LZC is stabilized (Gómez et al., 2006); m is the signal mean value.

302 Thereafter, the resulting binary sequence $s(n)$ is scanned from left to right, and the
303 number of different patterns is counted. The complexity value $c(n)$ is increased every
304 time a new pattern is encountered (Bachmann et al., 2018; Gómez et al., 2006; Lempel
305 and Ziv, 1976). Lempel and Ziv (Lempel and Ziv, 1976) showed that

306

$$307 \quad \lim_{n \rightarrow \infty} c(n) = b(n) = H \frac{n}{\log_k n} \quad (5)$$

308

309 where N is the segment length, k is the number of different symbols in the sequence (in
310 the binary case, $k = 2$, and H represents entropy (Equation (6)).

311

$$312 \quad H = \frac{-1}{\ln(k)} \sum_{i=1}^k p_i \log(p_i) \quad (6)$$

313

where p_i indicates the probability that a state i is obtained by counting the occurrences of each symbol, divided by the total number of symbols in the sequence.

To avoid the variations due to the segment length, normalized LZC values are calculated as follows:

$$C(N) = \frac{c(N)}{b(N)} \quad (7)$$

The more random the sequence, the greater the number of different patterns present in it; that is, its normalized complexity $C(N)$ approaches one. Meanwhile, $C(N)$ values that approach zero are associated with deterministic sequences. Normalized LZV - $C(N)$ was applied to artifact-free signal segments to investigate oscillatory, chaotic, and random components in bovine brain signals. To illustrate the relationship between $C(N)$ and signal characteristics, low- and high-complexity known sequences were calculated.

3. Results and Discussion

3.1. Equipment validation

Figure 1 presents the graph of a 3.90-Hz sine wave sampled at 100 Hz by the test equipment (TE) and control equipment (CE). In this case, SER was 39.35 dB.

333

334 *Figure 1 – 3.90-Hz sine wave sampled at 100 Hz by (a) test equipment (TE) and (b) control equipment (CE).*

335

336 Figure 2 shows the graph of a 30.2-Hz sine wave sampled at 100 Hz by the test

337 equipment (TE) and control equipment (CE). In this case, SER was 38.36 dB.

338

Figure 2– 30.2-Hz sine wave sampled at 100 Hz by (a) test equipment (TE) and (b) control equipment (CE).

Figure 3 compares the PSD of both sine waves obtained with the TE and CE.

Figure 3 – Power spectral density (PSD). (a) 3.90-Hz sine wave and (b) 30.2-Hz sine wave. Test equipment (TE) in black and control equipment (CE) in gray.

Figure 4 compares the brain signals acquired from a human volunteer by both the TE and CE. Figure 5 illustrates the PSD calculation for the signals in Figure 4. In this case, SER was 2.41 dB.

Figure 4 – EEG of a 26-year-old female volunteer recorded with (a) test equipment (TE) and (b) control equipment (CE).

Figure 5 - Power spectral density (PSD) for the human volunteer. Test equipment (TE) in black and control equipment (CE) in gray.

In the time domain, the signals were only slightly significantly different as compared to the signals obtained with the method proposed by Kavitha and Narayana

361 Dutt (1999). The main difference between the signals was due to equipment resolution,
362 which was 10 and 12 bits for the TE and CE, respectively. The PSD indicated the same
363 frequencies for both the generated sines and brain signals.

364 The first telemetric equipment developed to record EEG (West and Merrick,
365 1966) weighed approximately 0.450 kg, transmitted data up to 91.44 m, and was
366 completely analog. The equipment cost about US \$1500, including the receiver. The
367 literature has no other report on the development and use of other EEG equipment for
368 use in cattle.

369 The equipment developed herein weighs 180 g and transmits data up to 200 m.
370 In addition, it has a microcontrolled processing system that allows digital data
371 transmission and costs approximately US\$ 100.

372 *3.2 Signal monitoring*

373 Figure 6-a shows a head section of an adult bovine. This figure illustrates the distance
374 from the brain to the frontal region of the bovine head. This distance can be longer or
375 shorter, depending on the paranasal sinuses. Finding the best position for signal
376 acquisition involves locating the region where this distance is as short as possible.
377 Figure 6-b shows the layout of the monitoring electrodes at the three evaluated
378 positions.

379
 380 *Figure 6 – (a) Head section from adult animal. POS2 corresponds to the region where the brain is closest to the*
 381 *surface. (b) Layout of monitoring electrodes at three positions. Numbers 1, 2, and 3 indicate POS1, POS2, and*
 382 *POS3, respectively.*

383 Figure 7 depicts a disc electrode and electrode fixation with adhesive tape.

384
 385 *Figure 7 –Disc electrodes at POS2.*

386
 387 The animal module consisted of a box measuring 2.5 x 7.0 x 11.0 cm³ and
 388 weighing 180 g. To make it less uncomfortable for animals, the box was placed in a

389 fabric bag, which was attached to the neck with a soft ribbon. Figure 8-a presents the
 390 animal module placed at the animal's neck, whilst Figure 8-b shows the halter that was
 391 used to protect the electrodes and the electrodes fixed with super glue.

