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Bioelectrical activity demonstration in Marandu grass (*Brachiaria brizantha*)

Demostración de la actividad bioeléctrica en pasto Marandú (*Brachiaria brizantha*)

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ABSTRACT

Brachiaria, a genus of tropical grass, occupies a prominent place in Brazilian pastures. *Brachiaria brizantha, Brachiaria decumbens and Brachiaria humidícola* are widely grown as fodder plant. There have been made many researches about the physiology of these *Brachiaria* species, but information about their bioelectric activity is not available. The objective of this study was to demonstrate bioelectric activity in *Brachiaria brizantha* cv. Marandu. Plants were grown on individual pots, at greenhouse under 100%, 70% and 50% illumination levels. Watering and maintenance fertilization were supplied along grown phase. Prior to electrical measurements, plants were kept in Faraday box. For detecting electrical signals, electrodes were connected from plants to high impedance electrometer, with wireless technology. Collected signals were sent to microprocessor, processed in Matlab® device and analyzed following Welch model. It was possible to detect electric signals of Marandu grass through this electronic instrumentation. Marandu grass plants subjected to frequency range from 0 to 60 Hz generate variations in dB/Hz ratio. Welch Power Spectrum Density Estimate (WPSDS) generated from individual plants grown under same illumination conditions were similar, but WPSDS generated from plants growing on different illumination conditions were distinct for each one. More extensive and deeper research for better understanding of the bioelectric activity in plants is required.

Key words: bioelectric signals, *Welch model*, physiological response, pasture, cattle.

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RESUMEN

Brachiaria es un género de pasto tropical que ocupa un lugar destacado en los pastos brasileños. *Brachiaria brizantha, Brachiaria decumbens* y *Brachiaria humidícola* se cultivan ampliamente como planta forrajera. Existen muchas investigaciones relacionadas con la fisiología de estas especies de *Brachiaria*, pero no se dispone de información sobre su actividad bioeléctrica. El objetivo de este estudio fue demostrar la actividad bioeléctrica de *Brachiaria brizantha cv. Marandú*. Las plantas se cultivaron en invernadero, en macetas individuales bajo niveles de iluminación del 100%, 70% y 50%. El riego y la fertilización de mantenimiento se suministraron durante la fase de crecimiento. Antes de las mediciones eléctricas, las plantas se mantuvieron en una caja de Faraday. Para detectar las señales eléctricas, se colocaron electrodos en las plantas y se conectaron a un electrómetro de alta impedancia con tecnología inalámbrica. Las señales recogidas fueron enviadas al microprocesador, procesadas en el dispositivo Matlab® y analizadas siguiendo el modelo de Welch. Se detectaron señales eléctricas en el pasto Marandu mediante esta instrumentación electrónica. Las plantas sometidas a un rango de frecuencia de 0 a 60 Hz generan variaciones en la relación dB / Hz. El estimado de la densidad espectral de potencia Welch (WPSDS) obtenido a partir de plantas cultivadas bajo las mismas condiciones de iluminación fue similar, pero el WPSDS generado a partir de plantas que crecen en diferentes condiciones de iluminación fue distinto para cada una. Se requiere una investigación más extensa y profunda para comprender mejor la actividad bioeléctrica en las plantas.

Palabras clave: señales bioeléctricas, modelo Welch, respuesta fisiológica, pastos, ganado.

INTRODUCTION

In Brazil, the pasture-raised livestock sector and the formation of good grasslands are very important, being the latter the best option for feeding cattle, since it provides available and affordable food that offers all the nutrients needed for sucessfully development of animals. It is noteworthy that livestock represents a strong expressive of Gross Domestic Product (GDP) in agriculture. The cattle population in Brazil is aproximately 165 million animals, 72% is concentrated in the Southeast, South and Midwest (Moreira *et al.,* 2009; Dias-Filho, 2014).

Genre *Brachiaria* is part of pasture for cattle in Brazil. It has featuring as main characteristics: high drought resistance, good resistance to trampling, good development before fertilization, and aid in erosion control. Inadequate practices for grassland conservation increase economic losses, since it contributes in rising of diseases and weight loss in cattle (Criar e Plantar, 2009; Moreira *et al.,* 2009).

There are many researches related to the spread of *Urochloa decumbens* (Stapf) R. D. Webster (sin: *Brachiaria decumbens* Stapf), but they are lacking in literature information about the study of its bioelectric signals. Bioelectrical signals have been studied in plants to demonstrate that they are reliable and can be used to define parameters and generate models using an electronic signal acquisition system (Van Bel and Ehlers, 2004; Volkov, 2006; Fromm and Lautner, 2007). It has been shown presence of electrical signals in complex plants, which arise as result of normal physiological processes, or in response to photo electric and mechanical stimuli (Pickard, 1974).

Under biological focus, one of the biggest differences between plants and animals is that the latter can coordinate their activities through electrical stimulation known as action potentials - (AP), which propagate along a network nerve cell. In turn, most plants have no such coordination, however, there are species whose natural behavior is mediated by AP, such as *Mimosa pudica* L.: Fabaceae (sensory or poppy), whose leaves close in response to any small perturbation vibratory (Pickard, 1974).

Another example is the insectivorous plant *Dionaea muscipula* J. Ellis: *Droseraceae* (agile) whose leaves close trapping insects that touch them, in order to achieve additional sources of nitrogen in their nutrition. From biochemical stand point, in accordance with Lehninger (2006), the fast move (less than 0.5 m/s) of the leaf is a result of change in turgor pressure in mesophilic cells, due to the release of K^+ íons and consequent water efflux by osmosis.

Same reaction observed in *M. pudica* was presented by Sibaoka (1966, 1969) and Pickard (1973). They demonstrated that the propagation of signals in *M. pudica* presented similar characteristics to the nerve´s action potential and, therefore, demonstrated that plants use electrical signals to regulate their physiological functions. Plants exhibit characteristics of excitability, and this is used by the cells, tissues and organs to replace their internal and external conditions (Volkov *et al*., 2000).

Very important for the maintenance of grassland forage species, is the knowledge of their physiology, this opens doors to new and key studies and can contribute to the preservation of many ecosystems. This is fundamental to improve techniques for management of pastures and consequently to add economic value to livestock industry.

The objectives of this study were to capture and demonstrate bioelectrical signals in *Brachiaria brizantha* using a non invasive method, and appliying digital signal processing techniques to characterize them, also to perform a study in removal of artifacts and on the main frequency characteristics of the signals.

MATERIAL AND METHODS

Were used eight plastic pots with 9 Kg capacity for each seeded Marandu grass; the soil was corrected after analyses performed in the Agricultural Soil Sciences Laboratory and the Faculty of Animal Science and Food Engineering, University of São Paulo (FZEA/USP).

The experiment was conducted in a greenhouse at the Agricultural sector, located on the campus of FZEA/USP, in the municipality of Pirassununga - SP, located at latitude 21 º 59’ 46 “ South, 47º 25’ 33” West, and altitude of 627 meters. The climate is classified as Cwa (mesothermal) Köeppen Rating Scale (Carvalho Leite, 2008).

Field capacity (cc) of each vessel was calculated. With the values determined and properly recorded in each pot, these were just completed. During the signal collection days, the pots were irrigated only after all the work ended.

The harvest was made after signal collection, respecting the plant cycle (28-35 days), it was 15 cm (base of the plant) using gardening shears. Soon after, it was performed the maintenance of fertilization with potassium chloride and ammonium nitrate (33%). Fertilization was performed manually. After the last signal collection were harvested the aerial parts of the plants to perform analysis of foliar nutrient content (N), at the Laboratory of Soil and Agricultural Sciences College of Animal Science and Food Engineering, University of São Paulo.

The eight pots used were distributed as follows: two grown under a full sun situation (100% light) and receiving maintenance fertilization, two being grown under a situation of shading (70% light) and receiving fertilization maintenance, two being cultivated under a situation of shading (50% light) and receiving maintenance fertilization, and last two vessels grown in a full sun situation (100% light) without maintenance fertilization.

Three contact electrodes (noninvasive) were placed as follows: the first adjacent to ligule, the second 10 cm above the ligule, at the top surface of the last fully expanded leaf, and a third at the base of plants, as Figure 1 follows shown.

The bioelectric signals were sampled at 120 Hz, and each collection was made for 15 seconds. During the experiment the plants were kept in a Faraday cage made by the Computational Applied Physics Laboratory and the Faculty of Animal Science and Food Engineering, University of São Paulo – (LAFAC), in order to maintain stable source of electrical signals and without significant changes to the experiment. The electrical signals are detected using gold disks electrodes attached on the surface of the plant by means of an aqueous gel conductor type commonly used in electroencephalography.