392

393

394 *Figure 8 – (a) Animal module inside a fabric bag placed at animal's neck. (b) glue fixation of electrodes and*
 395 *protection with halter.*

396

397 The adhesive tape failed to keep the electrodes rigidly attached to the animal, so
 398 they often came loose. The glue (Figure 8-b) kept the electrodes in the right position for
 399 a whole day of acquisition. At the end of the day, the electrodes and the animal's
 400 forehead had to be cleaned for a new acquisition. Compared to the use of implanted
 401 electrodes, which can go on for a year (Perentos et al., 2017), this is a short period. It is
 402 important to highlight the possibility of noninvasive monitoring here, though.

Figure 9 contains signals acquired at the three selected positions for one of the two animals in this stage of the experiment, while Figure 10 corresponds to the artifact-free signal sections in Figure 9.

406

407

408 *Figure 9 – Signal at (a) POS1. (b) POS2. (c) POS3. Original signal in grey and selected signal epochs in black.*

409

410

411 *Figure 10 – Artefact-free signal sections in (a) POS1, (b) POS2, and (c) POS3.*

412

413

Figure 11 contains the PSD for the signal sections in Figure 10.

414

415 *Figure 11 – PSD for artifact-free signal sections at (a) POS1, (b) POS2, and (c) POS3.*

416

417 In Figure 9-a, the signal at POS1 presented many artifacts, most of which were
418 due to ear movement. POS2 provided longer artifact-free sections than POS1 and POS3
419 as a result of two main facts. First, POS3 was largely influenced by eye movement,
420 whereas POS2 is the region where paranasal sinuses were smaller and hence the surface
421 was closer to the cerebral cortex. This result was expected given the analyzed
422 anatomical head section (e.g., Figure 6). The three positions had similar PSD profile,
423 and the observed frequencies (2–10Hz; 13–27Hz) agreed with those obtained by Suzuki
424 et al (1990)(Suzuki et al., 1990) and Ternman et al (2012). Therefore, the metric to
425 choose the best electrode position was the length of the obtained artifact free-section,
426 and POS2 was selected as the best one to acquire brain signals in cattle.

Most studies in this area have been conducted in laboratory conditions (Suzuki et al., 1990) and used subdermal electrodes (Jones et al., 1988; Suzuki et al., 1990) in animals that were subjected to some type of containment (Jones et al., 1988; Small et al., 2019). The method presented herein allows brain signal to be recorded with surface electrodes in freely moving animals.

Figure 12 displays enthalpy values for the animals in climatic chamber or pasture during the first of the three days of experiment.

Figure 12 - Enthalpy for animals in (a) climatic chamber and (b) pasture.

In the climatic chamber, between 20:00 h and approximately 22:30 h and after 03:00 h, the animals experienced a heat stress situation, but most of the time, they were in a comfortable situation (30–70 J/kg of dry air).

The stress condition was due to animal agitation in periods of enthalpy above 70 J/g, which provided a greater amount of water vapor resulting from animal panting. This increased the internal relative humidity, which progressively became an obstacle to heat loss through breathing and the skin surface. Under these conditions, the temperature and

relative air humidity were above 32 °C and 60%, respectively. After a period of reduction in animal agitation and renewal of the chamber air, the condition of thermal comfort was established, with average values of 25 °C and 60 %. In the pasture, there were no conditions of thermal stress. In fact, during the analysis period, enthalpy decrease, as the incidence of solar radiation decreased, which modified the temperature and relative air humidity (Rodrigues et al., 2011).

451

452 3.3 Signal characterization

Figure 13 exemplifies a pre-processing stage when artifact was removed from the signal for one of the animals in the climatic chamber (13-a) and one unrestrained animal (13-b).

456

457

458 Figure 13 – Artifact removal. (a) Climatic chamber. (b) Pasture. Original signal in grey and signal selected epochs
459 in black.

460

The proportion of artifact-free data in relation to the original data seen in Figure 13 was observed for most of the database. In all the acquisitions, at least one artifact-free section could be selected.

461

462

463

464 Figure 14-a contains the PSD for the artifact-free signal section in Figure 13-a,
 465 while Figure 14-b shows the PSD for the second artifact-free signal segment in Figure
 466 13-b.
 467

468
 469 *Figure 14 – Power Spectral Density (PSD) for artifact-free EEG signal. (a) Climatic chamber. (b) In pasture.*

470
 471 We were not able to establish a direct relationship between enthalpy and any
 472 specific behavior observed during the experiments (eating, drinking water, chewing,
 473 standing, lying down, or walking). The stress experienced by animals in the cage
 474 overlapped with any other behavior. This reflected even on the duration of artifact-free
 475 epochs in the signal (less than 4 s for all the animals).