The electrodes were connected by shielded cables to a high impedance electrometer, with wireless technology, to collect and send acquired signals from the plant in real time.

The telemetry system used had low energy consumption and high reception capability. Transceiver used was BIM2-433-160, which is bidirectional and allowed to transmit data up to 200 m. This transceiver was previously selected, based on the fact their consumption (20 mA at 3.0 V) is smaller, and its ability to transmit data at 64 kbps. The transceiver is connected to the microprocessor to manage digital communications using the RS232 protocol. The communication protocol was the Floating Base

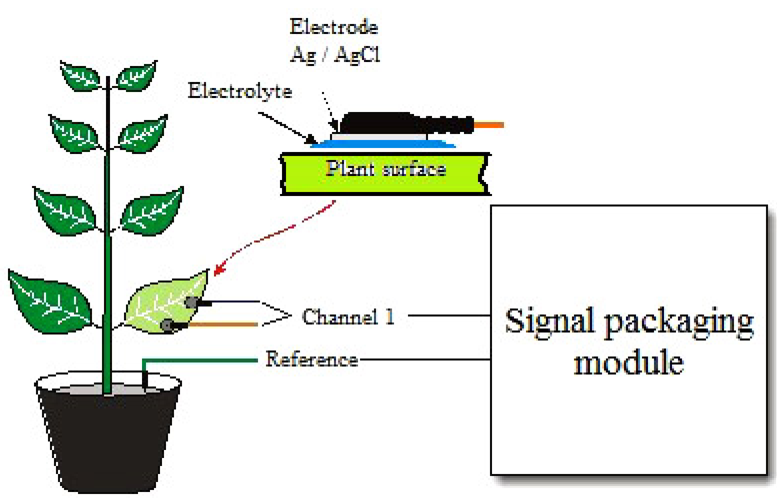

Figure 1. Electrodes location on plant surface to take bielectrical signals.

Sensor Network, described by Silva *et al*. (2005), and used wireless network.

To interpret the collected data, signal analysis methods were applied along with processing in Matlab® and were worked on the following models: Burg, Covariance, Modified Covariance, Thompson Multitaper, Periodogram, Yule-Walker and Welch.

The spectral analysis consisted of a standard tool in order to exploit the characteristics of a function (non-periodic) or practical terms of a time series. The periodgram is an expansion on sine and cosine of a time series sampled, and was obtained by applying the Fourier transform (Freitas, 1992). The signal collection was initiated after a noticeable morphological differentiation due to shade conditions (approximately four weeks). Previously, eight cycle of plants signal capture were performed to improve the method.

RESULTS AND DISCUSSION

In the Table are presented the results of foliar analysis for nitrogen content in Marandu grass (*Brachiaria*) and availability of lighting during experiment.

Fodder development falls with increasing of shading conditions. However, depending on the species, the higher feed yields can be checked in moderate shade conditions (Carvalho *et al*., 2001).

In this work, it is noted a higher rate of stretching in the leaves and narrowing of leaf surface of plants grown under more intense shade (50% light only) than in those grown in full sun and 70% of light; a fact similar to that seen by Paciullo *et al.,* 2008 in their work.

It has been found in *Brachiaria* a marked decrease in the rate of growth when the plants were subjected to severe shadowing (more than 50% light reduction) (Andrade *et al*., 2004). Increasing availability of various soil nutrients, under shading condition, may result in improvement of crude protein and mineral contents in the roughage, such as phosphorus, potassium and calcium, (Deinum *et al*., 1996) compared to full sun (Durr and Rangel, 2000).

Nitrogen (N) availability is important because is a dominant factor for controlling the processes of growth and development of the plant. It operates in the axillary buds formation and beginning of stems. Its deficit increases the dormant buds number, while adequate availability increases the stems number and allows maximum tillering (Nabinger and Medeiros, 1995).

According to Oliveira *et al.* (2007), nitrogen deficiency in Marandu grass can cause: reduced

Table. Foliar analysis results of Marandu grass (*Brachiaria*) and availability of lighting during experiment.

	N g/kg	100% (light)	70% (light)	50% (light)
Plastic pot 12	13, 69			X
Plastic pot 13	11,76		X	
Plastic pot 15	16,18			X
Plastic pot 20*	8,12	X		
Plastic pot 21	14,00	X		
Plastic pot 23	10,05		X	
Plastic pot 26	19,60	X		
Plastic pot 29*	8,51	X		

*These plastic pots were not receiving maintenance fertilization after each cut.

development in the plant, reduced tillering, early aging in the leaves, chlorosis (yellowing of the leaf blade) and reduction on values of nitrogen (N) in the aerial parts (13-20 g / kg). This study shows chlorotic leaves in plants where was not performed fertilizer maintenance, as well as less development of leaves than plants that received nutritional supplementation.

Moreover, plants which did not receive maintenance fertilization showed very low levels of N (+/- 8 g / kg). Although plants grown to 70% of light with maintenance fertilizer also showed N deficiency (< 13 g/kg).

The spectral analysis made from the Welch model, allowed grouping plants with frequency bands according to their culture conditions: plastic pots 12 and 17 (*Brachiaria brizantha* cv. Marandu grown under an avaliability of 50% light): 5 Hz to 10 Hz; plastic pots 23 and 25 (*Brachiaria brizantha* cv. Marandu grown under an availability of 70% light): 15 Hz to 20 Hz; plastic pots 21 and 26 (*Brachiaria brizantha* cv. Marandu grown under a 100% availability of light): 20 Hz to 40 Hz; plastic pots 20 and 29 (*Brachiaria brizantha* cv. Marandu grown under a 100% availability of light and without the maintenance fertilization): 15 Hz to 50 Hz.

Analysis made in plants grown under 100% light availability and without maintenance fertilization did not limit this group in a frequency range (Hz) as in the other groups of plants studied. Many are the hypotheses for this to have occurred by opening new thresholds for other studies.

An important part of the scientific support of this work is based on the digital processing of signals. This is nothing more than digital procedures, mathematics and algorithm manipulating those signals. The vast majority of them can be decomposed into a sum of frequencies; the frequency analysis of a particular signal involves resolution of its components, thus composing a frequency spectrum for the signal to be studied, is the process known as spectral analysis (Costa, 2006).

The Fourier transform is a basic transform that serves to support another processes, so it is consistent to say that it comes primarily from the issue plastic pots 20 and 29 (Marandu Grass grown under a 100% availability light and without the maintenance fertilization), and could use other more refined processing techniques as wavelets transform and Adaptive Gaussian Representation (AGR). The spectral analysis obtained by processing data is be displayed in the following figures.

Figures 2 and 3 show the plants spectra grown under a 50% availability of it light with maintenance fertilization.

Figures 4 and 5 show the plants spectra grown under a 70% availability of light with maintenance fertilization.

Figures 6 and 7 show the plants spectra grown under a 100% availability of light with maintenance fertilization.

Figures 8 and 9 show the plants spectra grown under a 100% availability of light without maintenance fertilization.

In accordance with Davies (1987), it is common the occurrence of action potentials in plants. This may be generated in response to light, as pointed out by Stolarek *et al*. (1984), and also due to heat, cold, chemicals, electrical stimulation and injury (Gradann and Mummert, 1980).

The generation and transmission of electrical signals are probably the initial response of plant to out side stimuli. During plant growth, the electrical signals migtht also shows different characteristics due to low light or high humidity. So there such electrical signals have potential for be used in diferent applications given, that indicates the physiological condition of the plant (Huang *et al*., 2009).

The conduction of electrical signals on specialized structures should be seen as a special and universal quality of living organisms, since any living cell receives information from its surroundings, and is appropriate to note that the surface of their membranes have numerous receptor proteins, which interact with virtually all vitaly important molecules (Volkov *et al*., 2000).

Gurovich and Hermosilla (2009) also reported that the effect of light and dark in the electrical excitation of the plasma membrane is related to the flow of H^+ ion during photosynthesis in plant cells.

The energy required for active transport is provided by ATP hydrolysis-In plants, the dependency was observed in potential membrane with respect