476 Figure 15 illustrates STFT for the second artifact-free signal segment in Figure
 477 13-b.
 478

479

480 *Figure 15 – STFT for the second artifact-free EEG signal segment in Figure 13-b.*

481

482 We were not able to associate the observed frequencies in the group with any
 483 specific animal behavior, such as eating, drinking water, chewing, standing, lying down
 484 or walking, mainly because of the amount of data that was acquired. However, the
 485 spectrogram clearly showed the predominant frequencies in cattle signals (Figure 15).
 486 As expected, the delta wave presented high amplitude between 1 and 3 Hz and was very
 487 expressive between 1 and 2 Hz. There were also well-delimited regions between 4 and 7
 488 Hz (Theta), 7 and 12 Hz, (Alpha), 12 and 30 Hz (Beta), and above 30 Hz (Gamma).
 489 Observed frequencies still agreed with the observations of Suzuki et al. (1990) and
 490 Takeuchi et al. (1998).

491 This methodology allowed us to acquire brain signals from animals that can
 492 move freely while minimizing the stress generated by confinement. It also allowed the

493 acquisition to occur noninvasively. However, the acquisition conducted in this way
 494 produced data with a greater number of artifacts, but it had the advantage of reducing
 495 the discomfort caused by using subdermal electrodes. Another important point to
 496 consider is the challenge imposed by the size of the animals. Considering the larger
 497 amount of data, this methodology could be applied to investigate changes in brain
 498 electrical activity during animal farming, to monitor brain pathologies and other
 499 situations.

500 Table 1 lists normalized complexity for seven knowledge sequences.

501

502 *Table 1 – Lempel & Ziv normalized complexity $C(N)$ for different time series.*

Sequence	Cn
$S1 = [0\ 1\ 0\ 1\ 0\ 1\ 0\ 1\ 0\ \dots\ 1\ 0\ 1\ 0\ 1\ 0\ 1\ 0\ 1]$	0.0202
$S2 = \sin(2\pi 30t)$	0.0559
$S3 = \sin(2\pi 0.5t) + \sin(2\pi 10t) + \sin(2\pi 18t) + \sin(2\pi 30t) + \sin(2\pi 30t)$	0.0129
$S4 = \sin(2\pi 0.5t) + \sin(2\pi 10t) + \sin(2\pi 18t) + \sin(2\pi 30t) + \sin(2\pi 30t) +$ (array of random numbers)	1.0000
$S5 = \text{white noise}$	0.9957
$S6 = \text{array of random numbers}$	1.0870
$S7 = \text{logistic map in chaos threshold value (Costa et al., 2017)}$	1.0000

503

504 Table 2 contains Cn for artefact-free signal sections in Figure 13.

505

506 *Table 2 – Normalized complexity C_n for artefact-free signal sections*

Sequence	Cn
----------	----

Climatic chamber 1 st section (size: 4 s)	0.9894
Climatic chamber 2 nd section (size: 4 s)	0.6596
Climatic chamber 3 rd section (size: 4 s)	0.7483
Climatic chamber 4 th section (size: 4 s)	0.7475
Climatic chamber 5 th section (size: 9 s)	0.5857
Pasture 1 st section (size: 240 s)	0.6078
Pasture 2 nd section (size: 15 s)	0.6163
Pasture 3 rd section (size: 3 s)	0.8656

507

508 We obtained values similar to the values in Table 2 for the other five animals we
509 evaluated. High normalized LZC C(N) values can be associated with the presence of
510 noise, random numbers, or the predominance of one of these events (Table 1). On the
511 basis of the values we observed for bovine signals (Table 2), we can consider that the
512 artifacts were not completely eliminated, and that cattle brain signals have some random
513 characteristics. Costa et al. (2017) used the logistic map in the chaotic behavior
514 threshold to obtain the entropic q-index for different values of initial conditions, to find
515 that C(N) always presented values around one. Thus, complexity values next to one
516 might indicate that bovine brain signals also have chaotic components.

517 Future improvements to this method could include increasing the number of
518 animals and intensifying signal acquisition during a specific behavior (eating, drinking
519 water, chewing, standing, lying down, or walking), so that said behavior could be
520 associated with signal features. Another point for the future is to handle artifacts
521 automatically, given that this is a time-consuming stage of signal processing.

522

523 **5. Conclusions**

524 EEG signals can be acquired from unrestrained cattle by using surface (noninvasive)
525 electrodes and telemetric equipment. There is an ideal position for electrode attachment
526 to the front of the head, so that longer artifact-free signal sections can be acquired. The
527 signal presents typical EEG frequency bands, such as the bands found in humans and
528 other animals. The signals also display random and chaotic characteristics. As expected,
529 the restrained animals present signals with more artifacts. The method developed herein
530 can be applied to understand several situations involving bovine (or other bigger farm
531 animals) brain signals ranging from normality to some brain pathologies.

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540 **Ana Carolina de Sousa Silva:** Conceptualization, Methodology, Investigation, Data
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