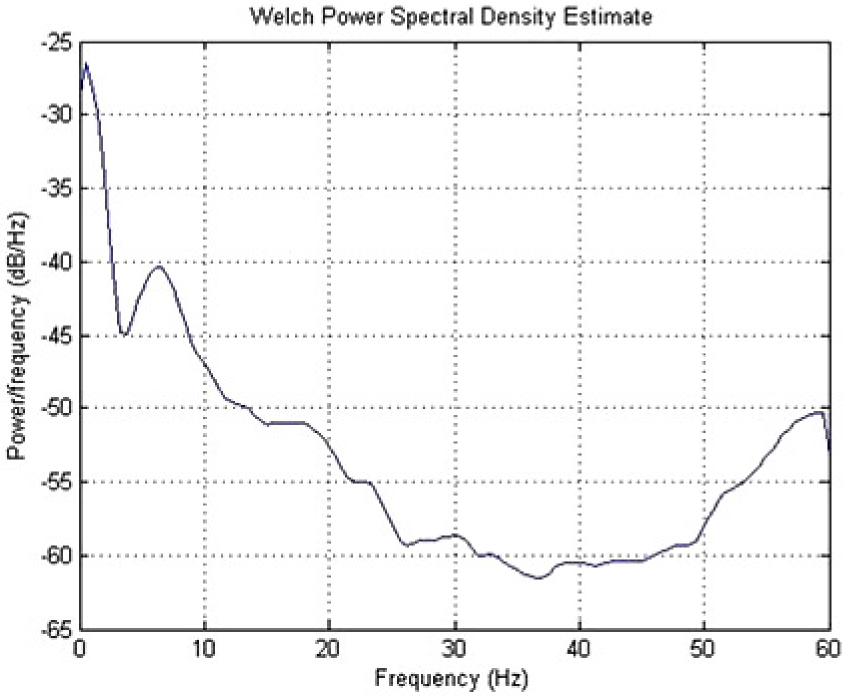

Figure 2. Plastic pot 12 - Amplitude spectrum calculated from the model Welch, Marandu grass (*Brachiaria brizantha)* grown under an availability of 50% of light.

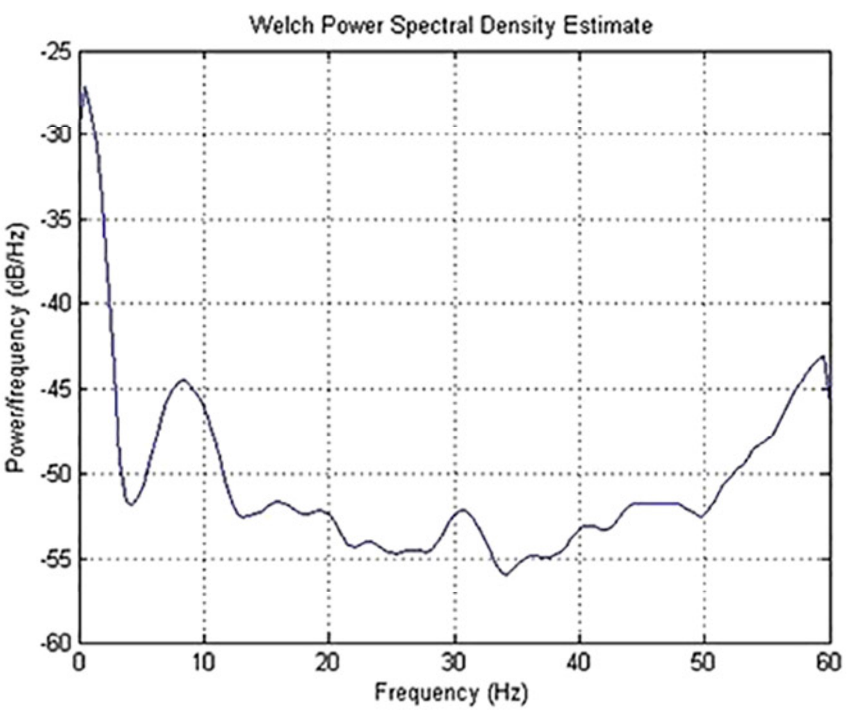

Figure 3. Plastic pot 17 - Amplitude spectrum calculated from the model Welch, Marandu grass (*Brachiaria brizantha)* grown under an availability of 50% of light.

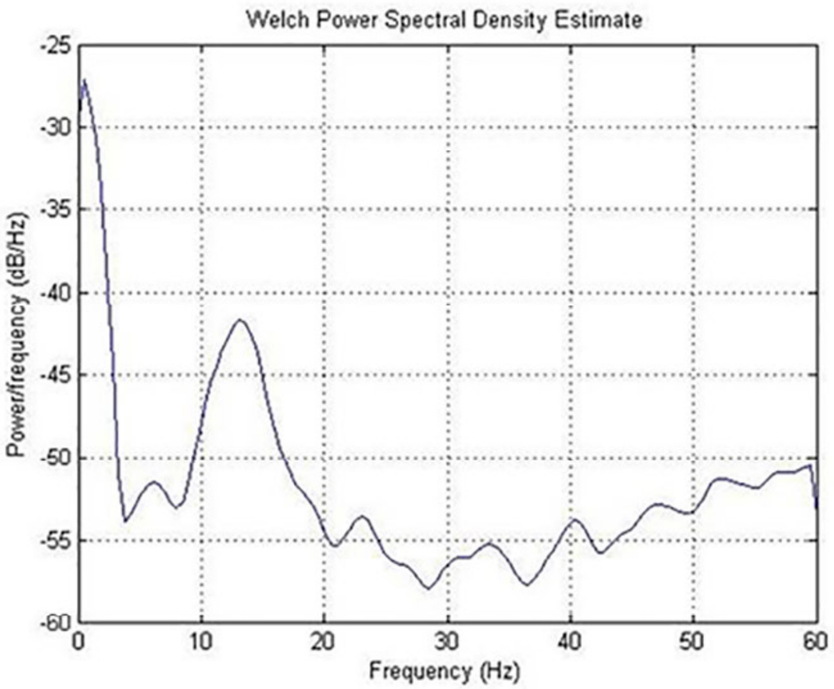

Figure 4. Plastic pot 23 - Amplitude spectrum calculated from the model Welch, Marandu grass (*Brachiaria brizantha)* grown under a 70% availability of light.

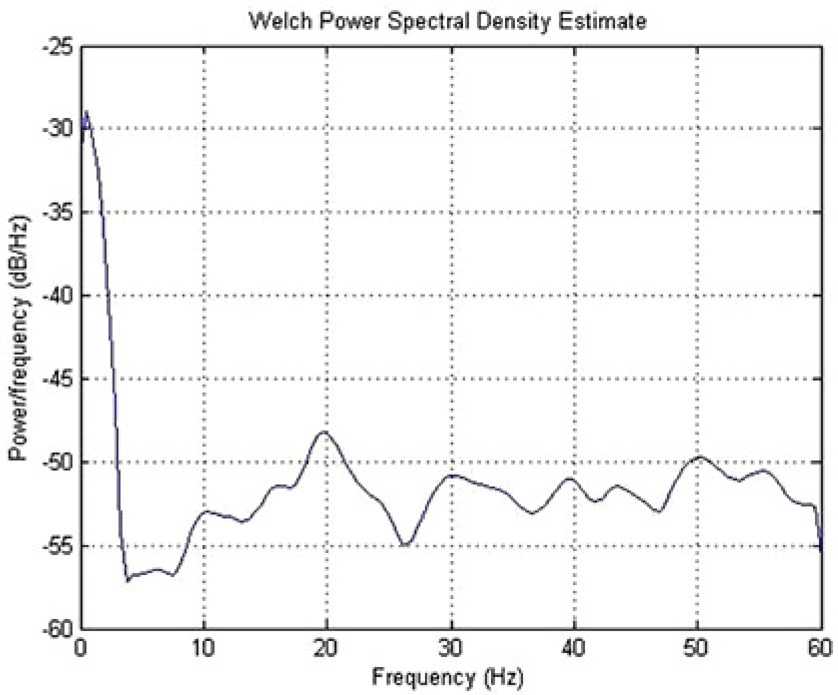

Figure 5. Plastic pot 13 - Amplitude spectrum calculated from the model Welch, Marandu grass (*Brachiaria brizantha)* grown under a 70% availability of light.

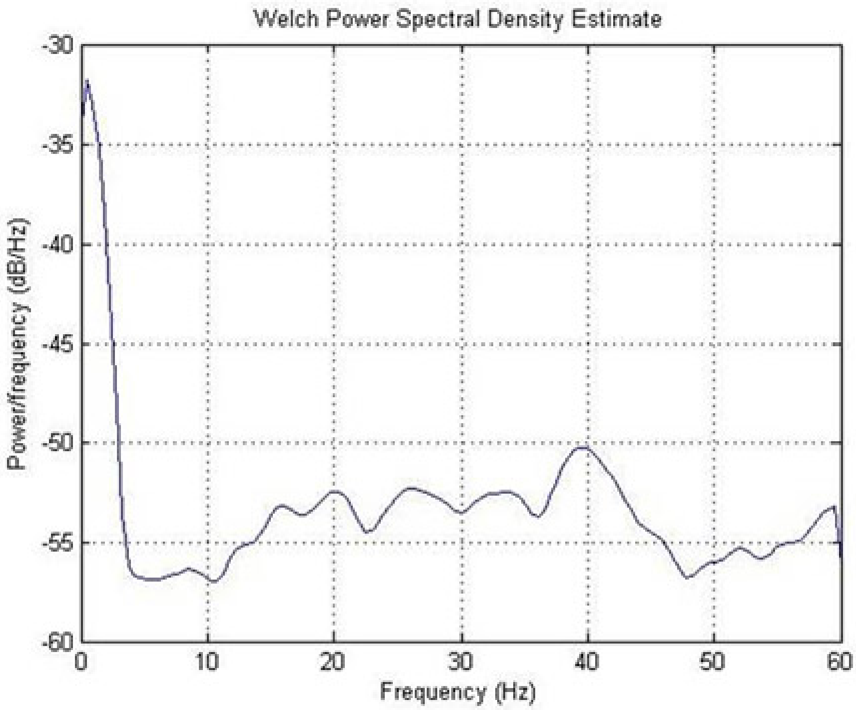

Figure 6. Plastic pot 26 - Amplitude spectrum calculated from the model Welch, Marandu grass (*Brachiaria brizantha)* grown under a 100% availability of light.

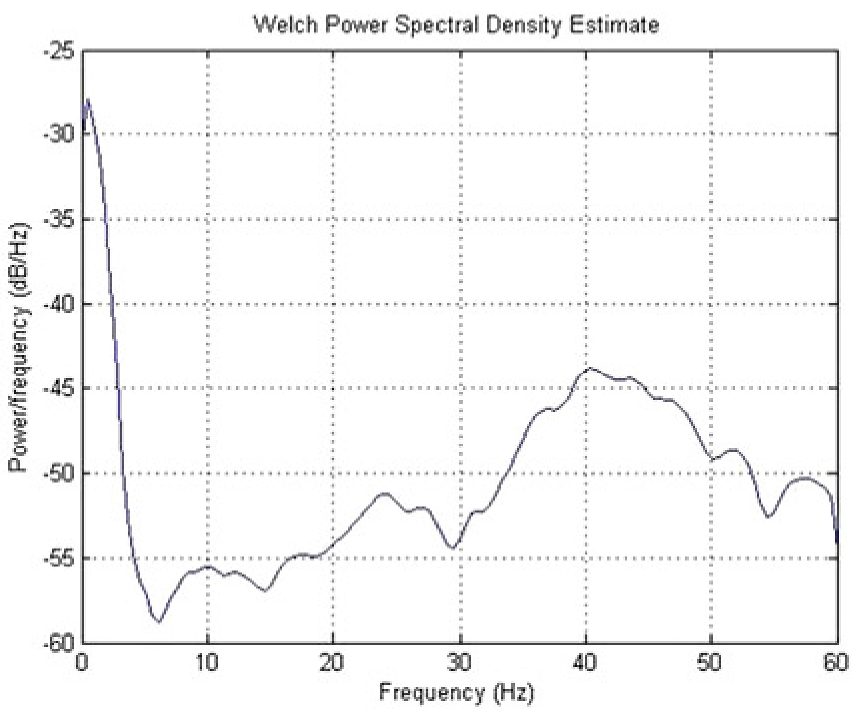

Figure 7. Plastic pot 21 - Amplitude spectrum calculated from the model Welch, Marandu grass (*Brachiaria brizantha)* grown under a 100% availability of light. (without fertilization).

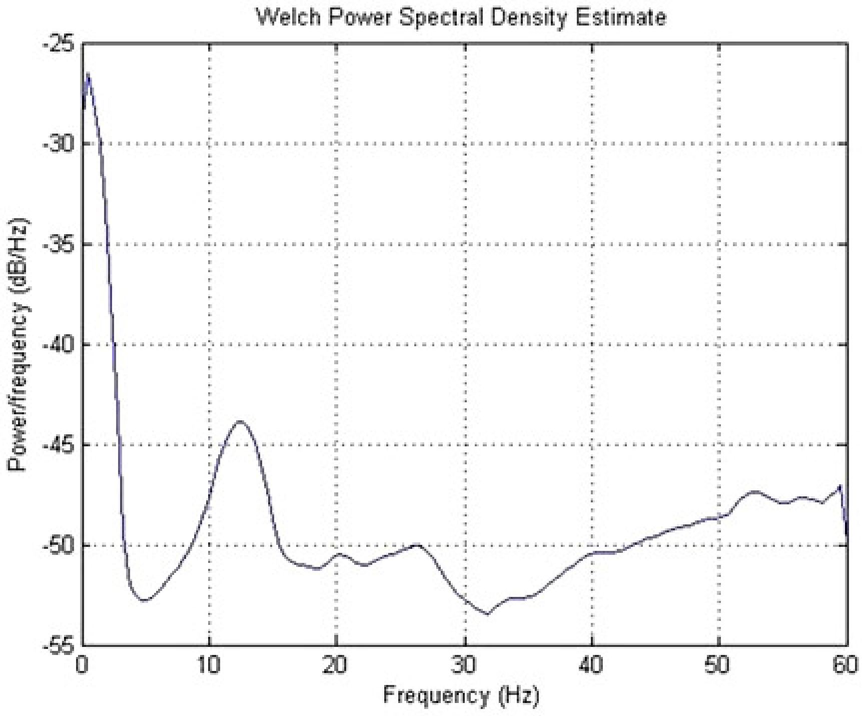

Figure 8. Plastic pot 20 - Amplitude spectrum calculated from the model Welch, Marandu grass (*Brachiaria brizantha)* grown under a 100% availability of light (without fertilization).

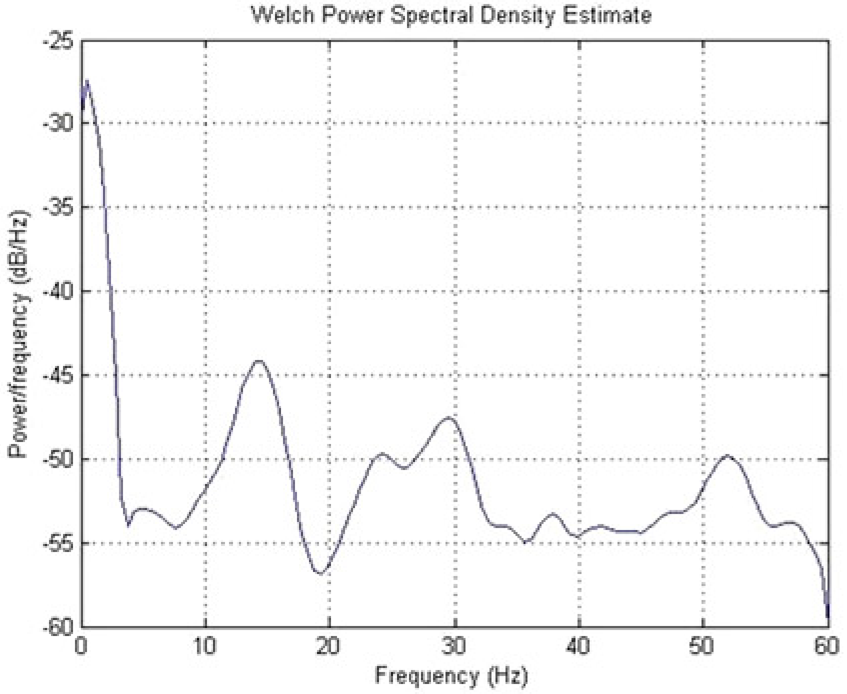

Figure 9. Plastic pot 29 - Amplitude spectrum calculated from the model Welch, Marandu grass (*Brachiaria brizantha)* grown under a 100% availability of light (without fertilization).

to ATP, by the effect of quickly mitochondria cyanide poisoning, that deplets the ATP. When the cyanide inhibit electrogenic ion transport, the pH of the external medium increase while the cytosol becomes acid due to the H+ ions that remains inside the cell. This fact demonstrated that the active transport of H+ out of the cell is electrogenic, that is to say able to generate an electric potential (Taiz and Zeiger, 2006).

Taken together, these results indicate that equipment used in this study showed efficacy to identify frequency ranges groups for cultivated plants in 100%, 70% and 50% light, nevertheless was not effective among cultivated plants in 100% light, without maintenance fertilization, after harvest. In view of these results it is required further studies. Note that frequency values obtained in this experiment should be considered like frequency bands and not single frequency.

CONCLUSION

It was demonstrated the capture of electrical signals in Marandu grass, growing freely and in distinct level of shading, through the use of electronic instrumentation techniques, as an important tool in the study of fodder physiology. However it is worth mentioning that this study is only the first step; more studies in plant electrophysiology, and deeper research in this field must be done. The Welch model was the most appropriate to calculate the spectral amplitude, because the frequencies observed showed characteristic peaks and were consistent with the literature and previous studies.

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